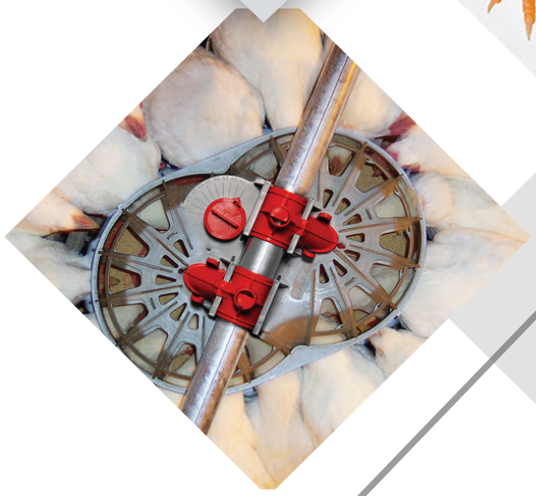
2018

INTERNATIONAL POULTRY SCIENTIFIC FORUM

JANUARY 29 – 30



ABSTRACTS
2018 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 29-30, 2018

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Milton Y Dendy Keynote Address

B-313 The Evolution of Broiler Production Programs since 1985 and Challenges in How Broilers Will Be Grown in Coming Years Michael Donohue, *Agri Stats, Inc.*

Broiler producers worldwide continue to benefit from annual gains in efficiencies in both broiler production and processing. These gains are a result of ongoing work in genetic selection programs, greater knowledge in poultry health management systems along with efforts to improve poultry housing and ventilation.

Some of the tools used to realize these gains are no longer available for use. Changes in consumer choices for the types of chicken they want to purchase demand that broiler producers adapt to the changes and adopt different management and feeding programs to meet these new requirements.

In broiler growout these changes include transitions in the use of many feed medications including antibiotics and ionophore coccidiostats, differences in the density of placement for birds and increased downtime between flocks in many operations. In the processing plant the importance of developing effective food safety programs to meet higher standards has led to changes in processing programs and in the products used to reduce the incidence of pathogens in the system.

Using industry data the magnitude of these changes will be explored with the goal of trying to understand in both production and economic terms the ongoing effects of industry evolution. Change is inevitable, how we respond to change is our option.

***Author presenting paper**

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Physiology, Endocrinology & Reproduction

M1 Effect of in ovo LAB and Gram-negative bacterial inoculation on the microbiome and GIT development of chicks Kim Wilson*[GS], Whitney Briggs, Audrey Duff, Kaylin Chasser, Liwen Zhang, Xiaolun Sun, Lisa Bielke *The Ohio State University-OARDC*

Improved GIT development of chicks may be influenced by exposure to bacteria during hatch. To promote early colonization of beneficial bacteria, *in ovo* administration is a potentially efficient means to influence pioneer colonizers of the GIT. Impact of different types of pioneer colonizers on the development of the microbiome community and GIT are not well-studied, especially direct comparison of Gram-negative and lactic acid producing (LAB) strains. Preliminary studies showed that exposure to *Klebsiella oxytoca* or a LAB-probiotic resulted in increased LAB recovery in the GIT of day of hatch (DOH) chicks on MRS agar. Therefore, it was hypothesized that *in ovo* inoculation by different species of bacteria may affect microbiota and tissue development of chicks. The objective of this study was to directly compare impact of two apathogenic Gram-negative isolates or a LAB-probiotic as pioneer colonizers on the microbiome and proteome of chicks. At ED18, embryos were inoculated with either saline (S), or ~ 10^2 CFU of *Citrobacter freundii* (C), *Klebsiella oxytoca* (K) or a LAB-probiotic (L). On DOH, the whole-GIT were collected and DNA from contents were processed for 2 x 300 paired-end Ilumina MiSeq for microbiome analysis. In addition, protein was extracted from the whole GIT and were subjected to a Fusion mass spectrometer and proteins were identified using the *Gallus gallus* reference in MASCOT. Ceca were collected at 3D and 10D for microbiome evaluation. Though richness and diversity did not differ between treatments ($p > 0.05$) at 3D and 10D, DOH host proteins were differentially expressed, particularly in stress-related proteins. There was a 0.4 fold down-regulation of HSP 70 and 3.4 fold up-regulation of peroxiredoxin in C compared to S ($p < 0.05$). In the K chicks, there was a 0.2 and 0.3 fold down-regulation of HSP 90 and heat shock cognate protein 90 compared to S ($p < 0.10$). Taken together, the opportunity to impact the bacterial community and GIT development is very early and different isolates may alter intestinal development before the chick leaves the hatcher and result in altered phenotypic changes involved in GIT growth and development.

Key Words: in-ovo, microbiome, proteome

M2 Blood physiology of broilers presented with breast myopathies and supplemented with dietary glutamine and arginine. Matthew Livingston*[GS], Peter Ferket, John Brake, Kimberly Livingston *North Carolina State University*

Wooden breast (WB) and white striping (WS) muscle myopathies are a *Pectoralis* major muscle condition found recently in broilers and has been characterized by degenerative necrosis, atrophy of individual muscle fibrils, and an infiltration of adipose tissue. It has been reported to occur in as much as 12% of commercial broiler flocks (Petracci et al., 2013). One causal theory is least-cost feed changes that include lower soybean meal and greater synthetic amino acid inclusions resulting in marginal dietary levels of conditionally essential amino acids such as glutamine and arginine. For this study, 2 levels of supplemental glutamine (0 and 1%) and 2 levels of arginine (1.25 and 1.5% of diet) were evaluated in a 2x2 factorial arrangement. Live production performance parameters including feed intake, BW, and mortality were analyzed using pen as the experimental unit. Blood chemistry (pH, pCO_2, pO_2, BEecf, HCO_3, tCO_2, sO_2, Na^+, K^+, iCa^{2+}, Glu, Hct, Hb) and carcass characteristics including WS and WB scores were determined on individual birds. Data were analyzed using the GLM procedure of SAS with means separated using the LS means procedure. Broilers fed supplemental glutamine exhibited greater BW (3.39 vs 3.20 kg; $P<0.001$) and improved FCR (1.66 vs 1.69 kg; $P<0.02$). Broilers fed supplemental arginine also exhibited greater BW (3.37 vs 3.23 kg; $P<0.001$) and improved FCR (1.66 vs 1.70; $P < 0.001$). However, supplemental glutamine also produced greater WS scores (2.64 vs 1.91; $P < 0.001$) and greater WB scores (1.83 vs 2.73; $P < 0.05$). Moreover, as the severity of WB and WS myopathies increased, blood HCO_3 levels increased ($P < 0.05$), hemoglobin increased ($P <0.05$), and oxygen saturation was numerically reduced ($P < 0.09$). These data suggested that myopathies may be associated with a physiological state of hypoxia. Broilers in this condition coupled with the availability of ketogenic amino acids (glutamine) and ATP demand may have resulted in the increased WS myopathy scores.

Key Words: Broiler Myopathies, Wooden Breast, White Striping, Glutamine, Arginine

M3 The effect of feed restriction on ghrelin concentration in male broiler chickens Nurudeen Taofeek*[GS], Fernando Vizcarra, Martha Verghese, Jorge Vizcarra *Alabama A & M University*

The role of ghrelin as a polypeptide of importance in the control of feed intake in male broiler breeders was evaluated in this study. Ten one-day old male broiler chicks were reared using normal feeding and lighting management as recommended by the industry. At 3 weeks of age birds were transferred to individual cages with continued free access to feed and water and a 23L:1D photoperiod. To facilitate continuous blood sampling, birds were catheterized in the jugular vein on experimental day 0 at a body weight of 2.0 ± 0.1 kg. Blood samples were collected at 1 h intervals for 6 h (time 1 to time 6) commencing the day after cannulation (d1) and on days 2, 3, 4, 5, 6 and 7 for a total of 42 samples/bird. On day 2, birds were placed in a feed restricted diet until the end of the experiment (d7). The restricted feeding regimen consisted of one-third of the quantity of feed consumed per day by birds during the week before cannulation. Restricted birds were fed immediately after the first blood sample was obtained (time 1). Concentrations of ghrelin were evaluated in plasma samples using a chicken ghrelin ELISA kit and concentrations of glucose were measured using a colorimetric assay. The effect of feed restriction on glucose and ghrelin concentration were analyzed using repeated measurements over time. The statistical model included the effect of day of treatment (d1 to d7), time within day (time 1 to time 6) and the day x time interaction. There was no day x time interaction for both glucose and ghrelin concentrations. There was a day ($P = 0.0392$) and time ($P = 0.0002$) effect on glucose concentrations. Concentrations of glucose decreased linearly from d1 to d7 and increased at time 2 and time 3 compared to time 1. Concentrations of ghrelin were increased on days 5, 6 and 7 as compared to day 1 ($P = 0.0016$). Taken together, feed restriction was associated with a normal physiological response to glucose concentrations. Ghrelin concentrations increased after four days of feed restriction and remain increased until the end of the experiment (d7). Our results indicate that concentrations of ghrelin are increased in feed restricted male broiler breeders.

Key Words: Ghrelin, Broilers, Restriction

M4 Effects of phytogenic feed additives on feed efficiency and hypothalamic neuropeptide gene expression in broilers Joshua Flees*[GS], Elizabeth Greene, Walter Bottje, Shivi Maini, Sami Dridi *University of Arkansas*

The use of phytogenic feed additives in animal nutrition is increasing due to prohibition of sub-therapeutic dosage of antibiotics. However their modes of action are not completely defined. The present study was undertaken to determine the effect of three phytogenic feed additives from Ayurvet Ltd. on broiler growth performance, feed efficiency (FE), and to identify their underlying molecular mechanisms. 384 healthy day-old broilers (Cobb500) were randomly assigned into 32 pens (12 chicks/pen) with 8 pens/treatment with *ad libitum* access to a corn-soy based diet. AV/HGR/16, Superliv concentrate premix, and AGR premix feed additives were mixed into the feed at 0.5kg/ton of feed at each starter, grower, and finisher phase of feeding with untreated pens used as control. Birds were grown under standard growing conditions for 42 d, individual body weights (BW) were recorded weekly, and feed intake (FI) was measured daily. At d 35, hypothalami were isolated from 1 bird per pen and RNA was isolated using TRIzol. RNA was then purified, quantified, and reverse transcribed into cDNA. Relative expression of target feeding-related hypothalamic genes was measured using real-time quantitative PCR. 6 birds per treatment were randomly selected for body composition analysis. Data were analyzed using a one-way ANOVA and means were compared by Tukey's multiple comparison test and considered significant at P<0.05. All treatments significantly reduced cumulative FI compared to the control group with no observed change in BW or body composition. The reduction in FI resulted in improved FE in the treated groups. Molecular analysis showed no significant differences in the expression of neuropeptide Y (NPY), agouti-related peptide (AgRP), pro-opiomelanocortin (POMC), cocaine and amphetamine regulated transcript (CART), or corticotrophin releasing hormone (CRH). This data indicates that these phytogenic feed additives can improve FE in broilers. The analysis of other neuropeptides associated with FI and energy homeostasis should be investigated to explain the changes in FE.

Key Words: growth performance, feed efficiency, phytogenic feed additives, neuropeptides

M5 Dietary supplementation with microalgae improves performance, serum composition, meat quality, antioxidant status and fatty acid profile of broilers Shenfei Long*[GS], Sheng Kang, Qianqian Wang, Yetong Xu, Long Pan, Jiangxu Hu, Xiangshu Piao *China Agricultural University*

Dietary intake of long chain (LC) n-3 polyunsaturated fatty acids (PUFA) is suboptimal in Western and Chinese diets. However, LC n-3 PUFA are crucial for human health. Manipulating fatty acid composition of poultry meat through altered fatty acid composition of broiler diets is considered to be an efficient approach to increase LC n-3 PUFA consumption in human diets. Microalgae (MA) contain a high concentration of docosahexaenoic acid (DHA) which may improve the deposition of PUFA in animal products. This experiment was conducted with 126 as-hatched male Arbor Acres chicks (1-d-old, weighing 45.3 ± 0.72 g) to determine the effect of MA on performance, serum composition, meat quality, antioxidant status and fatty acid deposition of birds. The birds were randomly allocated to 1 of 3 treatments with 7 replicate pens per treatment (6 birds per pen). The dietary treatments included a control diet [corn-soybean basal diet supplemented with 3% soybean oil (SO), CON], 1% MA diet (basal diet supplemented with 1% MA and 2% SO, 1MA), and 2% MA diet (basal diet supplemented with 2% MA and 1% SO, 2MA). Data was subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1996). Pen was the experimental unit. Differences among treatments were separated by Duncan's multiple range test. Significance was designated at $P < 0.05$. Compared with CON, birds supplemented with MA (1MA or 2MA) had greater ($P < 0.05$) average daily gain, feed efficiency, liver index (liver weight / body weight), serum glucose and lower ($P < 0.05$) abdominal fat index (abdominal fat weight / body weight) and total serum cholesterol. Moreover, MA increased ($P < 0.05$) concentration of eicosapentaenoic acid, DHA, superoxide dismutase, and total antioxidant capacity, and decreased ($P < 0.05$) n-6 PUFA to n-3 PUFA ratio, polyunsaturated fatty acid to saturated fatty acid ratio, and malondialdehyde in the breast and thigh muscle of birds compared with CON. In conclusion, dietary supplementation with 1% or 2% microalgae had positive effects on performance, serum composition, meat quality, antioxidant status and fatty acid profile in birds.

Key Words: microalgae, performance, antioxidant, DHA, broiler

M6 Live pigeon pox virus increases parthenogenesis of in vitro cultured Chinese painted quail eggs Reshma Ramachandran*[GS], Christopher McDaniel *Mississippi State University*

Avian parthenogenesis (PG), also known as 'virgin birth', is mostly an unorganized and abortive form of embryonic development that occurs without fertilization. Previously, *in vivo* exposure of poultry to live viruses increased the incidence of PG and parthenogen size. However, the mechanisms by which viruses enhance PG in poultry are unknown. Hence, the objective of this study was to determine the effect on PG of live pigeon pox virus directly administered over the germinal disc (GD) of *in vitro* cultured Chinese painted quail eggs. In this study, 151 eggs from 18 virgin hens, genetically selected for PG, were stored for 0-3 d at 20C. Albumen pH and GD size were measured before and after yolks were *in vitro* cultured for 48h at an incubation temperature of 37.5C. Eggs were macroscopically classified as initially exhibiting PG or containing no embryonic development before incubation as well as after incubation. Prior to incubation, GD were either treated with 50µl of commercially available live pigeon pox virus vaccine or not treated (control). Data were analyzed as a randomized complete block design with hen as block and a 2 (eggs initially with PG development and without embryonic development) x 2 (virus treated and control) factorial arrangement of treatments. Initial embryonic development and virus treatments interacted such that in eggs initially exhibiting PG, treatment with virus yielded a greater incidence of PG at 48h (P = 0.003), GD size at 48h (P = 0.04), and GD growth over incubation (P = 0.03) as opposed to eggs without initial development and virus treated or eggs not exposed to virus. In fact, for eggs that initially exhibited 100% PG development before incubation, virus treatment maintained embryo detectability after incubation at 91% as opposed to 50% for eggs not virus treated. Additionally, an interaction for albumen pH change over incubation also revealed that virus treated eggs with initial PG development exhibited the least increase in pH over time (P = 0.09) as compared to all other treatments. This low albumen pH was likely due to CO_2 production by viable parthenogens. In conclusion, it appears that live pigeon pox virus is capable of enhancing parthenogen liveability and increasing parthenogen size in eggs that initially exhibit PG.

Key Words: Parthenogenesis, poxvirus, invitroculture, quail, embryo

Processing & Products

M7 Instrumental texture analysis of chicken patties elaborated with broiler breast fillets exhibiting Woody Breast characteristics Juan Caldas-Cueva*[GS], Casey Owens *University of Arkansas*

Potential applications of chicken meat with woody breast (WB) condition in further processing products could provide processors alternatives to face this meat quality problem. The objective of this study was to evaluate the effect of the use of broiler breast fillets at varying degrees of WB severity on instrumental texture characteristics of chicken patties. A total of 54 breast fillets were collected from birds processed according to commercial practices and classified based on tactile evaluation in three WB categories (0 or 0.5 as normal-NOR; 1 or 1.5 as mild-MID, and 2, 2.5 or 3 as severe-SEV). Instrumental compression analysis was performed to validate subjective scores. Nine treatments with six replicates of chicken patties were prepared: 100% NOR (T1), 66.67% NOR + 33.33% MID (T2), 66.67% NOR + 33.33% SEV (T3), 33.33% NOR + 66.67% MID (T4), 33.33% NOR + 66.67% SEV (T5), 100% MID (T6), 66.67% MID + 33.33% SEV (T7), 33.33% MID + 66.67% SEV (T8), and 100% SEV (T9). Chicken breast muscles (cranial region) were ground (3-mm plate), kneaded, formed (diameter: 87 mm, thickness: 15 mm; 85 g), vacuum packed (stored at -22°C), and grilled (core temperature reached 75°C). Texture profile analysis (TPA: hardness, cohesiveness, springiness, and chewiness) was performed using a texture analyzer (TA.XT *Plus*, Texture Technologies Corp.). Additionally, cooking loss, reduction in diameter and thickness were evaluated in cooked patties. Data were analyzed using an ANOVA with treatment factor fit as fixed effects. With exception to T1, T2, and T4, hardness, springiness, and chewiness of chicken patties decreased (P<0.05) as WB severity increased in the meat added to the mixture. Hardness, chewiness, and cooking loss presented a clear difference (P<0.05) among T1 (100% NOR), T6 (100% MID) and T9 (100% SEV). Cohesiveness, springiness and reduction in diameter values varied (P<0.05) between the extreme treatments T1 and T9. These data indicate the use of broiler breast fillets with WB condition modifies significantly (P<0.05) instrumental texture characteristics of cooked chicken patties. However, the use of mixtures of chicken meat exhibiting normal and mild levels of WB severity could be considered by processors as an alternative in commercial chicken patty formulations.

Key Words: wooden breast, texture profile analysis, processing, poultry products, meat quality

M8 Meat quality attributes associated with Woody Breast and effect of location and freezing on fillet hardness Barbara A. Mallmann*[GS], Ashunti Jackson, Andy Mauromoustakos, Pramir Maharjan, Craig Coon, Casey Owens *University of Arkansas*

Woody breast(WB) is a major myopathy in broilers characterized by hardness of the breast fillet and can be evaluated by human palpation with a severity scale of 0(normal) to 3(severe). The objective of this study was to determine instrumental and meat quality factors that are associated with WB scores that may potentially be used in sorting programs. Additionally, this study was to determine if there is a location effect (breast side) or effect of freezing on compression force(CF) of fillets. After commercial style processing and deboning(3 h postmortem), 207 breast fillets were collected and scored for WB. Thickness and length (cranial, caudal, and keel regions) of the *Pectoralis major* were measured with a caliper. CF was measured using Texture Analyzer in four regions at the cranial part of the fillet on both the right(RS) and left(LS) sides. Color and pH were analyzed on the LS of the breast. The RS frozen at -20C for 48h and thawed for 24 h, and then CF was measured, along with cook loss, MORS, and BMORS. Pearson correlation coefficients and ordinal logistic regression were used. Paired t-tests were utilized to address the effect of side and effect of freezing on CF. Measurement responses were compared for the four categories of WB(0=normal,1=mild,2=moderate, and 3=severe) using Fit Model with JMP. The length measurements on the breast showed no difference (*P>0.05*) and small correlation. However, the thickness is correlated moderately(r=0.69) and could differentiate between the scores. A thicker breast denotes a higher severity of WB. In addition, CF of LS and RS sides of the breast fillet were significantly different(P<0.01), with the RS of the breast showing higher force. Freezing significantly decreased(*P<0.05*) CF of thawed fillets compared to pre-frozen fillets. Cook loss increased as severity for WB increased(*P<0.05*). Peak counts for MORS and BMORS were higher for the severe compared to the others(*P<0.05*). In conclusion, the thickness of the breast fillet may potentially be used for sorting purposes, possibly in combination with other sorting criteria. Freezing the breast meat improves the softness of the WB and differences exist between right and left fillets.

Key Words: Woody breast, Compression force, fillet thickness, location, freezing

M9 The effect of multiple levels Arginine and/ or vitamin C on performance, meat yield and incidence of white striping and woody breast when supplemented in combination with low digestible amino acid grower diets. Brooke Bodle*[GS], Yves Mercier, Rob Shirley, Jason Lee, Christine Alvarado *Texas A&M University*

Prior research indicates feeding vitamin C, a higher ratio of dArg: dLys or a grower diet with a lower digestible AA (dAA) content can potentially mitigate the presence and severity of white striping (WS) and woody breast (WB) in high-yielding broilers. The objective of the current study was to define the optimal level of dArg: dLys and vitamin C that could alter the incidence and severity of WS and WB. A total of 2,240 broilers were distributed between 7 treatments consisted of 10 replicate pens with 32 broilers per replicate. Relative to a commercial-type Control diet (Trt 1) that contained a four-phase program (starter, grower, finisher, and withdraw), the following nutritional strategies were investigated: Trt 2. Reduce the dAA in only the grower phase by 15%, and feed the same starter, finisher, and withdraw diets as in Trt 1; Trt 3. As 2, with a dArg: dLys of 124%; Trt 4. As 2, with a dArg: dLys of 136%; Trt 5. As 2, with 100 ppm Vitamin C; Trt 6. As 2, with 200 ppm Vitamin C; Trt 7. As 2, with 124 dArg: dLys and 100 ppm of Vitamin C. Performance was measured on days 15, 29, 43, and 50. On d 51, 9 birds per pen were processed to obtain boneless, skinless breast meat yields and breast fillets were examine for the incidence and severity of WS and WB. Comparing the Trts 1 and 2, reducing the amino acid density in the grower phase negatively impacted BW and FCR ($p < 0.011$). At the end of the growout (d 50), no differences in BW were observed ($p = 0.547$); however, the two supplemental dArg levels and the highest vitamin C level gave the best reduction in FCR when compared to Trts 1 and 2. No differences were observed in *Pectoralis major* weights ($p = 0.232$) or yield (as a % of live weight; $p = 0.149$). Compared to Trt 1, the WS score was reduced by 13 and 9% in Trts 3 and 7, respectively (p=0.011). Similarly, the WB score was reduced by 19 and 18% in Trts 3 and 7, respectively (p=0.006). While the reduction in dAA in the grower phase had no effect on WS or WB, increasing the dArg: dLys through L-Arg supplementation did increase the proportion of 0/1 scores ($p = 0.049$). These data suggest that nutritional strategies can be potentially used to mitigate the severity of WS and WB in high-yielding broilers while maintaining performance.

Key Words: Broiler, Arginine, Yield, Breast

M10 Application of advanced technologies to analyze modern meat quality issues, such as Woody Breast Avery Smith*[GS], Laura Bauermeister, Meredith Johnson, Ronald Beyers, Amit Morey *Auburn University*

Recently developed meat quality problems in broilers, such as Woody Breast, highlight the lack of quantifiable, nondestructive research techniques that can be used to effectively analyze emerging issues. Research

was conducted to determine the application of highly advanced technologies, such as Magnetic Resonance Imaging (MRI), as accurate and exploratory quality measurement method for not only Woody Breast, but also future meat quality issues.

A pilot study was performed using a severely affected woody filet and a non-affected filet. These were scanned using a 7-Tesla Siemens MRI device for 45 minutes. Filets were scanned at every 4mm and images were generated using T1 and T2 spin relaxation times. Images were enhanced using ImageJ and showed visible differences between woody and normal filets. T1 relaxation times were significantly different for woody and non-woody filets at a significance level of $p<0.05$. The success of this study prompted further investigation to increase sample sizes and examine mildly affected filets. Three filets were selected as representatives of non-woody, mildly woody, and severely woody breasts and scanned using the methodology given above. A total of 9 samples were analyzed during the study. Significant differences in T1 and T2 relaxation times between different woody breast severities was determined using ANOVA with Tukey's LSD at $p<0.05$.

Pilot study data indicated that average T1 relaxation time of woody breast was 845.88 ms, while the average T1 for the normal filet was 932.79 ms. These results demonstrate the potential to use highly advanced technologies, such as MRI, to study meat quality.

Key Words: Woody Breast, Magnetic Resonance Imaging, meat quality, MRI, Wooden Breast

M11 Investigating a Bioactive Ingredient with the Potential to Reduce Wooden Breast Toughness during Further Processing Meredith Johnson*[GS], Avery Smith, Jasmine Kataria, Ivey Wise, Amit Morey *Auburn University*

The poultry industry is facing a meat quality issue known as wooden breast (WB) which renders the meat tough in texture and leads to consumer complaints and economic losses. The poultry industry does not have any solution to improve the texture of WB meat and utilize it in whole muscle products. Therefore, research was conducted to determine the effect of adding external proteolytic enzyme to improve WB fillet texture.

Severe WB fillets were obtained from a local processor. Fillets (n=5 per trial; 3 trials) were grinded separately and divided into 25-gram containers. A commercially available proteolytic enzyme was dissolved in water to obtain a concentration of 2.5 mg/mL, 5 mg/mL and 10 mg/mL from which 1 mL was incorporated in ground WB samples (n=3/treatment x 3 trials). Ground WB without treatment and added with 1 mL of water served as control samples. All the samples were placed at 4C for 24 h and were frozen at -80°C. Ground WB samples (n=3/trial) were also frozen immediately upon grinding to determine initial collagen content. Samples were analyzed for total, soluble, and insoluble collagen content. Data was analyzed using one-way ANOVA with Tukey's LSD to determine significant differences.

Data indicated that the total, soluble, and insoluble collagen content in wooden fillets was 19.5, 4.9, and 14.6 mg/g, respectively. Insoluble collagen content reduced significantly to 5.8 mg/g after enzyme treatment (10 mg/mL). Thus, there is potential to improve texture of WB meat using proteolytic enzymes and help the poultry industry to utilize WB meat.

Key Words: protease, collagen, Wooden Breast, texture

M12 Acoustic Signatures of Woody Breast: Converting Woody Breast Scans into Sound as a Simple Diagnostic Tool Divya Srinivasan*[UG], Amit Morey *Johns Creek High School*

Woody breast is a novel meat quality issue leading to significant consumer complaints. One of the methods to reduce the incidence of woody breast is to detect and remove the affected fillets from the processing line. At present, hand-palpation is the standard method used to detect woody breast in the industry, however advanced methods such as ultrasound, elastography and MRI, generally used for tumor detection, are being studied at Auburn University. The images and scans obtained from the highly sophisticated methods are difficult to read and analyze, limiting the methods to lab scale. In order to make these techniques available in the field, there is a need to simplify the analysis of the images and make instant decisions.

For the High School Science Fair Project, a research was conducted to determine if ultrasound, elastography and MRI scans can be converted to sound and that the breast fillets with varying woody breast severities have different acoustic signatures.

Freshly deboned broiler (8-9 lbs live wt.) breast fillets with varying degree of woody breast severities were procured from a commercial slaughter facility. Each fillet was scored as below: 0-normal fillet; 1- mildly woody; 2-medium woody and 3-severe woody. Samples were subjected to ultrasound and elastography analysis at Auburn University Veterinary Clinic. Scans from each fillets were imported to a freely available software called PhotoSounder (1.9.0 Demo) and translated into sound frequency profile of each image and flipped 270˚. Sound patterns were generated for each fillet scan and heard to detect differences.

The sound frequency profile of normal and severe woody breast fillets exhibited distinct patterns. Sound patterns generated from the various woody breast scan indicated audible differences. The novel proof-of-concept research can be used as a simple tool in addition to advanced technologies to detect woody breast by field personnel without significant training.

Key Words: Elastography, Woody Breast, Sound, Data, Detection

M13 "Functional Ice", a Novel Sustained Antimicrobial Release Mechanism, to Improve Salmonella food safety of raw poultry parts during storage Jasmine Kataria*[GS], Meredith Johnson, Avery Smith, Laura Bauermeister, Amit Morey *Auburn University*

Antimicrobials used during poultry processing do not suppress *Salmonella* during storage and transportation thus potentially increasing food safety risks. There is a need to develop novel methods to deliver antimicrobials to ensure safety of raw poultry during storage and transportation. A novel sustained antimicrobial delivery mechanism termed "Functional Ice" (FICE) (Patent Pending) was developed and validated against *Salmonella* on raw chicken thighs during storage.

Aqueous solutions of sodium tripolyphosphate (2.5% and 5% w/v) and sodium lactate-sodium diacetate (1% and 2.5% v/v) were frozen to make FICE while tap water ice acted as a control. Nalidixic acid resistant (35µg/ml) *Salmonella* Typhimurium suspensions were inoculated (100 uL) on raw boneless, skinless thigh meat pieces, allowed 1 h attachment time (final inoculum 10^{6-7} CFU/sample), placed in FICE treatments and sampled at 0, 12, 24, 36 and 48 h. Samples (n=3/sampling point) were individually rinsed (1 min) with 30 ml of phosphate buffer saline solution, rinsates were serially diluted, spread plated on XLT4 with nalidixic acid (35µg/mL) and incubated at 42˚C for 24-32 h. Viable *Salmonella* colonies were reported as log CFU/mL of rinsate. Significant differences among treatments were determined using ANOVA with Tukey's LSD at $p<0.05$.

Salmonella levels were reduced ($p<0.05$) by >1 log within 12 h by FICE made with STPP 2.5 and 5% compared to ice during the 48 h refrigerated storage. FICE made with sodium lactate-sodium diacetate treatments were not as effective as STPP in reducing *Salmonella*. FICE demonstrated the potential to improve food safety during raw poultry storage.

Key Words: Functional Ice, Salmonella, storage, transportation, poultry

M14 The antimicrobial effects of Defenstat™ against Salmonella Typhimurium on boneless skinless ground chicken breast Clarissa Harris*[GS], Sally Williams *University of Florida*

One of the most effective approaches for reducing consumers' risk of foodborne illness is to treat the processed poultry products with antimicrobials. Biological interventions are efficient solutions for reducing foodborne illnesses. Acetic acid (vinegar) is generally recognized as safe (GRAS) and

is a potent antimicrobial that has been used to control *Salmonella* contamination in meat and poultry products. Defenstat™ is a proprietary blend of vinegar (source of acetic acid) and natural flavorings with applications in the meat and poultry industry. The experimental approach in this study evaluated the effects of Defenstat™ against *Salmonella* Typhimurium on boneless, skinless, ground chicken breast. Except for the negative control, the ground chicken meat was inoculated with *Salmonella* Typhimurium (ATCC 13311) and it was treated with Defenstat™. The five treatments for the chicken meat included no treatment (meat only negative control), meat plus inoculum (positive control), and treatments 1%, 2%, and 3% Defenstat™ added to the total weight of the meat. The ground chicken meat was packaged in sterile Whirl-Pak bags, stored at 3±1˚C and analyzed on days 0,1,3,5, and 7 for *Salmonella*, psychrotrophs, and pH. Compared to the control, the ground chicken meat treated with 1%, 2%, and 3% Defenstat™ resulted in a significant reduction in *Salmonella* Typhimurium on day 7 for 1% (1.14 log reduction), 2% (1.12 log reduction), and 3% (1.24 log reduction). There was also a significant reduction in psychrotrophs on day 7 with 1% (2.21 log reduction), 2% (1.86 log reduction) and 3% (1.26 log reduction) compared to the control. The results indicate that adding the Defenstat™ to the chicken will retard the growth of *Salmonella* Typhimurium and psychrotrophs over time. Defenstat™ has the potential to increase the shelf life of a poultry product without affecting the pH.

Key Words: Defenstat™, Salmonella

M15 Evaluation of the efficacy of electrochemically activated water against Salmonella Heidelberg isolates Daiane Wilsmann*[GS], Daiane Carvalho, Abrahão Martins, Gabriela Zottis, Thales Furian, Vladimir Nascimento *Federal University of Rio Grande do Sul*

Salmonella spp. is among the microorganisms of greatest concern in poultry and public health. According to 2017 data from the European Union Rapid Alert System for Animal Food and Feed (RASFF), *S.* Heidelberg was the most isolated serovar in poultry meat and poultry meat products exported to the EU until October 2017, representing more than 12% of all Salmonella isolates during the above mentioned period. Electrochemically activated water (EA) is a technology that makes it possible to produce a biocide from water, salt and electricity. Through membranes electrolysis, EA generators produce free radicals, hypochlorous acid and free chlorine, which are considered non-toxic and biodegradable compounds. In this context, the objective of this study was to evaluate the efficacy of EA against 30 *S.* Heidelberg isolates of avian origin. Concentration of 50 ppm of free chlorine was used at 4°C with the contact times of five and fourth minutes (simulating chiller conditions) and 200 ppm at 25º C with the contact time of ten minutes (simulating disinfection conditions) were tested. The viable cells count was performed using the dilution technique and Drop-Plate counting. The data were analyzed by T student test (PASW Statistics 18).The mean bacterial count of the treatment at 50 ppm with five minutes contact was 2.6 x 10^4 CFU / mL, and at fourth minutes obtained a count of 1.1 x 10^4 CFU / mL ($p<0,05$). The treatment at 200 ppm with 10 minutes contact reduced totally the bacterial count on 26 isolates. In four samples the mean bacterial counts showed a significant difference between treatments of 200 ppm for 10 minutes (1.3 x 10^2 CFU / mL) when compared to the control group (7.2 x 10^4 UFC / mL). These data show that a longer contact time reduces the microbial load demonstrating the importance of the permanence time of the microorganism in contact with the EA. All evaluated treatments resulted in a decrease in the mean bacterial count of the 30 strains of *Salmonella* Heidelberg. It was concluded that, in general, 50 and 200 ppm EA was efficient against S. Heidelberg, showing that this product has an antimicrobial potential to be used at the food industry.

Key Words: SalmonellaHeidelberg, ElectrochemicallyActivatedWater, Poultry

M16 Evaluation of poultry water treatments during feed and water withdrawal on water usage and Salmonella prevalence in broilers Caitlin Harris*[GS], Ming Teo, Larry Lu, Connie Mou, Kevin Gottilla, L Bartenfeld, Dianna Bourassa, Brian Fairchild, Brian Kiepper, R Buhr *University of Georgia*

Acidic water treatments during feed and water withdrawal were evaluated as a potential preharvest *Salmonella* intervention. The hypothesis for the study was the addition of acidified water treatments during feed and water withdrawal should impact the recovery of *Salmonella* from broiler crops and ceca. Previous data indicates that adding 50 ppm hydrogen peroxide in combination with citric acid (pH 5.0 or 6.2) to drinker lines during feed and water withdrawal may have depressed the number of *Salmonella* positive crops and ceca. On day 1, 6 wk old broilers were challenged with 1.0 mL of 10^8 CFU *Salmonella* Typhimurium marker strain, distributed into 12 pens (12 broilers/pen, 144 total), and provided feed and water *ab libitum*. On day 3 post challenge, pens were connected to carboys containing treatment or control (tap) water. Treatments included: hydrogen peroxide (50 ppm) pH adjusted to 5.0 or 6.2 with citric acid and sodium bisulfate (pH 3.2). Feed consumption and water usage were monitored by weight at beginning and end of trial. The 3 pens/treatment were assigned to one of the following feed/water withdrawal times: 12 h feed/ 6 h water withdrawal, 6 h feed/ 0 h water withdrawal, or 0 h feed/ 0 h water withdrawal. On day 6 after feed/water withdrawal, 6 broilers/pen were euthanized and crops and ceca were aseptically collected for microbiological analysis. Crops and ceca with buffered peptone water were stomached and the rinsate streaked onto brilliant green sulfur agar plates with nalidixic acid (200 µg/mL); plates and samples were incubated at 37°C for 24 h. Plates were restreaked from enriched rinsate if direct plating was negative then incubated. One-way ANOVA was used for analysis of water usage and feed consumption data and no significant differences were found. For *Salmonella* data, Fisher's exact test was used for statistical analysis and the water treatments did not differ significantly from the control for both crops (65% +) and ceca (79% +). There was significantly lower Salmonella recovered for enriched crops between full fed vs. 12 h ($P=0.0032$) and 6 h ($P=0.0010$) feed withdrawal. Results indicated that the acidic water treatments did not impact feed consumption, water usage, or *Salmonella* recovery from the crops or ceca after feed and water withdrawal.

Key Words: Salmonella, broilers, water, withdrawal

Metabolism & Nutrition - Enzymes

M17 Effects of mineral level, nutrient density, and phytase level on broiler performance and breast meat yield Austin Jasek*[GS], Kyle Smith, Jon Broomhead, Jason Lee *Texas A&M University*

The objective of the current study was to evaluate the impact of mineral level, nutrient density, and phytase concentration on male broiler performance and breast meat yield. A total of 2,304 Ross 708 male broilers were randomly assigned to 8 dietary treatments consisting of 8 replicate pens of 36 broilers each. The experiment was a 2 x 2 x 2 factorial design that included two nutrient densities (control and reduced density with a reduction of 2, 4, and 6% amino acids and 1, 2, and 3% in energy for the starter, grower, and finishers, respectively), two levels of phytase (1000 and 4,500 FTU/kg), and two mineral levels (control and reduced by 0.15% P and 0.12% Ca). Birds were fed a crumbled starter (d 0-14) and a pelleted grower (d 14-28), finisher (d 28-41), and finisher2 (d 41-47). Average body weight (BW), mortality corrected feed conversion ratio (FCR), and feed consumption (FC) were determined on d 14, 28, 41, and 47. FCR was also adjusted (aFCR) to a common BW through d 41 and 47. On d 48, 5 birds from each replicate were randomly selected for evaluation of carcass and breast yield. Data were analyzed via a 2 x 2 x 2 factorial ANOVA with main effect means deemed significantly different at $P \leq$

0.05. Through grower and finisher periods, reducing nutrient density decreased BW (P<0.001), increasing phytase level increased BW (P<0.001), while reducing mineral level only reduced BW at d 28 (P<0.05). Feed consumption was influenced by mineral and phytase level as the reduction in minerals reduced (P<0.05) FC and increasing phytase level increased FC (P<0.01). The decrease in BW associated with reduced nutrient density also negatively influenced carcass and breast weight (P<0.05). Overall and d 41 aFCR was negatively (P<0.05) influenced with the reduction in nutrient density but was improved (P<0.05) by reducing minerals or increasing phytase level. Reducing the mineral content improved FCR and aFCR in the control density diet but not in the reduced density diet resulting in a mineral by density interaction (P<0.05). These data demonstrate the interactions with mineral level, phytase supplementation, and nutrient density of the diet and the importance to consider all factors when formulating diets.

Key Words: Broilers, Phytase, Nutrients, Minerals, Yield

M18 Effects of energy density and enzyme inclusion strategy on 0-60 d Ross x Ross 708 male broiler performance, foot pad dermatitis, woody breast severity, and breast yield. Andrew Brown*[GS], Omar Gutierrez, Mark Lemons, Staci Loop, Kelley Wamsley *Mississippi State University*

Research on the impact of exogenous enzymes and their inclusion strategies in diets of differing nutrient densities on performance and carcass attributes is limited. Therefore, the objective of the current study was to examine the effects of varying energy density (ED) with phytase (FTU/kg) and xylanase (EPU/kg) at different inclusion rates on d 0-59 broiler performance, foot pad dermatitis (FPD), woody breast severity (WBS), and % breast yield. This study used a 2 (ED) x 3 (Enzyme Inclusion Strategy; EIS) factorial arrangement of treatments within a RCBD. Diet ED consisted of high or low, relative to breeder recommendations. One of three EIS were used: 1) No Enzyme (NE), 2) 1500 FTU/kg+1500 EPU/kg or 3) 1500 FTU/kg+3000 EPU/kg. A total of 1,200 Ross x Ross 708 male broilers were obtained from a commercial hatchery and equally allocated to 60 pens (10 replications/treatment). Birds were individually weighed and feed intake was recorded on d 14, 28, 46, and 59 for calculating average BW, BW gain, bird uniformity, feed intake/bird (FI), % mortality, and feed conversion ratio (FCR). On d 59, 4 birds/pen were selected for d 60 % breast yield relative to carcass weight (BY), FPD, and WBS. Overall data (d 0-59) demonstrated that broilers fed HD diets consumed 64 g less feed (P<0.01). Differences were shown for ED and EIS for d 0-59 FCR (P<0.05); broilers fed HD diets had an 18 pt reduction in FCR as compared to LD and broilers fed EIS2 had a 4 pt reduction in FCR in comparison to birds receiving NE. Also, ED significantly affected FPD (P<0.01) with broilers fed HD demonstrating a lower incidence of FPD on d 60. On d 60, WBS (P<0.05) was impacted by main effects of ED and EIS. Broilers receiving HD demonstrated lower WBS likely attributed to lower BY due to decreased FI. Birds fed EIS3 exhibited the lowest WBS in comparison to birds receiving EIS2; broilers fed NE were intermediate. An interaction between ED and EIS was also found; broilers had similar d 60 BY, except for broilers fed HD+EIS3, which resulted in a lower BY, this could be due to a decrease in FI as compared to LD+EIS3 (P=0.02). These data suggest that EIS will lower FCR and WBS, regardless of ED of diet. Use of EIS3 may further decrease WBS; however, BY may also be decreased, depending upon ED.

Key Words: Exogenous Enzymes, Energy Density, Broiler Performance, Woody Breast

M19 Evaluation of multiple calcium and phosphorus levels, nutrient density, and phytase concentration on male broiler performance and breast meat yield Kyle Smith*[GS], Austin Jasek, Jon Broomhead, Jason Lee *Texas A&M University*

The design of the current study was a 3x2x2 factorial to evaluate the impact of 2 levels of phytase (1,000 and 4500 FTU/kg) with 3 levels of nutrient densities (A, B, and C), and 2 levels of minerals (control and reduced by 0.15% P and 0.12% Ca) on performance and breast yield. Nutrient density A was formulated as an industry type diet, where Basal B had a 2, 3, and 4% reduction in amino acids (AA) and 1, 2, and 3% reduction in energy in the starter, grower, and finisher, respectively. Basal C had a 2, 4, and 6% reduction in AA in the starter, grower, and finisher and the same reductions in energy as Basal B. Each treatment included 9 replicate pens of 18 Ross 708 male broilers per pen. The dietary program consisted of 4 phases: starter d 1-14, grower d 15-28, finisher d 29-42, and finisher2 d 43-49. Broilers were weighed and feed consumption determined on d 14, 28, 42 and 49. On d 50, 5 broilers per replicate pen were processed to determine breast yield. Throughout the trial, significant two and three way interactions were observed in all evaluated parameters. Body weight (BW) and mortality corrected feed conversion ratio (FCR) was negatively impacted with reductions in nutrient density (main effect; P<0.05). Elevating phytase level increased d 1-28 and 1-49 feed consumption and BW, but decreased FCR (main effect; P<0.05). Improved FCR and BW with elevated levels of phytase were more pronounced in broilers fed the control vs. reduced mineral level resulting in a phytase by mineral interaction (P<0.05). Overall, reducing minerals resulted in an increased d 28, 42, and 49 BW (main effect; P<0.05), however this was not notable in Basal A resulting in mineral by density interaction (P<0.05). Mineral reduction reduced breast weight in Basal A and increased breast weight in Basal B and C resulting in a density by mineral interaction (P<0.05). Elevating phytase level increased breast weight overall (main effect; P<0.05) and was more notable in higher density diets (density by phytase interaction, P<0.05), which may be related to nutrient (feed) consumption. These data demonstrate the complex relationship between phytase level, nutrient density, and mineral level and that all need to be considered to maximize growth performance and breast yield.

Key Words: Phytase, Amino-Acids, Energy, Minerals, Broiler

M20 Effects of carbohydrase supplementation on growth performance in male broilers fed reduced energy diets Mallori Williams*[GS], Hunter Walters, Roshan Adhikari, Alfredo Manon, Jason Lee *Texas A&M AgriLife Research*

The objective of the current study was to evaluate the impact of supplementing alpha-galactosidase in diets containing variable energy levels fed to male broilers. The experimental design consisted of a 2 x 2 factorial arrangement of energy level (control or reduced) and enzyme inclusion (with and without alpha-galactosidase) resulting in a total of 4 dietary treatments with 10 replicates per treatment and 25 birds per replicate for a total of 1,000 Ross 708 males. Birds were fed a corn-soybean meal diet with the low energy level diet formulated to be a reduction of 88 kcal/kg in metabolizable energy (ME) compared to the control diet. Average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption, and mortality (%) were determined on days 14, 28, 35, and 41. Data was analyzed via a 2 x 2 factorial ANOVA with main effect means being deemed significantly different at P≤0.05. Although energy level and enzyme inclusion did not influence BW throughout the trial, flock uniformity as measured by coefficient of variation within each replicate was decreased (P=0.05) in the reduced energy diet. Feed consumption and FCR were impacted by both factors, energy level and enzyme presence. Reduced energy levels consistently increased feed consumption throughout the trial with significance being detected during the grower period and cumulatively through 28 days. Similar trends were observed with enzyme supplementation yielding a constant reduction in feed consumption with significance being observed during the starter phase. Impacts on feed consumption resulted in differences in FCR with both energy level and enzyme supplementation influencing (P<0.05) FCR during the starter, grower, and cumulatively through d 28, 35, and 41. Reduced energy levels increased (P<0.05) feed consumption resulting in a higher FCR compared to the control with enzyme supplementation reducing feed consumption and improving FCR (P<0.05). These data indicate that performance im-

provement associated with alpha-galactosidase supplementation is not dependent on dietary energy level.

Key Words: broiler, performance, low-energy, carbohydrase

M21 Meta-analysis of the effect of phytases on intestinal histomorphology of broilers. Diego Martinez*[GS], Carlos Vilchez *La Molina National Agrarian University*

The objective was to determine the overall effect size (OES) of phytase on morphometric characteristics of intestinal villus and crypts of broilers. Scientific papers including villus height (VH), crypt depth (CD) and villus to crypt ratio (VCR) as response variables in broilers fed phytases were collected. A pool from 2004 to 2016 was considered including 148 registries, 2704 intestinal samples, and more than 25,746 histological lectures. Nine independent meta-analysis (MA) were conducted to test the effect of the negative control diet (NCD) with lower dietary available phosphorus content on VH (MA1), CD (MA2) and VCR (MA3), and the effects of phytases when included in the diet using a nutritional matrix value on VH (MA4), CD (MA5) and VCR (MA6), or fed on-top without reformulating the diet, also on VH (MA7), CD (MA8) and VCR (MA9). The MA were performed with the Metafor package in R version 3.4.2 and heterogeneities (H^G) were quantified using random-effects models, by the test of homogeneity, the between-study variance (τ^2), and the H2 statistic. 95% confidence intervals (CI) are presented. Results showed that NCD significantly reduced the VH (OES -48.2 µm; CI -74.4 to -22.0; 5.7% lower; P<0.001) and VCR (OES -0.26; CI -0.45 to -0.07; 4.4% lower; P<0.001), but no effect was seen on CD (P>0.39). Phytases applied using nutritional matrix values produced no effect on VH, CD or VCR (P>0.40). The on-top application of phytases showed a positive effect on CD (OES -4.2 µm; CI -8.3 to -0.2; 2.7% lower; P<0.05) and VCR (OES +0.18; CI +0.05 to +0.30; 2.6% higher; P<0.01). Significative H^G were found (P<0.01) and factors identified responsible were: for the effect of NCD on VH were the reduction in available phosphorus (RAP) and the number of birds sampled (NBS) (P<0.01) (73% H^G); for NCD on VCR was RAP (P<0.001) (100% H^G); and for the addition of the phytase on-top on CD and on VCR were bird age (P<0.02) (17% H^G) and NBS (P<0.02) (10% H^G), respectively. Phytases doses (500-4000 FTU/kg) did not explain the H^G among studies on CD (P>0.60) or VCR (P>0.20). In conclusion, NCD affects negatively the VH and VCR. Adding phytases applying a nutritional matrix has no effect on the studied variables; however, if added on-top, positive effects on CD and VCR were found.

Key Words: meta-analysis, phytase, intestinal-morphometry, villus-to-crypt-ratio

M22 The effects of phytase super-dosing in combination with xylanase on the ileal microbial profile of market turkey hens Marissa Herchler*[GS], Samantha Black, Matthew Warren, Ramon Malheiros, Peter Ferket *North Carolina State University*

Xylanase and phytase are commonly used to reduce animal performance variation and improve nutrient utilization, but little is known about their effects on the microbial profile of the gut. A market turkey trial was conducted to evaluate the effects of phytase (Phy) super dosing in combination with xylanase (Xyl) on the microbial profile in the gut. Nicholas Super Select® turkey hen poults were randomly assigned among 16 litter floor pens containing 36 poults, each according to a randomized complete block design. Four dietary treatments were manufactured consisting of a positive control (PC) or a negative control (NC) basal diet. These basal diets differed by 0.145%aP, 0.125%Ca, and 100kcal ME/kg. The two enzyme diets consisted of the NC basal diet with differing enzyme levels: the standard enzyme levels (ST: 250 FTU Phy/kg, 1500 EPU Xyl/kg) or the super-dosed phytase level (SD: 1500 FTU Phy/kg, 1500 EPU Xyl/kg). Birds were sampled for relative abundance (RA) of the microbial profile in the ileum digesta at wk 14. During the overall production phase (0 to 14wk), body weight (BW) was measured and used to calculate feed conversion (FCR), as well as breast muscle yield (BMY) as a percentage of BW. At wk14, the microbial profile of PC showed greater RA of lactobacillus (LAC) than NC (91 vs 41%); however, NC showed a greater diversity than PC. The RA of LAC increased in ST (57%) and was further increased by SD (71%). BW was improved (p=0.03) by ST and SD as compared to PC (9.26 to 9.55 and 9.56kg), but was not different from NC. FCR was unaffected by the dietary treatments (avg FCR 2.29). BMY was increased (p=0.01) by ST and further increased by SD as compared to PC (23.8 to 24.8 and 25.1%), but ST was not different from NC. Increased RA of LAC in PC may be due to increased dietary energy content, allowing more available calories for LAC fermentation than in NC. In contrast, decreased nutrient density of the NC diet allowed other organisms to compete with LAC, thus increasing the microbial population diversity in the ileum. Increased nutrient availability due to enzyme inclusion in ST and SD apparently allowed birds to allocate more resources to growth and muscle development and favored a shift toward increased RA of LAC in the ileum as observed in the PC treatment.

Key Words: market, turkey, xylanase, phytase, microbiome

M23 Assessment of a superdosage of phytase (Natuphos E) on broiler performance fed a reduced calcium, available phosphorus and metabolizable energy diet Jinquan Wang*[GS], Mike Coelho, Arnulf Troescher, Peter Ader, Woo Kim *University of Georgia*

Recent studies showed that supplementation of phytase beyond standard doses could furthermore reduce the anti-nutrient effects of phytate, break down most of the phytate and release energy from the inositol ring. An experiment was conducted to evaluate the effect of a superdosage of Natuphos E on broiler performance fed a reduced calcium, available phosphorus and metabolizable energy (ME) diet. A total of 1150 one-day old Cobb 500 male broilers were randomly allocated into 5 dietary treatments with 10 replicates and 23 birds each replicate. Dietary treatments consisted of positive control (PC) with all nutrients meet or exceed Cobb 500 nutrition manual; negative control (NC) with 90 kcal/kg metabolizable energy (ME), 0.15% calcium and available phosphorus reduction from PC; standard phytase dose control with 1000 FTU/kg Natuphos E supplementation on the top of NC; reduced ME control with 290 kcal/kg ME reduction from PC; superdosing of Natuphos E with a dietary supplementation of 3000 FTU/kg Natuphos E on the top of reduced ME control. Feed intake, body weight, and feed conversion ratio were recorded at d 14, 28, 42. For the results, ME reduced control diet decreased (p<0.05) body weight and bodyweight gain compared to PC at d 0-14, d 15-28, d 0-28, d 29-42 and d 0-42 period. Supplementation of 3000 FTU/kg Natuphos E increased (p<0.05) the body weight and bodyweight gain compared to the ME reduced control diet and reached (d 0-14, d 29-42 and d 0-42) or exceeded (d 15-28 and d 0-28, p<0.05) the level of PC. ME reduced control diet cause an increase (p<0.05) in feed conversion ratio compared to PC. Supplementation of 3000 FTU/kg Natuphos E decreased (p<0.05) feed conversion ratio to the same level with PC. In conclusion, supplementation of 3000 FTU/kg Natuphos E could compensate, on the performance wise, the reduction of 0.15 % calcium, 0.15% available phosphorus and 290 Kcal/kg metabolizable energy in the diet.

Key Words: phytase, phosphorus, energy, performance, broiler

M24 Evaluating the effect of feeding starter crumbles on the overall performance of broilers raised for forty-two (42) days Frank Idan*[GS], Chad Paulk, Charles Stark *Kansas State University*

The physical form of feed affects the growth rate and feed consumption of broilers. A 42-d experiment was conducted to determine the effects of starter feed form on the growth performance of broilers. A total of 900 1-day-old male broilers (Cobb 500; initial BW 38 g) were randomly assigned to floor pens with 25 birds per pen. Pens were randomly assigned to 1 of 4 dietary treatments within location block to provide 12 pens per treatment. Birds were fed dietary treatments during the starter phase for 21d followed by a common mash diet from 21 to 42d. Treatments were: 1) 7 d crumbles then 14 d mash (7C), 2) 14 d crumbles then 7 d mash (14C),

3) 21 d crumbles and 4) 21 d mash. Starter, grower, and finisher diets were formulated to meet or exceed the NRC recommendations. Pelleted treatments were conditioned to 80^{0}C for 45 seconds, pelleted and crumbled. Pen body weight (BW) and feed intake (FI) were measured and used to calculate FCR on d 7, 14, 21 and 42. The GLMMIX procedure of SAS (SAS Institute Inc., NC) was used to analyze the data and mean differences were separated by Tukey's Test at (P<0.05). Overall the BW, ADG and ADFI were increased (P<0.05) in birds fed crumble diets compared to mash diets. The BW and FCR at 21 d were 903 g, 975 g, 968 g and 1007 g and 1.25, 1.21, 1.22 and 1.21 for M, 7C, 14C and 21C, respectively. At 42 d, the BW and FCR were 2963 g, 3114 g, 3131 g and 3140 g and 1.53, 1.50, 1.51 and 1.50 for M, 7C, 14C and 21C respectively. From the data, maximum improvement in BW and FCR at 21 d and 42 d were observed in birds fed 21C. The ADFI from the current study were (50 g, 53 g, 54 g, 55 g) at 21 d and (106 g, 109 g, 111 g, 109 g at 42 d for M, 7C, 14C and 21C respectively. As expected the ADFI was significantly decreased (P < 0.05) in broilers fed mash diet compared to those fed the crumble diet from 21 to 42 d. However, the highest ADFI was recorded in 21C birds at 42 d. The data from the current study suggest that feeding broiler starters crumbles improved BW, ADG, ADFI and FCR as compared to feeding mash diets. However, the best performance in absolute terms was obtained when crumbles were fed for 21 d.

Key Words: Pellet, Crumble, Feed form, Broiler, Body weight

M25 The effects of dietary lysine level with and without added protease on growth performance of broiler chickens. Haley Wecker*[UG], Caitlin Evans, R Beyer, Charles Stark, Miguel Barrios, Chad Paulk *Kansas State University*

The objective of this experiment was to determine the effects of added protease to diets with varying levels of standardized ileal digestible (SID) lysine on the growth performance of broiler chicks. A total of 480 chicks (Cobb 500; initial BW 40.7 g) were housed in 4 Petersime batteries and used in a 20 day study. Treatments consisted of a 2 × 4 factorial with main effects of protease (0 or 125 g/MT) and 4 different levels of SID lysine (1.12, 1.15 1.18, 1.21%). Treatments were randomly assigned to 80 cages within location block resulting in 10 cages per treatment with 6 chicks per cage at placement. Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS analyzing for interactive and main effects of Lys and protease. Increasing Lys level linear and quadratic polynomials were used for main effects of Lys. Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$. From 0-14 d and 0-20 d, there were no Lys × protease interactions or Lys or protease main effects for ADG and ADFI. There was a Lys × protease interaction (quadratic, $P < 0.047$) for FCR. Chicks fed 1.12 and 1.21% SID lysine diets with added protease had improved FCR compared to chicks fed these diets without protease. There was no difference in FCR between birds fed diets with or without the protease when fed 1.15 and 1.18% SID Lys diets. There was no Lys × protease interaction for d 14 BW. Chicks fed increasing levels of SID Lys had increased (linear, $P < 0.014$) d 14 BW. Chicks fed diets with protease had marginally significant increased ($P < 0.096$) ADG and d 14 BW. Increasing the level of lysine in diets improved FCR; however, the rate of improvement was dependent on the inclusion of an exogenous protease.

Key Words: Broilers, Protease, SID Lysine

Pathology

M26 Effects of a water applied biopromotor and feed applied MOS on the prevalence of Colibacillosis and egg production in commercial heavy breeders Carlos Oliveira*, Justino Hernandez, Rodrigo Morales, Eduardo Camacho, Sherryll Layton *BV Science/ Vetanco Mexico*

Commercial poultry production farms are under increasing pressure to reduce the use of antibiotics while maintaining health standards and pathogen control. Currently, there is an increased need for the development of effective products that serve as alternatives to antibiotics. We have developed a water applied biopromoter through careful selection of different types of prebiotics strategies (inactivated fermented *Bacillus subtilis* fragments and yeast cell wall extracts) that improves mucosal integrity and controls gram negative pathogens, while increasing productivity parameters and health status of the birds. A commercial poultry farm (Ross 508 heavy breeders), Cuenavaca, Mexico, with a historical problem of increased mortality, increased diarrhea and reduced egg production attributed to Colibacillosis beginning at week 28 was divided into two treatment groups (n=2 houses). House 1 (n=20000 heavy breeders) served as the untreated control and was fed a commercial basal diet without AGP and House 2 (n=20000 heavy breeders) was treated with 2 doses of the biopromoter (0.2ml/bird) in the drinking water at week 24 and 18 days later and MOS incorporated into the standard commercial basal diet (2kg/ton) free of AGP during weeks 25-34. Results show significantly increased mortality in the untreated house beginning at 28 weeks and continuing until 34 weeks, comparable with historical farm data, while birds in the treated house presented normal mortality consistent with stress associated during the peak of production. Microbiological analysis confirmed that the increased mortality in the untreated house was consistent with Colibacillosis. Through peak of production the treated house achieved 84.48% production while the control house achieved a maximum production of 67.03%. Analysis further showed egg production in the treated house was significantly higher (n=899,230) when compared to the untreated control house (n= 497,425). These data indicate that treatment with two doses of the biopromoter and temporary incorporation of MOS into the commercial basal diet can moderate the effects of *E. coli* in heavy breeders. Furthermore, this strategic approach of combining prebiotics provides an excellent alternative intervention strategy to chemotherapeutic agents.

Key Words: Biopromotor, Colibacillosis, Breeders, MOS

M27 Efficiency of a carvacrol formulation at liberation delayed on Campylobacter jejuni: in vitro in vivo and electronic microscopy approaches Marion Allaoua[1], Elsa Bonnafe[2], Virginie Noirot[1], Pierre Etienne[1], Jean Francois Gabarrou*[1], Michel Treilhou[2] *[1]Laboratoires Phodé; [2]University Champolion*

Campylobacter is well known as the leading cause of foodborne diarrheal disease worldwide, with *Campylobacter jejuni* and *Campylobacter coli* representing the most frequently involved species. The main source of infection is the meat from poultry origin mostly contaminated during evisceration. Thus, reducing *Campylobacter* concentrations in the intestinal tract and particularly in the caeca may help decrease flesh colonization, and in this way reducing human infections by the bacteria. Some natural substances have interesting antimicrobial properties. Studies have for example reported the antibacterial effect of carvacrol against *Campylobacter*.

As essential oils compounds are often absorbed before they reach the last part of the intestinal tract, they do not get to the site of *Campylobacter* growth. A new galenic formulation (Phodé Sciences, France) has been created to resolve this issue. This product contains a liquid formulated core based on carvacrol, and a specific solid carrier.

1- In the present study, we compared the efficacy of carvacrol and the formulated carvacrol, against *Campylobacter jejuni* ATCC 33291 using a broth microdilution method. The new formulation of carvacrol has the same efficacy as carvacrol alone (P>0,05).

2- We also compared the mechanism of action of both products by Scanning and Transmission Electron Microscopy. The new galenic formulation still showed the same results as pure carvacrol. Treated cells showed wrinkles, clefts and blisters. We also noticed, large membrane blebs caused by separation of the plasma membrane from outer membrane, with leakage of the cytoplasmic content into the intermembrane space.

3- We then undertook a follow-up of carvacrol in the digestive tract of chickens to confirm that our galenic formulation allowed delayed release of carvacrol in the caeca. The majority of the administered carvacrol was found in the caeca, the colon and the droppings (at least 85%).

These results suggest that the new galenic formulation of carvacrol permit to vectorise the active compound into the caeca and allow a reduction of Campylobacter contamination.

The next step of our study will be to test the new formulation on chicken *in vivo*.

Key Words: Campylobacter, broiler, caeca, carvacrol

M28 The role of enteric inflammation and loose stool on the rate of Histomonas meleagridis transmission Katherine Cupo*[GS], Jason Payne, Robert Beckstead *North Carolina State University*

Histomonas meleagridis, the causative agent of blackhead disease, is a protozoan parasite that colonizes the ceca of turkeys and spreads to the liver causing infectious enterohepatitis. Turkeys are capable of laterally transmitting the protozoan directly between birds by means of cloacal uptake of contaminated feces. In turkeys, flock mortality associated with blackhead disease is primarily less than 10%; however, in some cases, entire flocks succumb to the disease. Though blackhead disease has been correlated with diarrhea, poor sanitation, and secondary pathogens, research has not demonstrated why these factors cause variation in flock mortality. To identify factors that may affect the rate of blackhead disease transmission between birds, a lateral transmission trial was performed. 396 day-old poults were placed on clean shavings in 2ftx2ftx1.5ft battery cages with 9 birds per pen and 7 pens per treatment. Treatments consisted of a standard turkey starter ration (diet 1), diet 1 supplemented with MgCl2 or MgSO4, diet 1 with an oral administration of 0.45g dextran sulphate 3 and 4 days post inoculation, diet 1 formulated with coarse corn and diet 2 which was formulated with excess sodium and an amino acid imbalance. These treatments were designed to increase fecal moisture (MgCl2, MgSO4, diet 2), stimulate reverse peristalsis (coarse corn), or induce enteric inflammation (dextran sulphate). The infection rate for directly infected birds were 74% diet 1, 83% diet 1 MgCl2, 76% diet 1 MgSO4, 94% diet 1 dextran sulphate, 83% coarse corn, and 94% diet 2. Diet 1 and diet 1 MgSO4 rates of infection were statistically different from diet 1 dextran sulphate and diet 2. The rate of lateral transmission between birds were 0% diet 1, 4% diet 1 MgCl2, 4% diet 1 MgSO4, 8% diet 1 dextran sulphate, 8% coarse corn, and 21% diet 2. The rate of lateral transmission was statistically different between diet 1 and diet 2. This data suggests that enteric inflammation or loosening of the stool affect the rate at which birds contract blackhead disease after a direct inoculation. Further research is needed to better understand the relation between intestinal health and the transmission of blackhead disease.

Key Words: Histomonas meleagridis, blackhead disease, transmission

M29 Rescue and establishment of chicken models for spontaneously occurring Hashimoto's thyroiditis and systemic sclerosis/scleroderma Joseph Hiltz*[GS], Gisela Erf, Sara Orlowski, Nick Anthony *Department of Poultry Science, Division of Agriculture, University of Arkansas*

Chickens selected for spontaneous and predictable development of autoimmune disease have contributed to the understanding of complex, noncommunicable diseases. Two such research lines include the Obese strain (OS) developed at Cornell University and the UCD-200 originating from the University of California Davis. The OS is valued for studying spontaneously occurring Hashimoto's thyroiditis. The UCD-200 chicken line is the only model for spontaneously occurring fibrotic disease (systemic sclerosis/scleroderma) with similar symptoms as those observed for humans. Following their establishment as biomedical research models at Medical Schools in Austria (Innsbruck) and Sweden (Uppsala), US maintenance of the OS and UCD-200 lines was discontinued. In 2015, urgent requests were sent from both Innsbruck and Uppsala to adopt and rescue these valuable animal models at the University of Arkansas. A relocation plan was implemented which included importing pedigreed hatching eggs. The eleven chicks hatched from the UCD line originated from 4 unrelated sires and 4 unrelated dams. The OS line hatched 36 chicks from 10 unrelated sires and 11 unrelated dams. A minimum of 90 healthy viable birds per line were generated from these parents. Purity of the offspring was characterized by MHC-typing with OS being *B*-13 and the UCD segregating for *B*-2 and *B*-15. To examine whether the lines retained all of their unique autoimmune disease characteristics, we characterized the disease incidence, time-course of expression, severity and immunopathology. The UCD line has good fitness and clearly shows the self-dubbing phenotype predominantly in males. Although the OS phenotype is clearly observed, the OS line is very difficult to manage and reproduce. It is unclear whether the observed fertility issues are due to males only or both genders. Future matings will combine phenotypic and molecular assessment with careful mating structure to ensure sustainable breeding populations of both avian models.

Funding: Arkansas Biosciences Institute

Key Words: autoimmune scleroderma, Hashimoto's autoimmune thyroiditis, genetic selection, conservation genetics

M30 Evaluation of a water applied biopromotor to improve production status and control Salmonella spp in commercial broiler chickens Igor Praxedes-Campagnoni*[GS], Fabrizio Matte, Fabio Gazoni, Felipe Chiarelli, Sherryll Layton *University of Santa Catarina State, Department of Animal Sciences*

Currently, the world's population is more conscious than ever about diseases associated with animal production especially foodborne pathogens that can directly affect public health. There has long been a need to reduce Salmonella contamination in commercial poultry production while maintaining favorable production parameters. We have developed a water applied additive biopromotor composed of inactivated bacillus strains, selected for their ability to produce molecules which improve mucosal integrity and reduce gram negative bacteria, and yeast cell wall extracts as an alternative strategy for controlling foodborne pathogens and improving production parameters. The biopromotor was evaluated in two separate large scale field trials on commercial broiler farms located in Santa Catarina, Brazil to determine if there was any effect on mortality, feed conversion and Salmonella positive samples (environmental and processing plant). The biopromoter was administered to all chickens (Cobb 500) orally through the drinking water (0.2ml/bird) on d3 and 17 of life for all farms in both zones: evaluations were carried out in Zone A for 3 consecutive production cycles (n=9,138,074; May-August) and in Zone B for 4 consecutive production cycles (n=1,381,981; April-September). Following each production cycle productive parameters were calculated for each lot and Salmonella positive samples were recorded, at the conclusion of the evaluation period the results were averaged for the total treatment period (per zone) and compared with results during the same months of

the previous year in the same zone. Zone A results indicated: reductions in total mortality (4.33% to 2.89%), improved feed conversion (1.633 to 1.615 a difference of 18g feed/kg bw) and reduction of Salmonella positive samples (18.4% to 4.02%). Zone B results indicated: reductions in total mortality (3.67% to 2.21%) improved feed conversion (1.718 to 1.659 a difference of 59g feed/kg bw) and reductions in Salmonella positive samples (3.04% to 1.07%). These data from two separate commercial field trials indicate that the biopromotor provides a promising alternative for increasing commercial poultry performance parameters and reducing important food safety pathogens.

Key Words: Biopromotor, Salmonella, Production

M31 Effect of Acetic Acid Supplementation in Broiler Chickens Experimentally Infected With Salmonella Enterica Serotype Gallinarum Biotype Gallinarum Gulbeena Saleem*, Umar Farooq, Asim Aslam, Muhammad Javed, Zafar Hayat, Iram Liaqat *University of Veterinary and Animal Sciences, Lahore Pakistan*

The present study was conducted to see the ameliorarting effects of acetic acid in in broiler chickens experimentally challenged with Salmonella *enterica* serotype *gallinarum* biotype *gallinarum* (S. *gallinarum*). A total of two hundred and twenty five experimental birds were divided into five groups each replicated three times with 15 birds each. Group A acted as control, Group B was infected with S. *gallinarum*. Antibiotic and acetic acid was given respectively to the challenged Group C and Group D. Birds of Group E were given acetic acid supplementation only. Clinical signs were observed on daily basis. Postmortem examination was done on two birds from each group on days 7, 14, 21 and 28 revealed sinusoidal congestion, hepatic cord necrosis, intestinal nephritis and tubular necrosis in kidneys. Microbial investigations showed that liver had the highest count ($P > 0.001$) followed by spleen, lungs, intestine and caeca and least in heart and kidney. Immunohistochemical findings showed localization of bacteria in different organs except in the heart. Acetic acid supplementation reduced the severity of gross pathological and histopathological changes as compared to other groups. Moreover fecal excretion of S. *gallinarum* significantly reduced with acetic acid supplementation and antibiotics. It can be concluded from present study that acetic acid supplementation to broiler chickens can be helpful in minimizing the severity of gross and histopathological lesions in infected chickens.

Key Words: S. Gallinarum, immunohistochemistry,, post-mortem, broiler chickens

M32 Protection conferred by a subunit Salmonella vaccine against Salmonella Infantis in broiler chickens P Joaquim, Sherryll Layton, Bruno Vecchi, Ariel Sugezky, Pablo Chacana* *Instituto de Patobiología, CICVyA, Instituto Nacional de Tecnología Agropecuaria*

Salmonella infection is a major cause of bacterial enteric illness in both humans and animals. This foodborne pathogen is often associated with poultry production and contaminated poultry products. Currently, vaccination is one of the main strategies to control *Salmonella* in commercial poultry farms avoiding the use of antimicrobials while improving food safety for consumers. We have previously tested the efficacy of a commercially available subunit vaccine (Biotech Vac Salmonella) to protect against *Salmonella enteritidis*. The aim of this study was to evaluate the performance of the same vaccine against *Salmonella infantis* (SI). 1 day old broiler chicks were randomly assigned to one of two treatment groups: a vaccinated group (n=22) or an unvaccinated group (n=27). Chickens in the vaccinated group were individually administered by oral gavage Biotech Vac Salmonella (0.2ml/bird) at day 3 and 17 of life and chickens in the unvaccinated group received PBS (0.2m/birdl). At day 30 all birds were challenged by oral gavage with 10^9 CFU of a virulent strain of SI. Individual cloacal swab samples were taken at 4 and 8 days post-infection (dpi) to determine the excretion of SI. Briefly, samples were enriched overnight in tetrathionate broth and streaked on XLD agar plates for recovery of the challenge strain. Additionally, on the 8th dpi enumeration of *Salmonella* in cecal contents was performed (n=5/group). Results showed 4 dpi 21/27 (77%) and 8/22 (36%) birds respectively from unvaccinated and vaccinated groups were positive for SI; whereas at 8 dpi SI was recovered from 7/27 (26%) in the unvaccinated group and none in the vaccinated group 0/22 (0%). SI was isolated from 4/5 of the cecal samples in the unvaccinated birds (avg: $10^{2.4}$CFU/gram) but no SI was recovered in the vaccinated group. Data indicates the vaccine was able to reduce the excretion and cecal colonization of SI. These results were similar to those obtained previously when chickens were challenged with SE, demonstrating the efficacy of the vaccine to control several serotypes of *Salmonella*, even when they belong to different serogroups. The vaccine combined with biosecurity management may be a useful tool to control *Salmonella* by reducing the infection in the birds and in the environment.

Key Words: Salmonella, vaccine, Immunity

M33 H9N2 in the Middle East/ NA countries and efficacy of vaccines Husam Al-Bakri*[1], Entisar Al-Hallaq[2] *[1]Vaxxinova; [2]Private researcher*

Avian influenza (AI) is a highly contagious disease caused by type A influenza viruses that are members of the family Orthomyxoviridae in the genus Influenza virus A.

Poultry industry in Middle East (ME) area and North Africa (NA) is a continuous growing industry due to increase in local investors; a growing the mind towards local production for quality, safety and slaughtering methods (Halal products). Increase awareness to future growing consumption of white meat.

The population in ME and NA ~ 4 billion broilers, 160 million commercial layers and 40 million breeders.

The region faced different health problem since 2010 such as drop in egg production, close to peak production up to up to (40-50%) during 2-3 weeks thin – shelled, rough and misshapen eggs, moderate mortality and respiratory signs in commercial Layer and Breeder production; while in broiler it was noticed severe mortality (over 40%) after 3 weeks of age) and in some cases the signs appeared earlier than 3 weeks. In addition to severe respiratory signs, high percentage of condemnation due to secondary bacterial infections.

Different serum, tissues, and FTA cards samples were taken from breeders, layers and broilers from different countries and analysed by serology, virus isolation and PCR.

The results showed the presence of AI H9N2 virus in the Middle East/ NA regions combined with IB or ND; different investigations were done using reduction in mortality and improvement in production as parameters.

By using AI vaccines, the farmers were able to control the economic impact of the infection and reduce the mortality.

The usage of AI vaccines, improve the production in breeders/layers and decrease the mortality and condemnation in broilers taking into consideration other respiratory vaccines like ND and IB variants ones should align AI vaccines.

Key Words: H9N2, ME, NA, AI, vaccines

SCAD

M34 Salmonella Infantis biomapping in broiler chicken farms and processing plant: Identification and persistence of an emerging serotype in a broiler vertical integration Martha Pulido-Landinez* *Mississippi State University*

During 2016 and 2017, *Salmonella* Infantis (*S.* Infantis) was identified in a broiler vertical integration in South America as the predominant *Salmonella* serotype. In 2013, this emerging serotype had caused high mortality and poor productive performance in broilers older than 14 days that suffered concomitant infection by infectious bronchitis virus and were housed with high density in the same integration. To determine if *S.* Infantis was still prevalent in the same company, samples of litter, broiler chickens, feces and carcass rinses were collected from broiler farms and from the processing plant. A total of 55 *Salmonella* isolates were spotted in FTA cards and submitted to the PRDL to perform Salmonella Genotyping by Intergenic Sequence Ribotyping (ISR). S. Infantis was identified in all types of samples including litter, chickens of different ages, feces collected from the transportation cages, and carcass rinses collected post chiller. The importance of these results was to determine that S. Infantis persisted throughout the broiler vertical integration. *Salmonella* Infantis biomapping showed the increase in the isolation of this bacterium at different stages of the broiler production, with the most important isolation at the carcass postchiller stage. The high prevalence of *S.* Infantis at this level may represent a food safety problem. The widespread isolation of this serotype in broilers may cause further public health problems.

Key Words: Salmonella Infantis, Emerging serotype, Broiler processing plant, Biomapping

M35 Live ST vaccination trial comparing salmonella prevalence, load and serotype distribution of broiler rinsates at rehang Kalen Cookson*, Manuel Da Costa, Jon Schaeffer *Zoetis*

Live *salmonella typhimurium* (ST) vaccines are a production-side intervention sometimes used to help reduce colonization and shedding of salmonella. Carcass rinsate sampling at rehang is considered a good index of the salmonella levels that are coming into the processing plant. The purpose of this study was to measure the impact of live ST vaccination on the frequency, load and serotypes recovered from carcass rinsates over several weeks. Study Design: A broiler complex in the Southeastern United States ran a 2-week on/off trial for a total of 14 weeks including 6 weeks of Poulvac® ST vaccinated flocks. Each week, 3 carcass rinsates per lot were taken from several lots for salmonella enumeration (MPN method's detection limit is 4 organisms) and serotyping. Percent positive birds and lots were calculated as well as mean salmonella counts and serotype distribution. Flock performance was also captured. Results: Control lots were 3 times more likely to test positive (26.8%) compared to vaccinated lots (8.7%). Mean salmonella counts (with individual scores capped at 40) were 4.61 in controls compared to 2.02 in vaccinated lots. 16.2% (33 of 204) of control rinsates were positive compared to only 6.4% (9 of 141) of vaccinates. Of the 33 salmonella positive non-vaccinated controls, the top three serotypes were *S. kentucky* (52%; Group C3), *S. typhimurium* (18%; Group B) and *S. infantis* (15%; Group C1). Of the 9 positive samples in the vaccinated flocks, 7 were *S. kentucky* and 2 were *S. muenster* (Group E). Vaccinated flocks were 12 points heavier which translated into an adjusted feed conversion ratio improvement of 1.3 points. Discussion: Poulvac® ST vaccinated flocks were 3 times less likely to test positive using the MPN method, had lower mean salmonella counts and no isolations of *S. typhimurium*—the second most common serotype recovered from the non-vaccinated control flocks.

Key Words: salmonella, vaccination, broilers, rinsates, serotypes

M36 The effect of Saccharomyces cerevisiae yeast products In reducing direct colonization and horizontal transmission of Salmonella heidelberg in broilers Charles Hofacre*, Roy Berghaus, T. Kiros, T. Gaydos, Ruth Raspoet, J. Corley *Southern Poultry Research Group*

A recent report of meat processing plants by the USDA-FSIS and FDA NARMS for 2002-2012 found *Salmonella kentucky*, *S. enteritidis* and *S. heidelberg* had the highest prevalence in poultry meat. *S. enteritidis* and *S. heidelberg* were the two most commonly associated with human illness associated with poultry. A study was conducted to evaluate the effectiveness of a *Saccharomyces cerevisiae* yeast cell wall (YCW) product at different use rates and a yeast culture for reduction in *S. heidelberg* prevalence and load in the ceca. There were eight replicate floor pens per treatment with *ad libitum* feed and water. All treatments had one-half of chicks in each pen challenged at 1 day of age with a Nalidixic acid resistant strain of *S. heidelberg.* The treatments were: none, YCW (125 ppm), YCW (250 ppm), YCW (500 ppm), yeast culture (1250 ppm). At 42 days five ceca of challenged (direct) and ten ceca of penmates horizontally challenged were aseptically removed. Tetrathionate with iodine was added to weighed ceca bags, 0.1 ml was removed for Most Probable Number (MPN) enumeration, then bags and deep well MPN in whirl pack were incubated overnight at 42°C. The 10-fold dilution MPN was done in deep well blocks with tetrathionate per method of Berghaus et al., 2013. The bags with ceca and MPN were either struck or spotted (MPN) onto XLT-4 agar with 25 µg/ml Nalidixic acid. Delayed secondary enrichment was performed for bags S.H. negative. There was a clear dose effect on S.H. reduction of ceca prevalence with the YCW at 500 ppm having 41.7% positive vs. control 54.2%. However, these differences were not statistically significant. The effect of the 500 ppm YCW on reducing Salmonella prevalence in the horizontal or indirect challenged was 32.5% ($P = 0.09$) vs. 57.5% in untreated control. The MPN counts also followed a similar dose trend with 500 ppm YCW at 1.7 *Salmonella* MPN/g vs. control 2.7 MPN/g. The horizontal challenged for the 500 ppm YCW at 1.4 MPN/g which was a significant reduction ($P = 0.03$). In conclusion, YCW at 500 ppm demonstrated the trend to decrease prevalence of S.H. positive ceca and reduce *S. heidelberg* colonization levels in the ceca.

Key Words: Prebiotic, Salmonella, heidelberg, Saccharomyces, cerevisiae

M37 The role of genes encoding for tetrathionate respiration, SPI-1, and SPI-2 on the cecal colonization and systemic spread of Salmonella Typhimurium in chickens, with or without coccidia coinfection Claire-Sophie Rimet*[GS], John Maurer, Roy Berghaus, Brian Jordan, Luciana Antoniassi da Silva, Lisa Stabler, Kasey Johnson, Laura Tensa, Karen Segovia, Monique Franca *The University of Georgia*

Reactive oxygen species produced during intestinal inflammation react with thiosulfate present in the intestinal lumen to form tetrathionate. In the mouse colitis model, tetrathionate respiration confers a growth advantage for *S.* Typhimurium over the competitive microbiota in the lumen of the inflamed intestine. SPI-1 and SPI-2 are major virulence factors involved in *Salmonella* intestinal invasion and survival within phagocytic cells respectively. In poultry, coccidiosis promotes infiltration of inflammatory cells in the intestinal mucosa. We hypothesized that the inflammatory infiltrate induced by coccidiosis may provide a growth advantage for *Salmonella* over the intestinal microbiota and may enhance its mucosal invasion and systemic spread.

Our objective was to evaluate intestinal inflammation induced by *Eimeria* spp. and *S.* Typhimurium challenges. We also wanted to determine the fitness of *S.* Typhimurium strains deficient in tetrathionate reductase, SPI-1, and SPI-2 genes for cecal colonization and dissemination in tissues, in the presence or absence of *Eimeria* infection.

One-day-old chickens were orally inoculated with a sham inoculum or with 400 oocysts of *Eimeria* spp. Five days later, birds were orally administrated with a combination of *S.* Typhimurium wild type and mutant strains (3.5 to 4.0 x 10^8 CFU/bird). Ceca, liver, and drumstick were collected 3, 7, 14, and 42 days post *Salmonella* inoculation for bacteriology. Intestinal inflammation was scored by histology.

Without coccidia coinfection, *Salmonella* counts in ceca were higher for the tetrathionate mutant than for the wild type strain; differences in cecal counts were not significant between tetrathionate mutant and wild type strains in the presence of coccidia coinfection. Compared to the wild type strain, *Salmonella* prevalence in liver was lower for the SPI-1 mutant only. Coccidia coinfection did not significantly increase *Salmonella* prevalence in liver and drumstick.

We conclude that the tetrathionate reductase gene of *S.* Typhimurium may not be required for cecal colonization in chickens. Deficiency in the SPI-1 region had a detrimental effect on *S.* Typhimurium systemic spread in liver. Finally, low dose of *Eimeria* may not increase *S.* Typhimurium dissemination in tissues of infected chickens.

Key Words: Salmonella Typhimurium, Chicken, Tetrathionate respiration, SPI-1, SPI-2

M38 Interactions of environmental temperature and dietary protein levels with coccidia vaccination Jarred Oxford*[GS1], Hector Cervantes[2], Ivan Alvarado[3], Andres Montoya[3], Ricardo Nunes[4], Jomara Bronch[4], Gene Pesti[1] *[1]University of Georgia; [2]Phibro Animal Health; [3]Merck Animal Health; [4]Universidade Estadual do Oeste do Paraná*

The objective of this study was to investigate the effects of environmental temperature and dietary protein levels in commercial broilers given coccidia vaccine at d of hatch. Two thousand one hundred and twelve, male d-old chicks were used in this study. This experiment was designed in a 2x2 factorial arraignment consisting of 2 environment temperatures (ET), standard (S) or reduced (R; 3℃ cooler after 3 d of age), and 2 dietary protein levels, high (HP) or low (LP). Two trials were conducted with the room treatments alternated between trials to account for room effects. Each treatment group contained 528 chicks, 24 pens of 22 chicks each. There were two dietary phases: starter (0-16d, HP 23% CP, LP 18% CP) and grower (16-29d, HP 23% CP, LP 19% CP). Two birds per pen were randomly selected for cloacal temp. measurements at 8, 15, 22, and 29 d of age. BW was recorded at 0, 16, and 29 d and FCR at 16 and 29 d. Significant effects (P= 0.041) on BW based on ET were only observed at 16 d with the S room having higher mean BW (589.5 vs. 573.5g). At 16 and 29 d, there were significant differences in BW (P< 0.001) and BWG from 16-29 d (P= 0.005) based on protein level. HP fed chicks were heavier at 16 (596.3 vs. 566.7g) and 29 d (1742.6 vs 1652.1g) and had higher BWG from 16-29 d (1146.3 vs. 1085.3g). At 16 d, there were significant differences observed on FCR based on ET, with the S group having lower FCR, (P< 0.001, 1.332 vs. 1.405) and differences (P< 0.001) in FCR seen at 16, 29, and 16-29 d based on protein level. ET affected (P< 0.001) percent mortality at 16 and 29 d, with the cooler room having more (9.19 vs. 20.50%, 18.04 vs. 29.04). No protein level effects (P> 0.625) were observed on mortality during any part of the experiment. The chicks in the cooler room had higher mean cloacal temps. (P= 0.018), however there were no other significant (P> 0.354) observations on cloacal temps. throughout the trial. No interactions between ET and protein level were observed for BW, BWG, FCR, or mortality. It is important to note that most of the mortalities observed during the trial, had clinical necrotic enteritis lesions. This observation shows the importance of proper brooding conditions for broilers when administering coccidia vaccines.

Key Words: Coccidiosis, Temperture, Protein

M39 Evaluation of different coccidia vaccines, with or without a bioshuttle, for control of a pathogenic E. tenella Laura Tensa*[GS], Grace Albanese, Brian Jordan *University of Georgia*

Coccidiosis is an economically significant enteric disease caused by *Eimeria spp.* One species in particular, *E. tenella,* infects the ceca of chickens causing bloody feces, thickening and sloughing of the cecal wall, and mortality. For the past several years, a commercial poultry integrator has had a pathogenic *E. tenella* challenge on multiple broiler complexes. These challenges have been unresponsive to multiple interventions including the use of multiple coccidiosis vaccines and anti-coccidial treatments. The purpose of this trial was to evaluate the protection properties of two coccidiosis vaccines used alone or in combination with an ionophore against the pathogenic isolate. For this trial, two challenge times were used, an early challenge to coincide with when lesions and mortality were seen in the complexes, and a later challenge to determine if further cycling of vaccinal oocysts would improve immunity. Litter samples were collected from all groups to determine vaccinal oocyst cycling. Body weights, gross lesion scores, and oocyst count scores were collected to determine if birds were protected compared to non-vaccinated challenged and non-challenged controls. Additional birds were grown to 35 days to determine the lasting effects from the challenge on processing weights. Oocysts per gram of litter counts showed that both vaccines were infective and all species of *Eimeria* were shed in the first cycle, though at different levels from each vaccine. In the early challenge, the vaccinated birds were not protected when compared to the non-vaccinated challenged control. In the late challenge, all vaccinated groups had significantly lower lesion scores than the non-vaccinated challenged control. After the early challenge, the groups vaccinated with vaccine A had significantly decreased weight gain compared to the other groups. Before the late challenge, all vaccinated groups had significantly lower body weights than the non-vaccinated groups. Results from this trial indicate that for the early challenge neither vaccine, with or without the ionophore, protected the chickens against challenge from this pathogenic field isolate of *E. tenella*. Once the vaccine completed cycling to induce immunity, both commercial vaccines were protective against the isolate.

Key Words: Coccidiosis, Vaccination, Bioshuttle, tenella

M40 Impact of necrotic enteritis disease models on the growth curve of broilers Kaylin Chasser*[GS], Kim Wilson, Whitney Briggs, Audrey Duff, Ramesh Selvaraj, Lisa Bielke *The Ohio State University, OARDC*

Necrotic enteritis (NE) has major relevance to the broiler industry with both short and long-term effects on body weight (BW). Coccidiosis is commonly associated with NE, though some reports suggest NetB+ *Clostridium perfringens* (CP) can cause NE in the absence of *Eimeria*. Our lab has previously characterized the impact of various strains of *E. maxima* (EM) and CP on BW and lesion scores during clinical disease. These studies evaluated the immediate effect on BW and impact on the growth curve of broilers to d57 or d56 when NE was induced by various methods. Two strains of EM (Guelph or M6), NetB- CP (TXAM), and NetB+ CP (NetB1, NetB2, or NetB3) were tested. In Exp 1, treatments were non-challenged control (NC), Guelph or M6 + TXAM (GCP or M6CP), TXAM, NetB1, NetB2, NetB3, mixed NetB+ Supernatant, or Washed NetB+. On d16, all birds were weighed, and EMCP groups were challenged with EM Guelph or M6 oocysts. On d17-20, NetB+ groups were challenged with 50mL of 10^6 to 10^8 CFU/mL CP once daily on feed. On d20, NetB- groups were challenged with 10^8 CFU/bird CP via oral gavage. Body weight was measured d22, then weekly through d57. In Exp 2, treatments were NC, GCP, M6CP, NetB1 Low, NetB1, Supernatant, and Washed. All BW were recorded d16, and EMCP groups were orally challenged with EM Guelph, or M6. Days 17-20, NetB1 Low received 100mL of 10^8 CFU/mL once daily in the feed, while NetB1, Supernatant, and Washed received 1L of 10^8 CFU/mL CP twice daily in the feed. The EMCP groups were challenged with 10^8 CFU/bird CP on d20. Body weight was measured d22, then weekly through d56. In Exp 1, BW was

significantly lower ($p<0.05$) than NC on d22 for M6CP, and remained nearly parallel with NC through d57, though not significantly lower. In Exp 2, d22 BW for NetB1 Low and Supernatant were not significantly lower than NC, but GCP, M6CP, NetB1, and Washed had a significant reduction in BW that required a week or more to meet NC growth. EM played a role in clinical NE, while extremely elevated levels of CP alone were required to produce subclinical NE, where BW was impacted without notable clinical signs. This separation in BW can be used to measure effectiveness of intervention tools against NE.

Key Words: necroticenteritis, growth, Eimeria, Clostridium

M41 Characterization and Quantification of Toxin Genes in Necrotic Enteritis (NE) Producing and Non-NE Producing Clostridium perfringens Isolated from Chickens Wenyuan Yang*[GS], Chung-Hsi Chou, Chinling Wang *Mississippi State University*

Necrotic enteritis (NE) caused by *Clostridium perfringens* is a re-emerging disease in chickens in recent years, contributing to enormous economic losses to the poultry industry. Toxins produced by *C. perfringens* as well as other predisposing factors are responsible for the onset and development of the disease. However, the importance of plasmid encoding toxins NetB, CPB2, and TpeL, to clinical NE cases is controversial. Therefore, we compared the toxinotype and the presence of *netB, cpb2,* and *tpeL* genes, in 15 NE-producing and 15 non-NE producing *C. perfringens* isolates using the conventional PCR and the quantitative PCR (qPCR). Results indicated that all 30 isolates were characterized as toxinotype A, and all were negative for *cpe* gene which is associated with human food poisoning. The *netB* gene was detected in 6.7% of NE-producing isolates by the conventional PCR, whereas 70% by the qPCR. In 15 non-NE producing isolates, no *netB* gene was detected by the conventional PCR but 60% of isolates were positive for *netB* by the qPCR. The presence and copies of *netB* gene were not significantly different between NE and non-NE producing isolates. No difference was observed between NE and non-NE producing isolates in the presence of *cpb2* or *tpeL* gene. The results suggest that neither the presence of *netB, cpb2* and *tpeL* nor the copy number of *netB* genes in *C. perfringens* is correlated with clinical NE. The qPCR should be used to detect the presence of *netB* gene instead of the conventional PCR.

Key Words: NE, Clostridium, toxin, qPCR, netB

M43 The effectiveness of lauric acid and Akkermansia muciniphila against necrotic enteritis in chickens Chinling Wang*, Hsin-Yi Lu, Wei-Yun Yang, Yue-Jia Lee, Scott Branton *Mississippi State University*

Necrotic enteritis (NE) caused by *Clostridium perfringens* (CP) is a multifaceted disease and requires many predisposing factors to facilitate the development of the disease. *C. perfringens* grows favorably in the alkaline and mucin-rich intestinal conditions. *Akkermansia muciniphila* (AM), a mucin-degrading anaerobe and a probiotic supplement, has anti-inflammatory and the improvement of gut integrity effects. Therefore, the objective of this study was to test if lauric acid or *A. muciniphila* can protect chickens against *C. perfringens challenge*. Chickens were divided to nine groups, CP, CP+ *Eimeria*, CP+*Eimeria*+ lauric acid, CP+*Eimeria*+AM, CP+AM, *Eimeria*+AM, AM, *Eimeria* or placebo. Results showed that the NE lesion score in lauric acid or AM treatment group was not significantly different from the CP or the CP+*Eimeria* challenged group. Our qPCR assay also confirmed that chickens with necrotic enteritis lesions had significantly higher numbers of CP in the jejunum, ileum and cecum than the CP challenged birds without NE lesions. In conclusion, neither lauric acid nor *A. muciniphila* supplement prevents chickens against the CP challenge. Interestingly, the composition of gut microbiota seems to influence the development of NE lesions in chickens.

Key Words: Clostridium, enteritis, acid, chickens, probiotics

M44 Effect of zinc sources and doses on the presence of Necrotic Enteritis in broilers caused by C. perfringens Greg Mathis*, Brett Lumpkins, Agathe Romeo, Stephane Durosoy *Southern Poultry Research, Inc.*

Clostridium perfringens-induced Necrotic Enteritis (NE) has become a great concern to the poultry industry, which has resulted in a significant decrease in growth performance, poor feed conversion, and increased mortality. A trial was conducted with 72 cages starting with 8 chicks each, which was divided into 3 groups of 24 cages: one group fed with 80 ppm of Zn from zinc sulfate ($ZnSO_4$) and the others with Zn from potentiated zinc oxide (ZnO) source (Hizox®, Animine), at 80 ppm or at 120 ppm. Then, each group was subdivided into 3 groups of 8 cages: non-challenged birds, challenged birds with non-medicated feed and challenged birds with virginiamycin at 20 ppm. A randomized block design with 8 replications of 8 chicks per cage was used, and $P<0.05$ was used to determine the level of significance. All diets were fed experimental diets *ad libitum* throughout the study. On D14, all the broilers were orally inoculated with a low dose of *E. maxima*. On days 19, 20 and 21, the birds in the challenged groups were orally dosed with *C. perfringens* ~10^8 cfu/mL. Birds and feed were weighed by cage on D0, 14, 21, and 28, and performance parameters were measured. On D21, 3 birds from each cage were sacrificed and examined and scored on the degree of severity of necrotic enteritis lesions, from 0 (normal) to 3 (sloughed and blood small intestine mucosa). The NE model was successful in producing a heavy NE infection. The therapeutic level of the antibiotic Virginiamycin (VIR) improved the performance of all of the challenged birds. Challenged birds fed either level of the potentiated ZnO had improved growth performance compared to ZnSO4 fed birds. The 120 ppm level had the most significant feed conversion ratio and lowest NE mortality of the challenged non-antibiotic groups. These results emphasize the benefits of a potentiated ZnO source in reducing Necrotic Enteritis compared to $ZnSO_4$.

Key Words: Enteritis, zinc, broilers

M45 Characteristics of Gallibacterium anatis isolated from poultry samples Jessica Hockaday*[GS], Alejandro Banda, Jay Kay Thornton, Lifang Yan, Martha Pulido-Landínez *Mississippi State University CVM Poultry Research and Diagnostic Laboratory*

Over the last decade the Poultry Research and Diagnostic laboratory (PRDL) has seen an increase in clinical cases of *Gallibacterium anatis*. *G. anatis* is a naturally occurring commensal bacterial of the upper respiratory system in poultry that has been shown to have pathogenic effects in many avian species. Clinical presentations associated with *G. anatis* include upper respiratory signs, decreased egg production, salpingitis, peritonitis, oophoritis, and airsacculitis and has been isolated in broilers, broiler breeder and commercial layer flocks. Field strains of *G. anatis* collected throughout the Southern US in 2016 and 2017 were evaluated for this study. Samples from layers, broiler breeders, and broiler chickens were cultured, microbial sensitivities performed along with molecular genotyping and phylogenetic analysis. As an emerging pathogen the understanding of appropriate techniques to isolated and further evaluate *G. anatis* is useful to many aspects of poultry medicine and diagnostics.

Key Words: Gallibacterium anatis, Field samples, Chickens

Metabolism & Nutrition - Amino Acids

M46 Metabolisable energy of wheat for broilers Muhammad Azhar*[GS1], Stephen Rose[1], Alexander Mackenzie[1], Michael Bedford[2], Vasil Pirgozliev[1] [1]*Harper Adams University;* [2]*AB Vista*

Wheat is primarily used in broiler feeds for its content of available energy but there is considerable variation in the apparent metabolisable energy (AME) of different wheat samples. The aim of this study was to examine the differences in the chemical composition and AME of seventeen current UK wheat samples.

Seventeen UK wheat cultivar samples were selected to formulate broiler grower diets. Samples were a mix of soft, milling (hard) wheat, soft feed wheat and hard feed wheat. Samples were analysed for dry matter, protein, ash, fat, gross energy, starch, soluble and insoluble non-starch polysaccharides (NSP), endosperm hardness, hagberg falling number, specific weight and water extract viscosity. Seventeen diets were formulated including 670 g/kg of each wheat sample and 330 g/kg of a balancer. Three additional diets containing 470, 570 and 770 g/kg of one wheat sample were formulated to determine AME in basal diet by the substitution method. All diets were pelleted. The diets were made iso-nitrogenous by adding wheat protein isolate. Eight hundred Ross 308 male Ross broilers were allocated to 160 raised floor pens. Each diet was replicated 8 times and birds were fed *ad libitum* from 0 to 21d age in a randomised complete block design. Excreta were quantitatively collected for the last three days and AME was determined following standard procedures. Data was statistically compared by randomised block one-way ANOVA.

The mean AME of the wheat samples ranged from 13.68 to 14.63 MJ/kg DM. There were no significant differences in AME of wheat cultivars samples. There was no relationship between broiler growth performance and AME. The mean FI of broilers fed the 17 wheat samples ranged from 37.5 to 43.1 gram/bird/day DM and mean WG 30.4 to 34.6 g/b/d. There were differences in FI and WG ($P < 0.05$) between some of the 17 individual wheat samples. There were no differences between wheat samples in analysed starch, non-starch polysaccharides (NSP), soluble NSP, insoluble NSP, ash, fat and protein. There was no relationship ($P > 0.05$) between broiler growth performance and determined AME. Multiple linear regression indicated that AME is predicted by the combination of protein, ash, soluble NSP ($P<0.05$; $r^2 = 0.55$; SEO = 0.200).

Key Words: Wheat, AME, starch, NSPs

M47 The effect of amino acid density on growth performance, processing yield, and the incidence of white striping and woody breast Kyle Brown*[GS], Brooke Bodle, Rocky Latham, Roy Brister, Rob Shirley, Christine Alvarado, Jason Lee *Texas A&M University*

In a randomized complete block design, the growth performance, meat yield and incidence and severity of white striping (WS) and woody breast (WB) in high-yielding male broilers was investigated. The factorial arrangement consisted of 2 digestible amino acid densities (dAA; normal industry vs. 10% higher dAA) and 2 modifications of the grower phase diet (normal industry level vs. a 15% reduction in dAA). Each treatment had 10 replicates, and each replicate contained 25 male broilers. The following production parameters were evaluated on days 13, 28, 42, and 49: body weight (BW) and mortality-corrected feed conversion ratio (FCR). On d 50, 10 birds per pen (400 birds total) were processed to determine boneless, skinless breast meat yields, and breast fillets were evaluated for the incidence and severity of WS and WB. Data were analyzed as a 2 X 2 factorial, with main effect means deemed significant different at $p \leq 0.05$. Feeding a higher level of dAA improved BW on days 13 and 28 ($p < 0.028$), reduced FCR at all ages, and increased breast filet weight ($p = 0.01$). Reducing the dAA during the grower phase reduced BW and increased FCR on day 28 ($p < 0.001$); this effect continued throughout the remainder of the study, resulting in lower breast filet weight ($p < 0.01$). Although no differences in WS were observed, the WB profile was affected by the dietary treatments. Compared to the Control, feeding 10% more dAA reduced the proportion of 0/1 WB scores from 60% to 45%, respectively ($p = 0.005$). Conversely, when compared to the Control, reducing dAA by 15% during the grower phase increased the 0/1 WB scores from 47% to 58%, respectively ($p = 0.047$). As expected, dAA density significantly impacts growth performance and processing yield. A targeted reduction in dAA level may be used as a strategy to mitigate WB severity.

Key Words: Broiler, Performance, Breast, Yield

M48 Effects of reduced crude protein diets formulated with or without added glycine fed to broilers from 15 to 34 days of age Ruben Kriseldi*[GS], Paul Tillman, Zhirong Jiang, William Dozier, III *Auburn University*

An experiment was conducted to evaluate effects of formulating reduced crude protein (CP) diets with or without Gly supplementation on growth performance and meat yield of broilers from 15 to 34 d of age. One thousand six hundred Ross × Ross 708 broiler chicks were placed in 64 floor pens at 1 d of age (25 birds/pen; 0.09 m^2/bird). Broilers received a common starter diet from 1 to 14 d of age formulated primarily with corn and soybean meal to contain 1.25% digestible Lys and 3,053 kcal/kg metabolizable energy. Dietary treatments were fed during grower (15 to 25 d of age) and finisher (26 to 34 d of age) phases with 1.10 and 1.00% digestible Lys and 3,086 and 3,119 kcal/kg metabolizable energy, respectively. Dietary treatments (8 reps per treatment) were arranged as 4 × 2 factorial with 4 CP concentrations each with or without added Gly. Four dietary CP contents were obtained by sequentially adding feed-grade amino acids from DL-Met to L-Trp to reduce CP content by approximately 2.4 percentage points between the highest and the lowest CP diets. Glycine was added to maintain total Gly + Ser to digestible Lys ratio of 1.80, whereas a minimum ratio of total Gly + Ser to digestible Lys in diets without added Gly was not included. From 15 to 25 d of age, a reduction in dietary CP content from 21.8 to 20.1% did not affect ($P = 0.33$) feed conversion but resulted in 3.3% lower ($P < 0.01$) body weight gain and feed intake of broilers. When dietary CP content was reduced to 19.8%, body weight gain, feed intake, and feed conversion of broilers were not different ($P > 0.05$) with those consuming the 21.8% CP diet. Cumulative growth performance and meat yields of broilers from 15 to 34 d of age were not affected ($P > 0.05$) by dietary CP content either with or without Gly supplementation. These data confirm that sequential addition of amino acids from DL-Met to L-Trp could enable dietary CP reduction of 2.4 percentage points without compromising growth performance of broilers from 15 to 34 d of age. The addition of Gly to obtain 1.80 total Gly + Ser to digestible Lys ratio in reduced CP diets may not be necessary to obtain optimal growth performance or meat yields of broilers from 15 to 34 d of age.

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

M49 Protein turnover and performance parameters on modern broiler strains fed varying levels of dietary amino acids and metabolizable energy. Garrett Mullenix*[GS], Craig Coon, Victor Naranjo, Antonio Kalinowski, Justina Caldas, Michael Schlumbohm, Katie Hilton, Judith England *University of Arkansas*

The objective of this study was to determine how modern broiler strains synthesize protein when fed varying dietary amino acid and metabolizable energy levels. Protein turnover was determined at 22d, 35d and 42d through intravenous flooding-dose of 15N-phenylalanine by fractional synthesis rate (FSR) and fractional breakdown rate (FBR). Body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), and protein turnover were evaluated. Two trials with two thousand twenty-five Cobb MX x Cobb 500 and Ross YP x Ross 308 were placed in 90 pens (n=45 birds/pen) for the study. Commercial starter and grower feed were fed from 1-10d and 11-22d, respectively. Five experimental finisher diets

were fed from 22-42d in pellet form (9 reps per strain/diet) in each trial. The varying AA finisher diets were isoenergetic (3125 kcal/kg) and formulated to the ideal amino acid recommendations of AMINOChick® 2.0: 0.80%-, 0.90%-, 1.00%-, 1.10%- and 1.20% dLys, respectively. Other first limiting amino acids were held at a constant ratio to dLys level: Met+Cys, 0.76; Thr: 0.65; Val: 0.80; Ile: 0.71; Arg: 1.05, and Trp: 0.16. The finisher metabolizable energy diets were iso-nitrogenous and formulated to different TMEn levels: 2800, 2925, 3050, 3175 and 3300 kcal/kg. All diets were formulated to AMINOChick® 2.0 recommendations, with dLys set at 1.00% and other amino acids set as a ratio to dLys. There were significant differences from strain (p=0.0479) (p=0.0022) and diet (p<.0001) for BW at 42d in both trials. Both lines synthesized (>40%d) and broke down (≈35%d) protein similarly until the experimental diets start. Line A had a higher FSR and FBR in the energy trial (17.01%d & 13.22%d) while Line B did in the amino acid trial (18.31%d & 15.27%d) although not significantly at 35d. There was a 7.29%d FSR and 7.15%d FBR difference between the lowest and highest energy diets at 42d. FSR and FBR changed (24.42%d & 26.38%d) from 22d to 42d. The AA trial suggests that FSR increased 6.73%d to the 110% AA diet (20.44%d) then both lines slightly decreased at the 120 AA level (18%d) at 35d. These trials suggest that both broiler strains synthesize and degrade more protein when fed increasing levels of AA while protein accretion was not affected by varying levels of dietary energy.

Key Words: Amino Acid, Protein Turnover

M50 Performance of broiler chickens fed diet with or without exogenous methionine and raised in conventional or organic production environment James Foutz*[GS], Marie Milfort, Alberta Fuller, Woo Kim, Romdhane Rekaya, Samuel Aggrey *University of Georgia*

According to the National Organic Program, synthetic amino acids are not permitted in organic production although low levels of synthetic methionine (MET) is permitted. The current National Organic Standard Board (NOSB) recommended levels (0.1% MET) pose issues and concerns for some organic producers citing lack of commercially available natural sources of methionine, and consideration pertaining to poultry health and welfare. We examined the effects of complete DL-methionine (DLM) replacement by Brazil nut meal in organically raised broilers. A total of 800 Ross 308 chicks were hatched and randomly divided into 4 treatment groups with 4 replicates of 50 chicks. Treatments included birds fed a conventional corn and soybean meal (SBM) diet while raised organically (Conv_Org), NOSB diet raised organically (NOSB_ORG), an organic diet utilizing Brazil nut meal to provide an adequate amount of MET raised organically (Org_Org), and a conventional corn and SBM diet raised conventionally (Conv_Conv). Comparisons were made by examining performance and carcass yields. Body weight at 8 weeks were 3,960, 3,995, 3,981 and 3,676 g (SEM=44) for Conv_Org, NOSB_Org, Org_Org and Conv_Conv, respectively. The BW of the Conv_Conv was significantly lower (P<0.05) than all the other three treatments. The cumulative FCR was 1.76, 1.87, 1.76 and 1.82 g/g (SEM=0.02) for Conv_Org, NOSB_Org, Org_Org and Conv_Conv, respectively. The Org_Org diet has significantly lower (P<0.05) FCR than the NOSB_ORG diet. There were no significant differences in carcass yield and composition across all treatments. It was concluded that the use of Brazil nut meal is a viable substitute for DLM in organically raised broilers, exhibiting no difference in growth and carcass yield and better FCR compared to the current NOSB diet recommendation.

Key Words: Methionine, Brazilnuts, Organic, Conventional, Broilers

M51 Digestible leucine requirements of female turkeys from ten to 21 days post hatch Joao Ferreira*[GS], Jeffre Firman, David Ledoux *University of Missouri*

Leucine (Leu) is one of the three branched chain amino acids. It is not a limiting amino acid in a corn-soybean meal basal diet; however, it is important to know the required level of Leu because it shares the same enzymes in the first three steps of metabolism with isoleucine and valine. An oversupply of leucine is known to affect isoleucine and valine metabolism. The objective of this work was to establish the level of digestible Leu required by female turkeys from hatch to 21 days of age. Two experiments were conducted in battery cages. In the first experiment, a total of 400 female poults 10 days post-hatch were randomly allocated into battery cages and feed and water were provided ad libitum. Poults (8 replicate cages of 5 birds per cage) were fed nine supplemental levels of digestible leucine from 1.20 to 2.2% with incremental increases of 0.125% between treatments. A control treatment using NRC (1994) recommendations was used to check the performance. Data were submitted to GLM and NLIN procedure of SAS to fit a linear and polynomial quadratic (PQ), and quadratic broken line (QBL), respectively. Average gain (AvGn) and feed conversion (FC) was not statistically different (P>0.05) among digestible leucine treatments. The second experiment utilized a similar experimental design and statistical analysis as in the first experiment. The levels of digestible Leu ranged from 0.85 to1.25% (with incremental increases of 0.05%). Results of the PQ analyses indicated digestible Leu requirements of 1.14% and 1.13% using AvGn and FC, respectively. Results of the QBL analyses indicated digestible Leu requirements of 1.06 and 1.03% using AvGn and FC, respectively. The digestible lysine (Lys) level utilized in this experiment was 1.44% and the Leu:Lys ratio was 73% using PQ and 79% using the QBL equation. The NRC (1994) suggestion for the Leu:Lys ratio is 119%, which is much higher than our results. This work suggests a lower and adequate Leu:Lys ratio of 73% and 79% using PQ and QBL, respectively.

Key Words: Turkey, requirement, digestible amino acids

M52 Effects of dietary amino acid density and feed allocation during the starter period on 41 d growth performance and processing characteristics of broiler chickens given coccidiosis vaccination at hatch Sara Cloft*[GS], Samuel Rochell, Ken Macklin, William Dozier, III *Auburn University*

Coccidiosis vaccinations illicit a performance depression while developing immunity in the broiler through a mild enteric challenge. A study was conducted to determine if modulating digestible (dig) amino acid (AA) density or the amount of starter feed can ameliorate the negative effects of vaccination. Sixteen hundred Ross × Ross 708 male broilers were placed into 64 floor pens (0.08m^2/bird) with each pen assigned to 1 of 8 treatments representing a 2 × 3 factorial arrangement of dig AA density [Moderate (1.15% dig Lys) and High (1.25% dig Lys)] and starter feed allotment (0.45, 0.73, and 1.0 kg/bird) with 2 positive control diets, and 8 replicates per treatment. Diets were formulated to similar dig ratios of Thr (0.67), TSAA (0.78), Val (0.70), Ile (0.67), Arg (1.05) and Trp (0.17) to dig Lys. Vaccinated birds received a 1x dosage of Coccivac®- B52 prior to placement, whereas non-vaccinated birds in the positive control groups were fed diets containing Diclazuril throughout the starter and grower periods. Following consumption of the starter diets, birds were provided common diets. At 21 d lesion scoring was conducted on 4 birds per pen to confirm vaccine efficacy. At 42 d, 12 birds per pen were processed for measurement of carcass attributes. At 21, 28, and 41 d of age, broilers fed the high AA diet during the starter period had higher BWG and lower FCR (P < 0.05) than those fed the moderate AA diet. Broilers fed the high AA diet had heavier carcass and total breast weights than birds fed the moderate AA diet ($P \leq 0.005$). A starter allotment of 1.0 kg/bird produced heavier broiler carcass weights than did lower allotments ($P \leq 0.006$). Additionally, broilers fed the high AA diet exhibited no performance depression and had a greater proportion of 0 scores for all intestinal regions during scoring ($P > 0.05$). A 1.1 g increase in dig Lys intake during the starter period resulted in a 0.64% increase in total breast yield for birds fed the high AA diet compared with the moderate AA diet fed broilers. Results from this study indicated that feeding a high AA diet during the starter period and feeding a higher starter allotment support the bird through the

challenge period of the vaccine without compromising performance and improved the overall growth of the bird.

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

M53 Growth performance and carcass characteristics of MV × Cobb 700 broilers fed diets varying in amino acid density from 1 to 46 days of age Stephanie Philpot*[GS], Justina Caldas, William Dozier *Department of Poultry Science, Auburn University*

Dietary amino acid needs of MV × Cobb 700 broilers for optimum growth performance and meat yield responses are sparse. An experiment was conducted to determine the effects of digestible amino acid density on growth performance and carcass characteristics of MV × Cobb 700 broilers. Fourteen hundred and eight broilers were vent sexed and randomly distributed by gender into 64 floor pens (22 birds; 0.10 m²/bird). A 2 × 4 factorial arrangement of treatments was employed with male and female broilers fed diets varying in amino acid density at 95, 100, 105, and 110% of Cobb 700 dietary amino acid recommendations. Each treatment was represented by eight replicate pens. Broilers were fed a four-phase feeding program (1 to 18, 19 to 28, 29 to 38, and 39 to 46 days) formulated to the primary breeder guidelines. Corn, soybean meal, DDGS, and animal protein meal sources were the primary ingredients. Body weight gain, feed consumption, feed conversion ratio, and mortality were determined at days 18, 28, 38, and 45. At day 46, 12 birds per pen were processed and the weights and yields of the abdominal fat, carcass, breast fillets and tenders were assessed, and breast quality was ascertained based on prevalence of wooden breast and white striping. From 1 to 18, 1 to 28, and 1 to 38 d of age, feed conversion ratio of broilers was reduced quadratically ($P \leq 0.01$) by dietary amino acid density with feed conversion being optimized at 105% of the Cobb 700 dietary amino acid recommendations. Broilers fed diets formulated to 105% dietary amino acid density had lower ($P \leq 0.05$) cumulative feed conversion ratio than broilers provided diets at 100% but no difference in cumulative feed conversion occurred with birds consuming diets formulated at the 95% vs. 105% amino acid density. Increasing dietary amino acid density from 95 to 100% of Cobb 700 amino acid specifications resulted in higher ($P = 0.002$) total breast meat yield (26.52 vs. 27.31%) of broilers. Throughout experimentation, males had higher body weight gain, lower feed conversion ratios, and higher incidence of wooden breast than females ($P \leq 0.001$). These results indicate that MV × Cobb 700 broilers are not as sensitive to increasing dietary amino acid density as previous research with Cobb 500 broilers.

Key Words: Broiler, Performance, Amino acid, Yield

M54 Effects of dietary amino acid regimens on live performance and processing characteristics of Cobb MV × 700 male and female broilers Craig Maynard*[GS], Rocky Latham, Roy Brister, Casey Owens, Samuel Rochell *University of Arkansas*

The timing of broiler responses to amino acid (AA) density can vary with genetic line and cross. Therefore, an experiment was conducted to evaluate the effects of dietary AA regimens on the growth performance, carcass composition, and *Pectoralis major* myopathies of Cobb MV × 700 broilers reared sex separately. Six dietary regimens were created by varying diets with high (H), medium (M), and low (L) AA density across 4 feeding phases: starter (0 to 14 d), grower (15 to 28 d), finisher (29 to 36 d), and withdrawal (37 to 46 d). Twelve treatments consisting of a factorial arrangement of dietary AA regimen (HHHH, HHHM, HHMM, HMMM, MMMM, and HHLL) × sex were distributed across 12 replicate pens of 12 birds (1,728 total). Digestible Lys levels of M and H diets were 1.18 and 1.26% in the starter and 1.07 and 1.14% in the grower phases, respectively, with ratios of other AA relative to Lys held constant. Similar relative differences in AA density were maintained between L, M, and H diets in the finisher and withdrawal phases. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined for each period. At 47 d, 4 birds per pen were processed for evaluation of carcass composition and scored for woody breast (WB) and white striping (WS) in *P. major* fillets. No diet × sex interactions were observed for any measurement. Males had higher BWG and FI ($P < 0.01$) and lower FCR ($P < 0.01$) at 28, 36, and 46 d than females. Dietary treatment impacted FCR at 28, 36, and 46 d ($P < 0.01$), with the highest ($P < 0.01$) overall FCR (0 to 47 d) observed for birds fed the HHLL regimen and lowest FCR observed for birds fed the HHHH regimen. Males had higher parts weights ($P < 0.01$) for all parts except fat pads and higher yields for wings ($P < 0.01$), whereas females had higher yields for tenders ($P < 0.01$) and fat pads ($P < 0.01$). Fat pad yield was highest ($P = 0.03$) for birds fed the HHLL regimen and lowest for birds fed the HHHH regimen. Breast fillets from male birds had a higher severity of WB than those from females birds ($P < 0.01$), but myopathies were unaffected by diet. Changes in AA density within dietary regimens used in this study impacted FCR and carcass fat, but were not sufficient to influence BWG or breast meat yield or myopathies.

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

M55 Determination of additivity of apparent and standardized ileal digestibility of amino acids in wheat, canola meal, and sorghum distillers dried grains in mixed diets fed to broiler chickens Saheed Osho*[GS], Olufemi Babatunde, Olayiwola Adeola *Purdue University*

An experiment was conducted to investigate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of crude protein (CP) and amino acids (AA) in mixed diets. Using the determined AID or SID for CP and AA in wheat, canola meal, or sorghum distillers dried grains (DDGS), the AID or SID for 2 mixed diets based on a wheat-canola meal or wheat-canola meal-sorghum DDGS were predicted and compared with determined AID or SID, respectively. A total of 480 Ross 308 male broilers were used in this study. Birds received a starter diet from d 1 to 16 post-hatching, thereafter grouped into 8 blocks and randomly allotted to 6 dietary groups in a randomized complete block design. The 6 experimental diets consisted of a nitrogen-free diet to estimate the basal endogenous loss of AA, 3 semipurified diets to determine the AID and SID of CP and AA in the 3 ingredients, and 2 mixed diets to test the additivity of AID and SID. Chromic oxide was added as an indigestible marker. On d 21 post-hatching, digesta from the distal two-thirds of the ileum was collected. To test the additivity of digestibility values, the difference between measured values in mixed diet and predicted values calculated with measured values in each ingredient were examined. The results revealed that the predicted SID were consistent with determined values, except for Arg, Phe, Asp, Cys, and Ser in the wheat-canola meal diet, and all the predicted CP, AA and total AA were consistent with the determined values in the wheat-canola meal-sorghum DDGS diet. The determined AID for Ile, Met, Thr, Val, Ala, Cys, Ser, and Tyr in the wheat-canola meal diet were greater ($P < 0.05$) than predicted. For the wheat-canola meal-sorghum DDGS diet, the measured AID was greater ($P < 0.05$) than the predicted AID for Trp, Cys, and Glu. However, the extent of the difference between the measured and predicted values in AID values for wheat-canola meal diet and wheat-canola meal-sorghum DDGS were higher than SID values for both diets. In conclusion, the results confirmed that standardized ileal CP and AA digestibility values were more additive than apparent ileal CP and AA digestibility values when a diet containing wheat, canola meal, and sorghum DDGS as protein sources were used.

Key Words: additivity, aminoacid, apparent, broilers, standardized

M56 The impact of feeding varying starter digestible lysine levels to Cobb MV × Cobb 500 male broilers on growth performance and processing yields Rosana Hirai*[GS], Leonel Mejia, Cesar Coto, Justina Caldas, Christopher McDaniel, Kelley Wamsley *Mississippi State University*

Increasing digestible Lysine (dLys) levels of diets may be effective in the starter phase if overall performance and economic benefits are realized throughout a complete growout period. Therefore, the objective of this study was to evaluate the carryover effect of feeding varying levels of dLys during the first 14 days of age to Cobb MV × Cobb 500 male broilers

on growth performance and carcass yields during a 42 d grow-out. Two basal diets comprised of mostly corn and soybean meal were formulated: LLys (Treatment 1) 0.88% dLys and HLys (Treatment 8) 1.44% dLys. The other 6 experimental diets ranged between 0.96 to 1.44% dLys in increments of 0.08%, which were obtained by blending in different proportions the LLys and HLys diets, and a positive control diet containing 1.28% dLys. Dietary treatments were provided to 1,344 male broilers randomly distributed in 96 pens from 0-14 d. Common grower and finisher diets were provided from 15-28 and 28-42 d, respectively. For this experiment, 1,344 day old chicks were randomly distributed in 96 pens placed at 14 chicks/ floor pen (0.08 sq m/bird) in a RCBD with 11 blocks. Feeding increased dLys levels resulted in higher ($P < 0.05$) BW gain and lower ($P < 0.05$) FCR during the first 14 days of age. No significant differences ($P > 0.05$) were observed in BW gain or carcass weight and yields at 41 days of age when birds were fed a minimum of 1.20% dLys during the first 14 days of age. In conclusion, benefits in BW, BW gain, and processing characteristics were found when birds were fed a minimum of 1.20% dLys during the starter period. Further research should evaluate the response on female Cobb MV × Cobb 500 female broilers and longer growout periods.

Key Words: amino acids, compensatory gain, processing yield

M57 The effect of supplementing herbal Methionine through feed and water on growth performance and liver metabolism in broilers Fernanda Castro*[GS], Brian Fairchild, Woo Kim *University of Georgia*

Methionine (Met) is the first limiting amino acid for poultry fed corn/ soybean meal diets. The most common source of Met is DL-Met, obtained from petrol-derived products chemical synthesis. However, with the rising demand for the production of organic animal protein, the development of organic sources of this amino acid has increased. The aim of the study was to evaluate the effects of herbal Met (H-Met), provided through feed or water, on growth performance, abdominal fat and liver nutrient metabolism in broilers. A total of 600 one-day old broiler chicks (Cobb500) were distributed in a completely randomized design, with five treatments and six replicates of 20 birds, from one to 42d. H-Met was provided either through feed or drinking water, using carboys connected to the nipples. Two positive control treatments were used, one connected to the regular water line (W) and the other to carboys containing only water (C). The treatments were T1=100% of DL-Met + W; T2=100% of DL-Met + C; T3=Negative control (no Met supplementation); T4=60% DL-Met + 40% H-Met through feed; and T5=50% DL-Met + 50% H-Met through water. The carboys were weighed weekly to compare the water intake (WI) between T2 and T5. Liver metabolic markers in blood, such as aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), total protein (TP), albumin (ALB) and globulin (GLO), performance traits and abdominal fat pad were evaluated at 21 and 42 days, respectively. The means were subjected to ANOVA and, when significant, were compared by Duncan test ($p \leq 0.05$). There was no difference in WI between T2 and T5. Serum AST levels were significantly higher for T3 and T4 when compared to T2 and T5, and T1 showed intermediate values ($P=0.0318$). T3 showed lower body weight gain, feed intake and higher feed conversion ratio when compared to the other treatments ($P<0.0003$). Abdominal fat was higher in T3 when compared to T1, T2 and T5, with T4 showing intermediate values ($P=0.02$). In conclusion, when compared to the positive control diet, the supplementation with 50% of H-Met through water resulted in similar growth performance and liver function assessed through blood markers, indicating that H-Met is a potential supplementation source of Met to organic production systems.

Key Words: Herbal, Methionine, Broilers

M58 Comparison of two net energy calculations of two broiler strains fed varying levels of amino acids and metabolizable energy in two different temperatures Katie Hilton*[GS], Garrett Mullenix, Michael Schlumbohm, Antonio Beitia, Pramir Maharjan, Judy England, Victor Naranjo, Antonio Kalinowski, Jordan Weil *University of Arkansas*

Two experiments were conducted. The purpose of experiment one was to determine the effect of diets varying in amino acid levels, in two broiler strains on net energy (NE) from d 22 to d 43. The purpose of experiment two was to determine the effect of varying levels of metabolizable energy (ME) on NE from d 22 to d 43. Three experimental diets were introduced on d22. Experiment one, diets were iso-caloric and formulated to AMINOChick® 2.0 recommendations for dLys and were formulated at 80, 100 and 120% of dLys. All other amino acids were formulated as a ratio to dLys. In experiment two, diets were iso-nitrogenous and true metabolizable energy (TME_n) values of diets determined *in vivo* were 2819, 3137, and 3452 kcal/kg. Birds were moved to the respiratory chambers 1d before evaluation for a period of adaptation. Heat production (HP) Kcal=3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974) was measured for 1d. After HP was measured, fasting heat production (FHP) was measured for 24h. Heat increment was determined (HI)=HP – FHP (Farrell, 1974). Body composition was measured on d 22 and d 42 by dual energy X-ray absorptiometry (DEXA) to determine net energy gain (NEg). Two NE equations were compared, NE_1(kcals)=ME-HI versus NE_2(kcals)=NEg + NEm, where NEm (net energy maintenance)= HP – HI. Both experiments indicated a linear relationship between Line A and Line B with Line A gaining more NEg ($p<0.0001$) than Line B. Furthermore, experiment one showed a significant difference between hot and cold temperature for NEg ($p<.0001$) and for NE_2 ($p=0.0180$), with cool temperature gaining 148 kcals/kg FI more than hot temperature and cool having overall 575 more kcals/kg FI of NE_2. Experiment two showed no significant differences between genetic lines. However, temperature effects indicated significant differences for NEg and NE_2. Hot temperature birds had 251 kcals/kg FI more NEg compared to cool temperature birds ($p<.0001$). Lastly, cool temperature birds had more ($p=0.0180$) NE_2 compared to hot temperature birds. These experiments indicate that NE expressed as NEm + NEg provides more information about the broilers genetics, body composition and environment.

Key Words: Amino Acids, Metabolizable Energy, Net Energy, Heat Production, Body Composition

Metabolism & Nutrition - Vitamins & Minerals

M59 Total betaine content in organic feed ingredients and betaine digestibility of organic feed ingredients in broiler chicks Darlene Bloxham*[GS1], Ali Boone[1], Jomara Broch[2], Gene Pesti[1] *[1]University of Georgia; [2]Universidade Estadual do Oeste do Parana*

Betaine (BET) is a compound that can be naturally occurring in plants, especially under drought conditions. BET can be important for animals as well. Animals can benefit from BET under heat stress or low methionine (MET) diets. This is especially important in organic poultry diets, where synthetic MET is limited by law, and the diet would not supply adequate MET. The objective of this study is to evaluate total BET in feedstuffs and the digestibility of BET of organic feed ingredients in broiler chickens. Fifteen ingredients were used to determine total BET content in each feedstuff: alfalfa meal (n=2), soybean meal (n=2), wheat midds, hominy corn, barley, canola meal, field peas, sorghum, sunflower meal, oats, flaxseed, and millet. Some ingredients had no BET detected these were: alfalfa meal (1), soybean meal (1), hominy, corn, field peas, and flaxseed. Some ingredients had a low BET content of approximately 1 mg/kg which is limit of detection: soybean meal (2), canola meal, and oats. The ingredients that had quantifiable levels of BET were alfalfa meal (2) (759± 67mg/kg), barley (399±86 mg/kg), sorghum (32±12 mg/kg), wheat midds (4174±123mg/kg), millet (1069±120 mg/kg) and sunflower meal (337± 82mg/kg). Feed ingredients that had enough feedstuff and contained quantifiable BET were fed to broiler chicks to determine digest-

ibility of BET (barley, millet, and sunflower meal). Two hundred fifty-six Cobb 500 male broilers were fed three organic feed ingredients diets in two trials. There was also a basal diet to determine endogenous losses for BET. Test diets were fed between 10 and 14 d. Total ileal contents were collected on 14 d. Barley had the lowest digestibility of 43.7±12.1%, followed by sunflower meal with a BET digestibility of 67.4±8.4%. Millet had the greatest digestibility of 95.7±0.4%. All digestibility values were statically different from each other. In conclusion, BET content in feed ingredients and digestibilities are highly variable. BET content with feed ingredients is variable as well.

Key Words: Betaine, Digestibility, Broiler, Organic

M60 Effects of supplementation of 1-alpha-hydroxy vitamin-D3 and calcium levels on broiler live performance of Ross 708 male chicks up to 14 d Hernan Cordova-Noboa*[GS], Edgar Oviedo-Rondon, Viviana San Martin-Diaz, Santiago Alvarez-Muñoz, Ingrid Martinez-Rojas, Albaraa Sarsour, Deben Sapcota, Juan Fernández *North Carolina State University*

Calcium (Ca) and Vit-D3 are two important nutrients involved in overall body function and physiology. Metabolites of Vit-D3 with increased biopotency may ameliorate the effects of variability of dietary Ca content and availability in poultry feeds that affect live performance and productivity. One experiment was conducted to evaluate the effects of dietary Ca content and 1-alpha-hydroxy Vit-D3 supplementation on broiler live performance and flock uniformity in male broiler chicks up to 14 d. Ten treatments were evaluated resulting from a factorial arrangement of five levels of dietary Ca (0.8; 0.95; 1.10; 1.25; and 1.40 % respectively), and the supplementation or not of 1-alpha Vit D3 (0 *vs*. 0.00125%). Dietary treatments were obtained from a basal diet and Ca content was adjusted by including limestone, dicalcium phosphate and sand in the diet depending on the treatment. A total of 400 males Ross-708 d-old chicks, were placed in groups of 5 chicks per cage in 80 battery cages distributed in 4 Petersime batteries with a capacity of 20 cages each. BW and feed intake were obtained, and BW gain and FCR were calculated at 7 and 14 d. Flock uniformity (CV%) was evaluated at 14d. Data were analyzed in a randomized complete block design with dietary Ca level and 1-alpha-hydroxy-Vit-D3 supplementation as main factors and battery was considered as random effect. Interaction effects (P<0.05) on BW, BW gain, feed intake, and FCR were observed at 14 d. BW and BW gain were reduced (P<0.05) in chicks fed with supplemented diets when dietary Ca content was 1.4%. Moreover, the supplementation with 1-alpha Vit-D3 improved FCR up to 7 points, when dietary Ca content was 0.80% compared to male-chicks fed non-supplemented diets. In addition, a quadratic effect (P<0.05) due to dietary Ca level in the diet was observed on FCR at 14d. An interaction effect (P<0.05) was detected on flock uniformity (CV%) at 14d. Chicks fed dietary Ca levels of 0.95%, 1.10% and supplemented with 1-alpha Vit-D3 showed the best flock uniformity (5.88% and 6.56%, respectively). In conclusion, the supplementation of 1-alpha-Vit-D3 ameliorated the negative effect of low dietary Ca content (0.8% Ca) on FCR up to 7 point at 14d, however it reduced BW and BW gain at the highest dietary Ca level (1.40% Ca).

Key Words: Broiler, 1-alpha-hydroxy-Vit-D3, Calcium, Uniformity, Performance

M61 Effects of 1 alpha-hydroxy-vitamin-D3 on bone development, and mineral retention in diets containing different Ca levels for Ross-708 Viviana San Martin-Diaz*[GS], Edgar Oviedo-Rondon, Hernan Cordova-Noboa, Santiago Alvarez-Muñoz, Ingrid Martinez-Rojas, Albaraa Sarsour, Deben Sapcota, Juan Fernández *North Carolina State University*

Two experiments were conducted to investigate the effects of calcium (Ca) content in diets supplemented with 1-alpha-hydroxy Vit-D3 on mineral retention, bone relative assymetry (RA) weight and length. Ten treatments were evaluated resulting from a 2 x 5 factorial arrangement of diets supplemented or not with 1-alpha Vit-D3 (0 *vs*. 0.00125%) and 5 levels of Ca (0.8, 0.95, 1.10, 1.25 and 1.40 %, respectively). For the first and second experiment a total of 480 females and 400 males Ross-708 d-old chicks, were allocated in groups of 6 and 5 chicks/cage, respectively in 80 cages. At 11 and 16 d, three chicks/cage were selected for tibia, femur and shank collection. Samples were weighed and measured to determine the RA weight and length, the right tibia was used for ash analysis. Fecal samples were collected from 11 to 13 d and mineral retention evaluated only in the second experiment conducted with males. Data were analyzed in a randomized complete block design with dietary 1-alpha Vit-D3 supplementation and Ca level as main factors and battery was considered as random effect. The RA weight of tibia, femur and shanks were not affected ($P > 0.05$) by dietary Ca content in female chicks. In contrast, RA weight of tibia and shank were affected ($P < 0.05$) by dietary Ca content in male chicks. In addition, for both experiments the supplementation with 1-alpha reduced ($P < 0.05$) the RA length of tibia. An interaction effect ($P < 0.05$) was observed on tibia ash in the experiment with male chicks. Broilers fed non-supplemented diets with 1.25 % dietary Ca had higher tibia ash compared to chicks fed non-supplemented diets containing 0.80 and 1.40 % dietary Ca. Supplementation with 1-alpha Vit-D3 resulted in intermediate responses, therefore supporting bone mineralization regardless of the dietary Ca level. Interaction effects ($P < 0.05$) were detected on Ca, P, Mn, and Zn retention. The supplementation of 1-alpha Vit-D3 in diets containing 0.95 % dietary Ca improved Ca and P retention, whereas in diets containing 0.80 % dietary Ca enhanced Mn retention up to 2%.. In conclusion, 1-alpha- it-D3 supplementation improved Ca, P, and Mn retention in diets containing low dietary Ca and reduced the RA length of tibia in males and female chicks, improving early bone development.

Key Words: Assymetry, Bone, Mineral, 1-alpha-hydroxy-vitamin-D3, Calcium

M62 Role of long-term supplementation with 25-hydroxyvitamin D3 on layer bone 3D structural development, and laying performance Chongxiao Chen*[GS], Bradley Turner, Todd Applegate, Woo Kyun Kim *The University of Georgia*

High production laying hens suffered from osteoporosis during a late laying period, which has been a production and welfare concern for the industry. A study was conducted to evaluate the role of dietary 25-hydroxyvitamin D_3 (25HD) on layer bone 3D structural development and laying performance from 0 to 95 weeks. A total of 390 1-day old Hy-Line W36 pullets were randomly allocated to 3 treatments with 10 replicates. Dietary treatments were: 1) vitamin D_3 at 2,760 IU/kg (D); 2) vitamin D_3 at 5,520 IU/kg (DD), and 3) vitamin D_3 at 2,760 IU/kg plus 25HD at 2,760 IU(69µg)/kg (25D). Body weight and feed intake were recorded at the conclusion of each stage: starter1 (0-3wk), starter2 (4-6wk), grower (7-12wk), developer (13-15wk), prelay (15-17wk), peaking (18-38wk), layer2 (39-48wk), layer3 (49-60wk), layer4 (61-75wk), and layer5 (76wk-95wk). Egg production was recorded daily, and femurs were scanned using Micro-CT for 3D structural analysis at wk17, 60 and 95. At 17wk, 25D showed higher volume in cortical bone, trabecular bone, and bone marrow, along with greater cortical porosity, which resulted in a lower cortical bone mineral density (BMD), but without alerting bone mineral content (BMC). At wk60, 25D recovered from low cortical BMD, in turn, had highest total BMC, and highest cortical volume, and trabecular bone connectivity. At wk95, 25D showed the highest volume and lowest porosity in cortical bone, along with highest total BMD. The DEXA scanning results showed similar trends with highest whole birds BMD and BMC in 25D treatment at both 60wk and 95wk. For laying performance, DD showed lower feed intake at layer2, but higher at layer3; lower hen day production (HDP) from 18 to 48wk, but a trend of higher HDP during layer5 (P<0.1) compared the others. Meanwhile, 25D had better FCR(FI/dozen) at layer2, and highest HDP during peaking, layer 2, but no changes during the later period, which resulted in a higher overall (18-60wk) HDP compared with DD, but no difference for the overall period of 18-95wks. In conclusion, supplementation with dietary 25HD could increase bone volume in pullets, providing more space for mineral deposition during the

laying period, as well as improved FCR, cumulative HDP and had positive effects on laying hen bone quality.

Key Words: 25-hydroxyvitamin D3, pullet, laying hen, bone 3D structure, egg production

M63 Protective effects of vitamin E on laying hens under chanllenged with Salmonella Enteritidis Yaojun Liu*[GS], Qiugang Ma, Lihong Zhao, Jianyun Zhang, Cheng Ji *China Agricultural University*

This study was conducted to examine the effects of vitamin E (VE) on production performance, egg quality, serum antioxidant capacity, intestinal villus morphology and immune function of Jinghong commercial laying hens under normal and challenged with Salmonella Enteritidis (SE). At age of 32 weeks old, 80 hens with negative effect after SE were randomly allotted to 4 treatments with 20 replicates (each replicate 1 bird, single cage rearing). Through a 2×2 randomized trial design, 2 vitamin E levels and whether challenge with SE. At age of 44 weeks old, challenge with SE 1×10^9 CFU and normal saline 1ml respectively. After 7 days and 21 days select 4 hens from each treatment and collect samples. The result showed that: SE significantly decrease average egg weight（P<0.05）, VE significantly decrease rates of death（P<0.05）, SE and VE have significantly interaction effect on feed egg ratio and daily egg mass（P<0.05）. SE significantly decrease hough units （P<0.05), VE significantly increase york color and york weight（P<0.05). At age of 44 weeks old, SE, VE and their interaction have no significant effect on serum antioxidant capacity (P>0.05); SE significantly increase IL-1β and IL-6(P<0.05); VE and SE have significant interaction effect on IgA (P<0.05). SE significantly decrease duodenum villus height, increase crypt depth and decrease V/C value (P<0.05); SE significantly decrease jejunum villus height (P<0.05); VE have significantly decrease jejunum crypt depth (P<0.05). At age of 46 weeks old, SE have significant increase MDA (P<0.05), VE and SE have significant interaction on MDA (P<0.05); SE, VE and their interaction have no significant effect on serum cytokines (P>0.05); SE have no significant effect on serum immune globuline (P>0.05), VE have significant increase IgA, C3 and C4 (P<0.05), VE and SE have significant interaction effect on IgM (P<0.05); SE significantly decerease jejunum villus height, increase crypt depth and decrease V/C value (P<0.05). In conclusion, the obtained results indicate the vitamin E as a functional feed additive contributes to alleviate SE infection.

Key Words: vitaminE, layinghens, SalmonellaEnteritidis, immunity

M64 Effects of Mn and Se sources on growth performance, carcass yield and occurrence of breast myopathies in broiler chickens Cristina Simões*[GS], Sergio Vieira, Alba Fireman, Liris Kindlein, Catarina Stefanello, Gabriela Santiago, Bernnardo Xavier *Federal University of Rio Grande do Sul*

An experiment was conducted to investigate the influence of inorganic and organic sources of selenium (Se) and manganese (Mn) at different levels on growth performance, carcass yield and breast myopathies of broilers. A total of 2,880 one-day-old male Cobb X Cobb 500 chicks were distributed in a completely randomized design with 8 treatments, 12 replicates and 30 birds per experimental unit and fed corn-soy diets. A 2 x 4 factorial arrangement with 2 Mn sources (80 ppm Mn sulfate; 40 ppm Mn amino acid complex and 40 ppm Mn sulfate) and 4 Se sources [0.30 ppm selenite; 0.15 ppm selenite and 0.15 ppm zinc-L-selenomethionine (Zn-L-SeMet); 0.30 ppm Zn-L-SeMet, or 45 ppm Zn-L-SeMet] was used. At 43 d, 6 birds per pen (n = 576) were processed to evaluate carcass and commercial cuts yields, occurrence and severity of wooden breast (WB) and white stripping (WS). Deboned breast fillets (*Pectoralis major*) were submitted to a panel evaluation in order to detect the presence of myopathies, as well as to provide scores of WB (0 – normal muscle; 1 – toughness in the cranial/caudal portion of muscle; 2 – widespread toughness in muscle; 3 – extreme toughness in all muscle) and WS (0 – normal muscle; 1 – less than 1 mm stripes and easily visible; 2 – more than 1 mm stripes). Data were analyzed using the GLM procedures of SAS. No differences between Mn sources were observed on performance, carcass traits and myopathies. Supplementation of 45 ppm Zn-L-Se-Met significantly increased (P < 0.05) BWG from 1 to 7 d, 36 to 42 d, and 1 to 42 d when compared to the inorganic Se source. Additionally, FCR has been significantly improved in birds fed diets supplemented with 45 ppm Zn-L-SeMet from 22 to 35 d, 36 to 42 d and 1 to 42 d. Means of WB scores were higher (P < 0.05) in breast meat of broilers fed 45 ppm Zn-L-SeMet compared to selenite and other organic Se levels, which can be justified by the positive correlation (P < 0.05) among WB, BWG and breast weight (0.25 and 0.37 respectively) that was observed. In conclusion, broilers fed diets with zinc-L-selenomethionine presented improvement in growth performance with higher occurrence of WB myopathy when compared to those fed with inorganic Se, which can be explained for the positive correlation among WB, BWG and breast weight.

Key Words: broiler, mineral sources, selenium, manganese, myopathies

M65 The effect of four feeding programs in rearing to sexual maturity on heat production and body composition in broiler breeder parent stock Jordan Weil*[GS], Katie Hilton, Antonio Beitia, Justina Caldas, Garrett Mullenix, Michael Schlumbohm, Pramir Maharjan, Maria Mayorga, Judy England, Craig Coon *University of Arkansas*

A total of 84 day-old parent stock Cobb500 FF were divided into four feeding treatments, which consisted of full-fed (FF), feed restricted (FR-4:3), feed restricted every day (FR-ED) and feed restricted skip-a-day (FR-SK) to determine the effect of feeding programs on heat production (HP) and body composition. FF were fed broiler starter, grower and finisher for 2 wk each while FR-4:3, FR-ED and FR-SK birds were fed pullet starter and grower. FF birds were fed *ad libitum* until 6 wk of age, and after 6 wk, FF pullets were placed on the same pullet grower feed as FR-4:3. Pullets were reared in floor pens and later transferred to individual cages and light stimulated at 21 wk. All treatments were fed the same Breeder 1 diet from 24 wk to the end of the production period. Body composition was measured every 2 wk from 0 to 32 wk by GE® Lunar Prodigy (dual energy X-ray absorptiometry, DEXA). HP was also measured every 2 wk using respiratory chambers using indirect calorimetry. Volumes of oxygen and carbon dioxide were measured in the respiratory chamber, and HP calculated using HP (kcal) = 3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974). At wk 4, FF pullets had more body fat (97.35 g/kg) compared to other treatments (P<0.01). However, during the preparation to lay at wk 20, FF pullets had less body fat by a minimal of 21.39 g/kg compared to all other treatments (P<0.01). Results of HP at 4 and 8 wk, showed FF pullets had more heat production than all other treatments (P<0.01). However, FR-ED treatment had higher HP kcal/kg feed at 20 wk (P<0.01). At this age, FR-ED birds also had the highest protein mass g/kg BW by 3.36 g/kg (P<0.01). Additionally, full fed pullets during the first 6 weeks resulted in pullets with less body protein from 10-20 wk during the rearing period, resulting in more fat deposition. This experiment indicated FF pullets mobilize protein immediately after feed restriction begins. A closer examination of feed restricted birds revealed that FR-4:3 birds had greater fat mass (g) (P<0.01) when compared to FR-ED and FR-SK birds. Lastly, this study indicates rearing program has a significant impact on body composition in the modern broiler breeder.

Key Words: broiler breeder, heat production

M66 Effect of high concentrations of dietary Vitamin D3 on skeleton health and eggshell quality when fed to W-36 laying hens from day of hatch until 68 week of age Jinlei Wen*[GS], Kimberly Livingston, Michael Persia *Virginia Tech*

The objective of this experiment was to investigate the effects of various concentrations of dietary Vitamin D_3 (VD3) on eggshell quality and bone health in white laying hens from day of hatch until 68 week of age. Initially, 360 Hy-line W-36 day-old pullets were randomly assigned to 5 treatments with 2 replicates per treatment. Birds in each treatment were split into 4 replicates at 2 week of age and into 8 replicates at 6 week of

age to meet flock density requirement as body weight increased. At 17 week of age, pullets were moved into a multi-teared A-frame cage system resulting in 5 treatments with 12 replicates of 6 birds per experiment unit. Control diets contained 1,475 IU VD3/kg diet and the the four treatment diets were manufactured by the supplementation of the control diet with 6,667, 16,667, 33,333 and 66,667 IU of VD3/kg diet on top. During the laying phase, up to 95 to 97 g of feed were provided per hen per day. The egg shell specific gravity, shell breaking strength data were analyzed using ANOVA and repeated measured and means were separated by Tukey's adjustment. Keel bone scoring were analyzed by nonparametric test (α=0.05). Bone mineral density, bone mineral content and bone breaking strength were analyzed by ANOVA and means were separated by Fisher's LSD test (α=0.05). Specific gravity of eggs from hens fed 66,667 IU VD3 diet were increased in comparison to those from control and 16,667 IU VD3 treatments with the reaming treatments being intermediate ($P \leq 0.05$). Eggs from birds fed 6,667, 33,333 and 66,667 IU VD3 showed an increased shell breaking strength than those from control treatment ($P \leq 0.05$). No significant differences were observed on keel bone score across all treatments. Bone mineral content of tibias from 68 wk old birds fed 66,667 VD3 concentration diets were increased in comparison to those from birds fed control, 6,667 and 16,667 VD3 diets ($P \leq 0.05$). No significant differences were noted with bone breaking strength but a trend of increased bone breaking strength was observed when feeding hens higher VD3 diets (P=0.09). Feeding high concentrations of VD3 diets generally improved eggshell quality and bone health of first cycle laying hens.

Key Words: Laying hen, Vitamin D3, bone health, eggshell quality, first cycle

M67 Broiler breeder hens zinc requirement André Mayer*[GS], Sergio Vieira, Eveline Berwanger, Liris Kindlein, Cristina Simões *Federal University of Rio Grande do Sul - UFRGS*

The objective of this study was to determine Zn requirement of broiler breeder hens. One hundred and twenty Cobb500 25wk old hens were randomly allocated in individual cages. Hens were fed a Zn deficient diet (18.7 mg Zn/kg) for 8wks. After depletion period, at 33wk, hens were individually weighed and assigned to one of six dietary treatments of 20 replicates. Zn was added to the deficient diet at 30, 60, 90, 120 and 150 mg/kg as zinc sulfate heptahydrate. Response variables were evaluated at the end of each 28d period (33-36, 37-40, and 41-44 wk of age). Eggs were collected four times a day and classified in normal, cracked and deformed. Settable eggs considered only eggs classified as normal. Total and settable egg production were analysed using Proc Mixed (SAS) in a 6×3 factorial design with repeated measures. The number of eggs per housed hen was analysed by Proc GLM in a CRD. Optimal dietary Zn was estimated by broken-line with quadratic (BLQ) and exponential (EXP) regression models. There were significant interactions between period and dietary Zn level for total and settable egg production ($P < 0.05$), thus regressions were applied for each period separately. All regression models used to estimate Zn optimal level were statistically significant ($P < 0.0001$). Zn requirement for optimal egg production, estimated by the BLQ model, were 83.0, 61.0, and 60.6 mg/kg, for periods 33-36wk, 37-40wk and 41-44wk respectively. As for the EXP model, Zn requirement was estimated at 78.3, 52.3 and 51.9 mg/kg for the same periods. Considering settable egg production, Zn requirement estimated by the BLQ model were 59.2, 58.3 and 65.3 mg/kg for periods 33-36wk, 37-40wk and 41-44wk respectively. Similarly, Zn requirement estimated by the EXP model were 49.1, 46.3 and 60.5 mg/kg for the same periods. Maximum number of eggs per housed hen were obtained with 72.8 and 64.2 mg Zn/kg, estimated by the BLQ and EXP models, respectively. In conclusion, data from this study indicates that broiler breeder hens Zn requirement for egg production rang from 46.3 to 83.0 mg of Zn /kg of feed.

Key Words: breeder, egg, mineral, zinc

M68 Effects of different supplementation levels of choline and total sulfur amino acid on growth performance of broiler chickens Gabriela Santiago*[GS], Sergio Vieira, Liris Kindlein, Cristina Simões, Thiago Luiz Noetzoud, Marco Antônio Ebbing, Nathália Isabelle Cordeiro *Federal University of Rio Grande do Sul*

Choline is an essential nutrient for poultry and has roles in the methyl donation, membrane function, transportation of lipids, and neurotransmission. An experiment was conducted to evaluate growth performance of broilers fed corn-soy diets supplemented with increasing levels of choline from choline chloride (60%). A total of 525 one-day-old Cobb 500 chicks were distributed in a completely randomized design in 75 battery cages, 7 birds per cage. A 73% corn semi-purified basal diet (342 ppm of choline) was supplemented, using a 3 x 5 factorial arrangement (3 levels of digestible total sulfur amino acid (TSAA) ratio to digestible Lys - 70, 75 and 80%; and 5 levels of choline supplementation: 0, 700, 1,400, 2,100, and 2,800 ppm). A 2-phases feeding program was used and growth performance was evaluated until 21 d. At 21 d all birds were evaluated for valgus and varus deformities. Data were analyzed using the GLM procedures of SAS and performance data were fitted to linear and quadratic polynomial regressions and the maximum response of choline supplementation was estimated. No interactions between TSAA and choline were observed, also no differences among TSAA levels ($P > 0.05$). The body weight gain (BWG) of broilers fed diets with increasing levels of choline increased quadratically ($P > 0.05$) and FCR decreased quadratically ($P < 0.05$) from 1 to 7 d, 8 to 14 d, 1 to 14 d, and 1 to 21 d. From 1 to 7 d, quadratic regression estimated requirements as 2,836 ppm for BWG and 2,156 ppm for FCR. From 8 to 14 d, requirements were determined as 3,111 ppm for BWG and 2,339 ppm for FCR. From 1 to 14 d, quadratic regression estimates were 3,073 ppm for BWG and 2,318 ppm for FCR. From 1 to 21 d, requirements were estimated as 2,711 ppm for BWG and 2,165 ppm for FCR. Treatments with no supplementation of choline had higher valgus deformities ($P < 0.05$) compared to the other levels of choline. Choline level that provided better BWG and FCR responses were determined as 2,711 and 2,165 ppm for starter phases, respectively. Considering a corn-soybean meal common diet (1,500 ppm of choline), 1,211 and 665 ppm of choline inclusion are appropriate to improve BWG and FCR response in starter phases, which are above breeder's recommendation (400 ppm of choline for starter phase).

Key Words: choline, broiler, sulfur amino acid, requirement, performance

M69 The effects of a titration of trace mineral proteinates in broiler breeder diets on progeny performance Coltin Caraway*[GS], Yanming Han, John Brake *NC State University*

The carry-over effects of broiler breeder trace mineral (TM) supplementation on male and female broiler progeny were evaluated when the breeder flock was 28 wk of age. A titration of TM proteinates (organic-ORG) and a control premix containing TM sulfates (inorganic-INO) were fed to broiler breeders from placement through 28 wk. The treatments were as follows: 1) Cu – 6 ppm, Mn – 100 ppm, and Zn – 100 ppm in sulfate form (INO-100%), 2) Cu – 6 ppm, Mn – 100 ppm, and Zn – 100 ppm in proteinate form (ORG-100%), 3) Cu – 3 ppm, Mn – 50 ppm, and Zn – 50 ppm in proteinate form (ORG-50%), and 4) Cu – 2 ppm, Mn – 33 ppm, and Zn – 33 ppm in proteinate form (ORG-33%). There were 4 replicate pens/TM treatment in the growing and laying houses. In the laying house, each pen housed 64 females and 8 males. Male and female chicks from each breeder treatment were randomly assigned to one of 40 broiler pens in a randomized complete block design, resulting in 5 replicate progeny pens/sex/breeder treatment. Each replicate pen housed 4 chicks from each pen/breeder treatment for a total of 16 chicks/pen. Broiler chicks were fed common starter and grower diets. Broiler BW, feed intake (FI), and FCR were determined at 14, 28, and 35 d. Data was analyzed using the FIT MODEL platform of JMP 12.2. In general, broiler breeder TM treatment was observed to impact male progeny, but not female progeny. At 35 d, BW of male progeny from ORG-100% (2246 g) parents was greater

(P≤0.05) than BW of male progeny from ORG-50% (2128 g) or ORG-33% (2116 g) parents, with INO-100% (2184 g) intermediate to ORG-100% and ORG-50%. BW differences were a function of FI as from 14-35 d, male progeny from ORG-100% (2725 g) parents exhibited increased (P≤0.01) FI compared to male progeny from ORG-50% (2604 g) and ORG-33% (2554 g). FCR and carcass yield were not affected by parental TM treatment. This study was in agreement with previous results and it was apparent that parental TM nutrition may function to alter progeny FI and BW and may be a useful tool for optimizing broiler performance.

Key Words: breeders, minerals, nutrition, carry-over, progeny

M70 Effects of dietary inorganic trace minerals replaced by organically bound trace minerals on the performance and antioxidant status of broiler breeders Lujie Liu*[GS], Geng Wang, Bojing Liu, Minqi Wang, Gang Lin, Yan Xue, Tuoying Ao *College of Animal Science, Zhejiang University*

This study investigated effects of replacing inorganic trace minerals (ITMs) with organic ones (BIOPLEX PP, Alltech Inc.) on the performance and antioxidant status of broiler breeder hens. Six hundred ZhenNing yellow feather hens at 40 wks of age, were randomly allotted to five dietary treatments with four replicated pens of 30 hens for 10 wks. Dietary treatments were: (G1) basal + ITMs at commercial level; (G2) basal + ITMs at low level (equivalent to 1.0 kg BIOPLEX PP/Ton, about 50% commercial level or less except Se); (G3), (G4), (G5): basal + 0.75, 1.0, 1.25kg BIOPLEX PP/Ton respectively. Fertility and hatchability were evaluated at wks 3, 5 and 7 post treatment diets. At the end of the trial, blood and liver samples were taken to analyze antioxidant enzyme activities. Breeder hens fed G4 diet had higher (P<0.05) HDP and lower (P<0.05) feed:egg ratio compared to those fed G2 diet. Eggs from hens fed G1 diet and G5 diet had better (P<0.05) fertility and hatchability compared to those from hens fed other treat diets. The LH level of serum from G4 was higher (P<0.01) than that of serum from G2. The P4 , albumin, glucose, and TP levels of serum from G1 were higher (P<0.05) than the levels of serum from G2 and G3. The serum GSH-Px activity from G2 and G3 was lower (P<0.01) than that from other treatments. The serum SOD activity from G1 and G5 was higher (P<0.01) than that from G2 and G3 and the activity from G4 was higher (P<0.01) than that from G2. The serum Mn-SOD activity from G1 and G5 was higher (P<0.05) than that from G2. The liver GSH-Px activity from G1 and G5 was higher (P<0.01) than that from G2 and G3 and the activity from G4 was lower (P<0.05) than that from G1, but higher (P<0.05) than that from G2. The liver SOD activity from G2 was lower (P<0.05) than that from other treatments and the activity from G5 was higher (P<0.01) than that from G3 and G4. The liver Cu and Zn-SOD activity from G1 and G5 was higher (P<0.05) than that from G2. The MDA value of liver from G2 and G3 was higher (P<0.01) than that from other treatments (P<0.01). The results indicated that replacing ITMs with low level of BIOPLEX PP in broiler breeder diets was beneficial for the production and reproduction performance and antioxidant status of broiler breeders.

Key Words: minerals, performance, antioxidant, breeders

M71 Microbiota, growth performance, and processing characteristics of broilers fed a standard vs low vitamin and trace mineral diet Elle Chadwick*[GS], Ramon Malheiros, Peter Ferket, Robert Beckstead *North Carolina State University*

Limited research has examined if reducing vitamin and mineral (VM) levels will alter broiler gut microbiota, which could change growth and processing characteristics due to the microbiota's role in nutrient absorption and utilization. Two trials were conducted to determine variation between a standard and reduced VM diet. It is hypothesized that reduced VM will not shift the microbiota population significantly enough to cause change in growth performance, processing yield or meat quality. In Trial 1, Ross 708 male broilers were given 100% VM (standard) or 40% VM (reduced) diet (25 birds/pen, 2 pens/ treatment). Cecal contents for microbiota analysis were collected on D7 and 42 and sent to the UNC Microbiome Center for 16s RNA analysis. In trial 2, birds were given the 100% VM (standard) or 30% VM (reduced) diet (32 birds/pen, 6 pens/treatment). Feed and bird weights were recorded on D14, 28 and 42 for both trials. On D49, birds from trial 2 were processed and the following samples were collected: carcass and cut up weights, white striping and wooden breast scoring, drip loss, paw quality, and shank, skin and breast meat color (Minolta Colorimeter). Analysis of the data was performed in JMP Pro 13 via the student t-test to compare dietary effects (p≤0.05). Limited shifts in the microbiota population were identified. On D42, the Firmicutes: Bacteroidetes ratio showed the standard diet to be 1.7:1 and reduced to be 2:1. Phylum Firmicutes genus Lactobacillus had the greatest difference in abundance (D7: 32%, 48% and D42: 11%, 6%). Phylum Bacteroidetes genus Bacteroides showed differences in recovery on D7 (18%, 0.05%); but limited differences were apparent on D42. No significant differences were found in growth performance for either trial. For processing, significant differences were only identified in the b* values of the paw quality and breast meat, indicating more yellow pigment in the paws and less in the breast meat for the standard diet in comparison to the reduced diet. The few shifts identified in the microbiota population were not significant enough to alter performance. Only paw and breast meat color were altered by the reduced diet, indicating that reducing the amount of VM has limited to no effect on broiler ceca microbiota, growth performance and processing yields.

Key Words: broiler, microbiota, vitamin, mineral

Metabolism & Nutrition - Feed Additives & General Nutrition

M72 Evaluating the effect of a wheat-SBM-based diet and adaptation length on corn and wheat middlings digestible energy in broiler chickens Andrew Dunaway*[GS], Sunday Adedokun *University of Kentucky*

This study was conducted to evaluate the effect of feeding a wheat-based diet and adaptation length on digestible energy (DE) in 22 d-old male Cobb 500 broilers. A completely randomized design was used for this experiment with 6 birds/cage and 6 replicate cages/treatment. The study was conducted as a 3x3 factorial arrangement of treatments with 3 diets consisting of wheat-SBM (WS, reference), corn-wheat-SBM (CWS), and wheat middlings-wheat-SBM (WWS), and 3 adaptation lengths (4, 8, or 12 days). Birds were fed a corn-SBM-based diet that met or exceeded nutrient requirements for the first 10 days. Excreta was collected on day 21 and 22. The index method was used to determine the digestibility of dry matter (DM), nitrogen (N), and energy (En), while digestible energy (DE) of the feed ingredients (corn and wheat middlings) were determined using the difference method. Birds fed WS and CWS had higher (P < 0.05) DM, N, En digestibility, and DE when compared to WWS (DE: 3,447 vs. 2,836 kcal/kg). N digestibility and DE was higher (P < 0.05) in birds fed WS, but no significant difference was seen in DM and En (DE: 3,447 vs. 3,343 kcal/kg) between the two. The En and DE values were lower (P < 0.05) with increasing adaptation lengths (lower on day 12 vs. days 4 and 8; DE: 3,158 vs. 3,234 and 3,233 kcal/kg). Energy digestibility and the DE of the test ingredient were higher for corn (P < 0.05) compared to wheat middlings (DE: 3,288 vs. 1,614 kcal/kg; En 72.7 vs. 35.3%). Based on these results, the En and DE of corn and wheat middlings was not influenced by the adaptation length. However, corn had a higher DE compared to wheat middlings.

Key Words: Broiler, Corn, Energy, Wheat, Digestibility

M73 Effects of multiple direct fed microbial feed additives on broiler performance Corey Johnson*[GS1], Kyle Smith[1], Nathan Augspurger[2], Jason Lee[1] [1]*Poultry Science, Texas A&M University;* [2]*JBS United*

Two experiments were conducted to evaluate growth performance in broilers fed diets supplemented with two Bacillus spp. based direct fed microbial feed additives (DFM). In both experiments, birds were fed non-medicated diets in three phases (d 1-12, d 13-26 and d 29-35). All diets were corn-soybean meal based and contained corn dried Distillers' Grains with Solubles and meat and bone meal. Body weight (BW), feed consumption (FC), body weight gain (BWG) and mortality corrected feed conversion (FCR) were evaluated at d 12, d 26 and d 35 for both experiments. Replicates were arranged in a completely randomized block design with 50 male Cobb broilers per replicate. Experiment 1 consisted of four treatments and 13 replicates, including a non-medicated control and DFM A supplemented at 3.67×10^8, 7.35×10^8, or 1.10×10^9 cfu/kg, respectively. Feed conversion ratio was reduced ($p<0.05$) in broilers fed diets containing DFM A at all inclusion levels compared to the control from d 1-12, d 27-35 and d 1-35. Elevated inclusion of DFM A did not further improve any evaluated parameter as compared to the lowest inclusion level. These results demonstrate a performance benefit with the inclusion of DFM A. Experiment 2 consisted of three treatments and 12 replicates, including a non-medicated control, DFM A at 3.67×10^8 cfu/kg and DFM B at 7.35×10^7 cfu/kg. During this experiment, an elevated level of mortality was observed during the grower phase between d 15 and 19. Mortality was reduced ($p<0.05$) in birds fed DFM A compared to the control from d 15-26 (2.48 vs 9.99%) and d 1-35 (6.67 vs 15.61%). Feed conversion ratio was improved ($p<0.05$) in broilers fed diets containing each DFM premix from d 1-12 (1.24 vs 1.27) and DFM B from d 13-26 (1.55 vs 1.58) compared to the control. These data demonstrate the growth performance benefits observed when using either of the Bacillus spp. based DFM investigated in these experiments.

Key Words: DFM, Broiler, Performance, Mortality

M74 Dynamics of expression of tight junction protein encoding genes in the jejunum of broiler chickens induced to necrotic enteritis and supplemented with sodium butyrate and essential oils Cristiano Bortoluzzi*[GS1], Bruno Vieira[1], Juan Mallo[2], Monica Puyalto[2], Maria Villamide[3], Charles Hofacre[4], Todd Applegate[1] [1]*University of Georgia;* [2]*Norel;* [3]*Universidad Politecnica de Madrid;* [4]*SPR Group*

The objective of this study was to determine the effects of sodium butyrate protected with sodium salts of palm fatty acids (0.1% of N'RGY; SB), and sodium butyrate plus essential oils (carvacrol and ginger, 0.5% of each in the commercial product) protected with sodium salts of palm fatty acids (0.1% of NATESSE; SBEO) on jejunum morphology, and expression of TJ proteins encoding genes in the jejunum *Clostridium perfringens* challenged broiler chickens. One-day-old broiler chickens were assigned to 4 treatments with 8 replicates of 58 birds each. The treatments were: 1 - Negative control – NC (basal diet and no challenge), 2 - Positive control – PC (basal diet and challenge) 3 – PC+SB, and 4 – PC+SBEO. On d 1, all birds were vaccinated against coccidiosis by coarse spray (Coccivac®-B52). On d 13, the birds from treatments 2 to 4 were inoculated with ~5,000 oocysts of *Eimeria maxima* by oral gavage. On d 18 and 19, the same birds were administered a fresh broth culture of *C. perfringens* via drinking water. Jejunal samples were collected at 12, 18, 21, and 28 d of age to analyze morphology, microscopic lesion score, and expression of claudin 1, 2, and 4, zonula occludens 1, and occludin encoding genes. There was no effect of diet nor challenge on the microscopic lesion score ($P>0.05$). Jejunal morphology was affected by diet on d 12, wherein SB supplementation increased villus height ($P=0.001$) compared to the other groups. Additionally, on d 12, SBEO upregulated the expression of occludin ($P=0.02$), when compared to the other groups. At d 18, five d after the *E. maxima* challenge, SB supplementation upregulated claudin 4 expression ($P=0.02$); SB tended to upregulate claudin 1 ($P=0.08$), and partially attenuated the effect of the challenge on the expression of zonula occludens 1 ($P=0.07$). No effects were observed at d 21 on the expression of these genes. On d 28, SB supplementation partially restored the expression of claudin 1 ($P=0.02$), compared to PC or PC+SBEO. Sodium butyrate supplementation had beneficial effects on the expression of TJ genes. Future research is needed, however, to determine whether these changes would affect the leaking of plasma proteins into the intestinal lumen, and decrease the translocation of undesirable molecules and/or bacteria.

Key Words: Butyrate, broilers

M75 The effect of refined functional carbohydrates (RFC) and coarse corn in broiler diets on the prevalence of Salmonella in broiler ceca. Grayson Walker*[GS], Coltin Caraway, Sangita Jalukar, John Brake *North Carolina State University*

Enzymatic hydrolysis of yeast produces RFC that have activities against gram negative bacteria. Specifically, Aviator SCP (Arm and Hammer Animal Nutrition, Princeton, NJ) possesses sugars that interfere with *Salmonella* attachment to the intestinal lumen. The utilization of dietary coarse corn (CC) in addition to RFC was investigated in response to a need for holistic broiler microbial control programs that address nutrition and management aspects of broiler production. A total of 576 male broiler chicks were assigned to new litter pens following coccidiosis vaccination. Broilers were fed a 0 (RFC-0) or 100 (RFC-100) g/MT RFC diet. Broilers were further fed either a fine corn (FC) diet or a diet with increasing levels of CC (0% to 10 d, 15% from 11 to 21 d, 30% from 22 to 35 d, and 45% from 36 d) to 48 d of age to complete a 2 x 2 factorial design. Treatments were randomly assigned to 6 replicate pens of 24 broilers per interaction. At 48 d of age, 5 broilers were randomly selected from each pen and assayed for presence of *Salmonella* in the ceca using enzyme linked fluorescence assay methods. *Salmonella* were isolated from 10.00% of ceca from RFC-0/FC broilers and 16.67% of ceca from RFC-0/CC broilers, which was significantly greater than the RFC-100/FC broilers ($P<0.05$) where there was no *Salmonella* isolated. The RFC-100/CC broilers were statistically intermediate with 6.67% of ceca positive. These data demonstrated that RFC had a beneficial effect on the presence of *Salmonella* in the ceca of broilers.

Key Words: Salmonella, RFC, coarse, corn, broiler

M76 Performance, nutrient utilization and relative immune organ weights in broiler chickens fed corn-soybean meal diets without or with yeast nucleotides upon challenge with Eimeria Haley Leung*[GS], Rob Patterson, John Barta, Elijah Kiarie *University of Guelph*

Studies on the effects of yeast supplements report conflicting findings on performance, nutrient utilization and immune response in the presence or absence of an enteric pathogen challenge. To evaluate the effects of yeast nucleotides (YN) on performance, nutrient utilization and immune organ weights, 336 d old male broiler chicks (Ross 708) were used. The birds were housed in floor pens with fresh wood shavings and allotted to a corn-soybean meal based diet without or with YN (500 g/t; n=14). On d 10, birds in seven pens/ diet were orally given 1-mL of *Eimeria* culture (*E. acervulina* and *E. maxima* sporulated oocysts) and the rest were given 1-mL of distilled water. On d 15 and d 35, two birds/pen were euthanized for spleen, bursa and thymus weights. Apparent retention (AR) of nutrients and AME were assessed on d 30-34. There was no YN effect ($P < 0.05$) on performance pre-challenge. An interaction between YN and *Eimeria* ($P = 0.046$) was observed for feed intake (FI) such that FI increased in *Eimeria* challenged birds not fed YN. *Eimeria* decreased ($P< 0.01$) final BW by 7% (2,186 vs. 2,359 g), BWG by 8% (1,927 vs. 2,101 g) and FCR by 14% (1.858 vs. 1.630). On d 15, spleen weight was significantly increased by *Eimeria* (83 vs 99 mg/g BW, $P< 0.01$) and tended to be increased by YN ($P= 0.07$). On d 35, YN increased bursa weight (157 vs 178 mg/g BW, $P= 0.04$). There was a tendency for an interaction effect ($P= 0.09$) on d 35 thymus weight, such that in challenged birds, YN fed birds tended to show light thymus relative to non-YN fed birds. There was no interaction ($P > 0.05$) between *Eimeria* and YN or *Eimeria* on AR and AME. The main

effects of YN were such that birds fed YN had lower ($P< 0.01$) AMEn relative to birds not fed YN. There was no interaction ($P > 0.05$) between *Eimeria* and YN or YN on caloric efficiency (observed FI*AMEn/BWG). *Eimeria* decreased caloric efficiency (5,604 vs. 6488 kcal/kg, $P< 0.001$). In conclusion, *Eimeria* challenge without or with YN depressed growth performance and caloric efficiency. *Eimeria* increased spleen weight in d 15 birds and YN stimulated relative bursa weight gain in d 35 birds suggesting implications on short and long term immunity respectively.

Key Words: Eimeria, nucleotide, performance, immune, energy

M77 Evaluating the effect of rye versus corn as a source of energy on the microbiome in broiler chickens in a nutritional rehabilitation model Mikayla Baxter*[GS], Juan David Latorre, Si Hong Park, Steve Ricke, Sun Ae Kim, Billy Hargis, Guillermo Tellez-Isaias *University of Arkansas*

The purpose of this study was to evaluate changes in the microbiome after the consumption of a low digestible diet (rye) and/or a highly digestible diet (corn) in broiler chickens. There were four dietary treatments: 1) corn-based diet (corn-corn); 2) rye-based diet for 10 days, and then switched to the corn diet (rye-corn); 3) rye-diet (rye-rye); and 4) corn diet for 10 days, and then switched to the rye diet (corn-rye). At 20d-of age birds were euthanized and samples of duodenum, upper and lower ileum and ceca were collected for microbiome analysis with an Illumina Miseq platform. In duodenum and upper ileum there was no difference between the treatments at phylum, family and genus levels. In the lower ileum, the rye-rye treatment group had significantly higher amount of *Firmicutes* at the phylum level*; Lactobacillaceae* at the family level; and *Lactobacillus* at the genus level than the corn-corn group ($P < 0.01$). The rye-corn treatment group had significantly higher *Turicibacteraceae* at the family level and *Turicibacter* at the genus level than any other treatment groups. The corn-corn treatment groups had significantly higher percentage of *Erysipelotrichaceae* at the family level and *Ruminococcus* at the genus level than the other treatment groups. In the ceca at the level of the phylum, chicks consuming rye in the second phase of the experiment had significantly more *Cyanobacteria* and *Proteobacteria; Bifidobacteriaceae*, *Lactobacillaceae* and *Clostridiaceae* at the family level and *Bifidobacterium*, *Lactobacillus*, and *Clostridium* at the genus level than those chicks consuming corn. In contrast, chicks fed corn in the second phase of the study showed significantly higher levels of *Coriobacteriacea*, *Turicibacteraceae*, *Ruminococcaceae* and *Christensenellaceae* at the family level and *Faecalibacterium*, *Oscillospira*, and *Ruminococcus* at the genus level. It is evident from this experiment that changing the diet shifts microbial populations within the lower intestinal tract. However, there are also clear differences between microbial populations between each of the dietary treatments suggesting that diet can have lasting effects on the microbiome in the lower intestinal tract.

Key Words: Microbiome, Broiler, Rye, Corn

M78 Effects of 2-Nitropropanol on growth performance and intestinal lesion scores in Eimeria-challenged broilers Po-Yun Teng*[GS], Lorraine Fuller, Woo Kim *University of Georgia*

Coccidiosis is an avian disease infected by *Eimeria* spp. which causes economic damage worldwide in the poultry industry. Application of anticoccidial drugs is the conventional means for preventing coccidiosis, but emergence of drug-resistant strains underlies the need for developing alternative strategies for replacement. We conducted an experiment to study the effects of 2-Nitropropanol on growth performance and intestinal lesion scores in 14-day-old Cobb 500 broilers challenged with *Eimeria maxima*, *Eimeria tenella*, and *Eimeria acervulina*. A total of 60 12-day-old male Cobb 500 broilers were randomly allocated to four treatments (non-challenged control, challenged control, 100 ppm 2-Nitropropanol, and 200 ppm 2-Nitropropanol) and divided into 12 battery cages with 5 birds in each cage. A corn-soybean meal based control diet was formulated and experimental diets included 100 or 200 ppm 2-Nitropropanol. Broilers were fed experimental diets for 9 days and all birds except the un-challenged control group were challenged with *Eimeria maxima* (50,000 oocysts/bird), *Emeria tenella* (50,000 oocysts/bird), and *Eimeria acervulina* (250,000 oocysts/bird) on day 14. On day 20, growth performance and intestinal lesion scores were evaluated. The results showed treatments with *Eimeria* spp. challenge significantly decreased body weight and feed intake, and increased intestinal lesion scores compared to non-challenged control group. Birds fed 200 ppm of 2-Nitropropanol had poorer growth performance than both control groups, but significantly reduced duodenal lesion scores (2.6 vs. 1.3) compared to challenged control. Treatment with 100 ppm 2-Nitropropanol had no effects on growth performance and lesion scores. Further research is warranted to study the appropriate dosage of 2-Nitropropanol as a potential alternative to anticoccidial drugs.

Key Words: Coccidiosis, 2-Nitropropanol, broilers

M79 Effect of different inclusion levels of whole corn on productive and processing performance of broilers Andrea Rubio*[GS1], Juan Aranibar[2], Henry Fuentes[2], Wilmer Pacheco[1] *[1]Auburn University; [2]Panamerican Agriculture University Zamorano*

Previous research has shown that inclusion of whole cereal grains could be an alternative to improve performance and stimulate gastrointestinal tract (GIT) development of broilers. In addition, economical revenues could be generated from reducing the costs of grinding whole grains. The objective of this study was to evaluate the effect of different inclusion levels of whole corn before pelleting on live performance and processing yields of broilers from 1 to 42 d of age. A total of 1,000 male Ross x Ross 708 broiler chicks were randomly distributed among 4 treatments with 10 replicate pens per treatment and 25 birds per pen. The treatments consisted of different inclusion levels of whole corn (0%, 2.5%, 5% and 7.5%) that replaced ground corn. The starter diets were fed in crumbled form while the grower and finisher diets were fed in pelleted form. Feed consumption and BW were determined at 14, 28, and 42 d of age and feed conversion ratio (FCR) calculated by using the weights of the mortality. At 43 d, 10 birds/pen were processed for yield determination. After processing, carcasses were chilled in slush ice for 4 hours before chilled carcass weights were determined. At 44 d carcasses were deboned to determine breast meat weight and breast meat yield. Data were statistically evaluated using ANOVA procedure and means were separated by Tukey honestly significant different procedure. The inclusion of whole corn did not influence BW and feed consumption ($P>0.05$) at 42 d of age. However, birds that received diets with whole corn had better FCR from 28 to 42 d of age (1.94 vs. 2.00, $P<0.05$). Birds fed diets with 5% of whole corn had higher breast meat weight (292 vs. 284 g, $P<0.05$) than birds fed 7.5%, but similar to birds fed diets without whole corn. The results of this experiment indicated that up to 5% of whole corn could be used to replace ground corn during the starter, grower and finisher periods without a negative effect on broiler performance and carcass characteristics.

Key Words: carcass, corn, crumble, pellet, yield

M80 Parameters monitored during pelleting and their relationship to xylanase recovery JT Pope*[GS], John Brake, Adam Fahrenholz *North Carolina State University*

Three trials were conducted to investigate the effect of the level of mixer-added fat (MAF; 1, 3, or 5%), MAF source (canola oil, poultry fat, coconut oil), and pellet mill die length-to-diameter ratio (L:D; 8 or 10) on xylanase activity loss as measured by recovery throughout the pelleting process. For all three trials, 227 kg batches of broiler grower feed with and without xylanase for each factor were pelleted after conditioning for 30 seconds at 83°C. Samples were collected each minute of the pelleting run and included unconditioned mash, conditioned mash, and pellets to be analyzed for xylanase activity. Data collection during the pelleting process included the difference in temperature between hot pellets and conditioned mash (ΔT), pellet mill energy consumption (PMEC), and pellet samples for determining pellet durability index (PDI). These data were used to determine the relationship between ΔT, PMEC, PDI, and xylanase recov-

ery throughout the pelleting process. An inverse correlation between ΔT and xylanase recovery in pellets relative to mash and conditioned mash was observed (P<0.01). As ΔT increased, xylanase recovery decreased. It was also observed that PMEC and xylanase recovery in pellets relative to mash and conditioned mash were also inversely related (P<0.01). As energy input into the pelleting system increased, xylanase recovery in pellets relative to mash and conditioned mash decreased (P<0.01). Similar to ΔT and PMEC, as PDI increased, xylanase recovery decreased in pellets relative to mash and conditioned mash (P<0.01). In conclusion, methods employed to improve PDI that may result in increased ΔT and PMEC may also exacerbate xylanase activity loss during pelleting. Conversely, methods employed to reduce PMEC that result in reduced PDI and ΔT may spare enzymes during pelleting.

Key Words: xylanase, pelleting, PDI, thermostability

M81 Evaluation of spray-dried plasma in broiler diets with or without bacitracin methylene disalicylate Hunter Walters*[GS], Thomas Lester, Joy Campbell, Jason Lee *Texas A&M University*

The objective of the current study was to evaluate the impact of dietary inclusion of spray-dried animal plasma (SDAP) during the starter phase with or without bacitracin methylene disalicylate (BMD) on performance and processing yield. The experimental design consisted of a 2 x 2 factorial arrangement of SDAP (0 or 2% during the starter phase only) and BMD (0 or 50 g/ton to market) resulting in a total of 4 dietary treatments with 9 replicates per treatment and 22 birds per replicate for a total of 792 male broilers. Prior to placement, broilers received a coccidiosis vaccine via a commercial spray cabinet and placed on built up litter for a 41-d assay period. Average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption, and mortality (%) were determined on days 10, 28, 41. On d 41, birds were individually weighed to determine flock uniformity by calculating replicate coefficient of variation. Data were analyzed via a 2 x 2 factorial ANOVA with main effect means being deemed significantly different at P≤0.05. As expected, the addition of BMD positively influenced growth performance with elevated BW on d 10, 28, and 41, increased carcass yield and reduced FCR during the finisher phase and cumulatively throughout the entirety of the trial. Inclusion of SDAP during the starter phase increased d 10 BW (P<0.001). This increase in BW continued on d 28 and 41 and improved (P<0.05) flock uniformity on d 41. Additionally, starter diet inclusion of SDAP reduced starter FCR (P<0.001), while grower and finisher FCR were not impacted. The improvement in FCR observed when SDAP was present resulted in cumulative d 1 to 41 FCR being reduced (P=0.02) as compared to control fed broilers. These results demonstrate the benefit of SDAP supplementation when included in the starter phase with benefits in BW and FCR in diets with or without BMD.

Key Words: broiler, performance, animal, plasma

M82 Structural resilience and alterations of the gut microbiota in hens under dietary fat perturbations Cong Liu*[GS], Meiling Li, Lihong Zhao, Cheng Ji, Jianyun Zhang, Qiugang Ma *China Agricultral University*

It is well known that dietary fat levels may affect the gut microbiota structure and metabolism in mice and human, but changes in hens are still unclear. This study was conducted to investigate impacts of dietary fat levels on the gut microbiota community in hens. A total of 450 1-day-old BJ-Pink hens were randomly assigned into 3 groups (LFD, SD, and HFD group), in which hens fed the diet containing 0% , 1.2%, 2.4% soybean oil, receptively. Each group included 150 hens in five replicates. After 6 weeks, the hens were switched to a SD diet. The body weight (BW) of 20 hens which were randomly selected in each group was measured every 2 weeks. The Plasma was collected at weeks 6 and 25 to examine GLU, TC and TG; the cecal contents were collected at day 1, weeks 6 and 25 to examine the gut microbiota by using Illumina MiSeq sequencing. In the brooding time, the BW of LFD and HFD group was significant heavier than SD group (P<0.001), the levels of GLU, TC and TG of LFD group were higher than SD group (P=0.070, P=0.076, P=0.078, respectively). After reverting to SD feeding, the gap between LFD group and SD group narrowed (P>0.05), while there still had significant differences between SD and HFD group (P<0.05); the TC level in HFD group was higher than others (P<0.05). With age, the index of Chao and Shannon was increased dramatically (P<0.001), but there were no significant differences among groups in the same stage (P>0.05). The PCoA revealed that the gut microbiota of hens showed obvious segregation in different stages, and we can saw distinct differences among three groups at weeks 25. The gut microbiota structure of LFD group was similar to SD group. The lowest Firmicutes/Bacteroidetes ratio was observed in SD group during the whole period (P<0.05). The proportion of Erysipelotrichaceae in LFD and HFD group was extremely higher than that in SD group (P<0.01) at 6weeks. The proportion of S24-7 and Rikenellaceae was the lowest (P<0.05) in HFD group. In conclusion, dietary fat levels can alter the gut microbiota component. Feeding LFD and HFD diet may increase the rate of obesity, while feeding SD diet can help hens keep a good condition. Although the effects can be alleviated by feeding SD diet in growth period, it may not reach the optimal condition.

Key Words: microbiota, fat, plasma, Resilience

M83 In feed supplementation of Bacillus subtilis C-3102 spores on broiler breeder performance Eveline Berwanger*[GS], Sergio Vieira, André Mayer, Liris Kindlein, Masaya Kato, Danny Hooge, Yosuke Kuwamura *Federal University of Rio Grande do Sul*

The objective of study was to evaluate the in feed supplementation of *Bacillus subtilis C-3102* (Calsporin®) effects on live performance, egg hatchability and hatching chick quality. Six hundred forty Cobb 500 SF breeders and sixty four males Cobb were placed in 32 pens using completely randomized design. Two treatments were tested: a control diet and a control diet added with 60 g/MT of *Bacillus subtilis C-3102 (*Calsporin®), with 16 replications per treatment. Resulting data were measured in 10 periods (25-28, 29-32, 33-36, 37-40, 41-44, 45-48, 49-52, 53-56, 57-60, 61-64 weeks of age). Incubation was done monthly using 2,600 eggs. Hatching chicks were weighed, lengthened, and had legs and navel button scored. The navel score were stipulated as 1 (normal), 2 (medium abnormal) and 3 (severely abnormal), and leg score was 1 (normal) and 2 (medium and severely abnormal). Numbers of scores were transformed into frequency of scores. Data were analyzed using the Mixed procedure of SAS 9.3 (2009) with repeated measures (period). Means were considered significantly different at $P < 0.05$ using the Tukey Kramer test. Different diets without and with Calsporin® supplementation to broiler breeders had neither beneficial nor adverse effect upon overall egg production and egg hatchability. However, hatching chick weight and length increased 0.7 g and 0.4 cm, respectively, when supplemented Calsporin® in the diet ($P < 0.05$). The frequency of the improperly healed navel (score 2) increased 6% and the frequency of the appearance of improperly healed and blackened navel (score 3) increased 3.4% in hatching chicks from breeders receiving diet without Calsporin® ($P < 0.05$). In addition, within the last evaluated periods the frequency of score 1 in the navel of the hatching chicks from breeders fed with Calsporin® was higher, which indicates healthier hatching chicks ($P < 0.05$). The *Bacillus subtilis* as Calsporin® can be supplemented to broiler breeders without impairing performance and could lead to better hatching chick quality, increasing chicks weight and length and reducing contamination of hatching chicks.

Key Words: egg, hatchability, probiotic, progeny

M84 Effects of antibiotics and probiotic additives on the intestinal morphology of d 28 male broilers Bo Zhang*[GS], Kacey O'Donnell, Xi Wang, Wei Zhai *Mississippi state university*

The effects of antibiotics and probiotics on the intestinal morphology of Ross × Ross 708 male broilers under coccidial challenge were determined. A randomized complete block design was applied. A total of 640 broilers were randomly allocated to 8 blocks in an environment controlled house.

Each block contained 5 treatments, 16 birds per pen. Birds in each treatment were fed one of 3 diets including negative control basal (N), antibiotic (A, 50 g bacitracin /ton of feed), or probiotic (P, 1.1×10^5 CFU of *Bacillus subtilis* /g of feed) diet in each of the 2 feeding phases (0-14 d, 14-28 d). The five treatments were NN, AA, PP, AP and AN. On d 14, all birds were orally gavaged with 10x commercial coccidial vaccine containing 5 living *Eimeria* species. Birds in the AA group also received anticoccidial (72 g narasin /ton of feed) between 14-28 d. On d 28, the duodenum, jejunum, ileum and cecum were collected from 1 bird per pen. Coccidial lesion scoring and gut morphology were determined. The lesion scoring was analyzed using nonparametric WILCOXON analysis using PROC NPAR1WAY of SAS 9.4. Morphology data were analyzed using one-way ANOVA using PROC GLM. Partial correlation between lesion and morphology were analyzed using PROC GLM. Coccidial lesions of jejunum, ileum and cecum were not affected by diets. However, the lesions of the duodenums of birds fed PP were lower than those fed NN group ($P < 0.05$), with the other three groups intermediate. Thus, we further studied the dietary effects on duodenal morphology. Diets did not affect duodenal villus length. Birds fed AN diet exhibited deeper crypt depth than those fed AA ($P < 0.001$), AP ($P = 0.001$), NN ($P < 0.001$) or PP ($P < 0.001$). The severity of lesions in the duodenum tend to be positively correlated to crypt depth ($r = 0.313$, $P = 0.063$). Duodenal muscle thickness of birds fed AN diet was thicker than those fed AA ($P = 0.001$), NN ($P < 0.001$) and PP ($P = 0.007$). In addition, birds fed AP exhibited thicker muscle than those fed NN diet ($P = 0.023$). In conclusion, if antibiotic was added in starter diet, but withdrawn from grower diet, birds exhibited higher turnover of their duodenal epithelial layer as indicated by deeper crypt depth and stronger propelling activity as indicated by thicker muscle layer.

Key Words: antibiotic, broiler, coccidiosis, probiotic, morphology

M85 Assessment of a phytogenic feed additive (Biomin P.E.P. 125) effect on broiler performance fed a standard or low protein diet Jinquan Wang*[GS], Chasity Pender, Basharat Syed, Raj Murugesan, Woo Kim *University of Georgia*

Due to the increasing concerns of antibiotic resistance among the publics, the demands of antibiotic free products are taking a large part of market and increasing continuously. Phytogenic feed additives have been used as an alternative to antibiotic with positive effects on growth performance. The aim of this study was to evaluate the effect of a phytogenic feed additive (Biomin P.E.P. 125) comprising a blend of oregano, anise and citrus peel on broiler performance fed a standard or low protein diet. A 2×2 factorial experiment (8 replicates/treatment, 30 birds/ replicate) was conducted with two levels of crude protein (standard or crude protein reduced by 1.5%) and with or without supplementation (125ppm) of P.E.P.. A total of 960 one-day Cobb 500 male broilers was randomly allocated into four dietary treatments in an environmentally controlled house. Feed intake, body weight and feed conversion ratio were recorded on a weekly basis (d 7, 14, 21, 28, 35, and 42). The low protein diet resulted in a significant decrease ($p<0.05$) in body weight (at d 35 and 42) and a significant increase ($p<0.01$) in feed intake (d 0-7) compared to the standard protein diet. The low protein diet also caused a higher ($p<0.05$) feed conversion ratio (d 0-7, d 29-35, d 36-42, d 29-42 and d 0-42) compared to the standard protein diet. Supplementation of P.E.P. resulted in a lower ($p<0.05$) feed conversion ratio (d 29-35, d 36-42, d 29-42 and d 0-42) compared to the control. In conclusion, during the 42 days grow-out period, the low protein diet caused a decrease in body weight and a higher feed conversion ratio, whereas dietary supplementation of P.E.P. significantly reduced feed conversion ratio with a comparative body weight to the control, indicating that P.E.P. could further save the cost of feed for growers.

Key Words: broiler, performance, protein, phytogenic

M86 Evaluation of chromium propionate and a butyric acid-zinc complex on male growth performance, corticosterone level, and meat yield Thomas Lester*[GS], Kyle Brown, Karen Vignale, Christine Alvarado, Jason Lee *Department of Poultry Science, Texas AgriLife Research*

The objective of the current experiment was to evaluate the effects of chromium propionate (Crprop) and a butyric acid-zinc complex (Baz) on body we ight gain, feed consumption, feed conversion, mortality, carcass characteristics, meat quality, and corticosterone level when fed to broilers during summer weather conditions reared to 53 days of age. The experiment was a complete randomized block design arranged in a 2 x 2 factorial design. The two factors evaluated were the inclusion or absence of Crprop (200 ppb) and Baz (29.1% butyric acid and 50 ppm zinc), totaling 4 treatments with 15 replicates per treatment and 28 male chicks per replicate for a total placement of 1680 Cobb 700 broilers. Broilers were reared in a tunnel ventilated broiler house during the summer months. During the day, rearing temperature beginning on day 10 was consistent at 29 ± 2°C for 12 hours and 24 ± 2°C for the evening temperatures. Feed intake and body weight was collected at day 14, 28, 35, 42, and 52. On d 42 blood samples from 2 broilers per pen were obtained to determine corticosterone levels. On d 53, 7 broilers per replicate pen were randomly selected to determine processing weights, yield percentage, and the presence and severity of woody breast and white striping in breast filets. All data were subjected to a 2 x 2 factorial ANOVA with main effect means deemed significantly different at $P \leq 0.05$. Supplementation of dietary Crprop reduced mortality adjusted feed conversion ratio (FCR) during the withdrawal phase ($P=0.05$) and cumulatively from d 1-52 ($P=0.03$) as compared to control fed broilers. Additionally, Crprop supplementation decreased mortality rate ($P=0.02$), decreased corticosterone level ($P=0.05$) and increased breast meat yield ($P=0.05$) as compared to control fed broilers. The addition of the Baz reduced the percentage of breast filets exhibiting moderate or severe woody breast (42.3 vs 34.1) and increased the percentage of filets that were absent or had mild woody breast (57.7 vs 65.9). In conclusion, benefits of dietary supplementation of Crprop and Baz can be observed through improvements in performance and potential reductions in woody breast severity.

Key Words: Chromium, Butryate, Broiler, Yield

M87 Effects of Eimeria challenges and dietary additives on mortality and growth performance of broilers Kacey O'Donnell*[GS], Wei Zhai *Mississippi State University*

Effects of various *Eimeria* challenges and dietary additives on mortality and growth performance of broilers were studied. A total of 2,016 Ross × Ross 708 male broilers were divided into 12 treatments with 8 replications. The treatments were arranged in a 3 (diets) × 4 (challenges) layout. The birds were fed one of 3 diets: control, antibiotic (50g bacitracin/ton of feed), and probiotic (1.1×10^5 CFU of *Bacillus subtilis*/g of feed) and were orally gavaged on day 14 with either *Eimeria acervulina* (EA, 20,000 oocysts/bird), *E. maxima* (EM, 10,000 oocysts/bird), a combination of EA and EM, or equivalent distilled water. Data were analyzed using two-way ANOVA of the PROC GLM using SAS 9.4. As expected, birds not challenged with *Eimeria* exhibited higher BW gain and lower FCR and mortality ($P = 0.002$, 0.003, and 0.02, respectively) from d 15-22 as compared to birds challenged with EM alone or both EA and EM. Challenge did not affect BWG, FCR, or mortality from d 23-29 ($P = 0.345$, 0.312, and 0.274, respectively). However, from d 30-39, birds not challenged exhibited a higher mortality than those challenged with EA alone or both EA and EM ($P = 0.009$), associated with elevated FCR ($P = 0.032$). This may due to the reason that birds challenged with *Eimeria* earlier might have recovered from the coccidiosis (caused by *Eimeria*) and built up immunity to *Eimeria*. Birds not orally gavaged with *Eimeria* might have picked up *Eimeria* oocysts from the used litter and developed coccidiosis during this period. From d 40-56, mortality was higher in birds challenged with both EA and EM as compared to EA or EM alone ($P = 0.038$), however, with only 1% difference. Antibiotics in the diet helped to lower the mortality from

d 15-39; however, probiotics in the diet did not provide same protection as antibiotics did. Antibiotics also lowered FCR from d 15-29 as compared to the control diet. Dietary treatment did not affect FCR during later feeding phases. In conclusion, *Eimeria* challenge affected FCR and BWG for a relative short time from d 15-22, however affected mortality for a prolonged period from d 15-56. Different *Eimeria* challenges exhibited different patterns on mortality at various growth stages, which revealed specific coccidiosis development caused by different species of *Eimeria*.

Key Words: antibiotic, broiler, Eimeria, probiotic

M88 Effects of supplemental Azomite on the performance of chicks from 0 to 21 days of age Matthew Jones*[GS], Humg-Yu Hsiao, Adam Davis *University of Georgia*

As consumers become more conscious about the ingredients that go into poultry diets, it is important to explore natural products that may enrich performance. One such product, marketed as AZOMITE(R), a volcanic ash deposit containing multiple trace mineral substances, has been reported to promote growth in various production systems (poultry, aquaculture, swine, cattle, horticulture). In the current research, Cobb 500 male chicks were fed a basal diet or this diet supplemented with 0.125, 0.250 or 0.500% Azomite from day of hatch to 21 days of age. Each dietary treatment had 18 replicate pens distributed equally across 3 battery brooders and each pen contained 5 chicks. The feed to gain ratio from 0 to 21 days of age was significantly ($P < 0.05$) improved in the broilers fed all three diets containing Azomite relative to those fed the basal control diet. Weight gain was significantly improved in the broilers fed the lowest level of Azomite relative to those fed the control diet. Plasma alpha 1 acid glycoprotein (AGP) levels and apparent Ca and P digestibility coefficients were determined in the broilers fed the diets containing 0 and 0.5% Azomite. The concentration of AGP was significantly decreased in the broilers fed the Azomite supplemented diet. The apparent digestibility of both minerals was improved in the broilers fed the diet containing 0.5% Azomite. In a subsequent experiment, with 49 day old broilers apparent Ca digestibility was also improved in birds fed a diet containing 0.25% Azomite. The results suggest that the addition of Azomite to a broiler starter diet may improve bird performance by decreasing inflammation.

Key Words: broilers, calcium, AGP

M89 Intestinal microflora stabilization with combining prebiotic effect and antimicrobial properties of herbal plant extracts Zsofia Bata*[GS], Laszlo Poppe *Budapest University of Technology and Economics, Department of Organic Chemistry and Technology*

Phytobiotics, as it is demonstrated under both *in vitro* and *in vivo* conditions, can address gut health challenges in the poultry industry and can be used as novel growth promoters. Traditional herbs play an important role in eliminating pathogenic infections, represent a novel solution for antibiotic resistance at an affordable price and serve the consumer's need for antibiotic free production. Combining herbal plant extracts with an optimal prebiotic allows products to work through multiple modes of action and ensures normal growth of the commensal microflora while inhibiting the growth of undesired pathogens and enhancing production parameters. The aim of this research project was to develop a natural growth promoter which has a selective inhibiting effect on the Gram positive pathogenic bacterial infections (*C. perfringens*); without limiting the multiplication of the commensal flora. 20 herbs were selectively screened individually and in combination for anti-clostridial activity *in vitro* using minimal inhibition concentration assay to determine combinations which inhibit the growth of *C. perfringens* at low concentrations; while having no effect on the normal microbiome. Additionally, due to the synergistic effect of the phytobiotic and prebiotic combination enhanced bioactivity was observed *in vivo*. *In vivo* testing to determine product efficacy was done on commercial broiler chicken farms in both Europe and Latin America. Results showed that in experimental trials production parameters (BWG, mortality and FCR) were significantly improved and incidence of necrotic enteritis was reduced in groups that received the phytobiotic/prebiotic included as part of the commercial basal broiler diet when compared to the non-treated groups. Metagenomic analysis revealed that the phytobiotic/prebiotic inhibits the growth of *C. perfringens* in the small intestine, the product does not influence the growth of *C. perfringens* in the large intestine, and therefore ensures optimal butyric acid production in the large intestine. These results suggest our novel phytobiotic/prebiotic efficiently controls *Clostridium perfringens* and improves production parameters when used in broiler chickens; providing a promising alternative to antibiotic growth promoters.

Key Words: phytobiotics, prebiotics, C.perfringens

M90 Comparison of three different oils sources added to broiler breeder diets and evaluated on egg size, hatchability and progeny performance Antonio Beitia*[GS], Justina Caldas, Michael Schlumbohm, Garrett Mullenix, Jordan Weil, Pramir Maharjan, Maria Mayorga, Judith England, Craig Coon *University of Arkansas*

The purpose of the study was to evaluate the effects of three different oil sources added to feeds of parent stock hens' (PS) on egg size, hatchability and progeny performance. Corn and canola oils have a different fatty acid profile compared to poultry oil. Previous research at University of Arkansas has shown benefits to embryonic development and early chick performance due to fatty acid supplementation. Additional research is needed to determine if feeding diets with canola oil or corn oil change egg size, egg composition, hatchability and progeny performance. 1008 Pullets were reared in floor pens and later transferred to individual cages and light stimulated at 21 wks. From 1-21wk, pullets were fed to meet Cobb 500FF target BW for parent stock pullets. All treatments were fed a common pullet starter 0 to 28d; pullet grower I 5 to 15wk; pullet grower II 16 to 24 wk. At 24wk, a total of 162 pullets were randomly assigned to 3 different dietary treatments consisting of 54 hens per treatment. The first treatment, which represented a standard broiler breeder diet, served as a control and contained poultry oil. The second treatment contained corn oil as the oil source, while the third treatment contained canola oil. Egg size, hatchability and early progeny performance was determined at 29, 32, and 39 weeks of age. An ANOVA analysis was performed using JMP Pro 13 (SAS, 2016). Egg size significantly increased from hens fed corn oil ($P<0.01$) in comparison to canola oil and poultry oil at 29 weeks; however, there was not an effect on egg size in week 32 ($P=0.338$). Progeny from 29 weeks old breeders showed at d1 body weight significantly higher for hens fed corn oil ($P<0.01$) in comparison to other treatments; however, there was no effect on progeny at 7d ($P=0.585$) and 14d ($P=0.360$) body weight. Cull chicks, (%) ($P=0.014$), significantly decreased in 39-week old hens fed corn and canola oil. Unhealed navels (%), significantly decreased ($P<0.014$) in 39-week old hens feed canola oil. Results from this study showed corn and canola oil supplementation improved early progeny performance and egg size in parent stock broiler breeders.

Key Words: Oil Sources, Progeny Performance, Hatchability, Egg size, Broiler Breeder

M91 Use of 2-Nitro-1-propanol as an intervention strategy in laying hens O. Yasir Koyun*[GS], Douglas E. Cosby, Nelson A. Cox, Woo K. Kim *University Of Georgia*

The presence of *Salmonella* in laying hens continues to be a problem in the industry. A study was conducted to evaluate the effect of 2-nitro-1-propanol (NP) on recovery of *Salmonella* internal organs of laying hens. Thirty-four White Leghorns were orally challenged with a nalidixic acid resistant Salmonella Enteritidis (SE^{NR}). Hens were housed individually in wire cages and randomly allocated to one of seven dietary treatments: T1 = SE^{NR} unchallenged (negative control), T2 = SE^{NR} challenged with low dose (10^6 cfu/ml), T3 = SE^{NR} challenged with low dose (10^6 cfu/ml) + 100 ppm NP, T4 = SE^{NR} challenged with low dose (10^6 cfu/ml) + 200 ppm NP, T5 = SE^{NR} challenged with high dose (10^8 cfu/ml), T6 = SE^{NR} challenged with high dose (10^8 cfu/ml) + 100 ppm NP, and T7 = SE^{NR} challenged with

high dose (10^8 cfu/ml) + 200 ppm NP. Fecal samples were collected at 3 and 6 days post inoculation (dpi) and assayed for recovery of SE^{NR}. At 3 and 6 dpi, T4 had the least positivity (40%) in terms shedding, whereas T5 and T6 diets did not show any considerable effect on hens to reduce the presence of SE^{NR} in the feces. Hens were sampled at 7 dpi. Ceca, liver with gall bladder (L/GB), spleen and ovary samples were collected for recovery of SE^{NR}. Treatment 4 (SE^{NR} challenged with low dose (10^6 cfu/ml) + 200 ppm NP) significantly decreased ($p<0.05$) cecal SE^{NR} count, but there was no substantial reduction in T5, T6 and T7. The results were analyzed using one-way ANOVA and when significant, means were compared with Duncan's test ($p<0.05$). In addition, there was no significant difference in SE^{NR} reduction in the L/GB, spleen and ovary samples. In L/GB, 40% of the hens were positive in T2 and T7, while 20% were positive in T6. No SE^{NR} was recovered from hens ın T3, T4 and T5. When the spleens were sampled, 40% were positive in T4, T5 and T7, however, T3 (SE^{NR} challenged with low dose (10^6 cfu/ml) + 100 ppm NP) showed only 20% positive. In the ovaries, no recovery of SE^{NR} was detected in T4 and T6, however 20% were positive in T2, T3, T5 and T7. In conclusion, inclusion of NP into laying hen diets can serve as an effective intervention strategy to reduce Salmonella infection in hens.

Key Words: Salmonella, Hens, Intervention, Nitro-compounds

M92 Evaluation of encapsulated sodium butyrate with varying releasing times on growth performance and necrotic enteritis mitigation in broilers Jundi Liu*[GS], Brett Lumpkins, Greg Mathis, Justin Fowler *University of Georgia*

This study was conducted to evaluate the effect of an encapsulated sodium butyrate (Na-B) with targeted releasing times on growth performance and mitigating the impact of necrotic enteritis in broilers. Two Na-B products CMA (2 h releasing time) and CMP (3-4 h releasing time) were evaluated following a necrotic enteritis challenge model. The experiment consisted of 4 Na-B treatments (500 and 1000 ppm of each product) plus 2 control (non-challenged and challenged). A total of 336 Cobb-Cobb male broilers were placed 8 birds per pen into 7 replicate battery cages. On d 14, birds from challenge treatments were orally gavaged with ~5,000 oocysts of *Eimeria maxima*. On d 19, 20 and 21, the challenged birds received 1 mL of 10^8 cfu/mL *Clostridium perfringens*. Total pen and feed weights were assessed on d 14, 21 and 28 for weight gain and mortality-adjusted FCR. On d 21, 3 birds were randomly selected per pen and scored for intestinal lesions. Results showed no significant effect of Na-B on growth performance before the challenge on d 14. CMA at 500 ppm showed significantly higher BW and BW gain ($P < 0.05$) compared to the challenge control at d 21. Adding CMA at 500 ppm also improved the cumulative FCR to a level that was comparable to the non-challenged control. CMA treatments showed equivalent BW, BW gain and FCR after an additional seven days post-challenge on d 28. Both products at 500 or 1000 ppm had lower lesion scores compared to the challenged control ($P < 0.05$). However, among the different Na-B treatments, there was no difference in lesion scores. Adding encapsulated Na-B was able to mitigate the effects of necrotic enteritis in broilers. CMA, targeted to release in the anterior intestinal tract, shows beneficial effects on growth and feed utilization efficiency in challenged broilers.

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

M93 Production of heirloom turkeys with native, natural feedstuffs Arianna Ferguson*[UG], Lisa Kitto, Erica Rogers, Stephanie Bieber, Heather Sciubba, Christine Ruffin, Paul Patterson *The Pennsylvania State University*

The poultry industry, generally desires the most efficient, fast growing turkeys however, there is a growing consumer demand for heritage bred poultry. In order to more effectively promote heritage breed meat birds, this trial evaluated natural feedstuffs for their impact on growth performance as well as carcass yield.

This study evaluated natural feedstuffs on the production of Artisan Gold turkeys, in a pen trial with 3 replicate control pens and 3 replicate natural feedstuff (NF) pens with 13 birds per pen. Control (C) pens were fed only commercial turkey feed, while the NF pens were fed 85% of commercial as well as 15% natural feedstuffs. Each week (wk) the feed was rotated between earthworms, black soldier fly larva, mushrooms, alfalfa pellets, berries, and nuts. All birds were fed a crumbled pre-starter from wk 1-4, pelleted grower from wk 5-10, and pelleted finisher from wk 11-finish. The birds were weighed and feed intake (FI) was measured to determine feed conversion (FC) for each wk until 16 wk, Dec. 4, for the hens and 18 wk , Dec. 15, for toms, at which time they were processed.

The data were analyzed using a 1-way ANOVA (1-8wk) and a 2-way ANOVA from wk 8-finish using the GLM procedure of SAS (9.4) and p-value at 0.05, and Tukey's test for multiple mean comparisons. There were no significant differences between body weight (BW) of the NF birds and the C birds. However, numerically BW of the NF birds was consistently greater than those of the control birds for wk 1-7. Body weight gain (BWG) per bird was not significant. For wk 3-4, both FI and FC showed significance ($P<0.05$) where the NF birds ate more with poorer FC. For wk 6-7, the C birds ate more with poorer FC. In conclusion, the introduction of natural feedstuffs did not significantly change heritage breed BW or BWG, however the turkeys on the NF diet tended to weigh more, potentially, providing a more marketable bird. The data also suggest that NF turkeys' FI can be significantly impacted dependent upon the palatability of the feedstuff.

Key Words: heirloom, turkey, natural, feedstuffs, growth

M94 Nitrogen corrected apparent metabolizable energy and lipid digestibility of various oil sources fed to broilers from 10 to 20 days of age Trevor Lee*[UG], Kristjan Bregendahl, Klinton McCafferty, Ruben Kriseldi, William Dozier, III *Auburn University*

An experiment was conducted to determine nitrogen-corrected apparent metabolizable energy (AME_n) and lipid digestibility of various oil sources of broilers from 10 to 20 d of age. Seven hundred fifty-six Ross × Ross 708 male broilers were distributed in 84 battery cages at 1 d of age (9 birds/cage; 0.05 m^2/bird). Broilers were fed a common starter diet formulated to contain 3,053 kcal/kg AME_n and 1.25% digestible Lys with corn and soybean meal as primary ingredients from 1 to 10 d of age. Broilers received 1 of 7 dietary treatments (12 reps/treatment) consisting of 94% corn-soybean meal basal diet (3,053 kcal/kg AME_n and 1.25% digestible Lys) and 6% test energy source from 11 to 20 d of age. Oil sources included soybean oil, corn-soy blend acidulated soapstock oil, palm-soy blend acidulated soapstock oil, distillers corn oil, flax oil, and canola oil. The control diet consisted of 94% basal diet and 6% dextrose was used to determine the AME_n of the various oil sources. From 11 to 17 d of age, birds were allowed to adapt to experimental diets, followed by 3 (24 hour) total excreta collection periods from 18 to 20 d of age to determine lipid digestibility and AME_n of each oil source. Growth performance of broilers was not affected ($P > 0.05$) by dietary lipid sources. Values of AME_n were 8,869, 8,396, 7,997, 8,836, 8,588, and 7,871 kcal/kg respectively for soybean oil, corn-soy blend acidulated soapstock oil, palm-soy blend acidulated soapstock oil, distillers corn oil, flax oil, and canola oil. Both AME_n and AME_n to gross energy percentage of soybean oil (8,869 kcal/kg and 94.5%) and distillers corn oil (8,836 kcal/kg and 94.6%) were greater ($P < 0.001$) compared with other dietary lipid sources. Additionally, canola oil had 11.3 and 11.6% lower ($P < 0.001$) AME_n (7,871 kcal/kg) and AME_n to gross energy percentage (83.5%), respectively, compared with soybean oil. Lipid digestibility of soybean oil (94.5%) was higher ($P < 0.001$) than corn-soy blend acidulated soapstock oil (93.4%) and palm-soy blend acidulated soapstock oil (93.7%), but distillers corn oil (94.6%), flax oil (94.7%), and canola oil (94.5%) were similar ($P > 0.05$) to soybean oil. These data demonstrated these alternative lipid sources have acceptable AME_n for growing broilers.

Key Words: broiler, oil, apparent metabolizable energy, lipid digestibility

M95 Influence of basal diet type on metabolizable energy values of an expeller-extruded soybean meal determined in broiler chicks using the regression method Skyler West*[GS], Samuel Rochell *University of Arkansas*

Basal diets used to determine ileal amino acid digestibility values of feed ingredients typically contain more purified ingredients than those used to determine metabolizable energy (ME) values; however, it would be advantageous if both could be determined with a common basal type within a single assay. An experiment was conducted to evaluate if nitrogen-corrected ME (ME_n) values of an expeller-extruded soybean meal (EE-SBM) determined in broiler chicks using the regression method are influenced by basal diet type. Two diet types included a semi-purified (SP) basal based on corn, casein, and dextrose and a basal based on corn and soybean meal (CSBM). The EE-SBM was included at 0, 15, 30, and 45% at the expense of dextrose in the SP diets and at the expense of all energy-providing ingredients in the CSBM diets. Four-hundred and forty-eight male Cobb broiler chicks were randomly distributed among 64 battery cages (7 birds/cage) and fed a common starter diet for 14 d. At 14 d post-hatch, 8 replicate cages of chicks were provided 1 of 8 experimental diets until 21 d post-hatch. A 48 h total excreta collection was conducted from 19 to 21 d to determine the ME_n of each experimental diet. The ME_n of the EE-SBM within each diet was determined based on its inclusion level and the ME_n value of the dietary components it replaced. The EE-SBM associated caloric intake was regressed against amount of EE-SBM intake in kg to generate linear regression equations with slopes corresponding to the ME_n value of the EE-SBM within each basal type. As EE-SBM inclusion increased from 0 to 45%, ME_n values of SP and CSBM diets decreased linearly from 3,438 to 2,873 and 3,088 to 2,710 kcal/kg, respectively. Linear regression of EE-SBM associated ME_n intake in kcal against EE-SBM intake in grams resulted in the following equations: $Y = 2{,}388X - 18$, $r^2 = 0.98$ (SP) and $Y = 2{,}466X - 39$, $r^2 = 0.96$ (CSBM). Resulting EE-SBM ME_n values determined using SP basal diets (2,388 kcal/kg) were similar ($P > 0.05$) to those determined using CSBM basal diets (2,466 kcal/kg). These results indicate that both SP and CSBM basal diets may be reliably used to characterize the ME_n content of soybean meal for broiler chicks when using the regression method.

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

M96 Longitudinal characterization of coccidiosis control methods on nutrient utilization, oocyst excretion, and plasma carotenoid concentrations in male broilers Alyson Gautier*[GS], Juan Latorre, Phil Matsler, Samuel Rochell *University of Arkansas*

Coccidiosis vaccines prevent coccidiosis outbreaks in broilers but can induce damage to the intestinal epithelium and impair live performance. An experiment was conducted to quantify the timing and magnitude of potential losses in growth performance and apparent ileal digestibility (AID) of nutrients and energy (IDE) for broilers vaccinated for the control of coccidiosis compared with those provided a chemical coccidiostat. Experimental treatment groups consisted of 3 coccidiosis control methods: 1) unmedicated and unvaccinated (UU), 2) in-feed chemical coccidiostat (Clinacox, Huvepharma) administration (ACD), and 3) live oocyst vaccination (Coccivac®-B52, Merck Animal Health) (VAC) at day of hatch. Treatments were administered to male Cobb broilers in floor pens that were fed starter (0 to 14 d), grower (15 to 28 d), and finisher (29 to 36 d) diets. Body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were determined at 12, 16, 20, 28, and 36 d. Blood and ileal digesta were collected from birds in 10 replicate pens of each treatment at 12, 16, 20, and 36 d to evaluate plasma carotenoid concentrations and determine nutrient AID and IDE. Oocyst cycling in VAC birds was confirmed by increased litter oocyst counts and decreased plasma carotenoid concentrations ($P < 0.05$) when compared with ACD birds at 12, 16, and 20 d. From 0 to 20 d, BWG and FI were lowest ($P < 0.05$) in VAC birds, intermediate in UU birds, and highest in ACD birds, with no differences in FCR ($P > 0.05$). By 28 and 36 d post-hatch, FCR was higher ($P < 0.05$) for VAC and UU birds than for ACD birds, but BWG and FI of VAC birds were similar ($P > 0.05$) to ACD birds. At d 12, IDE and AID of nitrogen and fat were 261 kcal/kg and 5.2 and 10.4 percentage units lower ($P < 0.05$) in VAC birds than in ACD birds, respectively. At d 16, AID of nitrogen was similar ($P > 0.05$) between ACD and VAC birds, whereas AID of fat remained 5.1 percentage units lower in VAC birds than in ACD birds. No differences in AID of nutrients or IDE were observed ($P > 0.05$) between VAC and ACD birds at 20 or 36 d. In conclusion, these data demonstrated that coccidiosis vaccination elicited a transient reduction in digestibility of energy and nutrients, particularly for fat, that diminished by 20 d post-hatch.

Key Words: broiler, coccidiosis, vaccination, nutrient digestibility, energy

Environment, Management & Animal Well-Being - Behavior & Incubation

M98 Evaluation of conventional and precision feeding systems on egg production in broiler breeder hens Sheila Hadinia*[GS], Paulo Carneiro, Martin Zuidhof *University of Alberta*

The effect of conventional daily restricted feeding (CON) and precision feeding (PF) systems on hen-day egg production (HDEP), egg weight (EW), and egg mass (EM) of Ross 308 broiler breeder hens was evaluated. A total of 480 hens were randomly assigned to two treatments CON and PF in 16 pens of 30 birds each from 23 to 34 wk of age. The CON birds were fed daily every morning whereas the PF system allowed access to feed for 60 seconds any time that BW was less than the target BW. Photoperiod was increased from 8L:16D to 14L:10D at 23 wk of age with an increase in light intensity from 10 to 30 lux. Eggs were collected and average EW was determined daily for each pen. The HDEP for each pen was calculated by dividing the number of eggs produced on a day by the number of hens present on that day. Egg mass (g/d) per hen for each pen was calculated as laying percentage multiplied by EW. Age at 50% production was calculated for both treatments using the NLIN procedure. The other traits were analyzed as a 2–way ANOVA using the MIXED procedure in SAS. For CON and PF hens, respectively, from 23 to 34 wk of age, ME intake was 366 and 354 kcal/d (P=0.006); BW was 3,357 and 3,138 g (P<0.0001); HDEP was 65.5 and 55.2% (P<0.0001); EM was 38.6 and 33.3 g/hen per d (P<0.0001); EW was 57.4 and 57.6 g (P=0.61) and age at 50% production was 193 and 201 d (P<0.05). The CON hens had higher ME intake, increased BW, and greater fat pad percentage relative to PF hens (2.03% and 1.76% respectively, P=0.005). Consequently CON hens likely had more lipid resources for vitellogenesis, which may have advanced the age at 50% production and increased egg production relative to PF hens. The PF hens had lower EM compared to the CON hens because they had lower egg production. Production related feed increases for PF hens occurred only after they laid an egg, whereas feed allocation increases for CON hens resulted in increasing feed intake for all of CON hens at the same time. Resulting differences in nutrient availability likely affected egg formation. Therefore an increase in feed intake around the time of sexual maturation is likely needed to increase productivity of PF hens.

Key Words: Hen, egg, feeding, productivity

M99 Evaluation of the effect of placing paper under drinkers on chick water usage and performance in commercial broiler houses in the first seven days Connie Mou*[GS], Michael Czarick, Brian Fairchild *University of Georgia*

A number of studies have documented a correlation between early chick performance and the time it takes for chicks to begin feeding and drinking after placement. Several poultry companies are placing paper under drinker lines in an effort to attract chicks to drinker lines. The noise cre-

ated by paper is an attractant for chicks to move towards drinker lines more rapidly and believed to increase drinking activity, feed consumption and performance. However, there has been no known documented field data to evaluate the effectiveness of this practice in terms of early chick performance.

A field study was conducted on five commercial broiler farms to examine the effect that placing paper under drinkers has on chick water usage and performance. Two houses were monitored on each farm from Day 0 to 7, one house with an 18" strip of paper under each drinker line (treatment) and the other without (control). Chick water usage was measured using high accuracy water meters that are capable of measuring on a minute to minute basis. In each house, 400 individual chick weights were taken on Day 0, 1 and 7. Overall mortality was recorded as well.

During the first two hours after placement, an average 40% increase in chick water usage occurred in the treatment house on three out of five farms. The difference in water usage diminished eight hours after placement as the average difference in chick water usage between treatment and control was less than 9%. While the treatment had an observable influence on chick water usage in the first few hours, the data indicated that the response was short term and did not heavily impact later chick performance. At Day 7, total chick water usage in treatment houses were an average 6% higher than control on two out of five farms, which was considered negligible difference. Total chick water usage on the other four farms had no difference between treatment and control. In terms of chick weights and mortality no apparent differences were found on any farm between treatment and control. Other factors such as parent breeder age were found having a more influential impact on first week performance than the effect of paper under drinkers.

Key Words: water-usage, chick-performance, brooding

M100 Temperatures experienced by commercial broiler chickens during transportation to processing Douglas Aldridge*[GS], Yi Liang, Colin Scanes, Kaushik Luthra, Karen Christensen *University of Arkansas*

The study reports on the temperatures experienced by broiler chickens during transportation to processing. It is important to assure poultry welfare throughout their lives. Without data of temperatures, it is not possible to assess fully whether or not conditions during transportation are satisfactory for bird welfare. Temperatures within transporters have been previously reported in European and Canadian systems but only briefly in the systems employed in the Southeast United States of America. Temperatures were recorded in 45 locations (3 vertical and 3 left to right and 5 locations from front to back) within trailers during transportation of broiler chickens in Arkansas, Oklahoma and Missouri. In addition, the external environment temperature was recorded. The temperatures varied on average by less than (3.7°C) within the transporter irrespective of location, external temperature or type of transporter. Data on changes in temperature relative to external environmental temperatures during transportation were statistically analyzed using LS-means from The GLIMMIX Procedure (SAS9.4). Trips were grouped by the trailer configuration used for the seasonal temperature mitigation. Configurations consisted of Heat Mitigation (HM 29.44 °C to 36.11°C) Open (Opn 12.22 °C to 29.44 °C), Single Board (Sngl 2.22 °C to 30.00 °C), Double Board (Dbl -16.39 °C to 10.56 °C), and Plastic Wrap (PW-16.39 °C to 2.78 °C). When HM was used over the average of the transport period temperatures showed a small increase of 0.35 °C from ambient. When PW was used an average increase of 9.34 °C from ambient environmental temperature was observed. It is concluded that the transporters employed commercially within the United States provide adequate temperatures for the transport of broiler chickens under the most commonly experienced conditions but there is still room for improvement.

Key Words: Transportation, Temperature, Broilers, Live-haul

M101 Effect of a high energy-low protein diet on broiler breeder reproductive performance, mating behavior, and feather cover Carla Aranibar*[GS], Jeanna Wilson, Ashley Owen *University of Georgia*

Feathering is often a concern on broiler breeder management with the assumption that feathering is directly correlated to mating behaviors and fertility of a flock. Nutrition plays a critical role to reach adequate energy and protein levels for egg production, feathering, activity and maintenance. Elevating energy levels has the potential to increase egg size and egg production but can potentially affect feather cover of hens. Based on industry observations, hens subject to elevated energy levels have the tendency to have increased feather loss. Objective of the study was to determine the effect of an increase in energy and decrease in protein level would have on reproductive performance and feathering condition among hens. A total of 420 Ross 708 broiler breeder hens were randomly assigned to 12 pens (n=35, 0.287 m^2/bird) with 6 of those pens allocated to one of two feeding treatments: high energy and low protein (HL, 2980 Kcal/kg, CP=13.1%) or low energy high protein (LH, 2900 Kcal/kg, CP=15.5%). Egg production, feather cover scores, mating behaviors, breast weight, abdominal fat pad weight and oviduct/ovary weights were measured for the laying period (21-62 weeks). Feather cover was visually evaluated and given a score of 1 to 5, with 1 being bare and 5 full cover. Data were analyzed using PROC GLM (SLICE, SAS 9.4). Dietary treatments did not impact overall body weights or fertility from 21 to 62 weeks. The HL diet significantly increased egg production ($p<0.001$) compared to the LH diet, without having a significant impact on egg weights. There were no significant differences in ovary/oviduct weights, abdominal fat pad, or breast weight at 62 weeks. Feather cover scores were not significantly different between treatments ($p=0.09$). In this study, a slight increase in dietary energy increased egg production with no measured negative impact. The reduction in crude protein appeared to have little impact of the hen performance or body measurements.

Key Words: Breeder, Feathering, Performance, Behavior

M102 Effect of early incubation temperature variation on broiler chicken growth performance and carcass yield Oscar Tejeda*[GS], Kathryn Meloche, Jessica Starkey *Auburn University*

Large variations in hatching egg incubation temperatures have been previously shown to negatively impact growth and FCR in post-hatch broiler chickens. Therefore, our objective was to determine whether small incubation temperature variations (± 0.5°F) from embryonic d 4 to 11, which encompasses both primary and secondary myogenesis, could significantly impact post-hatch broiler growth performance and carcass yields. Broiler hatching eggs were obtained from a 40-wk-old commercial broiler breeder flock and incubated in a single stage incubator at a commercial hatchery running a temperature profile designed to generate a 0.5°F temperature differential among trays placed in the top (TOP), middle (MID), and bottom (BOT) of the racks (n = 4 trays per rack location). Eggs were incubated at similar (< 0.3°F) temperatures from d 1 to 3 and 12 to 18. From d 4 to 11, average internal egg temperatures were 99.6, 99.9, and 100.1°F in the TOP, MID, and BOT trays, respectively ($P > 0.05$). Chicks hatched from the 3 incubator tray location (LOC; n = 240 per LOC) were vent sexed, vaccinated, and separate-sex reared with 12 birds per pen for 41 d on a common corn and soybean-meal based diet in a floor-pen facility. At d 41, all birds (n = 720) were processed to determine carcass and parts yields. No significant differences were observed in the growth performance of broilers incubated in different incubator tray locations ($P > 0.05$). Yet, broilers from the MID trays had significantly lower breast meat yield as a proportion of carcass weight (24.54%) than TOP (24.88%) and BOT (25.00%) broilers ($P < 0.05$). There was a significant LOC x Sex interaction for d 14 BW with MID females being lighter than those from the BOT and TOP trays ($P < 0.05$). LOC also significantly impacted broiler carcass weight and proportion of carcass yield. Interestingly, males from MID trays had heavier carcasses than BOT and TOP broilers ($P < 0.05$) as well as greater carcass yield as a proportion of d 41 BW ($P < 0.05$).

As expected, male broilers had significantly heavier carcass, tender, wing, and drumstick weights than females ($P < 0.05$). Overall, these data suggest that incubation temperature variation of as little as 0.5°F during myogenesis can significantly impact broiler carcass and breast meat yields.

Key Words: incubation temperature, broiler, myogenesis, carcass yield, muscle growth

M103 Effect of embryonic thermal manipulation on heat shock protein 70 (HSP70) expression and immune system development in pekin duck embryos post-lipopolysaccharide injection Revathi Shanmugasundaram*[GS], Micheal Lilburn *OARDC, The Ohio state university*

The initial induction of HSP70 in response to thermal manipulation (TM) during avian embryogenesis is one alternative to improving the response to post-hatch stressors such as heat stress or bacterial challenge. We previously identified that embryonic thermal manipulation from ED 11-21 at 38ºC increased the HSP70. The increased HSP70 as induced by TM suggested that the ducks might have improved response to an inflammatory challenge during post-hatch. Hence the objective of this this study was to identify the effects of high incubation temperature on the hatchability, body weight and immune response against lipopolysaccharide (LPS)-challenge on post-hatch pekin ducks. Pekin eggs (n=200) were incubated at two temperature profiles, a control or standard temperature (S: 37.5°C) or a high (H: 38.0°C) from either embryonic day(ED) (ED1-10) or (ED 11-25). The 3 experimental groups are as follows; S temperature ED1-10 and ED11-25 SS); S (ED1-10) and H temperature (ED11-25; SH); H temperature (ED1-10 and ED11-25; HH). Upon hatching, 32 ducklings (n = 6) from each treatment were injected with or not injected with *Salmonella* Enteritidis 500µg/Kg BW LPS intra-peritoneally. Body weigh,thymus,spleen and bursa samples were collected at 1, 3 and 5d post-LPS injection from each treatment for HSP70, IL6 and IL10, $CD8^+/CD4^+$ ratio, macrophage nitrix oxide production and T-cell proliferation assay. A one-way ANOVA was used to examine the difference between effect of incubation temperature on immune organs and the effect of LPS. Among the ducklings that were injected with LPS, ducklings hatched from SH group had significantly higher body weight compared to the ducklings hatched from SS group, ducklings hatched from SH and HH group had significantly lower splenic HSP70 mRNA compared to the ducklings hatched from SS group. Ducklings hatched form SH-LPS and HH-LPS groups had higher thymocyte proliferation and less amount of macrophage nitric-oxide than ducklings hatched from SS-control group with no LPS injection. In conclusion, our study shows that the expression of HSP70 in thermally manipulated ducklings attenuated by a decrease in HSP70 transcription during recovery period after LPS challenge and long term benefit on resistance to on a post hatch bacterial challenge in ducklings.

Key Words: Embryonic thermal manipulation, HSP70, LPS-challenge, Pekin ducks

M104 Effects of egg storage temperature on pheasant and quail production Joshua Deines*[GS], Doug Yoho, R Bramwell, Samuel Rochell *University of Arkansas*

Commercially produced pheasant and quail are among many poultry species available to consumers. Production systems for non-chicken species successfully utilize a lot of the same management practices, equipment, and business models as chicken production. However, each species has unique biological characteristics that present the necessity for species specific research in order to specialize production practices and increase efficiency. As such, the objective of this study was to investigate the effects egg storage temperature has on two commercially produced species: Ring-Necked (Common) Pheasants, *Phasianus colchicus*, and Coturnix Quail, *Coturnix coturnix japonica*. Eggs from each species were randomly assigned a storage temperature treatment (50, 55, 60, 65, or 70°F), then maintained within two degrees for seven days of storage. There were two pheasant trials where each treatment consisted of 354 eggs, while the quail study used 1,000 eggs per treatment. At the conclusion of the storage period, eggs were randomly distributed in the same incubator. Hatchability, fertility, and stage of embryonic loss were recorded for each temperature treatment. Chicks were randomly distributed by treatment throughout pens in the same house, with equal number of pens per treatment. Feed and water were provided *ad libitum*, all other specifications provided according to industry standards. Bird weight (by pen) was recorded at placement, day seven, and day ten (for quail). Feed consumption was measured per pen on the same schedule with mortality, culls, and associated weights also recorded. Hatch percentages were evaluated using chi-square tests and performance means were compared using Tukey's Post Hoc Test. Percent hatch of fertile was highest ($P<0.0001$) for pheasant eggs stored at 55°F (80.5%), quail eggs at 50°F (90.43%), and lowest at 70°F (63.2% for pheasant, 83.60% for quail). Incidence of splay leg in pheasant chicks was lowest ($P=0.0052$) for 55°F (0.77%) and highest for 70°F (6.28%) storage temperatures. This experiment was conducted at the University of Arkansas Poultry Research Farm. Pheasant eggs were provided by MacFarlane Pheasants, Inc. (Janesville, WI) and quail eggs by Quail International, Inc. (Greensboro, GA).

Key Words: pheasant, quail, egg, storage, temperature

M105 Egg production performance of laying hens provided lighting during incubation Nilakshi Abeysinghe*[GS], Janice MacIsaac, Karen Schwean-Lardner, Bruce Rathgeber *Dalhousie University*

Environmental conditions during incubation can significantly affect the production performance of laying hens. In this study, it was hypothesized that provision of light during incubation can affect the egg production of layers. A total of 2400 hatching eggs from 2 hen lines (Lohmann Lite & Lohmann Brown) were incubated in 6 incubators. Three lighting treatments were assigned to 2 incubators each. Control eggs (n=300 eggs/hen line) were incubated in a dark environment (D21). For the first 18 days, 600 eggs from each hen line were incubated under red LED light (ONCE Innovation dim to red). For the last 3 days of incubation, one half of these eggs were transferred to the dark (R18) and the other half remained incubated in red light (R21). The remaining eggs were incubated in white LED light (4100K) for 21 days (W21). For lighting treatments, a day-night cycle of 12h light:12h dark (12L:12D) was used. Hatched female chicks (n=512) were housed in 64 cages at 8 birds/cage and provided 2 different light regimes. Control cages (n=32) were given a standard photoperiod of 23L:1D from day 1-3 & 20L:4D from day 4-14. Short day treatment cages (n=32) were given 18h of light including two 30 min phases during the dark period in first 3 days. From day 4-14 light was reduced to 17h of continuous light with two 30 min phases. For both regimes, day length was reduced over time and had 9h of light at 7-16 weeks of age. Day length was increased at 17 weeks by 1h of light/week to 14h of light by 21 weeks of age. Birds were moved to laying hen cages at week 18, placing 5 birds/cage. Egg production was monitored until each treatment combination (incubation, hen line & grow out photoperiod) reached 60% of hen day egg production for 3 consecutive days. At this point hen housed egg production (HHEP) results (period of 47 days) did not have interactions among incubation treatments, commercial hen lines & grow out photoperiod. However, HHEP was different among incubation treatments ($P<0.05$). It was highest for layers from R21 (27.6±0.67 eggs) & lowest for W21 (25.1±0.67 eggs) treatment. Two other treatments were intermediate (26.00±0.67 eggs). Based on the results it can be concluded that provision of red LED light throughout the whole incubation period improves HHEP of laying hens.

Key Words: Incubation, hen housed production, laying hens, photoperiod, LED light

M106 Comparison of surface and subsurface eggshell microbial counts in washed and sanitized commercial duck and turkey eggs. Karely Cantú*[GS], Gregory Archer, Craig Coufal *Texas A&M University*

Duck and turkey hatching eggs are often highly soiled, and therefore are normally washed to remove the organic matter and reduce the microbial loads that could lead to poor hatchability or hatchling quality issues. Previous chicken and duck hatching egg studies have determined that egg sanitization with the combination of hydrogen peroxide and ultraviolet light (H_2O_2/UV) decreases the total microbial load on the eggshell surface and results in improved hatchability. The objective of this study was to compare the effects of H_2O_2/UV to traditional commercial egg washing methods on the surface and subsurface eggshell microbial loads. One trial was conducted with White Pekin duck eggs and one trial with commercial turkey eggs. The treatments consisted of 1) control (untreated), 2) washed with a commercial hatching egg washer by the supplier of the eggs, and 3) sanitized with a prototype egg sanitizer which applies the H_2O_2/UV treatment. Ten eggs were aseptically sampled per treatment and evaluated for eggshell surface and subsurface microbial counts via a modified crush and rub technique. Samples were evaluated for total APC. Samples were also plated on various selective and differential media to further evaluate the microbial loads. A GLM using the model $y_{ij}=\mu+t_i+\varepsilon_{ij}$ was used to analyze all data ($P<0.05$). Results for duck eggs demonstrated that the control had higher total surface APC than the washed and sanitized treatments (5.63, 3.45, 3.93 $\log_{10}$ CFU/ml, respectively). Duck subsurface APC for control and washed were higher than the sanitized (4.34, 4.31, 3.09 $\log_{10}$ CFU/ml, respectively). For turkey eggs, a difference between all treatments was observed for total surface APC (5.67, 4.47, 2.89 $\log_{10}$ CFU/ml, respectively). The sanitized turkey eggs were lower for subsurface APC compared to the washed and control (1.50, 2.41, 2.82 $\log_{10}$ CFU/ml, respectively). Eggs from both species had significant reductions between the sanitized and control treatment in total coliforms, fungi, yeast and mold, *Enterococcus*, *Staphylococcus aureus*, and *Staphylococcus enteritidis*. The results of this study suggested that the combination of H_2O_2 and UV light as a method of egg sanitization effectively reduced surface and subsurface eggshell microbial loads on duck and turkey eggs.

Key Words: hatching, sanitization, ultraviolet, hydrogen, peroxide

M107 Analysis of commercial growth data from Cobb 500 and Ross 308 broiler breeder flocks and its effects on egg production Francisco Tovar-Martinez*[GS1], Edgar Oviedo-Rondon[2] [1]*University of Tolima;* [2]*North Carolina State University*

Growth and egg production (EP) data from broiler breeder flocks of Colombian companies were analyzed to evaluate association between these parameters and estimate optimum BW to achieve the best EP. This data was recorded between 2013 and 2017. The Cobb 500 database had 51 flocks for a total of 894,855 hens and the Ross 308 database had 159 flocks representing 2,996,322 hens. Flocks were classified using decision tree analysis into *superior* and *inferior* according to cumulative hen-housed EP at 60 wk of age. All statistical analyses were conducted in JMP 13. Ross 308 flocks laid on average 185.7 (29 flocks) and 167.9 eggs (37 flocks) while Cobb 500 breeders produced on average 171.2 (10 flocks) and 159.2 eggs/hen (14 flocks), for *superior* and *inferior* groups, respectively. The cumulative EP was different ($P<0.05$) between these groups. The groups labeled in this study as *inferior* EP were similar to the performance objectives for Ross 308 (2016), while it was 5 eggs/hen lower for the Cobb line (2013). The weekly average BW and BW gain of these groups were compared by one-way ANOVA, detecting differences ($P<0.05$) on growth during rearing for both genetic lines. In Ross 308, flocks with *superior* EP were heavier at 6 (704 *vs.* 687g) and 19 wk (2,148 *vs.* 2,117g), and lighter at 12 wk (1,241 *vs.* 1,268g) than those with *inferior* EP or the genetic line recommendations. Differences on average daily BW gain ($P<0.05$) for *superior* and *inferior* EP in Ross 308 groups were observed at 5, 8, 12, 13, and 22 wk of age. In Cobb 500, the best EP was associated with heavier BW between 9 and 19 wk and half of BW gain (200 *vs.* 406g) between 24 and 26 wk of age compared to *inferior* EP flocks. The average daily BW gain for Cobb 500 was different ($P<0.05$) between the two groups at 6, 9, 16, 21, 25 and 26 wk of age. Cobb *superior* EP flocks had similar BW to the recommended by the genetic line company, before photostimulation, and were up to 2.3% heavier from 21 to 24 wk. In contrast, *inferior* EP Cobb flocks were always below genetic line recommendations until 19 wk of age. In conclusion, based on commercial data, *superior* EP for both Ross 308 and Cobb 500 breeder hens is associated with a range of BW and BW gains before sexual maturity and slightly heavier BW before onset of egg production.

Key Words: pullets, breeders, reproduction, weight, growth

Environment, Management & Animal Well-Being - Stress Responses

M108 Development of a rainwater harvesting model for broiler farms to estimate on-farm storage needs Carson Edge*[GS], Jeremiah Davis, William Batchelor, Eugene Simpson, Joseph Purswell *Auburn University*

Access to water is critical for poultry production and can present a significant cost to poultry farmers depending on water supplies available to them. Many farmers rely on low-yield and poor water quality wells or costly municipal (city or county) water sources. Implementing a rainwater harvesting (RWH) system may reduce reliance on insufficient or costly water supplies for farmers in high precipitation areas across the U.S. Current uses of RWH systems have been primarily focused on reducing stormwater runoff in urban areas and providing sources of potable and non-potable water based on daily average water consumption values. Due to the variability in total water consumption values for a poultry farm because of evaporative cooling in summer months, sizing a RWH system for a poultry farm based on average consumption values is insufficient. The objectives of this research were to develop a RWH model to estimate the main water consumption sources for a poultry farm on a daily basis; bird water consumption (BWC) and evaporative cooling make-up water consumption (EWC) and to evaluate the performance of the model over a 25-year period at varying storage sizes for a simulated farm in nine locations across the U.S. representing high poultry production areas and varying climates. Equations for BWC and EWC were calibrated and evaluated using data from a poultry farm in East Alabama. Estimates of daily water used from RWH and water purchased from other sources were calculated over a 25-year period for storage capacities ranging from 189 m^3 (50,000 gal) to 1,136 m^3 (300,000 gal) in increments of 189 m^3 (50,000 gal). A simple economic analysis was performed to calculate the cost savings for each location over the 25-year test period for municipal water costs ranging from \$0.79 m^{-3} (\$3.00 per 1,000 gal) to \$3.17 m^{-3} (\$12.00 per 1,000 gal) in increments of \$0.26 m^{-3} (\$1.00 per 1,000 gal). Results suggest there are positive economic benefits for RWH systems implemented in high precipitation areas of the U.S., but there may be limited benefit in excessively large storage capacity and at low municipal water costs, which may limit economic feasibility of RWH systems on poultry farms.

Key Words: Modeling, Rainwater, Harvesting, Storage, Water

M109 The impact of poultry management and stress factors on lameness, mucosal permeability, and bacterial translocation in broilers Audrey Duff*[GS], Denise Rodrigues, Johel Bielke, Whitney Briggs, Billy Hargis, Kaylin Chasser, Kim Wilson, Lisa Bielke *The Ohio State University, OARDC*

Previous research indicates stress and enteric inflammation play an influential role in intestinal permeability which can predispose poultry to bacterial translocation (BT), subsequent lameness and bone pathologies

such as enterococcal spondylitis (kinky back). Various enteric stressors have been shown to increase mucosal permeability and can be measured by increased Fluorescein isothiocyanate-dextran (FITC-d) in serum and BT to liver and spleen (L/S). In Exp 1, treatments consisted of negative control (NC), 8h water withdrawal prior to sampling (WW), Dexamethasone in feed (DexF; 0.57ppm; d4-11), rye-based diet (RBD; d7-11), or 15% dried distiller's grains with solubles (DDGS). Liver BT and serum FITC-d were measured on d11. Dexamethasone, RBD, and WW resulted in numerically higher levels of serum FITC-d and percent incidence of positive BT relative to DDGS and NC. In Exp 2, all chicks were exposed to a mild cold stress (30°C for 6h) on d3, permeability-inducing treatment, as in Exp 1, and an *Enterococcus cecorum* (EC) challenge on d11 to all but NC. Serum FITC-d levels were measured on d11, EC was cultured from free thoracic vertebrae (FTV) region on d15, and birds were monitored for lameness through d70. Serum FITC-d and EC recovery were highest ($p<0.05$) in DexF, and by d70, no differences were observed in lameness, occurrence of kinky back, or incidence of ascites relative to NC. In Exp 3, treatments included NC, DexF (d14-24), DDGS, and feed restriction (FR; d23 for 24h). All birds were challenged with EC on d24. No differences were observed between treatments for BT in the L/S on d24 nor the L/S and FTV on d28. Piling up, resulting in hip scratching and an inflammatory process, and incidences of lameness were observed in multiple pens before d28. Birds were observed through d41, and no differences in total lameness were observed. These data suggest that, while DexF and dietary treatments increase measured mucosal permeability, low level stressors such as temperature change, water withdrawal, and injury may be sufficient for causing a meaningful decrease in enteric integrity. These experiments stress the importance of early management on development and maintenance of intestinal and long-term health of flocks.

Key Words: Stress, Gut-permeability, Spondylitis, Kinkyback, E.cecorum

M110 Characterizing the effects of heat Stress and Coccidial Infection on histological changes in immune tissues in broiler chickens Bryan Aguanta*[GS], Alberta Fuller, Susan Williams, Marie Milfort, Romdhane Rekaya, Samuel Aggrey *University of Georgia*

Heat stress and coccidial infection are among stressors that affect performance in broiler chickens, but the biological mechanisms that underlie these stressors are not fully understood. We investigated the histological changes in broiler chickens under heat stress and/or coccidial infection. Fourteen-day-old Cobb500 chicks were assigned randomly in a 2x2x3 factorial design experiment with two temperatures (25°C or 35°C), two infection levels (infection with 2.5 x 10^5 *Eimeria* oocysts or non-infection), and three levels of anticoccidial treatment (no coccidiostat, 100g monensin/1,000 kg of feed, or 113.5g nicarbazin/1,000 kg of feed). There were 12 treatments, 5 replicates per treatment and 8 birds per replicate. Coccidiostat administration began at 14 days of age, and *Eimeria* infection and heat treatment began at 15 days of age and ended at 28 days of age. Five birds per treatment were euthanized at 14, 21, and 28 days of age. The bursa, spleen, and thymus tissues were collected and fixed in 10% buffered formalin. Following fixation, samples were trimmed, routinely processed, embedded in paraffin, sectioned at 4 microns, and stained with hematoxylin and eosin. Slides were examined by light microscopy. Tissues were scored on a scale of 0 to 3 based on lesion type and severity, with the exception of follicular atrophy in bursal tissue, which was scored on a scale of 1 to 4. Statistical analysis using the PROC CATMOD procedure in SAS revealed significantly increased lesion severity in birds housed in higher temperatures. Compared to the control birds, heat-stressed birds developed significantly more severe lymphoid depletion of the bursa and thymus at 28 days of age. There were no significant changes in the spleen between treatment and control birds. Interactions between heat stress and the broiler immune system may exist warranting continued investigation of these interactions at the histologic, immunologic, and molecular levels.

Key Words: Eimeria, Immunity, Histology, Temperature, Lymphoid

M111 Evaluating the effects of water quality on broiler breeder production Ashley Ailara*[GS], David McCreery, Joey Bray *Stephen F. Austin State University*

The objective of this study was to determine the effects of water quality on commercial broiler breeder production performance. A total of 19 broiler breeder farms, currently in production, were evaluated on the water quality provided to the birds. Three water samples were taken from each farm, a well water sample, a sample from the nipple drinkers, and a swab sample from the water line itself. The swab samples were all taken internally at the flush end of the water lines. The water was analyzed for nitrate, nitrite, pH, hardness, sulfate, chloride, calcium, magnesium, aluminum, copper, iron, potassium, manganese, sodium, phosphorous, and lead. We evaluated the effects of these water quality parameters on total mortality, percent production total eggs, total eggs per hen housed, total eggs set, feed conversion per hatching eggs, total cull and double yolk eggs by the dozen, percent production cull eggs, cull eggs per hen housed, feed conversion per total eggs and hatched percent. After analyzing the data, we determined there was a significant interaction with total eggs per hen house and total eggs set for both nitrite and nitrate. The levels of nitrite in the water samples affected the percent production total eggs. Significant interactions were determined for total cull and double yolk (dozens), percent production culls eggs and cull eggs per hen house for pH. Sulfate, aluminum, copper, iron, potassium, manganese, phosphorous and lead were not at sufficient levels to have an interaction with the production parameters. These results suggest that nitrite, nitrate, and pH levels do have an effect on broiler breeder production performance.

Key Words: Water quality, Broiler Breeder, Nitrite, Nitrate

M112 Effect of stress induced by dexamethasone and two sodium sources on biological functions in broilers Opeyemi Olojede*[GS], Tuoying AO, Anthony Pescatore, Sunday Adedokun *University of Kentucky*

The physiological and behavioral indicators of the presence or absence of stress, can be used as an informative indicator of an animal's welfare. In the present study, the effect of dexamethasone (DEX) as a stressor, was examined in chickens. Day-old broiler chicks were fed a standard basal diet supplemented with sodium from two different sources (4.0 g/kg NaCl or 2.37 g/kg NaCl and 2.37 g/kg$NaCHO_3$). At d 16, the broilers were either orally gavage with 0.6 mL of DEX at 1 mg/kg BW (CHA) or the same volume of distilled water (NCH) on d 16, 18, and 20. On d 21, birds were weighed individually and sampled. The administeration of DEX in the CHA group from d 16 - 21, decreased ($P < .0001$) BW gain/bird (27%) and feed efficiency with no effect on feed intake. The DEX challenge resulted in elevation ($P < 0.05$) of blood alanine aminotransferase, albumin, creatinine, P, and Ca however; glucose, urea, and total bilirubin levels were not affected. Wet and dry liver weight, as well as Ca, P, and N contents of the liver showed an interaction ($P < 0.05$) between sodium source and DEX challenge, where the NCH group fed diets containing NaCl had the lowest values. Likewise, DEX administration increased ($P < 0.05$) liver weight, Ca, P, and N, as well as Zn concentration in the liver. No significant effect of DEX challenge was observed for villi height, crypt depth, and villi height:crypt depth ratio. Interactions ($P < 0.002$) between sodium source and DEX however, resulted in a lower crypt depth in the CHA group fed diet containing NaCl. Challenging the birds with DEX resulted in a decrease ($P < 0.033$) in phalanges bone breaking strength while the inclusion of NaCl and $NaHCO_3$ in the diet resulted in higher ($P = 0.041$) bone breaking strength for the femur. The percent bone ash was lower (tibia and femur; $P < 0.05$) in the CHA group. Furthermore, an interaction ($P < 0.05$) was observed for Ca and P total tract utilization where the CHA group fed diets containing NaCl had the lowest values. Results from this study showed that normal biological and physiological functions, can be adversely affected by induced stress, and the effects of acid-base balance cannot be overruled especially since the effect of partial replacement of NaCl with $NaHCO_3$ increased mineral utilization.

Key Words: broilers, stress, sodium source, dexamethasone

M113 Consequences of heat stress on broilers experimentally infected with Eimeria oocysts and treated with Nicarbazin or Monensin Eduardo Ortega*[GS], Alberta Fuller, Marie Milfort, Susan Williams, Romdhane Rekaya, Samuel Aggrey *University of Georgia*

The effects of heat-stress (HS) and Eimeria infection and coccidiostats on the growth performance of broilers were investigated. We conducted a 2x2x3 factorial design experiment with two temperatures (25°C) or (35°C), two levels of Eimeria maxima infection (no infection or 2.5 x 10^5 Eimeria oocysts) and three coccidostat treatments (no coccidiostat, 113.5g nicarbazin/ton of feed (NIC) and 100g monensin/ton of feed (MON)). Fourteen day old Cobb500 chicks were assigned randomly to each of 12 treatments. There were 5 replicates per treatment and 8 birds per replicate. Treatment started when the birds were 15 days old and lasted for 2 weeks. Rectal temperature and BW were measured in individual birds whereas feed intake was measured per pen at 1, 7 and 14 days post treatment. We measured lesion scores from 5 individuals selected at random per treatment at 1, 7 and 14 days post treatment. Fecal oocysts counts were obtained from two collection composites consisting of dropping from days 5-7 and 12-14 post infection. The BWG between day 1 and 14 for NIC (580 ±10 g) was lower (P<0.001) than that of MON (622±8 g) regardless of infection and heat treatment. Rectal temperature was higher (P<0.01) in infected birds (42.48±0.02°C) compared to non-infected birds (42.40±0.02°C) regardless of temperature treatment day 1 day post infection. As expected, when comparing the mean oocyst counts between infected and non-infected birds raised at 25°C, non-infected birds remained oocyst-free while infected birds had mean counts of 52.93 ± 6.89 (P<0.0001) for the first composite, and 92.47 ± 9.49 (P<0.0001) for the second composite, respectively For the first fecal composite of infected birds, the oocyte levels were 0.80 ± 16.47 and 37.20 ± 16.47 for NIC and MON, respectively. For the second fecal composite of infected birds, the oocyte counts were 18.40 ± 22.54 and 61.00 ± 22.54 for NIC and MON, respectively. Oocyst counts were non-detectable in infected birds raised at 35°C regardless of treatment The data from this experiment suggest that, whereas NIC depresses growth, but it protects birds against coccidial infection better than MON and there also appears to be an association between elevated ambient temperatures and decreased oocysts development in Eimeria infected birds.

Key Words: Eimeria, Temperature, oocytes, Performance, coccidiostats

M114 The effect of stocking density on deep body temperature in broiler chickens Ming Lin Teo*[GS], Michael Czarick, Brian Fairchild *University of Georgia*

A study consisting of two trials was conducted to examine the effect of stocking density on deep body temperature (DBT) of broilers during summertime conditions. Birds were placed in pens in a room where all exhaust fans operated continuously enabling inside conditions to closely mimic outside conditions. No circulation fans or evaporative cooling was used during the study. Six-week-old male broilers were randomly selected and a temperature sensor was surgically inserted into the abdominal cavity of each bird.

The study consisted of pre-stocking baseline, density and post-stocking baseline periods. During the pre-and post-stocking baseline periods, birds were placed at a control stocking density of 2 birds/m^2. At the end of the pre-stocking baseline period, birds were randomly assigned to four stocking densities; 2, 5, 7 and 10 birds/m^2. Deep body temperature and environmental conditions were measured every minute throughout the studies. The data were evaluated in Excel. Due to the fact that bird body temperatures tend to rise (41.1 to 42.2°C) when they sit down during a dark period which is unrelated to stocking density it was decided that body temperatures would only be analyzed between the hours of 4 am and 8 pm.

During trial one daytime temperatures ranged between 18 and 20°C and humidity ranged between 71 and 95%. Bird DBT increased as they were placed at higher stocking density. Birds (n = 4) at 2 birds/m^2 had the lowest DBT (41°C). The average DBT of birds (n = 5) at 5 birds/m^2 and 7 birds/m^2 was 0.1°C and 0.2°C higher respectively. The DBT of birds (n = 5) at 10 birds/m^2 was the highest (41.5°C).

Daytime temperature in trial two ranged between 23 and 27°C and humidity was between 72 and 93%. Birds (n = 8) at 2 birds/m^2 had an average DBT of 41.4°C. The average DBT of birds (n = 9) at 5 birds/m^2 was 41.9°C, whereas the average bird (n = 9) DBT at 7 birds/m^2 was 41.7°C. Birds (n = 9) at 10 birds/m^2 showed the highest DBT (42.1°C).

As the number of birds in a floor space increased, the space between them decreased, reducing their ability to dissipate body heat which led to an increased effective temperature. These data demonstrate the influence of stocking density on broiler deep body temperature.

Key Words: Stocking-density, Body-temperature

M115 Thermal performance of perforated pen panels for on-farm nutritional test trials Kelly Goneke*[UG], Jeremiah Davis, Joseph Purswell, Gary Chesser, Carson Edge *Auburn University*

Test pens are commonly used to segregate and geolocate birds in commercial-scale broiler houses to control for spatial variation in the environment. These pens should subject test birds to similar environmental conditions outside the enclosure, however pen design and materials can vary significantly and may affect environmental conditions within the pen. Evaluation of pen construction and materials is necessary to determine the optimum wall construction to maintain similar environmental conditions within the pen. Proportion of perforated area and air velocity were tested as main effects in a 7 x 4 factorial design. Treatments included seven levels of perforation area (100%, 90, 70%, 50%, 30%, 10%, and 0%) and four levels of air velocity (1, 2, 3, and 4 m/s). Two 0.75 x 1.5 m panels were installed in a wind tunnel to simulate the windward and leeward panels of a test pen. Sensible heat generation was simulated for 14 birds in the pen using enclosed incandescent bulbs. Five simulated birds were constructed outside the pen on both the windward and leeward side. A metal feeder was suspended in the center of the pen. Thermal changes in the simulated birds were analyzed with infrared thermography. Results showed that as open area was decreased, sensible heat increased both in the pen as well as on the leeward side. Proper design and construction of test pens is critical to accurately assess performance of test birds in a commercial broiler house when compared with the remaining flock.

Key Words: nutrition, pen, thermography, panels, thermal

M116 Effect of air velocity on broiler live performance, meat yield and breast meat quality up to 61 d Jimmy Cifuentes*[UG], Edgar Oviedo-Rondon, Hernan Cordova-Noboa, Albaraa Sarsour, Viviana San Martin-Diaz, Santiago Alvarez-Muñoz, Ingrid Martinez-Rojas, Francisco Tovar, Cristian Florez-Leguizamon, Lingjuan Wang-Li, Bin Cheng, Yijia Zhao *North Carolina State University*

Higher air velocity (AV) may improve broiler live performance and affect meat traits when outside temperatures indicate heat stress conditions. Two experiments were conducted in a broiler production simulation chamber complex with six-modular chamber systems that simulated typical conditions encountered in commercial broiler production facilities. The experiments were performed during early and late summer to evaluate the effects of two AV on live performance, carcass and cut up yields and breast meat quality in Ross 708-male broilers. At hatch, a total of 400 d-old male chicks were placed in floor pens considering groups of 10 chicks/pen for both experiments. At 28 d, 264 chickens were selected for each experiment and moved to six chambers. Broilers were raised up to 61 d for a final stocking density of 40 kg/m^2. The AV during each day were adjusted to target broiler comfort zone according to age. At 28, 42 and 61 d, BW and feed intake were recorded, and BW gain and FCR were calculated. Flock uniformity (CV%) was also analyzed at the end of each experiment. At 62 d, broilers were subjected to a 12-h feed withdrawal for processing. Carcass and cut up weights and yields (leg quarters, wings, breast meat and

rack) were obtained and calculated, and meat quality (pH, drip and cook loss, color, shear force, muscle myopathies) parameters were assessed. A completely randomized design with three replicates per treatment was used. Chamber was nested within treatment and considered as random effect. Results of the first experiment indicated that higher AV enhanced ($P < 0.05$) BW gain 277 g, and FCR tended ($P = 0.072$) to improve from 42 to 61d. In the second experiment higher AV increased ($P < 0.05$) BW gain 282 g from 28 to 61 d. Despite of no improvements ($P > 0.05$) on carcass yield due to AV in both experiments, breast meat yield was increased ($P = 0.0581$) up to 0.88 % points by higher AV in the first experiment only. No effects ($P > 0.05$) on meat quality or muscle myopathies were observed except for lightness (L*). Interestingly, for both experiments, higher AV decreased ($P < 0.05$) L* in breast meat. In conclusion, even small increases in AV could have potential improvements in BW, BW gain and breast meat yield in heavy broilers, mitigating heat stress effects.

Key Words: Air velocity, broiler, performance, meat quality, carcass yield

Physiology

M117 Production Factors Affecting Broiler Mortality: Implications for Animal Welfare & Food Policy Matthew Salois*, Justin Replogle, Kristi Baker *Elanco Animal Health*

This study examines the impact of key production and management related factors on mortality rates of broilers raised for meat consumption, and takes a macroeconomic, industry-wide approach to the analysis. Specific factors examined include body weight, average daily gain / growth rate, stocking density, downtime days between flocks, and percent of production that is raised without antibiotics.

Monthly aggregated complex-level production data from Agri Stats is utilized covering a time period between January 2009 through June 2017. To estimate the association of each factor on mortality a longitudinal econometric framework is used which controls for both time varying and individual complex specific effects. A double-logarithmic functional form is used which means regression coefficient estimates can be interpreted as elasticities (i.e., % change in X associated with % change in Y).

Results from the regression show that a 1% increase in average daily gain or growth rate is associated with 1.95% reduction in mortality rates. Conversely, a 1% increase in bird weight is associated with 1.7% increase in mortality rates. Additional results show that increased downtime is associated with lower mortality, while stocking density and the share of production raised without antibiotics is associated with higher mortality rates.

From a policy perspective, the issue of growth rates has been under scrutiny leading some animal welfare activist groups to advocate for slower-growing chickens. Results from the regression analysis models estimated consistently show that mortality is negatively associated with growth rates when size or weight is accounted for in the model. Moreover, growth weight or size is consistently related to higher mortality. Overall, findings suggest that the growth weight, rather than growth rate, is a more important factor when assessing mortality and overall animal welfare.

Key Words: animal welfare, broilers, panel data, slow grow, regression analysis

M118 Production and humoral immune effects of dietary Original XPC in layer pullets challenged with Mycoplasma gallisepticum Katie Elliott*, Scott Branton, Jeffrey Evans, Spencer Leigh, Elizabeth Kim, Hammed Olanrewaju, Gregory Pharr, Sharon Heins-Miller, Donald McIntyre, Hillary Pavlidis, Edgar Peebles *Department of Poultry Science, Mississippi State University*

Original XPC (XPC) has been shown to decrease adverse bacterial populations in broiler chickens. Therefore the objective of this study was to investigate the potential inhibitory effects of XPC on *Mycoplasma gallisepticum* (MG) bacterial infections in commercial layer pullets. Hy-Line W-36 pullets sourced from MG-clean breeders were fed either a basal diet (CON) or a basal diet with the addition of XPC (1.25 kg/MT) fed from hatch until 12 wk of age (woa). At 8 and 10 woa, half of the birds in each dietary treatment were challenged with R_{low} MG. Birds were raised in biological isolation units (n=16 units per diet with 18 birds per unit). Body weight was recorded at 3, 8, and 12 woa and select organs (ovary, ceca, and bursa) were weighed at 3 and 12 woa from a sample of birds (n=128). Blood samples were taken immediately prior to the initial MG challenge at 8 (n=96) and at 12 (n=288) woa to test for IgM (Serum Plate Agglutination (SPA) testing) and IgG (ELISA) antibody production against MG. All birds at 12 woa (n=288) were evaluated for MG lesion scores (Scale 0-3). There were no significant diet*challenge interactions for the parameters measured. Regardless of challenge, inclusion of XPC in the diet did not significantly alter BW or relative organ weights at 3 or at 8 woa. At 12 woa, BW for XPC-fed birds, regardless of challenge, was significantly (P=0.0038) heavier than CON by 25.7g. However, relative organ weights were not significantly impacted by XPC. All birds (from both diets) tested negative for MG antibodies prior to the 8 woa challenge. The respective percentage SPA and ELISA positive birds at 12 woa were 0% and 0% (CON, non-challenged), 1.4% and 0% (XPC, non-challenged), 100% and 47.2% (CON, challenged), and 100% and 50.0% (XPC, challenged). ELISA titers were not significantly different due to diet (P=0.9939) but were increased due to the challenge ($P < 0.0001$). Dietary treatment did not affect MG lesion scores (P=0.5289), but they were significantly higher for the challenged birds (P=0.0012). In conclusion, XPC increased BW gain in the layer pullets by 12 woa regardless if the birds had been challenged with MG. Feeding XPC did not alter the number of birds mounting a humoral immune response, their antibody titer levels, or the severity of MG lesions.

Key Words: Mycoplasma, XPC, layer, pullet, immunity

M119 The effect of an early infection of Histomonas meleagridis on layer performance. Christina Sigmon*, Robert Beckstead, Kenneth Anderson *North Carolina State University*

Blackhead disease is caused by a flagellated protozoan parasite, *Histomonas meleagridis*, and is known to infect a wide range of gallinaceous birds. In chickens, this disease is causing significant financial loss to poultry industry through mortality, morbidity, increased culling, and loss of flock uniformity. Commercial layers infected show a significant drop in egg production suggesting that blackhead may have a larger impact on the poultry industry. The objective of this study was to understand how an early infection with *H. meleagridis* can effect layer performance.

Commercial egg strain female chicks were obtained and placed in 4 replicate pens each containing 32 chicks for 2 treatment groups. Birds were weighed individually on days 14, 28, 42, 56 and 72. On day 18, the infected chicks were inoculated intracloacally with a virulent *H. meleagridis* strain. On days 5 and 10 post infection, 4 birds per pen from each treatment, were euthanized, scored, and sampled. The infection rate was 80%. Livability, body weight gain, feed consumption and flock uniformity were determined for both treatments. The control chicks had significantly higher body weights than the infected chicks at days 28, 42 and 56 days but not at day of hatch, 14 and 72 days. The control chicks had significant body weight gain during the 14-28 and 56-72 days. However, no significant differences were seen between treatments with feed consumption and livability.

On day 120, 24 hens per treatment were moved to a lay facility for production. Egg weight and quality, feed consumption, and body weight gain were determined for both treatments. Only one control and one infected

hen began laying before day 133. On day 147, 67% of control hens and 42% of the infected hens were laying. All hens survived and were laying upon termination of the trial on day 173. There were no significant differences between feed consumption, total eggs laid, egg weights quality. No infected hens showed signs of necrotic liver lesions or ulceration of the ceca upon termination. These results demonstrate that layers infected at an early age with *H. meleagridis* have slower body weight gain than control birds. Future research will determine if egg production drops in early infected birds kept for a longer production period.

Key Words: Blackhead, Histomonas meleagridis, Layers

Processing and Products

M120 Effect of marigold flower meal supplementation on production performance, egg quality, egg geometry and lutein concentration in egg yolks of laying hens Zafar Hayat*, Rehman Akram, Muhammad Nasir *University of Veterinary & Animal Sciences*

Lutein is a pigment belongs to xanthophyll family which can prevent age related macular degeneration (AMD). Bioavailability of lutein from egg yolk is highest as compared to other sources. The present study was aimed to examine effect of marigold flower meal supplementation on production performance, egg quality, egg geometry and lutein concentration in egg yolks of laying hens. A total of 500 W-36 laying hens were randomly divided into 5 treatment groups under Completely Randomized Design (CRD) in a way that each group had 5 replicates of 20 birds each. Marigold flower meal (MFM) containing 96% lutein, was supplemented in a basal laying hen diet at the rate of 0, 150, 300, 450 and 600 ppm to produce 5 experimental diets each fed to one group of hens for 6 weeks. Production performance (egg weight, egg production %, feed conversion ratio and egg mass), egg components (relative percentages of albumen, yolk, and shell to egg weight), egg quality (yolk index, shell thickness, albumen height, Haugh unit and yolk color) and egg geometry (egg volume, egg shape index and egg area) were determined. Concentration of lutein in egg yolk was estimated with the help of HPLC. Results of the present study showed that there was no effect (P>0.05) of different levels of MFM supplementation on production performance of laying hens as compared to control. Results also demonstrated non significant differences (P>0.05) for different parameters of egg components and egg geometry with supplementation of different levels of MFM. Similarly, shell thickness and yolk index was also not affected (P>0.05) in all dietary treatments by lutein supplementation. Albumen height, Haugh unit and yolk color were significantly different (P<0.05) in eggs from the birds fed diets with inclusion of different levels of MFM. In this trial higher accumulation of lutein in yolks was found in birds fed diets contained lutein (P<0.05). However, increase in lutein concentration in egg yolks was not linear with respect to supplementation of MFM in layer diets. It may be concluded that MFM has potential in increasing egg yolk lutein content without affecting production performance, egg quality and egg geometry.

Key Words: Marigold-flower-meal, lutein, production-performance, egg-quality, hens

M121 Assessing Commercial and Alternative Poultry Processing Methods using Microbiome Analyses Michael Rothrock Jr.* *USDA-ARS-U.S. National Poultry Reseach Center*

Assessing poultry processing methods/strategies has historically used culture-based methods to assess bacterial changes or reductions, both in terms of general microbial communities (e.g. total aerobic bacteria) or zoonotic pathogens of interest (e.g. *Salmonella, Campylobacter*). The advent of next generation sequencing technology has greatly expanded out ability to "dive deep" into the microbiota of poultry processing and assess how different processing methods or strategies may be affecting these poultry-associated microbiota. 16S microbiome analysis using the QIIME pipeline was used to look at strategies used in conventional and small-scale poultry processing plants effect the different types of microbiomes within those environments. Within the conventional poultry processing plant, microbiome analysis was performed to see how microbial populations change within the major processing water tanks (scalder, chiller) throughout a typical processing day, and how water sampling methods effect those microbiomes. Work performed within the small-scale poultry operation setting assessed how different processing/butchering methods u(e.g. small USDA-inspected facilities versus on-farm butchering, removing versus keeping on skin) affect the cecal and post-processing whole carcass rinse microbiomes, and how product storage method (fresh, refrigerated, frozen) affected the final product whole carcass rinse microbiomes. In all cases, microbiome data not only showed changes within the overall microbial community, but shifts in bacterial pathogens (e.g. *Salmonella, Campylobacter, Listeria*) were also able to be observed. Lastly, data will also be shown that demonstrates how taking a combination culture-based and microbiome approach can help determine the efficacy of new interventions within the processing plant environment. These results highlight the use and utility of microbiome analyses within the poultry processing environment, and how linking it with culture-based analyses can be an important tool for researchers and industry stakeholders in the future.

Key Words: Microbiome, Processing, Salmonella, Campylobacter

M122 Efficacy of neutralizing buffered peptone water on broiler whole carcass rinse Salmonella, Campylobacter, and Enterobacteriaceae following commercial treatment with peroxyacetic acid Dianna Bourassa*, Ivey Wise, Joshua Lapidus, Meredith Johnson, Amit Morey *Auburn University*

In 2016, the USDA-Food Safety and Inspection Service (FSIS) began using a neutralizing buffered peptone water (nBPW) to rinse broiler carcasses for *Salmonella* and *Campylobacter* performance standard testing. The nBPW contains chemical compounds, in addition to the standard buffered peptone water, to neutralize residual antimicrobials that may be transferred from the carcass to the sample rinsate. However, a direct comparison of using nBPW and BPW on carcasses commercially treated with antimicrobials (e.g. peroxyacetic acid, PAA) for the recovery of *Salmonella*, *Campylobacter*, and Enterobacteriaceae has not been conducted. An immersion chilling biomap of whole carcass rinse samples taken pre-chill, after pre-chill dip, after primary chill, after secondary chill, after post-chill dip, and after post-chill dip without the pre-chill dip was tested (n=30-50/site). Carcasses were rinsed with either buffered peptone water (BPW without neutralizer) or nBPW (400 mL / carcass) for 1 min. Rinsates were sampled for *Salmonella* and *Campylobacter* prevalence and both Enterobacteriaceae prevalence and count. No significant differences *(P*>0.05) were observed between sampling sites or rinse media for *Salmonella* due to an overall low prevalence (4/180). *Campylobacter* prevalence significantly decreased (*P*<0.05) from pre-chill through post-chill as anticipated, however, significantly *(P*<0.05) fewer *Campylobacter* positive carcasses were detected when nBPW was used in comparison to BPW. Both Enterobacteriaceae prevalence and counts significantly decreased *(P*<0.05) from pre-chill through post-chill. The use of nBPW versus BPW did not impact Enterobacteriaceae prevalence. Samples rinsed with nBPW had significantly higher counts at all biomap stages sampled. Results indicate that nBPW is necessary to neutralize PAA in carcass rinsates when sampling for EB counts, however, nBPW may inhibit the ability to detect *Campylobacter*. Further studies should be conducted to confirm the observed impact of nBPW on *Campylobacter* detection.

Key Words: nBPW, PAA, Salmonella, Campylobacter, Enterobacteriaceae

M123 The presence of enterotoxigenic Clostridium perfringens on broiler chicken carcasses along critical steps of the slaughter process: an equivalent risk for all abattoirs? Marie-Lou Gaucher*[1], Alexandre Thibodeau[1], Philippe Fravalo[1], Julie Arsenault[1], Marie Archambault[1], Sylvain Quessy[1], Ann Letellier[1], Sylvain Fournaise[2] *[1]Université de Montréal; [2]Olymel S.E.C. / L.P.*

The reduction approach for antibiotic use on poultry farms currently being implemented by many countries will most likely precipitate the industry in a new era in which *C. perfringens* will become a central player. A small proportion of the *C. perfringens* population produces CPE (*cpe* gene), an enterotoxin responsible for foodborne illnesses in humans. In Canada and in the USA, enterotoxigenic *C. perfringens* ranks as the second most prevalent cause of human foodborne disease and is responsible for nearly 1 million illness cases per year, respectively. No study has investigated the role of broiler chicken carcasses and their associated abattoirs as contributors. The objectives of the current study were to (i) estimate the proportion of broiler chicken flocks and carcasses positive for *cpe*, (ii) identify steps of the slaughter process critical for this contamination. A total of 379 broiler chicken carcasses from 79 flocks were sampled in two slaughterplants, Québec. For each flock, one bird was sampled at each of the five critical steps of the slaughter process: after bleeding (B), before evisceration (BE), before chilling (BC), after water (W) and air chilling (A). A total of 161 samples were recovered from slaughterplants environment, prior to slaughter activities, and during operations. Samples were screened for the presence of the *cpe*.

Results show that 25% of flocks, 10.0% of carcasses and 5% of environmental samples were *cpe*-positive. *cpe*-positive samples among critical steps in each abattoir were as follows: B)11.8% and 0%, BE)20.6% and 25.0%, BC)11.8% and 0%, W)26.5% and 50.0% A)29.4% and 25.0%. The prevalence of *cpe*-positive carcasses and environmental samples was 16.6% and 7.6% for one abattoir, and 2.3% and 2.7% for the other. Positive environmental samples were equally identified among the samplings conducted prior to slaughter activities and during the operations, indicating a possible persistence of the bacteria in those facilities. Up to 75% of the positive carcasses and 100% of the positive environmental samples were identified after chilling, suggesting that enterotoxigenic *C. perfringens* strains would reach the consumer.

Key Words: broiler, slaughterhouse, step, Clostridium, enterotoxin

Environment, Management & Animal Well-Being - Stress Responses

T124 Efficacy of sodium bisulfate or salt for reducing broiler house floor microbial populations. Susan Watkins, Samantha Beitia, Geetha Kumar-Phillips, Josh Payne* *University of Arkansas*

Sodium bisulfate (SBS) is often applied to acid shock poultry house dirt floors following litter removal. Some farms utilize salt as a floor treatment for microbial control. A field study was conducted to compare the efficacy of sodium bisulfate and salt for reducing broiler house floor microbial populations following litter removal. Commercial broiler houses were chosen as the test sites. Floor plots (0.6 m x 1.5 m) were established under feed and water lines throughout the house and treatments were assigned using a complete randomized block design. In trial 1, treatments consisted of (1) control; (2) sodium bisulfate at 45 kg/93 m^2 (100 lbs/1000 ft^2); (3) sodium bisulfate at 68 kg/93 m^2 (150 lbs/1000 ft^2); and (4) Farmer's Coop fine rock salt at 68 kg/93 m^2 (150 lbs/1000 ft^2). There were 4 replicate plots per treatment. In trial 2, treatments consisted of (1) control; (2) sodium bisulfate at 68 kg/93 m^2 (150 lbs/1000 ft^2); and (3) Farmer's Coop fine rock salt at 68 kg/93 m^2 (150 lbs/1000 ft^2). There were 5 replicate plots per treatment. Floor plots were aseptically swabbed using a sterile cellulose sponge pre-treatment and at 24 h and 72 h post-treatment. Samples were cultured to determine populations of total aerobic bacteria, *E. coli*, total coliforms, yeast, and mold. Floor plot surface pH was also measured. Additionally, samples in trial 2 were cultured for the presence of *Staphylococcus* spp. Data were analyzed using the ANOVA procedure of SAS. In both trials, salt had no effect on surface pH and populations of total aerobic bacteria, and yeast but did show reductions in mold populations in trial 2. Sodium bisulfate showed significant reductions in floor pH and populations of total aerobic bacteria, yeast, and mold in both trials. *E. coli* and total coliform counts were reduced over time regardless of treatment. *Staphylococcus* spp. presence in samples was reduced from 100% pre-treatment to 80%, 60%, and 0% positive 72 h post-treatment in control, salt, and sodium bisulfate treatments, respectively. Sodium bisulfate proved effective for reducing microbial populations on a broiler house floor while salt showed limited to no effect.

Key Words: bacteria, sodium bisulfate, litter, dirt pad

T125 Evaluation of biochar as a beneficial litter amendment for broiler production John Linhoss*, Joseph Purswell, Jason Street *Mississippi State University Agricultural & Biological Engineering Department*

Biochar (BC) was evaluated as a litter amendment for broiler litter over a thirty-five day growout. BC is a waste product created by the gasification process commonly used by lumber mills to heat kilns and has been shown in laboratory-scale studies to benefit litter quality. 960 birds were placed into sixteen 5.57 m^2 environmental chambers at a stocking density of 0.09 m^2. 5.44 kg of biochar was spread over clean pine shavings (PS) in eight of the chambers at the beginning of the trial. The remaining eight chambers contained only PS. Live performance parameters included BW, BW gain, feed intake, feed conversion, and mortality. No difference was found between treatments for the live performance parameters measured ($p > 0.05$). Mean foot pad scores were not different ($p = 0.1544$). Final moisture content was different for the BC and PS treatments ($p = 0.0107$). Future work to determine the effect of biochar on litter nutrient values (nitrogen, phosphorus, and potassium) and litter ammonia production will be conducted. Based on the results of this study, BC used as a litter amendment appears to have no negative effects on broiler live performance.

Key Words: biochar, litter, ammonia, nutrients

T126 A pre-slaughter welfare monitoring protocol for broiler chickens Leonie Jacobs*[1], Evelyne Delezie[2], Klara Goethals[3], Bart Ampe[2], Luc Duchateau[3], Frank Tuyttens[2] *[1]Virginia Tech; [2]Flanders Research Institute for Agriculture, Fisheries and Food (ILVO); [3]Ghent University*

A practical monitoring protocol is needed to assess the impact of the pre-slaughter phase on broiler chicken welfare. The aim of the study was to develop a user-friendly protocol with an integrated welfare score (IWS) based on literature, expert opinion and application on commercial flocks. Initially, 15 animal-based measures to assess at the slaughter plant were chosen based on literature. For those measures, the impact of each pre-slaughter stage (catching and loading, transportation, lairage) was tested on 81 Belgian commercially transported flocks. Measures were on individual level (n=132±3 birds/flock), crate level (n=88 crates/flock), or flock level (at the slaughter line; n=1785±33 birds/flock). Six measures were removed from the protocol either because of low scores on importance and methodology from 9 poultry experts surveyed during a workshop (n=3 measures) or because they were unaffected by the pre-slaughter phase (n=3 measures). The final protocol included recording the prevalence of fractures, bruising, panting/huddling, splay-legged birds, crowding, supine birds, stuck body parts, mortality and carcass rejections. Implementation took between 30-60 min per flock. Inter-observer reliability could not be calculated for mortality, carcass rejections or for measures at the slaughter line, but was reasonably good for the remaining 5 measures (≥73% agreement). The IWS, based on results from a digital survey among 19 poultry researchers, includes a two-step calculation. First, prevalences are used to

calculate measure scores (MS), all on a scale from 0-100, based on expert opinion. Second, MS are multiplied by 1) a weight (0.042- 0.172) based on the importance of each measure for welfare, and by 2) a compensation reduction factor (0.0005- 0.360) based on the relative rank of the MS. Thereafter, MS are summed to obtain a score between 0-100. The IWS is designed to give more weight to low MS, as experts indicated. Application of the calculation on 63 transported flocks showed a good spread of IWS and good sensitivity to extremely low scores. The protocol with IWS can be used in quality assurance schemes, to identify best practices, potential preventative actions and risk factors for broiler welfare issues during the pre-slaughter period.

Key Words: broiler, pre-slaughter, welfare, monitoring, protocol

T127 Evaluation of Viribus B at varying levels fed to broilers challenged with coccidiosis Brett Lumpkins*, Greg Mathis, Fredrik Sandberg, Julie von Hellens *Southern Poultry Research, Inc.*

This study evaluated the feeding of Viribus B (VB) on performance of broiler chickens that were challenged with mixed coccidiosis species. VB is an all-natural, no GMO blend product of botanical extracts, phytonutrients and minerals, which has been demonstrated to support improved feed intake and maintain proper health in swine. Therefore, the potential of VB was tested with broilers in a 42 day floor pen trial with built-up litter and was conducted using a randomized complete block (9 blocks) design. Cobb 500 male broiler chicks were used and no coccidiosis vaccination program was implemented. Each pen contained 50 chicks with 0.95 sq. ft per chick. All treatments consisted of a three phase dietary program that used based on commercial feed formulation standards; starter (ST) d0-21, grower (GR) d21-35, and finisher (FIN) d35-42. Treatments were: T1 no feed additives unchallenged control (NUC), T2 no feed additives coccidia challenged (NCC), T3 VB standard level at 2.5 lb/t (VB1x) coccidia challenged, T4 VB 2 x standard level at 5 lb/t (VB2x) coccidia challenged. All experimental diets were fed *ad libitum* for the duration of the study. Bird weights and feed consumption were measured on d21, 35, and 42. All birds, except NUC, were infected on d21 of age with a mixture of Eimeria species of coccidiosis. Oocysts per gram fecal material (OPG) were determined on d27. During the starter period, both levels of VB improved FI and BWG, and on d27 after the coccidia challenge birds fed VB regardless of level had improved live performance parameters ($p<0.05$) compared to the NCC birds, and BWG was similar to the NUC birds. However, there was no difference in OPGs between any of the treatments. On d35, FCR and BWG were observed to still be improved with either level of VB compared to the NCC birds. The birds on the VB2x diet had the best observed FCR and BWG of the coccidiosis challenged birds throughout the study. At d42 the VB2x birds also had BWG comparable ($p>0.05$) to the NUC birds and an 11pt improvement in FCR compared to the NCC birds. The feeding of Viribus B demonstrated significant improvements in performance following a coccidiosis challenge in broilers.

Key Words: natural, Viribus-B, broilers, coccidiosis

T128 USAID Feed the Future Innovation Lab for Genomics to Improve Poultry: Increasing food security in Africa by enhancing resistance to Newcastle Disease and heat stress in chickens Huaijun Zhou*, David Bunn, Rodrigo Gallardo, Susan Lamont, Jack Dekkers, Amandus Muhairwa, Peter Msoffe, Boniface Kayang, Augustine Naazie, George Aning, Terra Kelly *University of California, Davis*

Homestead and small-scale poultry production has tremendous potential for alleviation of malnutrition and poverty in climate-stressed rural communities in Africa. Poor animal health and husbandry practices limit village poultry production throughout Africa. Newcastle disease (ND) is the number one constraint of raising poultry in Africa, causing mortality as high as 80% among village flocks. The goal of this USAID-supported program is to utilize deep phenotyping combined with advanced genomic selection to sustainably enhance innate resistance to ND and heat stress in indigenous African chickens to improve production. Genes and signal pathways associated with genetic resistance to NDV infection and heat stress were identified by RNA sequencing in well-established ND-resistant and susceptible inbred lines in the US by challenging chickens with a lentogenic ND virus strain in temperate or hot environments. One commercial layer line (1100 birds) widely used in Africa and six African chicken ecotypes (500 birds per ecotype) were challenged with lentogenic NDV. Disease resistance phenotypes including viral load in tears at 2 and 6 dpi and antibody response in serum at 10dpi were measured. The chicken 600K SNP chip was used to identify SNPs or regions associated with resistance to NDV and heat stress. Estimated heritabilities for serum anti-NDV antibody levels, viral load in lachrymal fluid and physiological response to heat in blood were relatively low in commercial layer line, and were medium to high (0.14-0.49) in African ecotypes, respectively. The results indicate the ability to identify genomic regions with significant effects on the measured phenotypes of these important traits. The number of regions is very small and the effects are also small, suggesting that the genetic control of response to NDV and heat stress is distributed over many genes of small effect. Collectively, these studies demonstrate the feasibility of identifying components of the genetic control of response to NDV. This work serves as a foundation for our long-term goal of using genomics to improve disease resistance of local chicken ecotypes in Africa. The expected results will help achieve the USAID Feed the Future Program goals to reduce hunger and strengthen food security.

Key Words: Chicken, disease, genomics, African, heat

Environment, Management & Animal Well-Being - Behavior & Incubation

T129 Correlation of litter moisture results between traditional drying method and a commercial moisture meter Tsung-lin Lu*, Brian Fairchild, Michael Czarick *University of Georgia*

Litter moisture is a crucial aspect in poultry management as it influences both poultry performance and welfare. The traditional method to determine litter moisture content through drying samples is time consuming. Therefore, the purpose of this study was to evaluate the applicability of a commercial moisture meter as a measuring tool. The study was conducted in one house on three different commercial broiler farms raising birds to a market weight of 2.3kg. The farms used pine shavings as bedding material, where two of the farms reused litter and one utilized fresh pine shavings.

The data were collected on days 7, 14, 21 and 35. Nine areas along the house were selected for sampling. At each area measurements were taken approximately 0.3, 4.0 and 6.1 m from the sidewall. Three commercial moisture meters of the same model were used to measure the litter moisture. The result of each location was determined by the average of 10 measurements per meter. Three weights of 0.6, 1.5, 2.1 kg were placed on the meter to see if it would influence the correlation between the two methods. The litter samples were then dried at 85°C for 24 hours to determine the moisture level by comparing the difference between the weights. A correlation analysis was conducted between the two methods and a one-way ANOVA was used to compare the moisture readings between the three meters.

The litter moisture level ranged between 10% to 51% among the samples collected throughout the study. There was no significant difference between moisture readings of the three meters. There was a low correlation of the litter moisture results between the two methods (R^2=0.37). Although the weighted and the non-weighted meter readings were significantly different from one another ($P<0.001$), the accuracy did not improve overall, where R^2 value for the meter adding 0.6, 1.5, 2.1kg was 0.07, 0.11, 0.14

respectively and 0.12 for the non-weighted. While the added weight did influence the results from the meters, overall the data indicate that the moisture meter tested in this study did not measure litter moisture as accurately as the oven drying method.

Key Words: litter, moisture, broiler

T130 Ectoparasites (Ornithonyssus sylviarum) and sleep disruption in White Leghorn laying hens Leonie Jacobs*[1], Giuseppe Vezzoli[2], Bonne Beerda[3], Joy Mench[4] *[1]Virginia Tech; [2]College of the Desert; [3]Wageningen University; [4]UC Davis*

Northern fowl mites (*Ornithonyssus sylviarum*) are common parasites in caged laying hens in the US. Mites feed on blood and are pathogen vectors, with infestation resulting in reduced egg production and feed conversion ratio. Mite infestation causes skin irritation, possibly disrupting the hens' resting behavior. Unimpeded nocturnal resting is important for health and welfare, for example for energy conservation, tissue restoration, tissue growth and memory formation. Our aim was to study the impact of northern fowl mite infestation on nighttime behavior in laying hens. Sixteen beak-trimmed White Leghorn hens were individually housed in cages. They were experimentally infested with circa 35 mites at 25 weeks (wk) of age and observed for the behaviors "active", "preening", and the sleep-related states "dozing" and "sleeping". Focal continuous observations were based on infrared video recordings from 22:00h until 06:00h (dark period, lights off) for two consecutive nights at wk 0 and wk 3 post-infestation. Data were analyzed with linear mixed models, testing for the fixed effects time of night and time from infestation on frequency and duration of behaviors.

Preliminary results from wk 0, when none of the hens had mites, and wk 3 (mean of 676 mites/hen) show that dozing was the most common behavior (85% of time; 13 bouts per night), followed by sleeping (6.3%; 7 bouts), preening (2.4%; 10 bouts) and active (0.9%; 8 bouts). Mite infestation increased preening from 7.6 (wk 0) to 10.4 bouts (wk 3, $P<0.01$) and dozing from 10.7 to 13.6 bouts ($P<0.01$). Amount of time spent preening increased with increased mite load ($P<0.01$) but the change was non-significant for dozing. Mite infestation increased the frequency and duration of preening in hens, and shortened their dozing bouts (increased frequency with unchanged duration), suggesting welfare impairment due to irritation.

Key Words: Rest, Sleep, Mite, Hen, Behavior

T131 Effect of flock age, frequency of turning and SPIDES during storage on embryonic development, and hatchability of long stored eggs Serdar Özlü, Ahmet Uçar, Tolga Erkuş, Kazım Bircan, Serap Yasun, Dinah Nicholson, Okan Elibol* *Department of Animal Science, Faculty of Agriculture, Universitiy of Ankara*

This study investigated the effect of broiler breeder flock age, frequency of turning, and SPIDES during 14d storage on the developmental stage of embryos and hatchability.

Hatching eggs were obtained from two Ross female line grandparent flocks at younger (29 wk) and, older (58 wk) ages and eggs were stored for 14 days at 15°C and 75% RH. During storage, eggs were either held continuously in the storage room (Control) or were subjected to a heat treatment regimen delivering 3.5 hours above 32°C, in a Petersime Re-Store machine at d 5 of storage and turned 0 or 4 times daily during storage. In each treatment, 15 eggs were opened in both flock ages at 5d of storage to examine the stage of embryonic development. All eggs were set in a single incubator and hatcher. A tray of 150 eggs constituted a replicate and 6 replicate trays (900 eggs) were set per heating treatment at each turning frequency and flock age.

Embryonic development was advanced by SPIDES, turning frequency of 4 times and in eggs laid by the older flock. Hatchability was significantly better for the younger flock compared to the older flock. Hatchability was improved by turning eggs 4 times daily compared to no turning during storage due to lower late embryonic mortality ($P<0.05$). This effect was more evident for older flock. SPIDES increased hatchability and reduced embryonic mortality and second grade chick compared to the control in both flock ages ($P<0.05$).

It can be concluded that the highest hatchability was observed in eggs both turned 4 times and given one SPIDES treatment at 5 days of 14d storage in both flock ages.

Key Words: hatching_eggs, SPIDES, turning, flock_age, hatchability

T132 In ovo green light photostimulation during different embryonic stages affect Somatotropic axis activity Liron Dishon*, Natalie Avital-Cohen, Joanna Bartman, Sagi Zaguri, Israel Rozenboim *The Robert H Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem*

In ovo green light (GL) photostimulation of meat type birds, elevated body weight and muscle growth at market age. The mechanism of this phenomenon was due to elevation of the somatotrophic axis activity. The objective of this study was to find the in ovo critical period for stimulating the somatotrophic axis by GL photostimulation.

Two hundred equal weight fertile broiler eggs (Cobb 500), were divided to 4 in ovo treated groups: 1. Incubated at dark condition (Negative Control, n=60), 2. GL photostimulated from embryonic day 0 (ED0) until ED20 (hatching day) (positive control, n=60), 3. GL photostimulated between ED10 and ED20, (n=50) and 4. GL photostimulated between ED15 and ED20 (n=30). At ED10 and every other day until ED20, 10 eggs from each treatment groups were sampled. Eggs were opened, blood samples were drown for GH ELISA assay, hypothalamus, liver and breast muscle samples were collected for mRNA gene expression of GHRH, GHR (Growth Hormone Receptor) and IGF-1 (Insulin Like Growth Factor 1), by Real Time PCR. After finding no significant interaction between treatment and ED, all statistical analyses were conducted with the JMP software using one-way ANOVA.

In-ovo GL photostimulation from ED0, caused a significant elevation ($p<0.05$) in plasma GH levels (between ED14-ED20) of between 35% and 100%, compared to the negative control. Hypothalamic GHRH mRNA gene expression significantly increased in 30% (on ED16 and ED20), and both liver GHR (on ED12 and ED16-18) and IGF-1 (on ED16-18) were significantly elevated by 20%-100%, compared to the negative control. In-ovo GL photostimulation from ED10 showed positive effect (compared to the negative control) on GH plasma levels, with no effect on mRNA gene expression. Green light photostimulation from ED15 showed elevation in somatotrophic axis activity similarly to the positive control group ($p<0.05$).

In ovo green light photostimulation of broiler embryos, from ED15-ED20 significantly elevated somatotrophic axis activity similar to the positive control group. We suggest that the critical period for GL photostimulation acceleration of somatotrophic axis is between ED15-ED20.

Key Words: broiler, incubation, photostimulation, somatotropic

T133 Effects of UV light provision on broiler chickens' feeding behavior, fear response and lighting preferences Antonia Patt, Rachel Dennis* *University of Maryland*

The ultraviolet (UV) spectrum is present in natural light systems but absent from many conventional systems. It is used by birds in identifying social cues, discriminating between foodstuffs and has also been shown to alter behavioral displays. Here, we investigate the impact of exposure to UV-A light on feeding behavior, fearfulness, and lighting preference. Birds were randomly assigned to one of 24 pens with half of the pens being exposed to UV light for 4 hours/day, while control birds were exposed to the same white lighting schedule as treatment birds. At 4 weeks, all birds were moved to a new pen, half were moved the same lighting treatment, and half to the opposite treatment, resulting in 4 treatments combi-

nations: continuous UV exposure, early UV exposure, late UV exposure, white light only. Consumption behaviors and activity levels were recorded throughout the 6 wks. At weeks 4 and 6, fear response was tested in social isolation and flight distance tests. Lighting preference was tested in spectral and intensity preference tests. UV exposed birds were found to eat more frequently and forage less when UV lights were on compared to non UV exposed birds at the same time of day. No difference in feeding behavior was evident when the UV lights were off. UV exposed birds vocalized at a higher frequency during the social isolation test than birds without UV exposure. In a 4 arm preference test, birds exposed to UV light spent more time in the arms that provided a source of UV light than birds only exposed to white light only. Birds exposed to continuous UV or late phase UV had shorter flight distances to a novel person than birds exposed UV early only or white light controls. When tested for their preferred intensity of both UV and white light, birds did not differ in their preference for UV light intensity. However, early UV exposure birds showed a preference for lower white light intensity, whereas late UV exposure birds showed a preference for higher white light. The results show that UV light exposure reduces fearfulness, increases frequency of feeding and reduces ineffective foraging away from the feeder, and alters white light intensity preference. Our results suggest a potential use of lighting in improving well-being and regulating feeding behaviors.

Key Words: ultraviolet, lighting, feeding, fearfulness, broiler

T134 Effect of Ration Plus with antibiotic-free diets on live performance and gut health of broiler chickens in heat stress environment Sean Yang*, Mick Roberts, James McNaughton, Michael Canady, Kasey Schuster, Adam Blaszczak, Elizabeth Wozniak *Cytozyme Laboratories, Inc.*

Ration Plus™ for Poultry (RP; AAFCO 36.11 Dried *Lactobacillus acidophilus* Fermentation Product) applied to antibiotic-free diets has been shown to improve performance and reduce mortality of broiler chickens exposed to heat stress. The objective of this study was to analyze the mode of action of RP on broiler gut health by evaluating various parameters including intestinal lesions, microbiota, gene regulation and antioxidant enzyme activity in the blood. A total of 832 one-day-old mixed sex (50% male and 50% female) Ross 708 broilers were randomly allocated into 2 treatments with 8 replicated pens per treatment and 52 broilers in each pen for 49 d on used litter. Dietary treatments included: (1) an antibiotic-free diet (CON) and (2) CON + 1 g/kg of RP (RPT). Heat stress was maintained at temperatures of 27-30 °C (d 0 to 7), 38 °C (d 8 to 14) and 41 °C (d 15 to 49). Coccivac B was used for all chicks with no coccidiostats added to the feed. The performance data in the current study is consistent with previous findings: broilers fed RPT had greater body weight gain than broilers fed CON during d 0 to 21 ($P < 0.05$), d 22 to 49 ($P < 0.05$) and overall (d 0 to 49; $P < 0.05$). Overall feed conversion ratio improved in RPT ($P < 0.05$) compared to that in CON and mortality decreased ($P < 0.05$) in both growth phases (d 0 to 21 and d 22 to 49) and overall (d 0 to 49). Gut health, as indicated by lowered intestinal lesion score, improved in RPT ($P < 0.05$) compared to CON at d 21. RPT also decreased *Escherichia coli* count ($P < 0.05$) at d 21, and *Salmonella* sp. incidence ($P < 0.05$) and *Clostridium perfringens* ($P < 0.05$) at d 21 and d 49. These findings were supported by microbiome analysis. In addition, regulation of gene expression was investigated in selected tissues using microarray and qPCR analysis. RPT also increased erythrocyte glutathione peroxidase activity ($P < 0.05$) as compared to CON. Results from the present study confirm previously reported performance benefits of Ration Plus™ supplementation to antibiotic-free diets and suggest the product's mode of action is through improving gut health and reducing oxidative stress in broilers under high temperature conditions.

Key Words: antibiotic-free, glutathione peroxidase, gut health, heat stress, Ration Plus

SCAD

T135 Case report: Lymphoproliferative Disease Virus in a wild turkey from Louisiana Leslie Johnson*[GS], Jessica Hockaday, Natalie Armour, Rusty Berry, Brittany Baughman *Poultry Research and Diagnostic Laboratory, College of Veterinary Medicine, Mississippi State University*

Lymphoproliferative disease virus (LPDV) is an oncogenic avian retrovirus capable of causing neoplastic disease in domestic and wild turkeys (*Meleagris gallopavo*). Since the first report of a naturally occurring LPDV infection in a wild turkey hen in the United States in 2009, studies have identified a high prevalence of LPDV infection in wild turkeys throughout the eastern United States. A deceased wild turkey hen from Louisiana presented to the MVRDL for necropsy examination with a clinical history of emaciation, weakness, crusted lesions on the head and neck, diarrhea, and severe bilateral conjunctivitis. Upon gross evaluation, multifocal to coalescing, yellow, umbilicated cutaneous nodules were present on the head, neck, and legs. The spleen was mottled and contained a focal ~1cm diameter, tan centrally necrotic nodule expanding the parenchyma. Skin lesions were identified histologically as sarcomas of mixed round cell lineage and the histologic features of the splenic nodule were consistent with a sarcoma of suspected reticuloendothelial cell origin. Fresh spleen and cutaneous nodule samples tested positive for Lymphoproliferative Disease Virus by PCR performed at the Southeastern Cooperative Wildlife Disease Study Virology Laboratory.

Key Words: Lymphoproliferative Disease Virus, Turkey, Avian Retrovirus

T136 Evaluation of immunity in SPF birds vaccinated with reovirus S1133 live, S1133 inactivated and Genotype 5 autogenous reovirus vaccines William Dawe*[GS], Erich Linnemann, Holly Sellers *University of Georgia*

In recent years, the incidence of reovirus-induced tenosynovitis has increased dramatically and commercial vaccines no longer provide adequate protection against the variant viruses emerging from clinical cases. The use of reovirus autogenous vaccines has increased, but the duration of immunity provided by the vaccines in the absence of a homologous live prime is unknown. In this study, the antibody response was evaluated following vaccination with a combination of commercial S1133 live attenuated/inactivated and an autogenous reovirus vaccine. Forty-five, day-of-hatch SPF chicks were divided into one of the following 3 groups: 1) inactivated S1133 plus autogenous genotype five (GT5) at 12 weeks-of-age then GT5 at 18 weeks-of-age; 2) S1133 modified live vaccine at day-of-hatch, 3 and 6 weeks-of-age then inactivated S1133 and GT5 at 12 weeks-of-age and 3) S1133 modified live vaccine at day-of-hatch, 3 and 6 weeks-of-age followed by inactivated S1133 plus GT5 at 16 weeks-of-age. Birds were bled every 2 weeks for 30 weeks and serum evaluated by ELISA and S1133 and GT5 virus neutralization (VN) assays. Birds in group 1, receiving only S1133 and GT5 inactivated vaccines, had low ELISA and VN antibody titers throughout the duration of the study. Group 2 ELISA geometric mean titers (GMT) were higher at 18 and 20 weeks of age compared to group 3, however S1133 VN GMTs were similar between both groups between 18-30 weeks. Starting at 16 weeks, S1133 VN GMTs were higher in groups 2 and 3 compared to GT5 VN GMTs, supporting the use of homologous live and inactivated vaccines for optimal antibody response. Group 2 GT5 VN GMTs were higher compared to group 1, providing evidence that some priming occurred with the live at-

tenuated S1133 vaccine. By 30 weeks, GT5 VN antibodies had decreased below the threshold of protective immunity. Results from this study provide direct evidence that use of homologous live and inactivated vaccines provide optimal antibody response. In this study, the timing and number of autogenous vaccinations did not extend the duration of immunity for the GT5 beyond 30 weeks. Many variations of reovirus live, inactivated and autogenous vaccinations are used in the field and should be evaluated to better understand reovirus immunity.

Key Words: Reovirus vaccines, Reovirus immunity, Variant reovirus

T137 Protection efficacy of a Herpesvirus of Turkeys (HVT) recombinant vaccine against Infectious laryngotracheitis virus (ILTV) in broilers administered in ovo at three standardized doses Daniel Maekawa Maeda*[GS], Gabriela Beltran Garza, Sylva Riblet, Maricarmen Garcia *University of Georgia*

Infectious laryngotracheitis (ILT) is a highly contagious respiratory disease of chickens that produces significant economic losses to the poultry industry. The disease is caused by the avian alphaherpesvirus commonly known as Infectious laryngotracheitis virus (ILTV). In recent years, the use of recombinant ILT vaccines has significantly expanded as it offers a safer vaccination alternative for the control of the disease. However, the protective efficacy of recombinant ILT vaccines can be compromised by fractionating the vaccine dose. The objective of this study was to determine the protection efficacy elicited by a recombinant HVT-ILTV vaccine when administered *in ovo* at 1000, 3000 and 6000 plaque forming units (PFU) per embryo. The protection induced by the recombinant ILT vaccine was assessed by the ability of vaccinated chickens to prevent clinical signs of the disease, to lessen challenge virus replication in the trachea, and to avoid body weight loss after challenge. Independently of the dose administered all vaccinated-challenge groups showed significant decrease in clinical signs and their body weight gain was maintained. Furthermore, no statistical differences in clinical signs or body weight gain was detected among vaccinated groups of broilers. Assessment of the viral load in trachea post-challenge is ongoing

Key Words: HVT, ILT, protection, recombinant

T138 Effect of ammonia on the immune response to infectious bronchitis virus vaccination and protection from homologous challenge in broiler chickens Emily Aston*[GS], Mark Jackwood, Robert Gogal, Jr., Maricarmen Garcia, Brian Fairchild, Deborah Hilt, Sunny Cheng, Brian Jordan *University of Georgia*

Commercial broilers in the grow-out stage are commonly exposed to poor air quality. A major contributor to reduced air quality is ammonia, an irritant gas that escapes from the litter when uric acid in feces is broken down by bacteria in the presence of air and water. Ammonia concentrations are higher in poorly ventilated houses and coincide with the elevated incidence of respiratory disease occurring during the winter months, yet the impact of ammonia on immunity against respiratory disease is unknown. Our study aims to determine the effect of ammonia on the immune response to infectious bronchitis virus (IBV) vaccination and protection against homologous challenge in commercial broiler chickens. One-day-old chicks were immunized with IBV Mass-type vaccine by oculonasal route and placed in a climate-controlled room containing 30-60 parts per million (ppm) of litter-sourced ammonia. At 28 days, birds were challenged oculonasally with homologous IBV M41, and protection was measured by viral detection in the choanal cleft, clinical signs, ciliostasis, and presence of airsacculitis. IBV-vaccinated birds in both ammonia and no-ammonia groups were completely protected from challenge and showed significantly reduced viral load, clinical signs, no ciliostasis, and no airsacculitis. Nonvaccinated controls were not protected from challenge, and 90% of ammonia-exposed controls had airsacculitis, compared to 40-50% of controls exposed to no ammonia. Our results indicate that commercial broilers exposed to moderate levels of ammonia are not more susceptible to IBV challenge if they are appropriately vaccinated. This data implies that ammonia exposure between 30 and 60 ppm alone may not increase susceptibility to IBV challenge in vaccinated broilers, but it is not clear whether higher levels of ammonia or if ammonia is combined with factors such as incomplete vaccine coverage or other environmental stressors, could exacerbate respiratory disease.

Key Words: Ammonia, Infectious, bronchitis, Immunity, Vaccination

T139 Attenuation characteristics of ArkGA, a new Ark-type IBV vaccine candidate Grace Albanese*, Dong-Hun Lee, Deborah Hilt, Mark Jackwood, Brian Jordan *University of Georgia*

The Arkansas serotype of IBV is the most frequently detected IBV type in the field. The current vaccine, ArkDPI, does not infect and replicate adequately to stimulate proper protection from challenge. Previously, our laboratory identified mutations in the S1 region of the spike glycoprotein of ArkDPI that are deleterious to its success as a vaccine. A new Ark-type IBV vaccine candidate, ArkGA, is a highly attenuated and protective vaccine derived from the former Ark99 vaccine. Initial work to develop ArkGA showed that the vaccine was highly pathogenic in one-day-old broiler chicks, however, 60 passages in embryonating eggs reduced the reactivity sufficiently, although the mechanism for attenuation of IBV and the changes occurring in the genome remain unclear. Whole genome Illumina sequencing was performed on ArkGA passages 1, 20, 40, and 60 to analyze the viral genome during attenuation. Additionally, viral material from choanal cleft palate swabs of broiler chickens vaccinated with the ArkGA passages was sequenced to identify mutations occurring during viral replication in the chicken. Illumina sequencing showed that 14 single nucleotide polymorphisms (SNPs) evolved between the pathogenic Ark99 sequence and the consensus ArkGA P60 sequence, including SNPs in the leader sequence, the nsp3 protein gene, and the spike protein gene. SNPs recorded within each passage indicated that the viral genetic population became more stable and homologous over subsequent passage. Although changes occurred in the S1 gene region between ArkGA passages, two amino acid mutations seen in the P60 vaccine were shown to revert to P1 sequence in virus obtained from vaccinated birds. When comparing the whole genome consensus sequences of the ArkGA passages with ArkDPI sequence, there are numerous amino acid changes seen in the spike gene, and many more seen throughout the genome that indicate that ArkGA and ArkDPI, although the same serotype, are highly different viruses. These changes seen in the ArkGA vaccine during embryo passage provide increased insight into the attenuation process of IBV.

Key Words: IBV, vaccine, Arkansas

T140 A severe outbreak of Avibacterium paragallinarum serovars A-2 and B-1 in coinfection with Gallibacterium anatis biovar haemolytica and the non-hemolytic biovar anatis in commercial layers. Nancy Christy*, Sergio Carrasco, Edgardo Soriano, Vladimir Morales *Boehringer Ingelheim*

Infectious Coryza is one of the highly infectious respiratory tract diseases of poultry caused by *Avibacterium paragallinarum*. It has emerged as a big problem of commercial poultry because increased number of culls and reduced egg production. (10-40%)

Gallibacterium anatis report a variety of signs such as respiratory problems, necrosis in livers, peritonitis, salpingitis, hemorrhagic and ruptured follicles and a drop in egg production.

In México, during the last years, the outbreaks were associated with *Avibacterium paragallinarum* serovar C1, however in this case we found serovar A-2 and B-1, this is important because the last report of serovar A-2 in México was in 2001.

Other important reason is that both agents produced egg drop, and there is not enough information about simultaneous infection.

During 2017, a suspected clinical case of Infectious Coryza in layers was confirmed by serological and molecular identification of *Avibacterium*

paragallinarum. The clinical signs were nasal discharge; conjunctivitis with swelling of the sinuses, face and wattles; decreased feed and water intake with reduced egg production. At necropsy, hemorrhages were in mucous membrane of nasal passage and trachea. *Gallibacterium anatis* was confirmed by Gram staining, motility, oxidation/fermentation, growth in Hugh and Leifsons' medium with glucose, and for urease, catalase and cytochrome oxidase activity and to confirm the identification; a section of the 16S rDNA gene was sequenced.

Samples were submitted to Universidad Autónoma del Estado de México for the analysis.

Results showed the simultaneous presence of *Avibacterium paragallinarum* serovar A-2, B1 and *Gallibacterium anatis* biovar *haemolytica* and *anatis* in layers.

This paper reports the simultaneous presence, the prevalence of serovar A-2, B-1 in layers and the importance to include a vaccine with *Gallibacterium anatis* and *Avibacterium paragallinarum* with specific serovars in the vaccination program in order to avoid egg drops caused by *A. paragallinarum* and *Gallibacterium anatis*.

Key Words: Coryza, serovarA-2, Gallibacterium, serovarB-1

Metabolism & Nutrition - Amino Acids

T141 Effect of dietary supplementation of arginine on feed efficiency and breast meat yield in fast-growing broiler chickens. Marco Zampiga*, Massimiliano Petracci, Giulia Baldi, Filiberto Ceccaroni, Adele Meluzzi, Sami Dridi, Federico Sirri *Department of Agricultural and Food Sciences – Alma Mater Studiorum - University of Bologna*

Understanding the amino acid requirement of modern broiler strains is fundamental to better exploit their genetic potential. Recent findings suggest that the recommended levels of dietary arginine might be suboptimal for the current fast-growing chicken hybrids. The present study aimed at evaluating the effect of the dietary supplementation of synthetic arginine on vital economic traits including feed efficiency and breast yield in broilers. A total of 1,168 1-d-old male chicks (ROSS 308) was weighed and divided in two experimental groups (9 replicates each) fed a commercial basal diet (CON group; Arg/Lys ratio 105-105-106-107 of each feeding phase, respectively) or the same basal diet supplemented with synthetic arginine (ARG group; Arg/Lys 115-115-116-117). Productive performance were recorded at the end of each feeding phase (12, 22, 33, 43 d). At slaughter (43 d), carcass and cut-up yields, and incidence of foot pad dermatitis (FPD) were assessed on each bird. Moreover, incidence and severity of breast myopathies were evaluated (n=150/group). Meat quality traits, as pH, color, drip and cook losses, AK shear force were assessed on 12 fillets/group. ARG group showed a significant lower feed conversion rate (FCR) than CON group at 12 d (1.352 *vs*. 1.401, respectively; P<0.05), 22 d (1.398 *vs*. 1.420, respectively; P<0.01) and 33 d of trial (1.494 *vs*. 1.524, respectively; P<0.05). Arginine supplementation tended to improve FCR in the overall period of trial (1.646 *vs*. 1.675, respectively for ARG and CON; P=0.09). Body weight was significantly affected by the dietary treatment at 33 d (1,884 *vs*. 1,829 g., ARG and CON respectively; P<0.05). At slaughter, ARG group showed higher breast yield than CON (30.4 *vs*. 29.3%). No significant effect of arginine was observed on breast meat quality traits, incidence of breast meat myopathies and FPD. Overall, these results indicate that the arginine/lysine ratio currently suggested for the modern broiler genotypes is not adequate to exploit their productive potential with particular regard to feed efficiency and breast meat yield. Ongoing molecular studies will add critical insights regarding the underlying mechanisms behind the improvement in feed efficiency and breast meat yield observed in the supplemented group.

Key Words: broiler chicken, arginine, productive performance, feed efficiency, breast yield

T142 The impact of particle size and pelleting temperature of ileal digestibility of soybean meal for broiler chickens. Antoniel Franco*[1], Alex Maiorka[2], Rex Newkirk[1], Henry Classen[1] *[1]University of Saskatchewan; [2]Universidade Federal do Parana*

The objective of this work was to determine if the impact of particle size and pelleting condition on the amino acid digestibility of soybean meal (SBM).Two 21d trials were conducted. Study one examined the effect of particle size on the digestibility of SBM at four different particles sizes (649, 824, 937 and 1234µm) in a maize-based diet. Study two studied the effects of pelleting temperature on SBM digestibility in a corn-soybean diet. The SBM was pelleted at 5 temperatures in the conditioner (55, 65, 75, 85 and 95°C). The resulting pelleted SBM products were ground prior to addition to the test diets which were pelleted at 55°C and crumbled prior to feeding. In both, Ross 308 birds were randomly divided into 5 groups in trial 1 and 6 groups in trial 2. Each treatment replicated with 4 birds/cage and 5 cages/diet. Regression was completed in JMP Pro 13. Diets were fed to broiler chickens to determine the ileal digestibility of various amino acids. The digestibility of methionine, lysine, threonine, arginine, isoleucine and valine had a positive quadratic response (p<0.05) as particle size increased from 649 to 824µm, but were reduced at 937 and 1234µm. Peak digestibility occurred with 824µm, except for lysine that responded with a linear decrease (p<0.05) as particle size increased. This may be related to gizzard development, which reflects intestinal motility, influencing the nutrient absorption. In trial two, amino acid digestibility responded to conditioning temperature in a quadratic fashion (p<0.05), with 75° C showing the highest ileal digestibility of all amino acids tests, with the exception of methionine (no effect). The lower digestibility at low processing temperatures may be explained by the presence of anti-nutritional factors that are intrinsic in the SBM. Higher temperatures likely reduced digestibility through severe denaturation of protein and the formation of indigestible protein fractions as well as Maillard reaction, that occurs between the amino acid and carbohydrate, in a presence of higher temperatures. In conclusion, a particle size around 824µm and processing temperature of 75° C are important to maximize amino acid digestibility of SBM in broiler chickens.

Key Words: Amino acids, Digestibility, Soybean meal, Particle size, Pelleting temperature

T143 The effect of a mono-component exogenous microbial protease on standardized ileal amino acid digestibility of soybean meal and full-fat soy for broilers Aaron Cowieson[1], Jose Otavio Sorbara*[1], Levy Teixeira[1], Sandra Bonaspetti[2], Anelcir Scher[2], Maurilio Xavier[3], Luiz Albino[3], Horacio Rostagno[3] *[1]DSM Nutritional Products; [2]Seara Foods; [3]Universidade Federal de Vicosa/UFV, Departmento de Zootecnia*

2128 male Cobb 500 broilers with an average weight of 890 g were used. Broilers were distributed in randomized blocks in a 19x2 factor arrangement (nine FFSBB or SBM samples - 40% included + a protein free diet (PFD) - 60%, without or with the addition of protease). Totaling 38 treatments and eight replicates with seven birds each. Celite was added at 1% to all experimental diets. The product used (RONOZYME ProAct) was added at a rate of 200 ppm. The effect of protease and soy source was run as a 2-way ANOVA with a full factorial arrangement of treatments and significance was set at P<.05. The contribution of various chemical characteristics of SBM or FFSB to the digestibility of the standardized ileal digestibility (SID) of amino acids was assessed using a mixed model multiple regression analysis. The SID of essential amino acids in general was higher in FFSB than in SBM (89.2 vs. 86.9%; P < .001). However, in the case of Val and Thr the digestibility was higher (P<.001) in SBM than in FFSB. The SID of Lys was increased by the addition of protease more in SBM than in FFSB resulting in a significant interaction between

diet and enzyme (P<.01). Conversely, the SID of Met was increased by the addition of protease more in FFSB than in SBM, resulting in an interaction (P<.05) between diet and enzyme for this amino acid. There were no interactions between diet and enzyme for any other essential amino acid and the addition of exogenous protease resulted in increase in the SID of Thr, Ile, Leu, Val, His, Phe and Arg of around 3-6%. In general, the inherent digestibility of amino acids in SBM was a stronger predictor of protease response than was the case for FFSB. However, in most cases (the only exception was SID Lys for FFSB), the inherent digestibility of the amino acid in the control group was a significant explanatory of protease effect (P<.05 to <.001 depending on the amino acid). The effect of protease on the SID of Met was particularly strongly predicted by the inherent SID of Met in the control. The usefulness of exogenous protease to enhance the nutritional value of soy products for broilers is clearly demonstrated.

Key Words: Enzymes, Soy, Protein, Quality, Nitrogen

T144 Broiler responses to varying balanced protein intake: economic optimization based on stochastic approach Matheus Reis*, Nilva Sakomura, Gabriel Viana, Fernando Antayhua, Rony Lizana *UNESP*

The Avinesp is a broiler growth model that considers the genotype of an average or a group of individuals as well as the influence of diet and environment factors, to predict nutritional requirements, performance, and economic responses. Simulations were performed in Avinesp model, using 25 representative individuals to estimate maximum broiler performance and profit based on different feeding programs. Five diets in the starter (1 – 21 d) and finisher (22 - 42 d) phases were formulated varying balanced protein (BP) content, resulting in 25 feeding programs. In the first phase, BP ranged from 23.87 to 20.51%, whereas in the following it ranged from 22.73 to 17.29 %. In both phases it was respected an interval of 0.10% in digestible lysine (Lys) between diets, and amino acid ratios with Lys were kept constant. The feed cost ranged from 0.77 to U$ 1.032 in the starter phase and from 0.705 to U$ 0.879 in the finisher phase. Broiler population were generated using a weighted sampling method, varying 2 genetic parameters (protein weight at maturity (1.041±0.062) and protein growth ratio (0.051±0.005)) and five weightings from normal curve (-2, -1, 0, 1, and 2 standard deviations (SD)), obtaining 25 individuals (25 different growth potentials). In total, 625 simulations were performed (25 feeding programs x 25 individuals) to predict feed intake, body weight (BW) and breast, thigh, drum, and wing weight, which in turn, had their market selling prices considered as 2.00, 5.00, 3.21, 3.80 and U$ 4.52, respectively. The feed cost was reduced from the revenue to calculate profit. On 42 d, BW (3358±528g), breast (859±169g), thigh (322±57g), drum (407±75g), and wing (253±43g) weights were all optimized by the BP level of 23.87 (starter) and 20.51% (finisher). Considering BW, the BP which optimized profit, was estimated in 22.75 (starter) and 19.06% (finisher), predicting a BW of 3161±410g. Based on the parts, the BP for maximum profit was estimated in 23.31 (starter) and 17.29% (finisher) which estimated breast, thigh, drum and wing weight in: 859±169g, 322±57g, 407±75g, 253±43g, respectively. These outcomes indicate that maximum performance is not always followed by maximum economic return and growth models are useful tools in nutrition and selling market decisions.

Key Words: Balanced-Protein, Chicken, Stochastic, Growth-model

T145 Muscle fiber growth, collagen deposition, and in vivo collagen synthesis in Pectoralis major at d 35 and d 57 broilers Pramir Maharjan*, Michael Schlumbohm, Garrett Mullenix, Katie Hilton, Antonio Guerra, Barbara Mallmann, Juan Cueva, Maria Cortes, Judith England, Casey Hanning, Craig Coon *University of Arkansas*

A study was conducted to understand muscle fiber growth over age, collagen deposition at age d57 and *in-vivo* soluble and insoluble collagen fractional synthesis rate (FSR) at two different ages in Cobb 700 broilers. Birds were fed standard Cobb 700 diet throughout the study except the amino acid levels (120% of recommended level). For muscle fiber study, birds (n=5) were killed at d-21,28, 29, 35,42, 49 and 57, and breast muscle tissue were collected and subjected to histomorphology study- H/E, and Masson trichrome (MT) staining. Fiber diameter and fiber numbers per unit area were determined. Collagen assay was performed quantifying hydroxyproline (HP) content in muscle tissue for d57 birds (n=5) using colorimetric assay. Synthesis rates for soluble and insoluble collagen protein were determined for birds at ages d35 (n=5, woody breast(WB) score < 1) and d57 (n=5, WB score >2) using stable isotope flooding method. FSR (% h^{-1}) was determined using precursor–product method: $\Delta Ep/A^* 1/t$, where ΔEp enrichment change between baseline tissue sampling and tissue sampling taken at time 't' post infusion of tracer, and A is the area under the curve derived from the decay in plasma enrichment over time 't'. Birds were infused with 1-^{13}C Proline as tracer amino acid, and isotopic enrichment ^{13}C-to-^{12}C ratio for muscle and plasma samples were measured using the ratio of fragments 287 to 286 derived from GC MS mass spectrogram. Results from the histomorphology showed linear increase (R^2=0.96) of fiber diameter as bird aged from 20.97 ±2.52µm at d21 to 68.39 ±3.68 µm d57. In a polynomial way, fiber numbers decreased (R^2 =0.95) from 1479 at d21 to 98 fibers per mm^2 at d57, and non-fiber space per mm^2 increased (R^2 =0.73) from 0.48 to 0.64 mm^2 suggesting replacement of fibers with connective tissue. MT staining showed greater accumulation of collagen in interfascicular and interfibrillar regions as bird aged. HP assay showed the collagen content of 0.28µg/mg of insoluble collagen on DM basis. FSR at d35 was 0.069 (±0.009) and 0.31 (±0.15) % h^{-1} for soluble and insoluble collagen respectively, whereas at d57, FSR was undetectable when measured hourly. This indicated a slower synthesis rate of collagen in breast muscle as bird ages and with progression of woody breast myopathy.

Key Words: collagen, synthesis, muscle, myopathy

Metabolism & Nutrition - Enzymes

T146 The Effect of Different Levels of Nano Selenium on Growth Performance, Meat Quality and Quantity Traits of Broiler Chickens Duration Starter Period Farhad Ahmadi*, Nooshin Azadpour *Department of Animal Science, Agricultural College*

This study was carried out to investigate the effect of different levels of nano selenium (Nano-Se) as a source of Se on growth performance, quantity and quality carcass of broilers in the starter period. A total of 240 one-day male broilers (Ross-380) distributed in a completely randomized design with four treatments. Four replicates with 15 birds were allocated to each experimental unit and birds were reared for 21 days. Experimental diets were T_1 (control, basal diet without Nano-Se), T_2, T_3, and T_4 supplemented with 0.2, 0.4 or 0.6 mg Nano-Se/kg, respectively, with ad-lib feed and water throughout the experiment. Body weight (BW), feed intake (FI) and feed conversion ratio (FCR) were recorded as weekly. At the end of the experiment, two birds from each experimental unit were selected and after slaughtering, separation of carcasses, thigh and breast samples were stored in- 20°C until analysis of meat quality traits. The results indicated that body weight, FCR, dressing carcass, breast and thigh weight were higher in birds fed diet T_3 (0.4 Nano-Se) than the other experimental groups (P<0.05). Dry matter and crude protein in the breast and thigh muscle was higher (P<0.05) in birds fed diet T_3 (0.4 Nano-Se). Regression analysis was performed to estimate optimal dietary Nano-Se level in the presence of linear or quadratic responses. According to the linear model, the optimal level Nano-Se from hatch to 21 d of age were 0.31 and 0.34 mg/kg for LBW and dressing weight respectively. In conclusion, the results of the present research showed that addition of Nano-Se (as a source

of Se) during the starter period could be improve performance, slaughtering yields and carcass quality of broilers.

Key Words: Broiler, Performance, Nanoparticles, Selenium, Carcass

T147 Corn particle size separation and hammer mill performance Lisa Kitto, Greg Roth, R. Hulet, Paul Patterson* *Pennsylvania State University*

Corn particle size (PS) is a relatively unexplored topic regarding its impact on commercial poultry performance. Additionally, there are potential feed fabrication aspects to consider, as mills could potentially save machine energy, wear, and money by modifying PS. Two hammer mills (100 and 150 HP Sprout, model 3818) were used to generate four ground corn treatments (Wenger Feeds, LLC) with goal geometric mean diameters (GMD) of 600, 900, 1200, and 1500 µm. Random samples of the corn treatments were taken for DM, ME, ether extract, crude protein, crude fiber, and ash determinations in triplicate. Particle size distribution was measured using a W.S. Tyler sieve shaker (ASABE procedure S319.4) and GMD and geometric standard deviation (GSD) were calculated. Energy expenditures of the hammer mills were investigated for electrical usage to grind the corn to each given PS treatment. Percent separation data was analyzed using the GLM procedure of SAS (9.4) with Tukey's test for multiple means comparison with application of an arcsine transformation on all percentage data and significance determined at a threshold of $P < 0.05$. Percent separation between treatments revealed the 3360 and 2380 µm screens held the greatest percentage of 1500 µm corn at 53.28% compared to the 600, 900, and 1200 µm corn ($P < 0.0001$), while the remaining screens held no more than 13.89% each. The 1190 and 841 µm screens trapped the most significant amount of the 600 µm treatment corn at 16.74%, and 11.41%, respectively. Feed mill measurements included power and amperage of the motors using during grinding, rate at which corn was ground in tonnes/hr (TPH), efficiency, cost ($/tonne and $/KWhr), and speed of (hr/tonne) grinding. The actual GMD of the corn PS treatments fell within 200 µm of the PS goal and the GSD was calculated to be very low between 0.30 and 0.47. Economic analysis indicated lower energy cost and higher TPH with greater PS. In conclusion, based on the results of hammer mill energy usage and TPH, feed mills would benefit from grinding larger PS corn for poultry diets whenever feasible.

Key Words: corn, particle, milling, separation, poultry

T148 In vitro assessment of peptide size fractionation with endogenous proteases or endogenous proteases supplemented with Axtra PRO Luke Barnard*, Janne Thorgersen, Shukun Yu *Danisco Animal Nutrition, DuPont Industrial Biosciences*

The objective of this work was to characterise the cleavage profiles of endogenous proteases and endogenous proteases supplemented with an exogenous protease using a soy protein isolate in an *in vitro* digestion model.

The endogenous proteases tested in this experiment were pepsin (Roche Applied Science) and pancreatin (Sigma Aldrich). The exogenous protease tested was Axtra® PRO (PRO: Danisco Animal Nutrition). Incubations containing pepsin were carried out at pH 3.7 and incubations containing pancreatin were carried out at pH 6.5. The experiment had 4 treatments: pepsin, pancreatin, pepsin + PRO and pancreatin + PRO. In all treatments, the soy protein isolate (SUPRO®760 IP) was incubated with the protease at a ratio of 20:1 at 37°C for 1 hour before being subject to liquid chromatography and mass spectroscopy. The size distribution of identified peptides was illustrated as the number of peptides within a range of m/z 100 Da, relative to the total number of identified peptides. Data were analysed using ANOVA and means separated with Tukey's.

Approximately 2600-2700 peptides were detected in each digest apart from the pancreatin treatment which generated 3278 peptides. The relative size distribution was similar between pepsin and pepsin + PRO. In both cases around 13% of the peptides detected were 0-1000 kDa, 58-59% of the peptides were between 1000-2000 kDa and 27-28% were >2000 kDa. Peptides generated by the pancreatin alone had a size distribution with a higher average mass (15%, 63% and 21% for 0-1000 kDa, 1000-2000 kDa and >2000 kDa) compared to the combination of pancreatin + PRO (27%, 66% and 7% for 0-1000 kDa, 1000-2000 kDa and >2000 kDa) indicating the ability of the exogenous protease to further hydrolyse soy protein on top of the endogenous protease. When comparing the peak areas of curves there was a significant difference between the pancreatin and the pancreatin + PRO treatments for the 700-1400 kDa range ($P<0.05$).

In conclusion, Axtra® PRO facilitates the hydrolysis of soy protein by pancreatin leading to a reduction peptide size compared to the endogenous protease alone. This supports the MOA of the protease to increase protein hydrolysis in the gastrointestinal tract, increasing the availability of protein for absorption by the animal.

Key Words: protease, enzymes, protein, in-vitro, soy

T149 Inclusion of an exogenous neutral protease enzyme in low amino acids diets formulated with different protein sources in broiler chickens Sergio Gomez-Rosales, Maria de Lourdes Angeles, Alejandro Diaz-Alonso* *CENID-Fisiologia. INIFAP.*

The objective was to evaluate the growth performance, carcass yield and the nitrogen balance of broiler chickens fed reduced AA corn-soybean meal (C-SBM), C-SBM + canola meal (C-SBM-CM) and C-SBM + DDGS (C-SBM-DDGS) diets added with an exogenous neutral protease (NPro). One-hundred and eighty, male Ross B308 broiler chickens from 14 to 42 d of age were allocated to individual crates, and at 14 of age, they were assigned to nine dietary treatments in a factorial arrangement of 3 diets (C-SBM, C-SBM-CM and C-SBM-DDGS) and 3 formulations (Positive control diet (PC) with a reduction of 5% in essential AA in regard to normal recommendation; Negative control diet (NC) with a reduction of 12% in essential AA; and, Same as 2 with the addition of 0.01% of a neutral protease (NPro). Starter (14-21 d), grower (22-35 d) and finisher (36-49 d of age) diets were formulated and each diet was fed to 20 birds. Productive responses were registered; excreta was totally collected at the last week of the trial to determine the nitrogen retention (NR); the carcass traits were recorded from all chicken. Results were subjected to ANOVA. In C-SBM diets the weight gain had a trend to be lower in broilers fed the NC diet, compared to those fed the NPro and PC diets; in the C-SBM-CM diets, broilers fed the NC diet showed lower weight gain and higher feed conversion ratio ($P < 0.05$) compared to broilers fed the NPro and PC diets; while in the C-SBM-DDGS, the weight gain tended to be higher in the PC compared to the NC and NPro. In broilers fed the C-SBM set of diets, the carcass and breast weight and yield were similar; in the C-SBM-CM diets, the carcass weight had a trend to be lower ($P < 0.08$) in the NC; while in the C-SBM-DDGS diets, the carcass and breast weight and carcass yield were lower ($P < 0.05$) in broilers fed the NC and NPro compared to the PC diets. In the three set of diets, C-SBM, C-SBM-CM and C-SBM-DDGS, the NR was lower ($P < 0.05$) in broilers fed the NC compared to broilers fed the NPro and PC diets; the NR was similar between the NPro and PC diets. In summary, the addition of a NPro to low AA C-SBM diet improved the weight gain and the NR, in the C-SBM-CM diet the NPro improved the weight gain, carcass weight and NR, and in the C-SBM-DDGS diets improved the NR.

Key Words: Broilers, Production, Nitrogen retention, Neutral protease, Protein sources

T150 Inclusion of exogenous protease enzymes in low amino acid diets formulated with different protein sources in broiler chickens Alejandro Diaz-Alonso, Maria de Lourdes Angeles, Sergio Gomez-Rosales* *VIMIFOS*

The objective of the study was to evaluate the growth responses and carcass traits in broiler chickens fed low essential AA diets based on corn and soybean meal (SBM) and DDGS, whole soybean meal (WSBM), canola meal (CM) or meat and bone meal (MBM) added with acid or/and neutral

protease enzymes. Three-hundred and seventy five male Ross B308 broilers from 21-49 d of age were allocated in individual crates, and at 21 of age, they were assigned to dietary treatments in a factorial arrangement of 5 diets (SBM, or SBM with one of the protein sources: DDGS, WSBM, CM and MBM) and 5 formulations: 1, positive control (PC) with reduction of 5% in essential AA in respect to the normal recommendations; 2, negative control (NC) with a reduction of 9% in essential AA in respect to the normal recommendations; 3, same as 2 with the addition of an acid protease (APro); 4, same as 2 with the addition of a neutral protease (NPro); and, 5, same as 2 with the addition of both, acid and neutral protease (ANPro). Grower (21-35 d of age) and finisher (36-49 d of age) diets were formulated and each diet was fed to 15 birds. Productive responses were registered; at the end of the trial, the carcass traits were recorded from all chicken. Results were subjected to ANOVA. In the SBM diets, the weight gain was lower ($P < 0.05$) and the feed conversion was higher in the NC and APro compared to CP, NPro and ANPro. In the DDGS and WSBM diets, the productive responses were similar among the treatments. In the CM diets, the breast weight ($P < 0.05$) and the carcass weight ($P < 0.10$) were lower in the NC compared to the CP, APro NPro and ANPro. In the MBM diets, the thigh, breast and carcass weight ($P < 0.05$) were lower in the NC, APro and NPro compared to the CP and ANPro. In summary, the addition of an APro and NPro to low AA diet improved the weight gain and feed conversion in the SBM diets, and the breast and carcass weight in CM and MBM diets.

Key Words: Broilers, Production, Acid protease, Neutral protease, Protein sources

T151 Impact of dietary phosphorus and calcium level on phytase efficacy in improving phosphorus and calcium digestibility and growth performance of broilers Roger Davin, Frances Yan*, Colwayne Morris, David Ledoux, Mercedes Vazquez Anon *Novus International, Inc.*

A battery trial was conducted to evaluate the effect of phytase on ileal P and Ca digestibility and growth performance of broilers. Five corn SBM based diets were formulated to contain different levels of non-phytate P (nPP) and Ca: 0.45 and 0.93% (normal), 0.30 and 0.93% (marginal P normal Ca), 0.30 and 0.78 (marginal P and Ca), 0.13 and 0.93% (deficient P normal Ca), and 0.13 and 0.78% (deficient P marginal Ca). The five diets were supplemented without or with 500 U/kg phytase (CIBENZA® PHYTAVERSE®, Novus International Inc, St Charles, MO) resulting in 10 treatments. Each diet was fed to 16 replicate pens of 5 birds from 17 to 22 d in mash form. On d 22, all birds were sacrificed to collect digesta from the distal 2/3 of the ileum to calculate ileal P and Ca digestibility. Data were analyzed by one-way ANOVA and means were separated by protected Fisher's LSD test with a value of ≤ 0.05 considered significantly different. In the absence of phytase, P digestibility decreased in a nPP-dependent fashion: 54% at nPP = 0.45, 49% at nPP = 0.30, and 32% at nPP = 0.13. Phytase increased P digestibility in all diets; the magnitude was greater for P deficient diets (22 and 18% increase for normal and marginal Ca) than for P marginal diets (7 and 13% increase for normal and marginal Ca) and P normal diet (7% increase). Without phytase, lowering nPP from 0.30 to 0.13% reduced Ca digestibility by 11 and 10% at normal and marginal Ca, whereas, phytase increased Ca digestibility by 15 and 18% respectively. Although birds fed 0.30% nPP performed similarly to birds fed 0.45% nPP, phytase increased weight gain regardless of Ca level, and improved FCR when dietary Ca was marginal. Broilers on 0.13% nPP had inferior growth performance compared to birds fed 0.30 or 0.45% nPP; phytase improved performance and the magnitude was greater when Ca was marginal. In summary, phytase improved growth performance when P level was marginal or deficient with the improvement being greater at deficient P levels, and marginal dietary Ca level could facilitate phytase response on growth performance of broilers. Phytase increased P digestibility regardless of dietary P level, with the magnitude being greater at the lower P level, and increased Ca digestibility only when dietary P was deficient.

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

T152 Comparison of two different phytase levels in combination with a xylanase to a multi-enzyme blend on male broiler performance and production costs from 1 to 42 days. Tara York*, Craig Wyatt, John Brake, Adam Fahrenholz, Gilson Gomez *AB Vista*

An experiment was conducted to compare various approaches on how feed enzymes are supplemented in today's broiler industry and the impact on bird performance. Male Ross 708 broilers (n=2,304) were allocated to 72 floor pens and fed one of 8 dietary treatments. Birds were fed a 3-phase feeding program (crumbled starter: 1-14 d; pelleted grower: 15-33 d; and pelleted finisher: 34-42 d). A positive control (PC) diet was formulated to meet or exceed nutrient recommendations without enzyme supplementation. The negative control 1 (NC1) treatment reduced the Ca, avP, Na, and AME from that of the PC diet by 0.165, 0.150, 0.035, and 30 kcal/lb, respectively. The NC1 diet was then supplemented with either 500 or 1500 FTU of an enhanced *E. Coli* phytase and 12,000 BXU/kg xylanase enzyme (NC1+ 500XYL and NC1+1500XYL). The negative control 2 (NC2) treatment reduced Ca, avP, and Na by 0.150, 0.170, and 0.020 in all dietary phases with varying AME and amino acid matrices applied to the starter, grower, and finisher feeds based upon the multi-enzyme supplier recommendation. The NC2 diet was then supplemented with a multi-enzyme blend to include a phytase enzyme (NC2+Blend). For the negative control 3 (NC3) treatment the same multi-enzyme blend was included to all feeds with a "downspec" of 0.10 for Ca and avP and 30 kcal/lb (NC3+Blend). In general, broilers fed the NC diets exhibited reduced ($P<0.05$) feed intake (FI), BW gain and poorer mortality corrected FCR when compared to all other treatments. Birds fed the NC2 diet had the poorest livability ($P<0.05$) that was attributed to a phosphorus deficiency. The European production efficiency (EPE) factor was calculated for all treatments and indicated significantly lower overall performance for the NC diets ($P<0.05$). The EPE was highest for birds fed the NC1+1500XYL and NC3+Blend. Overall, birds fed NC1+1500XYL diets had the numerically best performance, resulting in the lowest feed cost per pound of live bird.

Key Words: phytase, xylanase, protease, feed cost, European Production Efficency

T153 Phytases differ: In vitro phytate degradation ends up in different inositol phosphate isomers Stefanie Gabler*, Imke Kühn, Mike Bedford *AB Vista*

Phytases produce different inositol phosphate isomers when hydrolyzing phosphates from inositol 1,2,3,4,5,6-hexakisdihydrogen phosphate (phytate, $InsP_6$). The objective of this study was to use an *in vitro* broiler gastrointestinal tract simulation using feed as the substrate to investigate the evolution of inositol phosphates from the action of four different phytases. There is an assumption that the positions of remaining phosphates on inositol phosphate isomers effects their chelating behaviour towards cations, and can therefore influence bioavailability of minerals e.g. calcium. Thus, differences in degradation pathways are of interest. Phytases were applied to a corn-soy bean meal mix (60:40) and consecutively processed through a test simulation of the broiler`s crop, gizzard and small intestine in respect of temperature, pH, time and digestive proteases (according to Sommerfeld et al., 2017). Extraction with EDTA was used to allow enantiomeric analysis of total inositol phosphates with high performance ion chromatography. By the end of the simulation the four phytases tested produced the enantiomer Ins(1,2,3,4,6/1,2,3,5,6)P_5 when hydrolyzing the first phosphate from $InsP_6$. Diversity between phytases is demonstrated with the removal of the second phosphate: Phytase 1 and phytase 3 produced enantiomer Ins(1,2,5,6/2,3,4,5)P_4 and phytase 2 and phytase 4 produced both Ins(1,2,5,6/2,3,4,5)P_4 as well as Ins(1,2,3,4/1,2,3,6)P_4 by the end of the test. However, phytase 2 produced InsP4 enantiomers in a ratio of 23%

and 77%, respectively, and phytase 4 produced them in a ratio of 51% and 49%, respectively. The concentration of specific $InsP_4$ isomers and their ratio might be important in respect to the bioavailability of positively charged minerals, especially under alkaline conditions in the small intestine, as well as for the further $InsP_4$ degradation within the intestinal tract. Further investigation is needed to differentiate phytases by their pathway of $InsP_6$ degradation when applying them in animal feed to further increase bioavailability of minerals.

Key Words: inositolphosphate, phytase, invitro, gastrointestinal-simulation

T154 High phytase doses on real-time gizzard pH of broiler chickens and laying hens Sophie Lee*[1], Jenny Dunne[2], Toby Mottram[3], Mike Bedford[1] *[1]AB Vista; [2]Drayton Animal Health; [3]eCow Devon Ltd*

A novel capsule technique, currently used in human pH diagnostics, was used to measure real-time gizzard acidity in mature broilers and layers fed diets with and without phytase. Ten male 42-day old Ross 308 broilers and 8 mid-lay hens were used in this study. Birds were fed a nutrient-adequate diet with or without 2500 FTU/kg Quantum Blue phytase. Following a dietary adaption period, capsules (Heidelberg Medical) were administered to birds and pH readings monitored over a 24 h period. Subsequent to capsule dosing, birds were humanly euthanised and a spear-tip probe used to measure gizzard pH. All capsules were located in the gizzard. Results demonstrate that acidity can drop to as low as pH 0.5 in both layers and broilers. The frequency of pH drops below pH 1.0 may have huge implications on fibre and protein degradation, as well as phytase efficiency in the diet. Average pH capsule readings were lower ($P < 0.001$) in laying hens compared to broilers. Moreover, a bird type x phytase interaction ($P < 0.001$) indicated that phytase affects broilers and layers differently; phytase addition reduced gizzard pH in broilers but increased pH in layers, making the average gizzard pH of broilers and layers comparable. Different responses to phytase between broilers and layers may be due to the level and solubility of the Ca used in the diets. However, capsule results were not supported by pH probe measurements. Phytase addition to both broiler and layer diets resulted in an increase ($P = 0.01$) in gizzard pH as measured by the probe. Moreover, layers had higher ($P = 0.002$) gizzard probe pH than broilers. These results demonstrate that taking point-in-time measurements post-euthanasia may not equate to real-time readings. However, simultaneous measurement of pH by real time probe and spear tip post euthanasia would confirm whether these two methods are comparable, and if so, if there is a shift in pH post euthanasia.

Key Words: pH, Capsule, Broiler, Layer, Gizzard

T155 Effects of a commercial xylanase-probiotic blend on live performance, gut health, and environmental pathogens of broilers fed corn-soy-based diets Sarah Page*, James McNaughton, Basheer Nusairat, James Tyus, Jeng-Jie Wang *BioResource International Inc.*

A study was conducted to evaluate the effects of a proprietary blend of xylanase and probiotics (EnzaPro®, EP, BioResource International Inc.) supplemented to reduced-energy, corn-soy-based broiler diets on the live performance, severity of intestinal lesions, and litter *Clostridium perfringens* CFU count of broilers raised in floor pens on reused litter to 42 days. A total of 1,664 day-old mixed-sexed Ross 708 broiler chicks were assigned to one of four dietary treatments, with 8 replicate pens per treatment and 52 birds per pen. Diets were formulated in three phases (Starter d 0-21, Grower d 22-35, and Finisher d 36-42) at one of two levels of ME: 1) breeder recommended ME (standard energy) or 2) standard energy minus 130 kcal/kg (reduced energy). Dietary treatments included: 1) standard energy positive control (PC), 2) reduced energy negative control (NC), 3) NC + 100 g/MT EP, and 4) NC + 50 g/MT commercial antibiotic growth promoter (AGP). Data were analyzed in a randomized complete block design format. At 42 d, BWG and FCR were significantly improved ($P \leq 0.01$) in birds fed the PC diet compared to that of those fed the NC diet. EP supplementation increased ($P < 0.01$) 42-d BWG by 57 g/bird, compared to NC, and was comparable to birds fed NC diets supplemented with AGP. 42-d FCR was comparable among birds of PC, EP and AGP treatments, and were significantly lower ($P<0.01$) than that of NC birds. EP supplementation significantly reduced ($P < 0.05$) d-42 lesion scores by 66% and 44% compared to the unsupplemented controls and AGP, respectively. Supplementation with either EP or AGP significantly reduced ($P < 0.05$) the abundance of *C. perfringens* in the litter at d 21 and d 42 compared to the unsupplemented controls. The results of the current trial suggest that this blend of enzyme and probiotics improves broiler performance and gut health, reduces environmental pathogens, and is more effective than AGP at reducing the severity of intestinal lesions.

Key Words: Broilers, xylanase, probiotic, lesions, pathogens

T156 Fibre solubilisation and broiler gut health effects of xylanase combined with α-L-arabinofuranosidase debranching enzyme in a corn-based diet Ninfa Pedersen[1], Jonas Ravn*[1], Vibe Glitsø[1], Dan Pettersson[1], Richard Ducatelle[2], Filip Van Immerseel[2], Nelson Ward[3] *[1]Novozymes A/S; [2]Ghent University; [3]DSM*

Solubilisation of prebiotic arabinoxylooligosaccharides from complex arabinoxylans (in maize, for example) by xylanases may be increased by addition of auxiliary debranching enzymes. In this study, the hydrolysis and fermentation of maize fibre was investigated *in vitro* using a xylanase and an α-L-arabinofuranosidase. Compared to applying enzymes separately, combining the enzymes induced a higher ($P<0.001$) xylose solubilisation and higher ($P<0.05$) butyrate production during *in vitro* fermentation of maize fibre with broiler cecal inoculum. Subsequently, fibre degradation and fermentation was investigated in ROSS 308 broiler chickens supplemented with the enzyme combination to test the effects on gut morphology and microbiota composition along with performance. A total of 480 birds allocated in 8 pens/repeats per treatment were fed a maize/soy based diet with 100 g/kg maize DDGS and 50 g/kg canola meal. Enzyme supplementation increased ($P<0.001$) body weight (+5.4%) and improved ($P<0.001$) feed conversion ratio (-5.8%) after 29 days compared to control birds. Non-starch polysaccharide analysis and confocal microscopy of jejunum digesta visualised confirmed solubilisation of the insoluble maize (glucurono)arabinoxylan. Birds receiving enzyme supplementation had increased ($P<0.001$) duodenum villi length (+120 µm) and reduced ($P<0.002$) CD3 T-cell infiltration (-22.1%) after 29 days. Cecal butyrate levels were increased ($P<0.05$) compared to controls. Although the microbiota composition was not significantly altered, numerical increases in cecal *Ruminococcaceae* and *Lachnospiraceae* genera were observed in birds supplemented with enzymes. These experiments clearly show that the combination of α-L-arabinofuranosidase with xylanase improves the solubilisation and fermentation of corn fiber for broilers.

Key Words: xylanase, arabinofuranosidase, glucurono-arabinoxylan, prebiotic, fermentation

T157 Field observations: Neither KOH protein solubility nor urease activity are adequate quality predictors of commercial full-fat soybeans for poultry. Nelson Ruiz*, Fabiola de Belalcázar, Jorge Castillo *Nelson Ruiz Nutrition, LLC*

Over the years quality control methods have been developed to successfully evaluate and establish optimum parameters for soybean meal (SBM) quality. Two such methods are urease activity (UA) and KOH protein solubility (KOHPS). Direct application of these methods to evaluate the quality of full-fat soybeans (FFSB) has been historically used by industry without any research supporting their validity as quality indicators. Basically, the accepted assumption was that what applies to SBM applies to FFSB. However, Ruiz & Parsons [Poult Sci 94(E-Suppl. 1):76] reported that this assumption is not valid. Their data revealed that KOHPS is not an adequate predictor of FFSB protein quality because no correlation could be established between KOHPS values and *in vivo* coefficients of amino acid digestibility ($P>0.05$). The objective of this presentation is to document with field data that commercial FFSB quality is not able to be

consistently predicted by UA (pH-rise method). A total of 120 samples of commercial FFSB from different Andean Pact countries and derived from different manufacturing processes were analyzed for both UA (AOCS Method Ba 9-58, 2011) and trypsin inhibitor (TI) content (ISO 14902:2001). Seventy-eight (78) samples of the 120 were classified within UA range of 0.000-0.050 pH units (ave. 0.009±0.010 pH units) and the TI range of 0.50-2.99 mg TI/g (ave. 1.82±0.60 mg TI/g). However, 42 samples of the 120 displayed an ave. UA of 0.015±0.015 pH units, but with an ave. of 5.89±3.45 mg TI/g. Therefore, in 35% of the samples the low UA (<0.050 pH units) did not correlate with a low TI content (<3 mg TI/g). A close follow-up of 16 samples derived from 8 different lots of soybeans processed by either the pressure cooker or wet extrusion method revealed that in both methods low UA values were achieved (0.018±0.014 pH units for the pressure cooker, and 0.044±0.019 pH units for the wet extrusion). But, TI values were 3 times higher in the samples from the wet extrusion method compared to the pressure cooker method (5.64±1.99 mg TI/g vs. 1.76±0.34 mg TI/g, respectively). It is concluded from these data that in contrast to SBM, following processing UA is not a reliable predictor of the quality of FFSB, however the TI value is an acceptable predictor of FFSB quality.

Key Words: Full-fat-soybeans, Urease, Trypsin-inhibitors, KOHPS, SBM

T158 Efficacy of a NSPase enzyme, Natugrain TS®, dose titration on jejunum viscosity, IDE and Necrotic Enteritis lesion score on birds challenged with coccidiosis vaccine and Clostridium perfringens and 28-day bird performance on corn/soy diets Mike Coelho* *BASF Corporation*

The objective of this study was to evaluate the efficacy of a NSPase enzyme, Natugrain TS®, dose titration on jejunum viscosity (with a Brookfield digital viscometer), apparent ileal digestibility of energy (IDE) (with a TiO_2 marker) and NE lesion scores (0-5 visual) on birds challenged with coccidiosis vaccine and Clostridium perfringens (Cp) and 28-day bird performance on corn/soy diets. A total of 480 male Cobb 500 broilers were used in a randomized complete block design (8 birds/cage x 6 Natugrain TS doses x 10 replicates). Feed was pelleted at 89C, 45 sec., birds were coccidiosis vaccinated (Coccivac® B52) at day1 and alpha toxin and netB-positive Cp administered at d19, 20 and 21. The Natugrain TS doses were 0 (NC), 50, 75, 100, 125 and 150 g/MT. IDE, jejunum viscosity, jejunum lesion score and performance were measured at d28. Birds fed 125g Natugrain TS had a lower (P<0.05) jejunum viscosity versus 0, 50, 75 and 100g (2.41 versus 3.92, 3.82, 2.98 and 2.58 mPa.s). Birds fed 150 g Natugrain TS had lower (P<0.05) 28d necrotic enteritis (NE) lesion score than birds fed 0, 50, 75, 100, and 125 g Natugrain TS (1.87 versus 2.95, 282, 2.41, 2.24 and 2.13 NE lesion score). There was a close correlation (R^2=0.97) between jejunum viscosity and lesion score decline as Natugrain TS dose increased. Birds fed 125 g Natugrain TS had a higher (P<0.05) IDE versus 0, 50, 75 and 100g Natugrain TS (3177 versus 3106, 3112, 3138 and 3152 Kcal/kg). 125 g Natugrain increased (P<0.05) IDE by 71 Kcal/kg versus NC. Birds fed 125 g Natugrain TS had a higher (P<0.05) 0-28d broiler weight gain versus 0, 50 and 75g Natugrain TS (1523g versus 1450, 1464 and 1478g). Birds fed 125 g Natugrain TS had a lower (P<0.05) corrected FCR than 0. 50, 75 and 100 g Natugrain TS (1.436 versus 1.478, 1.472 and 1.464). In summary, Natugrain TS decreased (P<0.05) jejunum viscosity, NE lesion scores and FCR, increased (P<0.05) IDE and the optimum bird performance at 28 days was reached with 100 to 125 g/MT Natugrain TS

Key Words: NSPase, broiler, performance, viscosity, enteritis

Metabolism & Nutrition - Feed Additives

T159 Meat antioxidants, chemical composition and performance of commercial broilers is affected by Moringa oleifera leaves supplemented as feed additive Shakeel Ahmad[1], Anjum Khalique*[1], Talat Pasha[1], Shahid Mehmood[1], Khalih Hussain[2], Sohail Ahmad[1] *[1]University of Veterinary and Animal Sciences; [2]University of Punjab*

The present study was executed to determine the effect of Moringa oleifera leaf meal (MLM) dietary supplementation (at levels of 0, 0.5, 1.0 and 1.5% of MLM over and above of basal diet), on growth performance, giblet pack, serum biochemistry, and meat bioactive compound index. Two hundred day old broiler (Hubbard) chicks were assigned to four dietary treatment with five replicates of ten birds each in a Completely Randomized Design (CRD) and means were compared with Duncan's multiple range test (DMR). Data showed that feed intake and feed conversion ratio (FCR) were significantly (P≤0.05) decreased and lowest values were observed in MLM-1.5%. Dressing percentage and carcass weight were significantly increased and highest values were observed in the group MLM-1.0%. Relative heart, gizzard and liver weights showed no difference among the groups. Breast meat and feed bioactive compounds β-carotene, Quercetin and Selenium showed a linear significant increase and highest values (0.18µg/100g, 267.34µg/100g, 79.07µg/100g & 8.93mg/Kg, 48.96mg/Kg, 0.54mg/Kg) respectively resulting in significant (P≤0.05) improvement in antioxidant activity indicator DPPH (di-phenyl-picryl hydrazil) radicals scavenging value (36.66%). Breast meat cholesterol level (59.24mg/100g) and serum biochemical compounds Glucose, Cholesterol, SGPT and Creatinine values were linearly decreased. Antibody titters against Infectious bursal disease (IBD) showed significant improvement (P≤0.05). Outcomes of present study revealed that supplementation of MLM in broiler diets showed positive impact on growth performance, immunity and bioactive compounds of breast meat with best results at dose level of MLM-1.5% and may be an alternative to phytogenic feed additives.

Key Words: Moringa, Broiler, β-carotene, Quercetin, Selenium

T160 Egg antioxidants, chemical composition and performance of commercial layers is affected by Moringa oleifera leaves supplemented as feed additive Shakeel Ahmad*[1], Anjum Khalique[1], Talat Pasha[1], Shahid Mehmood[1], Khalid Hussain[2], Sohail Ahmad[1] *[1]University of Veterinary and Animal Sciences; [2]University of Punjab*

Phytogenic feed additives for animal feed production have got significant consideration during the last decade and numerous plants and their metabolites have been investigated for the said purpose. In the same context, present study aimed to evaluate effect of Moringa oleifera as feed additive on layer's performance and egg's bioactive compounds and nutrient profile. HyLine W36 layers (200 birds), age 50 weeks, were randomly segregated in to 4 groups, each containing 50 birds (five replicates and ten birds per replicate). Four iso-caloric (2725 Kcal/kg) and iso-nitrogenous (CP 16%) diets were formulated and supplemented with 0, 0.5, 1.0 and 1.5%, w/w of Moringa oleifera leaves. The data showed positive effect on egg production, egg mass and feed conversion ratio whereas egg quality was decreased (P ≤ 0.05). Bioactive compounds like β-carotene, quercetin and selenium content of feed and egg yolk were significantly (P ≤ 0.05) increased, and their higher respectively values were 8.90, 48.88 and 0.54 mg/Kg in feed and 4906, 241 and 56.82 µg/100g in yolk in the group receiving feed containing 1.5% supplementation level. Linear decrease in the serum creatinine, glucose and cholesterol levels (serum and eggs) at 4th and 6th week of supplementation was recorded (P ≤ 0.05). Antibody titters against Newcastle disease were significantly improved (P ≤ 0.05). It was concluded that Moringa oleifera leaf meal can be a potent phytogenic feed

additive and may affect egg production and quality along with immune status of layers.

Key Words: Moringa, β-carotene, Quercetin, biochemistry, performance

T161 Effect of AZOMITE supplementation in post-molt hens Ramon Malheiros*, Vera Moraes, Kenneth Anderson *Prestage Poultry Science Department, NC State University*

AZOMITE® is a uniquely natural material, mined in Central Utah, USA. For over seventy years, crop producers have used AZOMITE® to support plant growth and vitality. AZOMITE® is not manufactured or chemically prepared. It is 100% naturally-derived and is completely free from additives, synthetics or fillers. The objective of this work was to evaluate the AZOMITE supplementation in layer diets as a mineral additive, during molt and post molt performance. For that, 96, W36 Hy-Line hens at the age of 67 weeks, were house in a conventional cage system, in 48 cages, divided in 2 treatments diets (AZOMITE, AZ, and Control, Ct). After 2 weeks of adaptation, the birds were submitted to the treatment diets. All hens submitted to 16L/8D. The diets were formulated to attend the requirements, and AZ added as a supplementation in the dose of 2.5g/kg. The hens a non-anorexic molt protocol was used, and production data collected until 85 weeks of age. Egg internal and external quality was evaluated bi-weekly throughout the experiment. The last week, ileum content, excreta and tibia bone were collected to be evaluated. No differences were observed (P>0.05) in BW, Feed Intake, FCR, shell color, egg weight, Haugh Unit, Yolk color, and Shell thickness. Feed conversion (g egg/g feed) showed a slight improvement favoring the AZ birds (0.55 vs 0.51g). At 85 week, the HH% and HD% production was better in AZ hens (90.54 AZ, vs 77.41Ct). Tibia bone showed a lower Ca and P (P<0.005) content at 85 weeks of age, in AZ hens, but no difference in ash content, Bone Mineral Density, Break Strength, and Elasticity. The ileum digestibility was improved (P=0.0192) in AZ birds (79.67 AZ vs 61.98 Ct), together with AMEn (2,999 AZ vs 2,741Ct), and ANR (47.83 AZ vs 31.00 Ct). Based on our findings, we can conclude that supplementation after during molt and post-molt in laying hens with AZOMITE can improve the absorption of the Calcium, and probably delivery a better performance.

Key Words: Mineral, Azomite, Molt, Laying Hens

T162 Butyric acid during very late lay (86-100 wks of age) in Laying Hen Rations Effects on Shell Strength Sheila Purdum*[1], Josephine Foley[1], Richard Sygall[2] *[1]University of Nebraska; [2]Perstorp Feed and Food Corporation*

A novel butyric product – butyric acid bound to glycerol forming a triglyceride was fed to an older laying hen flock from 86 to 100 weeks of age to measure effects egg production parameters and shell quality. Source of butyric acid was ProPhorce SR from Perstorp Corporation. Two treatments were fed to Bovan White Leghorn hens (control or butyric acid (500 g/ton) housed in a traditional cage unit with 12 replicate cages (3 hens/cage) for a 14 week period. Basal diets were corn/soy based diets with 10% DDGS with a total Ca level of 4.6%. Parameters measured included daily egg production, feed intake, biweekly egg wts, eggshell %, eggshell breaking strength (texture analyzer) and Ca and P digestibility marker study utilizing titanium as the marker. Results show no significant effects on rate of egg production or feed intake during this late stage of egg production. Butryric acid supplementation increased overall egg weight during the study (67.04 vs. 65.84 grams) at p<.001. This increase was noted after 3 weeks on the product with pronounced weekly effects at 7 wks during the trial. Subsequent effects on % shell were not significantly affected by the treatment, but shell breaking strength (kg force) was positively affected overall by the treatment (p<.08). Breaking strength improved from 39.501 to 42.522 kg. This effect was most prominent at the end of the trial at wk 11 (p<.06) and week 13 (p<.01) with some indication of effectiveness after 3 weeks on the product. There was a slight improvement in Ca digestibility from 25.91 to 31.94% at the end of the trial and little improvement in P digestibility (25.75 vs. 28.43%) with butyric acid supplementation. Egg quality data at point of egg collection as showed a slight reduction in soft-shell eggs produced during the trial. Overall, butyric acid supplementation appears to improved nutrient digestion and potentially eggshell calcification and strength in end of cycle laying hens.

Key Words: Layers, Butyrin, Eggshell

T163 Effects of various concentrations of butyric acid on the performance, intestinal lesion scores, and body composition of broilers raised on used litter Nathaniel Barrett*, James Lewis, Michael Persia *Virginia Tech*

An experiment was performed to determine the effects of commercial and higher concentration of butyric acid (BA) on performance, lesion scores (LS), and body composition when fed to broilers raised on dirty litter. Fifteen hundred male Heritage broiler chicks were randomly assigned to one of five treatments (trt), a positive control fed a diet without BA and raised on clean pine shaving litter (PC), a negative control fed a diet without BA and raised on dirty litter (NC), and three trt raised on dirty litter which received the same diet containing 250ppm (250BA), 500ppm (500BA), or 1,000ppm (1000BA) BA. Dirty litter was generated directly before the experiment (one week down time) by housing 20 chicks that received a coccidiosis vaccine day of hatch, until 28 days of age. Each experimental trt was applied to 12 replicate pens of 25 chicks. Body weight and mortality corrected feed conversion ratio (FCRm) were reported from the 1-14, 1-28 and 1-42 day periods. On D14, 3 birds/pen were euthanized for LS. On D42, 5 birds /pen were euthanized and de-feathered for body composition analysis via dual-energy x-ray absorptiometry. Data were analyzed using ANOVA and a Fisher's LSD test to separate means (P≤0.05). On D14, 500BA resulted in the highest body weight, while the NC fed birds had the lowest (P≤0.01). At days 28 and 42, 1000BA resulted in the highest body weight (P≤0.01) while the NC and PC were the lightest. There was no differences in FCRm for the first 14 days, but from days 1-28 and 1-42, the 1000BA resulted in the lowest FRCm (P≤0.01). Lesion scores were only different for the duodenum, with 500BA trt resulting in the lowest score, 1000BA and NC the highest, and PC and 250BA intermediate (P≤0.01). Lean mass and total mass showed similar results to BW with the 1000BA fed birds resulting in the highest lean and total mass after DXA analysis (P≤0.01). In conclusion although the commercial dose (250BA) increased the body weight until D28, over the last part of the experiment this effect was lost. The higher doses had similar to better responses that resulted in the heaviest birds with the lowest FCR over the entire experiment. These data could indicate that including BA at a dose higher could be beneficial for growth of broilers on built up litter.

Key Words: Broiler, Butyrate, Performance, Coccidiosis, Body composition

T164 Yeast cell wall and hydrolyzed yeast as a source of nucleotides effects on immunity, gut integrity and performance of broilers Jose Rivera, Lúcio Araújo, Elizabeth Santin, Carolina Oliva, Liliana Borges, Melina Bonato* *Departamento de Zootecnia, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de Sao Paulo*

A study was performed to evaluate the effects of the yeast cell wall (YCW) and hydrolyzed yeast as a source of nucleotides (YNU) compared to zinc bacitracin (ZBC) effects on the immunity, gut integrity and performance parameters of broilers. For this, 840 male Hubbard® chicks (1 d) were distributed in a CRD with 5 treatments: 1–Control; 2–Control with ZBC (50 g/MT); 3–YNU1 (*Saccharomyces cerevisiae* hydrolyzed yeast, 1 kg/MT up to 7 d / 0.5 kg/MT from 8-42 d, Hilyses®); 4–YNU2 (5 kg/MT up to 7 d / 0.5 kg/MT from 8-42 d); 5-YCW (from *Saccharomyces cerevisiae* at 0.5 kg/MT, IMW50®), with 14 reps of 12 birds each. The diets were divided into pre-initial (1-7 d); initial (8-21 d); growth (22-33 d) and final (34-42 d). The birds were housed in pens with reutilized litter from a commercial farm (2nd time used). The BWG, FI, FCR and Production factor were measured at 7, 21 and 42 d. At 21 d, 8 birds per treatment were selected and slaughtered to collect the ileum. The samples were pre-

pared for histology and immunohistochemistry analyzes and were evaluated macrophages, CD4+, and CD8+ cells count; lamina propria (LP) and epithelial thickness; enterocytes proliferation; epithelial plasma infiltration; mixed inflammatory infiltration of LP; goblet cells; congestion and necrosis. These parameters were qualified by "I See Inside" (ISI) index methodology (Kraieski, 2017). The data were analyzed by GLM produced from SAS and the means compared by Tukey test at 5% of significance. The effects were also analyzed by orthogonal contrasts by F test at 5% of significance. Significant differences (P<0.05) were found for immunohistochemistry, where the treatment with YCW supplementation result in lower macrophages and CD8+ cells count compared to others treatments. For CD4+ cells count, the control group has the higher (P<0.05) number. Considering the ISI index, no statistical differences were found (P>0.05). Regarding the performance parameters, the YCW and YNU2 improved (P<0.05) the FCR compared to the Control and YCW has similar results to ZBC (P>0.05). The supplementation of YNU2 and YCW in the broilers diet improved the FCR at 42 d compared to Control group. However, the YCW supplementation resulted in the best response of the immune parameters analyzed.

Key Words: Saccharomyces cerevisiae, Antibiotic, Immunohistochemistry, Nutrition, Poultry

T165 The effects of a dietary nucleotides-containing product YT500 on IBV antibody production and intestinal mucosal barrier functions in SPF chickens. Che Wu*, Zhenwei Yang, Chao Liang, Yue Zhang, Qingmei Xie *South China Agriculture University*

Sixty 1-day-old SPF chickens with the similar body weight were randomly divided into four groups, each group has 3 replicates and each replicate with 5 chickens. The Control Group (CG) was fed with basal diet, and the Low Dose Group (LG), Medium Dose Group (MG) and High Dose Group (HG) were fed with basal diet supplemented with 1, 3 and 5g /Kg of a dietary nucleotides product (YT500™, Hinabiotech, China), respectively. The chickens were raised in isolated chambers, and the feed and water were given *ad libitum*. All chickens were vaccinated intranasally with one dose of IB-D90（SCAU, China）at $10^{3.5}EID_{50}$/0.1 ml at day 1 and 10. IBV Ab was measured at day 1, 10, 17, 24, 31, 38 and 45, respectively by IBV Ab Elisa test kit (MEDIAN Diagnostic Inc. S.Korea). Data were analyzed by one-way ANOVA (SPSS 22.0). The results showed: (1) YT500 speeds up the IBV Ab to the effective level for 7 days in LG, MG and HG. Both MG and HG had statistical significance compared to CG at day17 (P<0.05); (2) The ileal villus height of HG was higher than that of CG (P<0.05), but there were no differences in the ratio of ileal villus height/crypt depth in LG, MG and HG (P>0.05); (3) At day 17, the expression of ZO-1 and Occludin mRNA gave a linear relation with YT500, but only HG had significant difference (P<0.05); (4) At day 17, the expression of MUC2 and TFF3 mRNA showed higher value in LG, MG, and HG than those of CG, although not significant (P>0.05); (5) At day 17, reduced value of IFN-α mRNA in LG, MG, and HG compared to CG, but IL-22 and IL-17A mRNA had no significant difference in all groups; (6) The CFUs of *E. coli* and *S. enteritidis* in MG and HG were lower than those in CG (P<0.05), while the CFUs of *Lactobacillus spp.* in LG, MG, and HG were higher than CG (P<0.05). In summary, this study demonstrated one week faster of IBV Ab to the effective level in the presence of YT500 under the tested condition. It also showed an improved intestinal mucosal barrier in terms of ZO-1, Occludin, MUC2, TFF3, IFN-α, IL-22 and IL-17A mRNA expression. This study provides a promising research model for better understanding the biological and economical values of YT500 in poultry.

Key Words: Nucleotides, Chickens, IBV, Intestinal, Mucosal

T166 Effects of in ovo injection of L-ascorbic acid on early growth performance and systemic antioxidant capacity in broiler chickens Saman Fatemi*, Haijun Zhang, Katie Collins Elliott, Oluwaseun Durojaye, E. David Peebles *Mississippi State University*

Effects of the *in ovo* injection of various concentrations of L-ascorbic acid (AA) on early post-hatch broiler performance and antioxidant capacity were investigated. A total of 2,200 Ross 708 broiler embryos were randomly assigned to 6 treatments: non-injected control, saline-injected control, or saline containing 3, 6 ,12 or 36 mg AA per egg. At 17 d of incubation (doi), a 100 µL volume of sterile 0.85% saline alone or containing different levels of AA were injected by an Inovoject m semi-automatic multi-egg injector. Each of the 6 treatment groups were represented in each of 10 replicate groups (block) of floor pens. In each floor pen, 14 male and female chicks were randomly placed at hatch (21 doi). Growth performance was determined from 0 to 7, 7 to 14, and 0 to 14 d of age (doa). Total plasma superoxide dismutase (T-SOD) activity and malondialdehyde (MDA) content were determined at hatch and at 7 and 14 doa by colorimetric assay. The experiment was conducted as a randomized complete block design and data were analyzed using SAS 9.4. Birds hatched from the 3 mg AA/egg treatment group had a higher BW at 7 doa (179.8 g, P=0.046) and a higher ADG from 0 to 7 doa (19.1 g, P=0.039) compared with non-injected birds (169.1 g and 17.6 g, respectively), they also had a higher ADG from 7 to 14 doa (42.9 g, P=0.037) relative to saline-injected controls (41.4 g). Chicks that received 3 or 36 mg AA/egg had reduced plasma MDA contents at hatch by 39 and 31%, respectively, and at 7 doa by 19 and 13%, respectively, compared to the non-injected control (P<0.05). Plasma T-SOD activities increased in birds that received 3 or 6 mg AA/egg compared to non-injected controls by 34 % and to saline-injected controls by 62% at 7 DOA (P<0.05). These results suggest that the *in ovo* injection of AA (3 to 6 mg/egg) has the potential to promote the growth and increase the systemic antioxidant capacity of broilers during the early post-hatch phase.

Key Words: vitamin, in-ovo, growth, antioxidant, broilers

T167 Effects of dietary inclusion level of a phytogenic premix on broiler growth performance, nutrient digestibility, total antioxidant capacity and gene expression of antioxidant enzymes Konstantinos Mountzouris*, Vasileios Paraskeuas, Eirini Griela, Andreas Kern, Konstantinos Fegeros *Agricultural University of Athens*

The inclusion level of a phytogenic premix (PP) characterized by carvacrol and thymol (Anco®Fit – Poultry) was studied for its effects on broiler growth performance, nutrient digestibility and total antioxidant capacity (TAC) of meat and liver. In addition, gene expression of antioxidant enzymes CAT, SOD and GPx was profiled along the broiler intestine.

A total of 500 one-day-old Cobb broiler chickens were assigned into 4 treatments, with 5 replicates of 25 chickens each. Basal diets were formulated to meet starter (1 to 10d), grower (11 to 22d) and finisher (23 to 42d) growth phase requirements. Depending on PP inclusion level (0, 750, 1000 and 2000 mg/kg diet), treatments were: PP-0, P-750, PP-1000 and PP-2000. Feed and water were available *ad libitum*.

Data were analyzed by ANOVA, taking the treatment as fixed effect. Statistical significant effects (P≤0.05) were further analyzed and means were compared using Tukey HSD test. In addition, polynomial contrasts tested the linear and quadratic effect of PP inclusion levels.

Growth performance responses did not differ (P>0.05) between treatments. However, there was a trend (P=0.089) for improved FCR by 4.5% in PP-1000 compared to PP-0 at the finisher phase. In addition, trends for improved European production efficiency index were seen for PP-1000 during the finisher phase (P=0.087) and overall (P=0.057). Treatment PP-1000 had the highest carcass (P=0.030) and breast fillet yield (P=0.023). From the digestibility study, PP-1000 had higher AMEn (P=0.049) compared to treatments PP-2000 and PP-0.

The PP inclusion level resulted in higher breast (P=0.005), thigh (P=0.002) and liver (P=0.040) TAC. In particular, breast and thigh TAC increased in a quadratic pattern and reached plateu at PP-1000, whereas liver TAC continued to increase linearly. Gene expression of SOD was significantly up-regulated in the duodenum (P=0.027), jejunum (P=0.026) and ceca (P=0.023) in PP-1000 and PP-750 compared to PP-0. In ceca, CAT expression displayed a quadratic pattern of up-regulation (P_q=0.053) in the same direction with SOD (P_q=0.006).

Overall, this study provides evidence for potential PP-1000 benefits for carcass and breast fillet yield, energy sparing and overall antioxidant capacity in broiler gut and meat.

Key Words: Chicken, Phytogenic, Antioxidant

T168 Discovery and in vitro characterization of a novel muramidase for use in animal feed Marianne Cohn, Kirk Schnorr, Lars Skov, Esben Schmidt, Peter Olsen, Steen Buskov, Raffaella Aureli, Estefania Pérez Calvo, Rual Lopez-Ulibarri, Mikkel Klausen* *Novozymes A/S*

The microbiota in the gastrointestinal tract live in a complex ecosystem in equilibrium with the host. There, the microbial turnover (replication and death) naturally produces a diversity of microbial cell components that are released into the gut lumen. In this study, we describe the *in sillico* and *in vitro* selection of a novel microbial muramidase (EC 3.2.1.17) that degrades the peptidoglycan component of microbial cell wall fragments (or bacterial cell debris).

In general, the discovery of novel enzymes involves several iterations of *in silico* selection of genes, expression of enzymes and *in vitro* characterization before large-scale production of the enzyme for study in *in vivo* trials.

In this study, the first round of *in silico* gene selection, consisting of hundreds of muramidase sequences, was based on the natural microbial habitat of donor organisms, which were mainly selected from microbe-rich environments. *In vitro* screening of expressed candidates was applied by using a negative and positive screening approach. Candidates were deselected if clearing zones were detected in a bacterial radical diffusion assay, and were ranked based on efficacy of peptidoglycan hydrolysis, stability and expression yield. Selected candidates were tested *in vivo* in broiler chickens. The best muramidases were further characterized by efficacy of hydrolysis of peptidoglycan from gut-derived microbes. The single best muramidase found using this setup belongs to the glycoside hydrolase family 25 (GH25), isolated from the fungus *Acremonium alcalophilum* JCM 7366. The 3D structure of the muramidase has been determined using X-ray crystallography, and this can help to explain the unique catalytic properties of this muramidase.

Key Words: Muramidase, Discovery, Peptidoglycans, Characterization, Enzyme

T169 Effects of a novel muramidase supplementation on gastrointestinal functionality and growth performance in broiler chickens Mounira Sais, Susana Martin-Orue, Ana Barroeta, Rual Lopez-Ulibarri, Estefania Perez Calvo* *1Servei de Nutrició i Benestar Animal. Universitat Autònoma de Barcelona*

The aim of the present study was to investigate the effects of dietary supplementation of the novel muramidase (Muramidase 007) on gastrointestinal functionality and performance of broiler chickens. A 35 days study was conducted in a total of 408 day-old Ross 308 which were randomly distributed into 16 floor pens of 30 birds per pen. Treatments were: Control Diet (C) and C + Muramidase 007 (MUR; inclusion at 35 000 LSU(F)/kg feed). A two-phase feeding program (starter: day 0-21 and grower: day 21-35) was used in this study. Both diets included soybean meal, corn, wheat and rye as main ingredients; no other feed enzymes, coccidiostat or growth promoters were added in the diets. All birds were vaccinated against coccidiosis. Growth performance parameters were registered at day 0, 9, 21 and 35 of study. At days 9 and 35 of the study, nutrient apparent ileal digestibility, jejunal histomorphology (villus height and crypt depth), vitamin A in plasma, short chain fatty acids (SCFAs) in caecum and microbiota composition in crop, ileum and caecum was determined by plate counting and in caecum also by sequencing the 16S rRNA gene. The data obtained were analyzed using one-way ANOVA with a P<0.05 level of significance. Overall, birds fed diets supplemented with MUR improved significantly feed to gain ratio compared to control (1.43 vs 1.36). These results were consistent with the observed improvement in ileal digestibility of energy, dry matter and organic matter at day 35. At day 9, MUR increased the digestibility of fat and the level of vitamin A in plasma, suggesting a higher intestinal uptake capacity during this phase. Improvements on ileal digestibility were independent of intestinal histomorphology. While MUR supplementation did not modify SCFAs concentration in the ceca, the bacterial counting showed a trend for a reduction in *Clostridium* in the ileum (P= 0.13) and cecum (P= 0.07) at day 9. In addition, results of 16RNA analyses showed that birds supplemented with MUR had a more diversity at day 9, and a significant increase in *Lactobacillus* genus (3.28 vs. 4.90%) at day 35 compared to C. The present study shows that MUR supplementation exerts a positive effect on gastrointestinal functionality which results in a concomitant enhancement in growth performance.

Key Words: muramidase, gastrointestinal, functionality, microbiota, enzyme

T170 Evaluation of the efficacy of a novel muramidase on performance of broiler chickens Mojtaba Yegani*, Bradley Turner, Thomas Frost, Greg Mathis, Brett Lumpkins *DSM Nutritional Products*

Muramidases are a category of enzymes that could be used as a digestive aid to optimize availability of nutrients in the digestive tract of animals. They belong to the family of glycosyl hydrolytic enzymes with the ability to catabolize peptidoglycans. Peptidoglycans constitute a substantial portion of bacterial cell debris that exist in the digestive tract of poultry. It is hypothesized that dietary addition of a novel microbial muramidase (muramidase 007) can positively impact the functionality of the gut, resulting in improvements in performance of broiler chickens. A 42-day floor pen study was conducted to investigate the effects of addition of muramidase 007 in corn-soy diets on growth performance of broiler chickens. A total of 1,280 day old male broiler chicks (Cobb 500) were allocated to two treatments (control and muramidase). Each treatment was replicated 32 times with 20 birds per pen in a complete randomized block design with pens as the experimental unit. A three-phase feeding program (starter: day 0-21, grower: day 21-35, and finisher: day 35-42) was used in the study. All birds were vaccinated against coccidiosis with a commercial vaccine at the time of placement and were provided *ad libitum* access to feed and water throughout the study. Muramidase supplementation significantly ($P < 0.05$) improved body weight gain (2.246 kg vs. 2.138 kg) and feed conversion ratio (1.639 vs. 1.681). Mortality was unaffected ($P > 0.05$) by the treatment. Results of this study demonstrated that addition of muramidase to broiler feed significantly benefited body weight gain and feed conversion ratio, supporting the hypothesis that muramidase 007 benefits gastrointestinal functionality which is reflected in improved performance.

Key Words: Muramidase, enzymes, peptidoglycans, nutrients, broilers

T171 A novel muramidase improves broilers performance via higher nutrient utilization. Leticia Bittencourt*, Rafael Hermes, Vitor Fascina, Dino Garcez, Estefania Calvo *DSM Nutritional Products*

The objective of this study was to evaluate the effect of dietary inclusion of a novel muramidase (muramidase 007) on growth performance and nutrient absorption in broiler chickens, under mild challenging conditions, to mimic suboptimal farming conditions. A total of 600-day-old Ross 308, were distributed across 3 treatments, 8 replicates of 25 birds each, in a completely randomized design, using floor-pens with fresh litter for a 42d trial period. Treatments were: (1) Control (C); (2) C + Muramidase 007 level 1 (MUR 1); (3) C + Muramidase 007 level 2 (MUR 2). A three-phase feeding program (starter: day 0-21, grower: day 21-35, and finisher: day

35-42) in mash form was used in the study. Diets were based on corn and soybean meal, rice bran, and meat and bone meal were also included, to challenge the gastrointestinal function with low digestibility ingredients. All diets included phytase and a yellow carotenoid. At day 2, all birds were inoculated with a multiple dose of an anticoccidial vaccine, to create a mild challenge overall the physiological status of the animals during the starting phase. Growth performance parameters and mortality were recorded during the experimental period at 0, 21, 35 and 42 days. At day 28, blood samples were collected from 20 birds per treatment to measure total blood carotenoids content. All data were analyzed by ANOVA and means were compared by the Tukey test at 5% probability. Overall, treatments MUR 1 and MUR 2, resulted in better feed conversion ratio compared to control (1.59 and 1.59, respectively, vs 1.68; P=0.0151). In addition, broilers receiving MUR 2 had higher body weight gain compared to the control (3.40 vs 3.27 kg, P<0.05). MUR 2 supplemented broilers, showed higher levels of total carotenoids in blood, compared to control (4.38 vs 3.57 mg/L, P=0.0055), suggesting a better intestinal functionality and thus a more efficient uptake of carotenoids. These observations, collected on broilers placed under suboptimal growing conditions, confirm that muramidase 007 supplementation supports the digestive function by fostering nutrient uptake, increasing feed efficiency, and thereby contributing to sustaining growth.

Key Words: muramidase, nutrient, absorption, conversion, weight

T172 Lowering the incidence of Wooden Breast with a new, innovative additive Manu De Laet*, Rob Goedegebuure, Renato Costa *Nuscience*

In the past few decades, the demand for poultry meat has increased substantially, especially chicken and turkey breast fillet. This growth is related to the perceived healthy and nutritional content, suitability for further processing and the cheaper price compared to red meats. To fulfil the increase in demand there was a selection for higher growth-rate and breast-yield chicken hybrids. However, improved live performances have been accompanied by an increased incidence of muscle abnormalities. Among those is the occurrence of wooden breast: visually hard, out-bulging and pale areas on the ventral surface of the Pectoralis major muscle.

Nuscience developed and validated Q-prove®, a product to combat wooden breast without reformulating the diet and without losing performance. A field trial was carried out on a commercial farm in The Netherlands. The farm was suffering from high incidences of wooden breast. The trial included 60 000 birds fed the control feed while 30 000 birds were fed the control diet supplemented with Q-prove® (1 kg/MT in starter, 0.650 kg/MT in grower, 0.350 kg/MT in finisher). Nuscience tested in detail 300 carcasses from the control group and 150 carcasses from the treatment group in the slaughterhouse. The scoring was performed by a Doctor of Veterinary Medicine with more than 35 years of experience in poultry carcass quality (score 0 = no wooden breast, score 1 = starting wooden breast with no financial consequence, score 2 = severe wooden breast with financial consequence). For statistical analyses, Chi square test is used.

The results showed a clear effect of the treatment group on the occurrence of severe wooden breast. When Q-prove® was added to the feed, the amount of carcasses with score 1 remained the same (9.00% for the control group vs 10.00% for the Q-prove® group), but there was a significant decrease in score 2 carcasses from 13.37% (control group) to 2.67 % (Q-prove® group).

Q-prove® is an additive that decreases the incidence of wooden breast without the need to adjust the composition of the diet. For this reason, it is possible to make full use of the genetic potential and maintain the profit of poultry meat production.

Key Words: wooden, Q-prove®, potential, abnormalities

T173 The effects of Magni-Phi® in floor pen- and commercially-raised turkeys Kenneth Bafundo*, Mark Blakley, Greg Mathis *Phibro Animal Health Corporation*

Magni-Phi (MP, Phibro Animal Health Corp.) is a natural feed additive that has been used in broiler chickens to improve coccidiosis control, intestinal health and bird performance. Previous results have shown additive improvements in coccidiosis control and feed conversion when MP is combined with various ionophores administered to broilers. To determine whether similar responses are produced in young turkeys, MP combination studies were carried out with both monensin (MON) and lasalocid (LAS). In each case, 2 x 2 factorial designs were employed where MP (250 ppm) was fed both alone and in combination with MON (68 ppm) or LAS (75 ppm) under continuous coccidial challenge via exposure to oocysts in the litter. Treatments were replicated 8 times; each trial was conducted for 42 d. Bird performance and oocysts per gram of feces (OPG) were determined at multiple time points in each trial. Data were analyzed for main effects and interactions and mean separation was established at (P<0.05). Results indicated that at 21 and 28 d, MP alone significantly reduced OPG, and when combined with MON, further reduced oocyst production by an additional 10.5% compared to MON alone. Similar additive responses in OPG were observed in the LAS trial. In both tests, MP alone significantly improved FCR at 42 d and provided additive improvements in FCR when combined with either MON or LAS. To validate these responses, three commercial trials were conducted involving more than two hundred thousand tom turkeys reared to 141 d. Trials involved comparisons of bird performance using ionophore medication programs used in commercial practice. In each trial, approximately half of the birds were fed either MON or LAS alone until processing; the remainder were fed the same ionophore supplemented with MP for the duration of the test. Because MP improved final feed conversion in each of the trials (a minimum of 4 points), results were consistent with previous tests, and indicate that MP can be applied successfully in turkey production for improved performance.

Key Words: Magni-Phi, coccidiosis control, turkey performance, ionophores

T174 Poultry-specific synbiotic supports enhanced growth efficiency of turkeys raised in antibiotic-free conditions in experimental and field settings Chasity Pender*, Michaela Mohnl, G. Raj Murugesan *Biomin America Inc.*

As the poultry industry searches for solutions to reduce the use of antibiotics to alleviate concerns of antibiotic resistance and satisfy consumer and regulatory demands, probiotics have received increasing attention for their ability to improve enteric health in poultry. Two studies were conducted with the objective of evaluating effects of synbiotic (combination of probiotics and prebiotics) supplementation on performance of turkeys raised in antibiotic-free conditions. The first experiment utilized a total of 540 day-old poults that were randomly assigned to one of two groups, each consisting of 6 replicates (45 birds/pen). Groups consisted of a negative control fed a standard commercial diet free of additives or medications and group provided the same diet, but supplemented with a synbiotic (PoultryStar® sol^{US}, Biomin Holding GmbH) through the drinking water. The trial was conducted for a period of 98 days and performance measurements were taken on days 0, 14, 40, 68 and 98. For all measurements, significance threshold was set at P<0.05. Throughout the experimental period, body weight (BW) was higher in the synbiotic supplemented group with the final BW being significantly increased in the supplemented birds. Similarly, feed intake was also significantly increased in the treated group during the day 14 to day 98 period. No statistical difference was found among the groups for FCR. Mortality and culls were also numerically reduced throughout the trial with synbiotic supplementation. The second trial was conducted at a commercial farm and utilized 17,600 day-old poults, divided evenly among two treatment groups; a negative control fed a standard commercial diet free of additives or medications and group fed the same

basal diet supplemented with a synbiotic (PoultryStar® me; 500 g/t). The trial was conducted over a 118 day period and final performance parameters were measured. Final BW was increased by 5.1% while FCR was decreased in the synbiotic supplemented birds. Additionally, early poult (7 day and 14 day) and overall mortalities were reduced in the synbiotic treated group. Overall, these results suggest that supplementation of poultry-specific synbiotic may be a viable solution to augment performance of turkeys in antibiotic-free production systems.

Key Words: probiotic, prebiotic, poults, performance, synbiotic

T175 In depth mode of action studies of the unique probiotic strain Bacillus subtilis DSM 32315 Jessica Kleinboelting, Stella Molck, Lorena Stannek-Goebel, Kiran Doranalli, Rose Whelan, Adriana Barri, Claudia Borgmeier, Guido Meurer, Stefan Pelzer* *Evonik Nutrition & Care GmbH*

In commercial poultry production enteric disorders, such as necrotic enteritis caused by toxin producing *C. perfringens* strains, are leading to significant financial losses. Their impact will further increase once the use of antibiotic growth promoters (AGPs) has ceased. Alternatives like efficacious probiotics are urgently needed. In a multi-parameter screening approach *B. subtilis* DSM 32315 was recently identified. In numerous feeding trials *Bacillus subtilis* DSM 32315 consistently improved performance of broilers reared in various feeding conditions. The current study will highlight the results of multidisciplinary *in vitro* studies that indicate that DSM 32315 has multiple mode of actions.

In vitro cultivation studies showed that *B. subtilis* DSM 32315 supernatant has the ability to inhibit field isolates of *C. perfringens, E. cecorum. S. gallinacaeus, C. septicum, C. jejuni* as well as *C. coli*. By applying a multistep fractionation approach, distinct sub-fractions of the supernatant were identified showing *C. perfringens* inhibiting activities. Structure elucidations by NMR analyses confirmed that two compounds were responsible for the inhibition activity, one of them identified as the bacteriocin Subtilosin A. These results were successfully validated in experiments using novel knock-out *Bacillus* strains.

The supernatant of DSM32315 also displayed various enzyme activities, such as endo-cellulase activity, which could be involved in positive alterations to the cecal microbial populations shown in broiler trials. The supernatant also showed mycotoxin degrading activity, which eliminated 2.5 µg/ml Zearalenone within 12 h and reduced 50% of 2.5 µg/ml Alfatoxin B1 within 24 h from supplemented supernatant. Finally, superoxide dismutase and catalase enzyme systems were discovered which were shown to support the antioxidant system required for restoring homeostasis in birds after stress or disease challenge.

This study demonstrates that *B. subtilis* DSM 32315 is a unique probiotic strain displaying multiple modes of action which are beneficial for poultry in commercial production. The diverse activities shown may reduce the risk of gastrointestinal disorders and explain the observed consistent performance improvements of flocks reared under different conditions.

Key Words: Probiotics, DFM, Bacillus, Pathogen, MoA

T176 Bacillus subtilis 29784 improves performance and footpad conditions of broilers. J Teyssier, V Jacquier, L Rhayat, E Devillard, J Barton* *Adisseo France SAS, Centre of Expertise and Research in Nutrition*

Footpad dermatitis (FPD) is characterized by necrosis on the plantar surface of footpads of poultry (Shepherd and Fairchild, 2010). FPD became important for the poultry industry in the 1980s with development of an overseas market for paws. Moreover, FPD is recognized as an animal welfare issue in a number of countries. Although the causation is multifactorial, poor quality litter is highly correlated with footpad dermatitis. By maintaining good litter quality, producers can reduce losses and improve welfare (Taira et al., 2013). It is commonly observed that the heaviest broilers present severe FPD. Because probiotics and direct fed microbials (DFM) have been frequently described to increase growth, there is a potential to exacerbate footpad dermatitis . In the present study, the Bacillus subtilis strain 29784 was tested for its effects on performance and on footpad dermatitis.

A 35 days experiment was conducted to evaluate the effect of *B. subtilis* 29784 on growth performance of Ross PM3 male broilers fed corn-soy diets. 320 birds were randomly distributed into 2 treatments with 8 pen replicates of 20 birds per treatment. The 2 treatments were: 1) Control or 2) Control + *B. subtilis* 29784 (1.0E+08 CFU/kg of feed). Body weight gain, feed intake, feed conversion ratio and mortality were measured. FPD and litter quality were scored at the end of the experiment.

At 35 days, *B. subtilis* 29784 improved BWG (+4.8%, p=0.01), FCR (-2.9%, p=0.003) and FCR adjusted (-4.8%, p=0.004). Moreover, *B. subtilis* supplementation improved litter quality: 60% of pens in the Controls had highly degraded litter (score 4), whereas this condition was observed in none of the pens of the *B. subtilis* group (p=0.031). Footpad health scores were also significantly improved by the use of *B. subtilis* with a 21% decrease of pens which scored “highly necrotic” (p=0.008) and a 11% decrease of pens with combined scores “highly and visibly necrotic” (p=0.014) vs controls.

The incorporation of *B. subtilis* 29784 into feed led to an improvement of performance and animal well-being, as reflected by improvement of litter quality and reduction of severe FPD. These results illustrate the usefulness of DFM to improve welfare of broilers while assuring high production performance.

Key Words: Bacillus, broiler, footpad, performance, pododermatitis

T177 Formulation of Bacillus probiotics is key to product performance Karoline Brinch*, Geraldine Lafitte, Adam Nelson, Robert Plowman *Novozymes Animal Health & Nutrition*

Purpose: Probiotic-based products have received more attention in poultry productions since the focus on reduction of antibiotics has increased in recent years. Several Bacillus-based products are available but often producers find a lack of consistent effects. Much focus has been on selection of the right strain but this factor is only one of several which needs to be considered in order to develop a consistent probiotic product.

In this talk we describe how a correct formulation of the probiotic strain is one of the key factors to succeess.

Description: A probiotic strain screened and selected for performance in broilers was assessed in a range of assays related to formulation. A simple vs an optimized formulation and commercial products were compared and analysed with respect to demixing, flowability (angle of repose) and particle size distribution (PSD) to assess the impact on homogeneity in feed and in distribution to the broiler flock.

Results: The optimized formulation had several beneficial characteristics when compared to simple formulations: The PSD was 233 um (Dv50) where other products were at either <100 or >500. In assessments of simple and optimized formulations of product showed significant differences in the demixing set up: In comparisons of bacterial counts (CFU) between bottom, middle and upper layer of a feed formulation the difference between upper and lower layer was a significant 142% (p<0.0001) in the simple formulation, while the difference in the optimized formulation was insignificant at 7%. In recovery trials from nine in vivo studies the average in-feed recovery was above 80% and the CV below 20% in all proving excellent in-feed homogeneity.

Conclusion: A careful development of the correct formulation is an often overlooked key feature to ensure product performance. Our studies have shown that an optimized formulation with a correct PSD will result in less demixing, better flowability and thus higher in-feed homogeneity - as well as more hasslefree usage. Especially in the starter feed it’s crucial that the in-feed counts of Bacillus spores are equal in each feed pellet as the chicks

only ingest tiny amounts. Therefore product performance is closely linked not only to strain selection but also to formulation features of the product.

Key Words: Probiotics, Formulation, Bacillus

T178 Zootechnical efficacy of diets supplemented with GalliPro® Fit, a new multi-strain direct feed microbial (Bacillus subtilis DSM 32324, DSM 32325 and Bacillus amyloliquefaciens DSM 25840), in broiler chickens Alfred Blanch, Florence Rudeaux, Dorthe Sandvang, Zahid Nasir* *Chr Hansen AS*

GalliPro Fit® is a direct feed microbial (DFM) based on viable spores of two strains of *Bacillus subtilis* (DSM 32324, DSM 32325) and one strain of *Bacillus amyloliquefaciens* (DSM 25840). This study evaluated efficacy of GalliPro® Fit-suppplemented diets at two different dosages in broiler chickens: 1.6 x 10^6 and 3.2 x 10^6 CFU / g of feed. One-day-old healthy male broiler chickens (Ross 708) were allocated to 30 pens. There were 10 replicate pens per treatment group. Each pen had 10 chickens giving a total of 100 chickens per treatment group. The chickens were allocated to three dietary treatments (T1: Control without DFM, T2: 1.6 x 10^6 CFU /g, T3: 3.2 x 10^6 CFU / g) at day 0. Chickens were fed crumbled feed (starter diet) and pelleted feed (grower and finisher diets) *ad libitum* (Starter D0-21; Grower D21-35; Finisher D35-42). At the first day of the experiment (day 0 of study) and at defined intervals (days: 21, 35 and 42) productive performance (body weight, body weight gain, feed intake, feed conversion ratio) were determined per pen. The statistical analyses was performed with the software package Minitab and based on One-way Anova. All treatment least squares means were compared with each other by Tukey's HSD test. Differences among least squares means with a probability of $P < 0.05$ were accepted as statistically significant, whereas differences with P-values ranging from 0.06 to 0.10 are accepted as trends. Overall, no significant dietary effect was observed during the starter period. However, for 35 day growth period (D 0-35), GalliPro Fit-supplemented diets (500 and 1000 g/MT) reduced FCR, compared to the control group by 2.1% and 3.4%, respectively (T1: 1.570a; T2: 1.536ab; T3: 1.515a; $p < 0.05$). Likewise, in the whole experimental period (D 0-42), FCR was improved by in DFM-supplemented groups compared to the control group (T1: 1.661a; T2: 1.634ab; T3: 1.614b; $p < 0.05$). No significant difference was observed among dietary treatments in mortality. The results of the present study suggest that GalliPro® Fit-supplemented diets were beneficial in reducing FCR of broiler chickens under commercial-like conditions.

Key Words: DFM, multistrain, Bacillus, chickens, performance

T179 Effect of GalliPro® Fit, a new multi-strain direct feed microbial (Bacillus subtilis DSM 32324, DSM 32325 and Bacillus amyloliquefaciens DSM 25840), on growth performance and digestibility in broilers Dorthe Sandvang, Alfred Blanch*, Florence Rudeaux, Joren Verbeke *Chr Hansen AS*

The use of Bacillus species as probiotic supplements is expanding rapidly and demonstrating immune stimulation, enzyme production and competitive exclusion as the most prevalent modes of action. The objective of this study was to investigate the effect of three novel Bacillus strains in the GalliPro® Fit product on performance and digestibility in broilers under floor pen conditions. Study; 960 animals were placed in 64 pens and from D1 until D22, a starter feed was ad libitum administered which was replaced by a commercial grower diet until D42. Titanium oxide was added to the feed (0.3%) and single Bacillus strains were at dose 1.0 x10^6CFU/gram of feed. On D42, 6 birds per pen were euthanized and ileal content were pooled. The dry matter, crude protein, crude fat, energy, ash and titanium dioxide content were determined (Wageningen University) on the ileal content, in fecal droppings and the feed to analyse effects of the probiotics on apparent ileal digestibility. Data was analyzed with RStudio (Version 0.99.467, RStudio, Inc.). Digestibility data at bird level were analyzed using linear regression models with treatment group as fixed effect (procedure lm). Body weight and daily weight gain were analyzed using linear mixed regression models. Statistical significance was assessed at $P \leq 0.05$.

Performance results showed the average weight of birds(g) for all three bacillus strains were significant improved compared to the control group: for example at day 42 *Bacillus amyloliquefaciens* DSM 25840 average weight was 2525g (P- value 0.009) compared to non-supplemented group of 2390g. Digestibility results showed that birds supplemented with *Bacillus subtilis* DSM 32324 showed a significantly higher protein digestibility (76.3%, P-value 0.05) compared to non-supplemented birds (74.1%). Birds supplemented with *Bacillus amyloliquefaciens* DSM 25840 showed significant improved ash (40.8% P-value 0.039), protein (76.3% P-value 0.05) and energy digestibility (67.2% P-value 0.043) compared to control birds: ash (38.2%), protein (74.1%) and energy digestibility (65.0%) In conclusion, the diets supplemented with the new Bacillus strains showed significant effects on performance parameters correlating to apparent ileal digestibility in broilers under floor pen conditions.

Key Words: DFM, Bacillus, performance, protein digestibility, apparent ileal digestibility

T180 A one year field comparison between the effects of a blend of Quebracho and Chestnut tannins and a commercial antibiotic program on broiler live performance Enzo Redondo, Leandro Redondo, Juan Diaz Carrasco, Octavio Bruzzone, Claudio Cabral, Victorino Garces, Maximo Liñeiro, Michele Battaglia, Mariano Fernandez Miyakawa* *CONICET*

The removal of antibiotic growth promoters (AGPs) from animal diets has resulted in a search for cost and effective alternatives. A blend of Italian Chestnut (*Castanea sativa*) and Quebracho (*Schinopsis lorentzii*), Silvafeed® Nutri P (NP) has been shown to improve productive parameters and prevent infectious diseases (as necrotic enteritis). The objective was to compare the effect on broiler live performance of replacing a commercial program (COM) with NP (1.0 kg/MT) in a field trial on a commercial farm over a one year period. Each of six tunnel ventilated broiler houses (20,000 birds, Cobb®) was assigned to: 1) COM (AGP rotation), or 2) NP (tannins) during 6 production cycles. Body weight and mortality were obtained weekly, and feed consumption was taken at the end of each cycle. Necropsies, histomorphometry, footpad and intestinal lesions were studied in 10 birds from each group at 21 and 35, or, 42 days of age. Microbiota analysis was performed by 16S sequencing of caecal contents. Over the six cycles, average body weight gain was significantly ($p<0.05$) higher in NP fed birds as compared to birds fed COM diets. Differences between COM vs. NP treatments were not observed in overall mortality-corrected feed conversion ratio ($p>0.05$), total mortality (2.93 vs. 2.87 %) and first week mortality (0.81 vs. 0.95%). The average Production Efficiency Factor (PEF) was 1.4 % higher on the NP feed. The severity and the number of animals with gross lesions were reduced by NP compared to COM. Number of animals with duodenal lesions was not statistically different in COM vs. NP groups (6.6 vs. 4.4%) but differences ($p<0.05$) were observed in jejunum (18.8 vs. 12.2%) and ileum (6.6 vs. 3.3%). Footpad lesions recorded during field necropsies were lower for NP treatment but footpad inspection could not be repeated at the processing plant. Intestinal morphology was improved by NP ($p<0.05$). Firmicutes/Bacteroidetes ratio, Lactic Acid Bacteria and Bifidobacteria were increased by NP ($p<0.01$). In a one year field trial, compared to a commercial AGP program, the dietary inclusion of 1.0 kg/MT of tannins (Nutri P®) improved intestinal health and increased PEF, suggesting that addition of this specific blend can be an alternative for a healthy and sustainable poultry production.

Key Words: microbiota, poultry, tannins, antibiotic, growth promoter

T181 Dose depending performance improvement of 42 d broilers fed diets containing a mixture of carvacrol, cineol, cinnamaldehyde and capsicum oleroresin compared to antibiotic growth promotants
Rafael Cabrera*[1], Jess Walls[1], Mark Richards[1], Henning Gerstenkorn[2] *[1]EW Nutrition, USA; [2]EW Nutrition, Germany*

The purpose of this study was to determine the effects of supplementing different levels of a Secondary Plant Compound (Activo®) and antibiotic growth promotants on growth performance in broilers fed for 42 d in commercial conditions. Activo® is micro encapsulated in a fat matrix and contained carvacrol, cineol, cinnamaldehyde and capsicum oleoresin. The effect of 5 different dosages of Activo® (50,100,150,200 and 250 g/ton) on live weight, feed convertion ratio and livability of broilers was compared to an antibiotic group (50 g/ton of BMD in starter and grower feed, 20 g/ton of Stafac in finisher feed) and to an untreated control. All diets were mashed based on corn, soybean meal, meat and bone meal and dried distillers grains. Each treatment had 10 repetitions with 27 straight-run Ross 308 broilers per pen. For a natural challenge, 4 pound of used, homogenized litter have been added to each pen. All birds were vaccinated with Coccivac-B at day 0. Body weight and feed intake were measured at day 0,14,28,36 and 42. Feed conversions and mortality-adjusted feed conversions were calculated. Mortality was recorded daily and cumulative mortality was determined per treatment. Response variables (Live weight, Feed Conversion Ratios (FCR), and mortality) were evaluated by Randomized Complete Block ANOVA model and the means were separated by LSD model (Statistix 10, Analytical Software, Tallahassee, FL). The study was conducted at Virginia Diversified Research Corporation from February to April. Results at 42 d showed Average Daily Gains were significantly improved with 250 g/ton of Activo® when compared to control group. 200 g/ton of Activo® yielded a significantly lower mortality-adjusted feed conversion ratio when compared to the control group. For all the parameters measured, there was no statistical difference between the 200, 250 g/ton of Activo® and antibiotic treated groups. In conclusion, the overall results of the 42 d trial indicate that dosages of 200 and 250 g/ton of Activo® lead to improved performance parameters similar to those observed in the antibiotic shuttle of BMD and Stafac group. This means that Activo® safely replace antibiotics in broilers diets in commercial conditions®®. The results indicate a dose dependent effect of the Activo® product.

Key Words: Activo, broiler, BMD, Stafac, performance

T182 Oregano essential oil lessens the impact of enteritis in broiler chickens
Saksit Srinongkote[1], Wendy Wakeman[2], David Harrington*[2] *[1]Animal Research Consultant; [2]Anpario Plc*

Eubiotics such as those based on oregano essential oil (OEO) are potential tools to help manage gut health in a poultry industry under pressure to reduce antibiotic usage. A study to determine the performance of broilers fed an OEO product when challenged with *Eimeria* spp. and *Clostridium perfringens*. A total of 168 Ross 308 broilers were assigned to 2 treatment groups (14 birds/pen, 6 replicates/treatment): 1) Control (CON): basal ration and 2) OS: basal ration + Orego-Stim 300g/tonne of feed (Anpario Plc, UK). The basal ration contained meat and bone meal (3-5% depending on feeding phase) duration of 35 days. Birds were housed in floor pens on clean litter with access to feed and water *ad libitum*. On day 7, birds were orally administered a 1ml mixture of *Eimeria acervulina*, *E. maxima* and *E. tenella* (15,000 oocysts/ml) followed by an oral dose on days 12, 13 and 14 of *C. perfringens* ($3x10^9$ cfu/ml, 3ml/bird). Performance was determined for 0-10, 0-24 and 0-35 days. On days 18 and 35, 2 birds/pen were euthanased to determine clostridial lesion scores (LS). Data were analysed by ANOVA and significance declared at $P<0.05$. Body weight[HH1] gain was similar in the period 0-10 days (0.244 vs. 0.254kg, CON vs. OS respectively) but was significantly higher in OS for the p 0-24 (0.995 vs. 1.034kg, CON vs. OS respectively) and 0-35 days (2.150 vs. 2.225kg, CON vs. OS respectively). Similarly, FCR between groups did not differ in period 0-10 days (0-10, 1.22 vs. 1.22, CON vs. OS respectively), but was significantly lower in OS groups for periods 0-24 (1.00 vs. 1.03, CON vs. OS respectively) and 0-35 days (1.66 vs. 1.60, CON vs. OS respectively). LS were significantly lower in OS groups versus CON on day 18 and 35 (day 18: 0.86 and 1.22; day 35, 0.72 vs. 1.03, OS and CON respectively). In conclusion, an OEO eubiotic (Orego-Stim) significantly improved bird performance and reduced intestinal lesions associated with *C. perfringens*, the causative agents of necrotic enteritis, in an enteritis challenge model.

Key Words: Oregano, Broiler, Enteritis, Gut, Performance

T183 Performance of Broilers Fed Diets Supplemented with Bastion® and Challenged with Clostridium perfringens.
Nicholas Evans*, Theodore Karnezos, Ronald Dvorak, Curtis Novak, Mahmoud Masadeh, Michael Sims *PMI Nutritional Additives*

A 42-day, 30 bird/pen, 4 treatment, 10 replicate (40 pens) broiler study was conducted to compare the performance, mortality and lesion scores of straight-run Ross 308 broiler chicks, vaccinated with Coccivac®, and challenged with *Clostridium perfringens* on Day 17, 18, and 19. Dietary treatments were: 1) negative control (no additives); 2) positive control (challenge + no additives); 3) BMD®/Stafac® (challenge + BMD® 50 g/US ton in starter and grower, and Stafac® 20 g/US ton in finisher); 4) Bastion (challenge + Bastion® 1.25 lb./US ton in starter, 1 lb./US ton in grower, 0.75 lb./US ton in finisher). Day 21 necrotic enteritis lesion scores for Bastion® treated birds were significantly reduced ($p\leq0.05$) compared to the positive control birds and were similar to the negative control birds. Lesions scores from the positive control birds and BMD®/Stafac® birds were not significantly different from each other, but both were significantly increased ($p\leq0.05$) compared to the negative control birds. Day 42 body weights were not significantly different among the three challenge treatment groups (positive control, BMD®/Stafac®, or Bastion®); however, the feed conversion ratio was reduced ($p\leq0.05$) for both the BMD®/Stafac® and Bastion® treated birds compared to the positive control birds. All challenge treatment groups (positive control, BMD®/Stafac®, or Bastion®) had significantly lower ($p\leq0.05$) body weights and higher ($p\leq0.05$) feed conversion compared to the negative control. Day 0-42 mortality and necrotic enteritis associated mortality for BMD®/Stafac® and Bastion® treated birds were similar to the negative control birds and were significantly reduced ($p\leq0.05$) compared to the positive control birds. For this study, Bastion® was comparable to or better than the BMD®/Stafac® antibiotic growth promoter program in supporting performance, reducing mortality, and reducing necrotic enteritis lesion scores of broilers challenged with *Clostridium perfringens*.

Key Words: Clostridium perfringens, Necrotic Enteritis, Broiler, FCR, Mortality

POSTER SESSION

Physiology, Endocrinology and Reproduction

P184 Discovering the optimal concentration of Gallipro Hatch® that enhances broiler hatchability and live performance Claudia Castaneda*[GS], Dana Dittoe, Kelley Wamsley, Christopher McDaniel, Alfred Blanch, Dorthe Sandvang, Aaron Kiess *Mississippi State University*

Concerns about bacterial resistance to antibiotics has led to the search for alternatives such as probiotics. *In ovo* application of probiotics has been suggested as a strategy that may enhance broiler performance and welfare. Therefore, the objective of this study was to evaluate the impact different concentrations of GalliPro Hatch (GH), an *Enterococcus faecium* based probiotic, have on hatchability and live performance when *in ovo* injected into fertile hatching eggs. For the study, 2,078 Ross X Ross 708 fertile eggs were obtained, weighed, and incubated. Eggs were candled on D12 of incubation and on D18 injected with the following treatments: 1) 50 µL of Marek's vaccine, 2) Marek's vaccine and $1.4x10^5$ cfu GH/50µL, 3) Marek's vaccine and $1.4x10^6$ cfu GH/50µL, and 4) Marek's vaccine and $1.4x10^7$ cfu GH/µL. At hatch chicks were weighed, feather sexed and hatch residue analysis was conducted. Male chicks (640) were randomly assigned to 40 floor pens; water and feed were supplied *ad libitum*. On D0, 7, 14, and 21 of the grow-out, performance data were collected. Hatchability data were analyzed using a completely randomized design and live performance data were analyzed using a randomized complete block design with a split plot over time. Means were separated using Fisher's protected LSD ($P \leq 0.05$). Results demonstrated that no hatchability characteristic was impacted by *in ovo* injection of any GH treatment ($P > 0.2$). Live performance from D0-7 demonstrated a lower feed intake and FCR ($P = 0.05$ and $P = 0.01$, respectively) for the 10^5 and 10^7 GH treatments compared to Marek's vaccine alone. No differences were detected from D7-14 ($P > 0.05$). From D14-21, weight gain increased as the concentration of GH increased, but GH treatments were not different than the Marek's vaccine alone ($P = 0.05$). For D0-21, the 10^7 GH treatment had a lower FCR than the Marek's vaccine alone ($P = 0.03$). In conclusion, *in ovo* application of GalliPro Hatch, even at high concentrations, should not impact hatchability and can improve broiler live performance, at least through the first 21 d of a grow-out.

Key Words: Gallipro Hatch, probiotic, in ovo, hatchability, FCR

P185 Changes to the gastrointestinal tract and yolk sac of broilers previously in ovo injected with different concentrations of Gallipro Hatch® Dana Dittoe*[GS], Claudia Castaneda, Kelley Wamsley, Chris McDaniel, Alfred Blanch, Dorthe Sandvang, Aaron Kiess *Mississippi State University, Department of Poultry Science*

In ovo application of probiotics has been suggested as a novel strategy to improve broiler performance. Thus, the objective of this study was to evaluate the effect of *in ovo* injection of GalliPro Hatch (GH), an *Enterococcus faecium* based probiotic, on intestinal parameters. For this study, 2,078 Ross X Ross 708 fertile eggs were obtained and incubated. The following *in ovo* treatments were applied on D18 of incubation: 1) a 50µL injection including Marek's vaccine (MV) as a control, 2) MV plus $1.4x10^5$ cfu GH/50 µL, 3) MV plus $1.4x10^6$ cfu GH/50 µL, and 4) MV plus $1.4x10^7$ cfu GH/50 µL. At hatch (D0), 640 male chicks were placed into 40 floor pens. Water and feed were supplied *ad libitum*. On D0, 7, 14, and 21 of the grow-out, 1 bird per pen was used to obtain yolk, crop, gizzard, duodenum, jejunum, ileum, and ceca weights, lengths and pH. Data were analyzed using a randomized complete block design with a split plot over time. Means were separated using Fisher's protected LSD ($P \leq 0.05$). The results demonstrated that all GH treatments reduced yolk weight compared to MV alone ($P = 0.0003$). Differences in duodenum length were detected on D14 where the 2 higher GH doses had longer relative duodenum lengths than the other 2 treatments, but by D21 no differences in length were detected. On D0 all GH treatment tissues were heavier than control tissues; however, by D7 the opposite was detected and on D21 no differences were apparent ($P < 0.0001$). On D14, the crop pH was lower in the 10^5 cfu GH treatment compared to the other treatments; however, by D21 the pH of this treatment was only lower than the highest dose of GH ($P = 0.02$). On D14, duodenum pH for the 10^6 cfu GH treatment was lower than the other treatments; but by D21, the highest GH dose had a lower pH than the control and lowest GH dose ($P < 0.0001$). For jejunum pH, no differences between treatments were detected on D0 or 21; but on D 14, the 10^6 cfu GH treatment had a lower pH compared to all other treatments ($P < 0.0001$). conclusion, *in ovo* injection of GalliPro Hatch increases yolk absorption and decreases gastrointestinal pH. These changes may lead to improved live broiler performance observed in other GalliPro Hatch studies.

Key Words: GalliPro Hatch, in ovo, intestinal morphology, pH, broiler

P186 Broiler hatchability following in ovo injection of Lactobacillus animalis or Bacillus licheniformis Tiffani Allen*[UG], Aaron Kiess, Christopher McDaniel *Mississippi State University*

With the emergence of antibiotic resistant bacteria, the poultry industry has been investigating alternatives to antibiotics, such as probiotics. Although the poultry industry works diligently to avoid contamination in the hatchery, if probiotics can be administered to fertile hatching eggs an even greater benefit to the broiler may follow. The purpose of this study was to assess hatchability characteristics after *in ovo* injection with either *Lactobacillus* animalis (*L*. animalis) or *Bacillus* licheniformis (*B*. licheniformis). For each of these 2 probiotic bacterial species, a separate experiment was conducted in which 7 treatments were applied: no punch (no injection), dry punch, and diluent punch as well as 10^3, 10^4, 10^5, and 10^6 cfu probiotic in a 50µl injection volume. For each experiment, 1,050 eggs were acquired from a commercial breeder farm and incubated under standard incubation conditions. Eggs were candled on D10 to remove infertiles and early embryonic mortalities. On D18, 150 live embryos per treatment were *in ovo* injected using Inovoject® equipment and placed into a hatcher. On D21, chicks were counted and weighed, and hatch residue analysis was performed. Data were analyzed using a completely randomized design with means separated using Fisher's protected LSD at $P \leq 0.05$. The results indicated that both *L*. animalis and *B*. licheniformis ($P = 0.18$ and 0.75, respectively) did not impact hatchability of fertile eggs. However, late embryonic mortality was higher when embryos were injected with 10^4 cfu of *L*. animalis as compared to all other treatments except for the diluent punch ($P = 0.01$). For *B*. licheniformis, late embryonic mortality was greater for the 10^4, 10^5, and 10^6 cfu dosages when compared to the 10^3 cfu and dry punch treatments ($P = 0.05$). Additionally, chick hatch weight was less for the no punch and 10^6 cfu *L*. animalis treatments as opposed to the dry punch treatment ($P = 0.005$); but for the *B*. licheniformis experiment, the no punch and 10^6 cfu treatments produced heavier chicks than dry punch and 10^5 cfu treatments ($P = 0.002$). In conclusion, because hatchability was unaffected, both *L.* animalis and *B.* licheniformis are candidates for *in ovo* administration; however, average chick weights could be impacted when certain probiotic bacteria are used.

Key Words: InOvo, L.animalis, B.licheniformis, Broiler, Hatchability

P187 21 day broiler performance after in ovo injection of individual or multiple probiotic bacteria Chrysta Beck*[UG], Kelley Wamsley, Christopher McDaniel, Aaron Kiess *Mississippi State University*

As many poultry integrators transition to antibiotic free production, viable alternatives to antibiotics, such as probiotics are necessary. Through

in ovo probiotic inoculation, beneficial gut microflora development may occur prior to hatch without negatively impacting hatchability. Therefore, the objective of the current study was to investigate the role individual probiotic bacteria and their combination have on broiler performance and gut development after commercial *in ovo* injection. A total of 2,080 fertile broiler hatching eggs were obtained and incubated under standard conditions. On D18 of incubation, 4 treatments were applied to live embryos which included a Marek's Disease vaccine 50µl (T1), *L. animalis* (10^9 cfu/50µl; T2), *E. faecium* (10^6 cfu/50µl; T3), and the combination of *L. animalis* and *E. faecium* (10^7 cfu and 10^6 cfu/50µl; T4). On day of hatch, hatch residue analysis and hatch of fertile data were collected. Male chicks (720) were placed in a grow-out facility to obtain live performance and gastrointestinal samples over a 3 wk grow-out. The results indicated no difference ($P > 0.05$) for hatch of fertile or chick weights; however, T2 had less pipped eggs ($P = 0.04$) than all other treatments. No differences in live performance characteristics were observed over the entire 3 wk grow-out. Numerous treatment by day interactions were detected for weight, length and pH of the gastrointestinal tract. In general, T3 had a heavier jejunum and ileum on D14 compared to T1 and T2 ($P = 0.02$ and 0.003, respectively) and a heavier ceca on D7 compared to T1 and T4 ($P = 0.01$). The length of the ileum for T4 was found to be longer than T1 on D0, T2 on D7 and T3 on D21 ($P = 0.04$). For the ceca, T4 was longer than T1 and T2 on D0 ($P = 0.007$). On D14, T4 had a higher jejunum pH compared to all other treatments ($P = 0.05$), and the duodenum pH of T4 was also higher than T1 on D14 ($P = 0.05$). In conclusion, *in ovo* injection of L. animalis, E. faecium or their combination had no effect on hatchability and D21 live performance. However, changes to the gastrointestinal tract were detected due to probiotic treatment, which may impact live performance characteristics during later stages of a grow-out or at processing.

Key Words: InOvo, Probiotics, Hatchability, LivePerformance, GastrointestinalTract

P188 Identification of absorptive and stem cells in chicken small intestine and yolk sac Haihan Zhang*[GS], Eric Wong *Virginia Tech*

The chicken small intestine plays an important role for nutrient digestion and absorption during the late embryonic and posthatch stages. During the prehatch stage, however, the chicken embryo assimilates nutrients mainly by absorption through the yolk sac. The small intestinal villi and yolk sac share the same physiological structure of a single epithelial cell layer that directly contacts the nutrient contents. Although the intestinal absorptive cells and stem cells have been clearly identified in mammals, the location and origin of the absorptive cells in chickens are still unknown. The objective of this study was to identify the distribution of absorptive cells that express the peptide transporter PepT1 and stem cells that express Lgr5 (Leucine rich repeat G-protein coupled receptor 5) and Olfm4 (Olfactomedin 4) mRNA in the chicken yolk sac and small intestine using RNAscope in situ hybridization. Yolk sac and intestinal (duodenum, jejunum, ileum) samples were fixed in buffered formalin, embedded in paraffin and cut into 4-6 um sections. Cells expressing PepT1, Olfm4 and Lgr5 mRNA were identified using custom RNAscope® probes and detection kits. In the yolk sac, PepT1 mRNA was localized to the epithelial cells. Lgr5 mRNA was mainly detected in vascular endothelial cells. No cells expressed Olfm4 mRNA in the yolk sac. In the small intestine, PepT1 mRNA was localized to the epithelial cells lining the villi. Cells expressing Olfm4 or Lgr5 mRNA were predominantly localized to the intestinal crypts. These results demonstrated that both the yolk sac and small intestine have absorptive epithelial cells that expressed PepT1 mRNA. Additionally, the intestinal stem cell population, marked by Lgr5 and Olfm4, resided in the intestinal crypts. Only Lgr5 but not Olfm4, was able to mark putative stem cells in the yolk sac.

Key Words: Absorptive, Stem, PepT1, Lgr5, Olfm4

P189 Effect of whole corn inclusion in the diets of broiler chickens on ileal and cecal microbiota Cristiano Bortoluzzi*, Andrea Rubio, Juan Aranibar, Todd Applegate, Wilmer Pacheco *University of Georgia*

The objective of this study was to evaluate the effect of different inclusion levels of whole corn on the ileal and cecal microbiota of broiler chickens at 42 days of age. A total of 1,000 male broiler chicks were randomly assigned to 4 treatments with 10 replicate pens of 25 birds/pen. The treatments consisted of 4 levels of whole corn (0, 2.5, 5, and 7.5%) that replaced ground corn prior to pelleting. The starter diets were offered as crumbles while grower and finisher diets were offered as pellets. At 42 days of age, two birds per pen were euthanized, ileal and cecal contents were separated, and a pool of the two birds was obtained. Bacterial DNA was isolated, and the V3-V4 regions of the 16s rRNA gene was sequenced by Miseq Illumina platform. All sequence processing was performed using QIIME software. High-quality sequences were aligned against SILVA database, and a reference operational taxonomic unit (OTU) table was generated. Diversity indexes were calculated using QIIME. The main phylum and genera of each treatment group were analyzed by ANOVA, and compared by Tukey's test. Alpha diversity was not affected by dietary treatments. Weighted and unweighted distance matrices (beta diversity) indicated that community structure (weighted), and membership (unweighted) contributed to differences in both ileal and cecal microbiotas. The ileal microbiota was dominated by the phylum *Firmicutes* (over 99%). The main genera found in the ileal microbiota was *Lactobacillus* (84%), followed by *Streptococcus* (5.4%), and *Enterococcus* (4.7%), with no differences among the treatments ($P>0.05$). The cecal microbiota was dominated by the phylum *Firmicutes* (89%), consisting mainly of *Clostridiales* (19.3%), followed by *Ruminococcus* (12.3%), *Ruminococcaceae* (11.8%), and *Lachnospiraceae* (7%), with no differences among treatments. The inclusion of 7.5% of whole corn, however, increased *Faecalibacterium* ($P=0.07$), and decreased *Lactobacillus* ($P=0.08$) in the cecal microbiota, when compared to 5% of whole corn inclusion. Although similar microbial richness was observed among treatments, the community structure was significantly different, in both ileum and cecum. The cecal microbiota was more affected by the diet than ileal microbiota, which also showed higher microbial diversity.

Key Words: broilers, microbiota, corn

P190 Antioxidant status of broiler chickens fed acerola byproducts meal Thaina Landim de Barros*, Rodrigo Pereira Cassiano, Ariana Aparecida Ferreira Pereira, Anelise Bosco, Paulo Ciarlini, Manoel Garcia-Neto, Elisa Helena Giglio Ponsano *São Paulo State University (UNESP)*

In broilers, the oxidant substances coming either from metabolism as from diets are supposed to be neutralized by the intrinsic antioxidant system. However, when this system is unsuccessful, the oxidative stress starts damaging lipids, proteins and nucleic acids and being one of the main initiators of the inflammatory reaction. As a way of increasing the antioxidant system, synthetic antioxidants (SA) are commonly added to broiler chicken diets, despite of their possible toxicological effects to humans and animals. Due to the increasing demand for natural antioxidants, the aim of this study was to investigate the effects of acerola byproduct meal (ACM) on the broilers oxidative status. One hundred sixty one-day-old Cobb 500 male chicks were divided (ten birds per pen) to receive four experimental diets (four repetitions): positive control (PC), containing 0.007% colistin sulfate 8% (antimicrobial growth promoter - AGP) and 0.01% butylated hydroxytoluene (BHT) (SA) and no ACM; negative control (NC), without AGP, SA or ACM; diet with 5% ACM (AC 5%); and diet with 7.5% ACM (AC 7.5%). Productive parameters were measured weekly until day 42. At slaughter, the blood was collected for the analyses of total oxidant capacity (TOC) and total antioxidant capacity (TAC, measured by ABTS and CUPRAC methods) in the serum. Data were analyzed by ANOVA and when a significant effect ($P < 0.05$) was detected, the difference between means was analyzed by Duncan`s test. There were no differences among the treatments regarding to feed intake, body weight gain and feed conver-

sion (P > 0.05). Although the animals from PC group received SA in their diets, they did not show different results for ABTS (P = 0.018) and CUPRAC (P = 0.010), when compared to animals from groups AC 5% and AC 7.5%. The high antioxidant activity found in the serum of the animals fed ACM can be assigned to the variety of bioactive compounds present in this byproduct, like phenolic compounds, carotenoids and ascorbic acid. TOC did not detect any difference among treatments (P > 0.05). It was concluded that the addition of ACM did not harm the broilers performance and presented an antioxidant potential similar to the synthetic antioxidant.

Key Words: antioxidant

P191 Expression of neuropeptide Y in chicken muscle and its effects on mitochondria dynamics. Marco Zampiga*, Elizabeth Greene, Federico Sirri, Joshua Flees, Sami Dridi *Department of Agricultural and Food Sciences – Alma Mater Studiorum - University of Bologna*

Neuropeptide Y (NPY) is an orexigenic peptide mainly expressed in hypothalamus. Recent findings suggest that NPY might be expressed in other peripheral tissues. The aim of the current study was primarily to characterize the expression of NPY in chicken muscle and then to evaluate its metabolic function in this tissue. Six birds showing the same age, gender, genetic line, fed the same diet and raised in the same environmental conditions, were humanely euthanized to collect breast muscle, leg muscle, and hypothalamus. Total mRNA was extracted and NPY expression (294 bp fragment) was assessed with conventional PCR. To understand the physiological role of NPY on muscle tissue, quail myoblast (QM7) cells culture was treated either with 0 (CON), 1 (A) or 100 (B) nM of recombinant NPY, total mRNA extracted, and RT-qPCR analysis performed to determine the relative expression of mitochondrial biogenesis-, function-, and dynamics-related genes. Results from conventional PCR analysis indicate that NPY is expressed in both breast and leg muscle, and also in CON QM7 cells. NPY treatments increased mRNA expression of NPY gene and its related receptors NPYR1 and NPYR5. NPY administration increased the relative expression of mitochondrial mtDNA mtSSBP1, OPA1, COX5, and PGC1α. However, mRNA abundance of MFN1, MFN2 DNM1 OMA1 and TFAM was decreased. Taken together, these results suggest that: 1) NPY is expressed in chicken breast and leg muscle, 2) NPY regulates its own system, and 3) NPY may play a pivotal role in mitochondria function and dynamics. Ongoing studies will add critical insights on the role of NPY on muscle mitochondria metabolism.

Key Words: Neuropeptide Y, chicken, muscle, gene expression, mitochondria

P192 Infrared thermography (IRT) as a non-invasive indicator of leg health in broiler chickens Shawna Weimer*, Robert Wideman, Andy Mauromoustakos, Colin Scanes, Karen Christensen, Yvonne Vizzier-Thaxton *Purdue University*

Lameness is an important welfare issue in broiler chickens. Bacterial chondronecrosis with osteomyelitis (BCO) is a major contributor to lameness. Currently, the method to diagnose the presence of BCO is *post-mortem* necropsy. Our objective was to assess BCO non-invasively *pre-mortem* by measuring leg surface temperatures with infrared thermography (IRT) technology in broilers. Surface temperatures of the legs and feet were determined by IRT in two experiments (E1, E2) evaluating the effects of light intensity (5, 10, and 20 lux) and leg health status (lame vs. non-lame) on the incidence of BCO. It was hypothesized that IRT would predict the severity of BCO lesions in the proximal femoral head (femoral head necrosis; FHN) and the proximal tibial head (tibial head necrosis; THN) of lame and non-lame broilers. Day old chicks were placed into six environmental pens with litter flooring. Light intensity treatments began at 1 wk of age. At wk 4 of age, birds were moved to wire flooring pens (N = 6) with the same light intensity as the source litter pen. Clinically lame birds were culled daily from 29 to 56 days of age in both experiments. An image of each leg was taken with a thermal camera and anatomical leg areas were isolated within each image using the thermal camera software. The birds were humanely euthanized and the proximal femoral and tibial heads were macroscopically scored (on a scale ranging from 0 to 4) for necrosis severity. For both experiments, lame bird lesion scores were more severe (P < 0.001) than non-lame birds for FHN (E1: 1.4 non-lame vs. 4.2 lame; E2: 1.9 non-lame vs. 2.7 lame), THN (E1: 2.3 non-lame vs. 3.7 lame; E2: 2.5 non-lame vs. 2.9 lame) and Total necrosis (FHN + THN; E1: 3.7 non-lame vs. 7.9 lame; E2: 4.4 non-lame vs. 5.6 lame). Non-lame bird leg surface temperatures were higher (P < 0.001) for the hocks (E1: 37.0°C non-lame vs. 34.0°C lame; E2: 38.5°C non-lame vs. 36.2°C lame), shanks (E1: 37.2°C non-lame vs. 34.5°C lame; E2: 38.1°C non-lame vs. 36.5°C lame) and feet (E1: 36.3°C non-lame vs. 30.1°C lame; E2: 37.0°C non-lame vs. 33.9°C lame). These studies suggest IRT can be used as a non-invasive tool for detecting BCO lesions attributed to lameness in live broilers.

Key Words: broiler, BCO, lameness, IRT, welfare

P193 A comparative study of skeletal integrity between a 1995 and 2015 genetic line of broilers Rodrigo Lopez*[UG1], Sara Orlowski[2], Nicholas Anthony[2], Katy Tarrant[1] *[1]California State University, Fresno; [2]University of Arkansas*

As a result of genetic selection pressures, economically important traits continue to make gains in fast growing broilers. In order to better understand the impact that has taken place over time on profitable traits, such as live weight and breast yield, as well as indirectly selected traits, two randomly bred genetic broiler lines representing the 1995 and 2015 broiler are maintained at the University of Arkansas. In this study, we evaluate variation that exists in the legs of broilers from the 1995 and 2015 random-bred (RAN) lines over a grow out period of six weeks. Twenty birds were sampled from each line at three time points (d 14, d 28, and d 42), totaling 120 individuals. Birds were raised with access to ad libitum feed and were weighed prior to euthanasia. Left legs (including thigh, drum, paw, and skin) were removed for further analysis. Individual leg weights and femur head diameter (FHD) was recorded for each individual. A t-test was used to compare means for comparisons between the lines. As expected due to genetic selection gains in body weight over time, both male and female live weights and total left leg weights were significantly larger in the 2015 RAN line (p<0.01). The d 42 2015 RAN mean male weight was 462 g larger than 1995 RAN males, while 2015 RAN mean female weight was 399 g larger than 1995 females. Drive by a need to support a larger frame, 2015 RAN male and female FHD increased in size for each time point, relative to their 1995 RAN counterparts (p<0.01). No variation exists between 1995 and 2015 leg weight to live weight ratio, indicating a proportional increase in leg size between these two lines. Interestingly, both the 1995 RAN male and female mean ratio of FHD to live weight and the mean ratio of FHD to left leg weight are significantly larger when compared to 2015 RAN individuals (p<0.01). The lack of a proportional increase between FHD and weight when comparing the 1995 and 2015 RAN lines indicate this parameter may be important when considering the influence of selection pressures on leg related afflictions, such as lameness seen in modern populations of broilers.

Key Words: broilers, lameness

P194 Percoll density gradient centrifugation technique isolates rooster sperm with low, medium and high mobility or fertility potential Kathryn Harison*[UG], Muslah Ahammad, Zachery Jarrell, Andrew Benson *University of Georgia*

This study was designed to examine the feasibility of using a Percoll density gradient (PDG) centrifugation technique to isolate sperm with different mobility or fertilizing potential and then analyze these separate sperm populations with 2-Dimesional Electrophoresis (2DE). An inert cell separation medium Accudenz® was used to assess sperm mobility. The sperm-inner perivitelline layer (IPVL) assay was used to assess the fertilizing potential of sperm. Two narrow gradients of Percoll with 1.08 and 1.07 densities were prepared using 1.5 M NaCl solution. Semen was overlaid

on the lower gradient of the Percoll suspension and centrifuged at 1500 ×*g* for 20 min. This resulted in three distinctive upper, middle and lower layers of sperm which were then isolated and centrifuged at 1500 ×*g* for 10 min. The sperm pellets were then suspended in motility buffer and sperm concentration was determined using a Neubauer hemocytometer. Sperm suspensions from either upper, middle or lower layers containing $5x10^8$ cells/mL was overlaid on 1.5 mL of pre-warmed 6% (w/v) Accudenz solution, and incubated at 41 °C for 5 min. The spectrophotometric absorbance at 550 nm was recorded at 1min intervals. Aliquots of the sperm containing $4x10^6$ cells/mL from the three different layers underwent incubation with IPVL at 37 °C for 15 min. Sperm isolated from the upper, middle and lower gradients of the PDG exhibited markedly different ($P<0.05$) low (0.11± 0.03), medium (0.34± 0.01) and high (0.87± 0.03) mobility, respectively. The number of penetration holes per 0.25 mm^2 also differed ($P<0.05$) between the sperm isolated from the different gradients of the PDG. The sperm isolated from the lower layer of PDG produced the highest number of penetration holes (51.1± 1.2) followed by the middle layer (33.2 ± 1.4) and upper layer (24.3± 1.1). Differential proteomic profiles for the different sperm aliquots was observed following isoelectric focusing and 2DE analysis using Bio-Rad PDQuest 2-D analysis software. The findings of the present study demonstrate that the Percoll density gradient technique can be used to isolate sperm with high, medium and low mobility or fertilizing potential and that these variances in mobility and fertility are reflected by differing proteomic profiles.

Key Words: fertility, mobility, Percoll, IPVL

P195 Relationship of eggshell mammillary core numbers to eggshell pore numbers on the large end and equators of hen eggs. Will Hamilton*[UG], Wallace Berry *Auburn University*

This experiment was conducted to determine the relationship between the number of mammillary cores and eggshell pores in eggshells of commercial broiler breeder hens. Ten eggs were collected from a commercial strain of broiler breeder hens. All of the breeders were of the same age and same commercial line. Eggs were emptied of contents and eggshell coupons were cut from the large ends and equators of the eggs. Coupons were processed with dilute acetic acid to facilitate shell membrane removal. Membrane coupons thus obtained were stained with H+E stain to visualize the mammillary cores on the membranes. Eggshell coupons were stained with methylene blue to visualize pores. Mammillary core counts and eggshell pore counts were taken from the large end and equator of the eggs. For counting of mammillary cores and eggshell pores, micrographs of three areas of each sample were counted using ImageJ software. Mammillary core numbers were counted in 2000 x 2000 pixel areas in each micrograph. The average number of mammillary cores on the large end of eggs was not different from the number at the egg equator (313 +/- 12 vs. 309 +/- 9). The average number of pores in the eggshell at the large end likewise did not differ from the number of pores at the equator (72 +/- 4 vs. 71 +/- 6). The correlation coefficient of mammillary cores and pores was found to be 0.12 indicating a weak positive correlation between the number of mammillary cores and eggshell pores.

Key Words: mammillary core, eggshell pore, hen, correlation

P196 Effect of dietary supplementation with Enterococcus faecium on performance, egg quality, histomorphology and salmonella colonization in laying hens infected with S. enteritidis Shimeng Huang*[GS], Cong Liu, Lihong Zhao, cheng ji, Jianyun Zhang, Qiugang Ma *China Agricultral University*

E. faecium (EF) have been suggested as antibiotic alternatives in animals by improving growth performance and alleviating pathogens infection. This research was to investigate the protective effect of EF on production, egg quality, histomorphology and cecum salmonella colonization in hens exposed to *S. enteritidis* (SE).

400 45-week-old salmonella-free layers were randomly allotted according to body weight and egg production with 5 replications 20 hens per replicate. Dietary treatments were: (1) NC, basal diet; (2) T1, NC + 0.02% EF; (3) PC, NC + oral SE administration; (4) T2, NC + 0.02% EF + oral SE. Eggs were collected on a daily. Egg production and feed intake was recorded, and feed to egg mass ratio was calculated weekly. The quality of eggs was evaluated weekly. On day 1, days 7, 14 and 21 post infection, five randomly chosen birds from each treatment were euthanized. Cecum contents sample were aseptically harvested. Meanwhile, The intestinal segments determine the duodenum and jejunum morphometry.

Results showed that the egg production was higher ($P_{EF}<0.05$) in T1 and T2 than that in NC and PC supplied with EF respectively. The shell thickness ($P_{SE}<0.05$) was reduced in post *salmonella*-challenged birds, and the yolk color was significantly lower ($P_{EF}<0.01$) in T1 and T2 than that in NC and PC supplied with EF respecitively. In the jejunum, supplementation of EF significantly reduced crypt depth ($P_{EF}<0.01$), and significantly increased ($P_{EF}<0.01$) villus height: crypt depth ratio (VH:CD), however, the crypt depth was increased ($P_{SE}<0.05$) during post-challenged. In the ileum, the villus height, crypt depth, and villus width, as well as villus surface area were reduced ($P_{SE}<0.05$) in laying hens challenged SE. In the cecum, EF significantly reduced SE copies ($P_{EF}<0.05$) at dpi 1 and dpi 7, moreover, SE copies in cecum were reduced ($P_{EF}<0.05$) at overall experiment time.

In conclusion, whether or not the birds infected pathogens, EF can improve production, egg quality, the maturation of intestinal morphology by increasing villus height and decreased crypt depth, alleviated SE infection by improving intestinal microbial balance, and decreased SE copies in the cecum.

Key Words: E.faecium, hens, S.enteritidis, performance

P197 Transcriptome analyses of differential gene expression in atrophic ovary of broody chicken Lingbin Liu*, Elizabeth R. Gilbert, Qing Zhu *Sichuan Agricultural University*

Broodiness in laying hens results in atrophy of the ovary and consequently decreases productivity. However, relevant regulatory mechanisms of ovary development are complex and remain unknown. To address this challenge, we collected atrophic ovaries (AO) from 380-day- old broody chicken (BC) and normal ovaries (NO) from even-aged egg-laying hens (EH) for transcriptome sequencing. A total of 58.5 Gb clean reads from RNA sequencing and 71.33 Mb from small-RNA sequencing were generated. Overall, 49872 mRNAs, 8738 long non-coding RNAs (lncRNAs), and 2827 microRNAs (miRNAs) were detected in the whole transcriptome profile of the chicken ovary. We found 3480 mRNAs, 959 lncRNAs, and 116 miRNAs differentially expressed in AO of BC. Numerous genes and target genes for miRNA/lncRNA were significantly enriched in reproductive processes, and cell proliferation and apoptosis pathways such as the Estrogen, Oxytocin, PI3K-Akt, cGMP-PKG, TGF-beta, and Hippo signaling pathways. An miRNA-intersection gene-pathway network was constructed by considering target relationships and correlations of the expression levels between differentially expressed (DE) genes and miRNAs. We also constructed a competing endogenous RNA (ceRNA) network by integrating competing relationships between genes and lncRNAs, and found lncRNAs predicted to regulate the CASP6, CYP1B1, GADD45, MMP2, and SMAS2 genes. In conclusion, this study broadened our current understanding of the molecular mechanisms underlying reproductive systems of laying hens, and identified strong candidate genes, miRNAs, and lncRNAs as potentially important regulators of ovary development in broody chicken.

Key Words: transcriptome, regulatory network, atrophic ovary, broodiness

P198 The bone development pattern and the correlation with growth parameters in modern laying hens Chongxiao Chen*, Woo Kyun Kim *The University of Georgia*

Modern laying hens have dramatically high egg production, which requires a strong ability to maintain mineral homeostasis. Optimizing the development of hen skeleton to provide more consistent calcium source for egg production, while minimizing the bone fracture incidence and osteoporosis during late laying period attracts more attention. However, little is known about the growth-related changes in bone mineral density (BMD) and bone mineral content (BMC) of modern high egg production layers, as well as the relationship of bone development with the other growth factors. A study was conducted to explore the bone develop pattern of modern high egg production laying hens and the correlation of bone growth with other growth parameters. Day-old Hy-line W36 pullets were raised until 21 wk. They were fed with standard diets formulated based on Hy-line W36 guide. From wk2 to wk21, 10 birds per week were weighed and scanned using Dual energy x-ray absorptiometry for whole body composition analysis. Simple and polynomial regressions were carried out between bone parameters (BMD and BMC) and other growth-related variables using SAS software. From the results ($P<0.001$), we found a quadratic increase of body weight (BW, $R^2=0.980$) and BMC ($R^2=0.916$); a linear increase of BMD ($R^2=0.821$), fat weight (FW, $R^2=0.597$) and lean weight (LW, $R^2=0.919$). During experiment period, BMD and BMC reached the highest level at 20 wk, however, the BW weight peaked at 19 weeks. The best fit was obtained with a quartic regression between BMD and BW ($R^2=0.869$), as well as BMC and BW ($R^2=0.940$). The correlation between BMC and LW ($R^2=0.678$) or FW ($R^2=0.892$) showed a linear regression model. BMD showed similar correlation ($R^2=0.709$ and 0.795, respectively). However, among these growth factors. LW and FW have a linear relationship but with low prediction accuracy($R^2=0.373$). In conclusion, this study showed an overall picture of hen bone growth pattern, which is valuable information for future laying hen bone research. The bone growth pattern was significantly correlated with different tissues accretion patterns, which indicates that the body size of pullets could affect bone quality.

Key Words: laying hen, bone, correlation, growth parameter, DEXA

P199 Blood variables of commercial egg-laying pullets following a field-strain Mycoplasma gallisepticum challenge E. Peebles*[1], Hammed Olanrewaju[2], Scott Branton[2], Jeffrey Evans[2], Spencer Leigh[2], Elizabeth Kim[2], Gregory Pharr[1], Katie Elliott[1] *[1]Mississippi State University; [2]ARS-USDA*

The R_{low} strain of *Mycoplasma gallisepticum* (RMG) has been commonly used as a pathogenic strain in field-strain *Mycoplasma gallisepticum* challenge studies. The effects of an RMG challenge at 8 and 10 wk of age (woa) on 16 blood variables in Hy-Line W-36 layer pullets at 12 woa were determined. Pullets were fed a basal diet beginning at hatch and through 12 woa. At 3 woa, birds were transferred from floor pens to an isolation facility in which 18 birds were placed in each of 8 isolation units assigned to each treatment in each of 2 rooms. In each room, birds in 4 units assigned to each treatment were either non-challenged or were challenged with RMG at 8 and 10 woa by eye drop and tracheal gavage, respectively. All challenged birds were positive for MG antibody production at 12 woa. Although HCO_3^- blood levels were unaffected ($P = 0.3666$), RMG challenge decreased pH ($P = 0.004$) and pO_2 ($P \leq 0.0001$) values, and Cl^- ($P \leq 0.0001$), oxyhemoglobin ($P = 0.001$) and dissolved oxygen concentrations at 12 woa. It also increased pCO_2 ($P = 0.0006$), osmolality ($P \leq 0.0001$), and anion gap ($P \leq 0.0001$) values, as well as Ca^{2+} ($P = 0.001$), Na^+ ($P = 0.002$), glucose ($P \leq 0.0001$), and corticosterone ($P = 0.008$) concentrations at 12 woa. Corticosterone concentrations were 5,354 and 14,720 pg/mL in the non-challenged and RMG-challenged groups, respectively. In conclusion, layer pullets challenged with RMG undergo a stress response associated with changes in various physiological blood variables. Furthermore, a decrease in pH and increase in pCO_2, in association with a lack of change in HCO_3^-, is indicative of respiratory acidosis.

Key Words: blood, corticosterone, field-strain, Mycoplasma, pullets

P200 The effect of pullet weight at 17 weeks of age on the productive performance and egg quality during the laying period Madalena Lordelo*[1], Carolina Duarte[1], Margarida Barbosa[2], David Henriques[2] *[1]Instituto Superior de Agronomia, Universidade de Lisboa; [2]Zêzerovo - Produção Avícola do Zêzere, S.A.*

It is well established that, amongst the same breed, the body weight at point of lay influences subsequent egg weight. The goal of the current study was to evaluate the differences of other equally important performance parameters as well as egg quality between the subsequent laying period of pullets of different weights at point of lay. In this study, 360 laying hens were classified as small, medium or heavy at 17 weeks of age (≤1500g, 1501-1600g and >1600g, respectively) and each group was housed in 8 cages of 15 hens each until 41 weeks of age. Performance parameters were analyzed, such as body weight, feed intake, egg production rate, egg weight and mass. Eggs were analyzed for shell thickness, air cell height, Haugh units, albumen as well as whole egg pH, yolk color, shell cracks, meat spots and blood spots. A microbiological analysis was also performed. The results demonstrated that the differences in pullet weight between groups remained equivalent throughout the period of the trial, without any indication of compensatory growth. In addition, the heavier group of hens had a higher egg production rate ($P < 0.05$) only at the beginning of the laying phase and that this group had a higher percentage of large and extra-large eggs compared to the lightest group. However, average total egg mass during the trial was not different between groups. In addition, total feed consumption was higher in larger hens ($P < 0.05$). Regarding egg quality, no differences were found between groups with the exception of heavier hens having the highest percentage ($P < 0.05$) of eggs with meat spots, a characteristic that most consumers do not prefer. These results indicate that, despite producing larger eggs, heavier pullets may be less able to produce egg mass more efficiently.

Key Words: Hen, Weight, Pullet, Egg, Performance

P201 Egg quality evaluations of commercially available table eggs Cirenio Hisasaga*[UG], Katy Tarrant *California State University, Fresno*

Increase demand in consumer choice has resulted in a wide variety of egg selection available in the retail market. Specialty designer eggs, including those that have been nutritionally supplemented, or are produced from hens with various levels of enrichment in their environment, represent a portion of the table egg market, which is increasing in size. In this study, egg quality characteristics were analyzed using a one-way analysis of variance and Tukey's test to evaluate one commercially available conventional egg brand (Brand A) and five designer egg brands (Brand B, C, D E, and F). Data collection occurred 15 days prior to expiration, and eggs were sampled twice over a seven-month span. No significant variations exist between the same brand at different sample times, or between brands in yolk color, yolk tack force, egg width, and shell compression force. Brand A was significantly smaller in weight, weighing 4.22 g less than all other brands ($p < 0.0001$), while having a significantly larger egg yolk to total egg weight ratio when compared to all other egg brands at 29% ($p = 0.026$). Each brand met AA grade standards; however, Brand C Haugh units were scored lower within the AA grade compared to all other brands by at least 16.6 units ($p < 0.01$). Ultimately, the measures of quality used in this study is essential for evaluating the sustainability of the specialty egg market.

Key Words: Egg quality

P202 Live pigeon pox virus vaccination increases parthenogen size in virgin Chinese painted quail (Coturnix chinensis) Reshma Ramachandran*, Midian Nascimento dos Santos, Christopher McDaniel *Mississippi State University*

Parthenogenesis is the embryonic development of unfertilized eggs. Virgin quail hens exhibiting parthenogenesis have reduced reproductive performance following mating. Also, certain viruses were known to increase the incidence of parthenogenesis as well as parthenogen size in chickens and turkeys during the 1960s. However, no modern information is available on the effect of current virus vaccine strains on parthenogenesis in poultry. Thus, the objective of this study was to determine the effect of live pigeon pox virus vaccination on parthenogenesis in virgin Chinese Painted quail hens. Two selected lines of quail, 1 line selected for parthenogenesis (P-line) and 1 line not selected for parthenogenesis (C-line), were utilized in a 2 x 2 factorial arrangement of vaccinated and non-vaccinated hens to create 4 treatments. The vaccinated hens were administered a commercially available live pigeon pox virus vaccine via wing web route at 5-6 wk of age. Eggs were collected daily, stored for 0 to 3 d at 20C, and incubated at 37.5C for 10 d. Eggs were weighed before and after incubation to obtain percentage egg weight loss. After incubation, parthenogen size, albumen pH, and shell thickness as well as albumen, yolk, and shell weights were obtained. Data were analysed as a completely randomized design. Hens from the P-line yielded larger parthenogens (P = 0.02) and lower albumen pH ($P = 0.01$) versus C-line birds. Vaccinated birds exhibited a larger absolute parthenogen size (P = 0.08) as well as a greater parthenogen size relative to egg (P = 0.06), yolk (P = 0.03), and albumen (P = 0.08) weights. Additionally, vaccinated birds had a lower percentage egg weight loss ($P = 0.001$) versus non-vaccinated birds. In conclusion, it appears that vaccination of virgin hens with live pigeon pox virus has the potential to increase parthenogen size either by acting directly or indirectly on the ovary. As vaccination for pox is a routine practice in the modern poultry industry, it is possible that pox vaccination of birds carrying the parthenogenetic trait will have a negative impact on overall fertility and hatchability.

Key Words: Parthenogenesis, pox, vaccination, quail, embryo

P203 Influence of environmental conditions upon the occurrence of locomotor problems in heavy male turkeys Vinícius Nickel*, Thais Pereira, Tamara Ferreira, Isabella dos Santos, Carla Leite, Liris Kindlein *UFRGS*

Environment can influence the occurrence of leg abnormalities in poultry farms. This study aims to assess the influence of population density (PD), litter condition (Reuses of Litter – RL and Litter Moisture Content – LMC) and environmental temperature (TºC) and moisture (MO) upon the Gait Score (GS) and the occurrence of Footpad Dermatitis (FPD), Arthritis Degree of both legs (AD) and Arthritis Occurrence (AO) in heavy male turkeys. To this, 18 turkey farms were visited and in each one of them it was obtained information about PD and RL. Environmental temperature and MO were checked with a digital thermo-hygrometer and the litter was graded according to the LMC, as follow: 1 – dry litter; 2 – mild moisture content; 3 – wet litter. Gait Score was randomly assessed in 30 toms per farm (n=540) based on the following criteria: 0 – absence of any reluctance to move; 1 – small reluctance to move; 2 – same changes of degree 1 plus mild swelling or mild valgus or varus; 3 – staggered movement, dropped keelbone, able to stand or move for more than 30 s; 4 – failure to stand or move for more than 30 s; and 5 – complete recumbency or only able to stand or move for a few seconds. After, all toms were evaluated for FPD, as follow: 0 - no footpad dermatitis; 1 - <25% of footpad affected; 2 - ≥25% and ≤50% of footpad affected; 3 - >50% of footpad affected. It was assessed the AD in the hock joints in 3 toms per farm (n=54), using the following criteria: 0 – absence of volume increase; 1 – volume increase with no color change; 2 – volume increase and focal color change; 3 – volume increase and extensive area of color change. Means of PD, RL, T°C and MO were analyzed by 1-way ANOVA and t-test (SPSS) and were significantly different when $P \leq 0.05$. Association ($P \leq 0.01$) between LMC and GS, FPD, AD and AO was assessed by chi-square test (SPSS). The Gait Score 0 was associated with lower PD and RL and higher MO. The Footpad Dermatitis 0 (in both legs) was associated with lowest PD and RL and highest MO. The Litter Moisture Content 1 was more associated to GS 0,1 and 2 and with right leg AD 0 and FPD 0 (both legs); also was less associated with AO. In conclusion, PD, RL, MO and LMC seems to play an important role in development of locomotor problems in heavy male turkeys.

Key Words: environmental conditions, Arthritis Occurrence, turkey, locomotor problems, Footpad Dermatitis

P204 Evaluation of body weight, leg and ultrasonographic measures in heavy male turkeys with different degrees of gait score and arthritis Vinicius Nickel*, Thais Pereira, Isabella dos Santos, Carla Leite, Liris Kindlein *UFRGS*

The prevalence of locomotor problems such as arthritis in turkeys has become a major concern for the producers and is leading to condemnations at the slaughterhouse. The aim of this study was to evaluate the hock joints (HJ) ultrasonographical measurements (Synovial cavity diameter – SCD and Joint Surface Thickness – JST), as well as Body Weight (BW) and leg measurements (Hock Joint Width – HJW and Hock Joint Thickness – HJT) in heavy male turkeys according to Gait Score (GS), Arthritis Occurrence (AO) and Arthritis Degree (AD). To this, 18 male turkey's farms were visited and 30 male turkeys of each farm (n = 540) were randomly evaluated for GS based in: 0 – absence of any reluctance to move; 1 – small reluctance to move; 2 – same changes of degree 1 plus mild swelling or mild valgus or varus; 3 –staggered movement and dropped keel bone, being able to stand or move for more than 30 s; 4 – failure to stand or move for more than 30 s; and 5 – complete reluctance or only able to stand or move for a few seconds if pushed to walk. After this, 54 male turkeys were selected for BW, HJW and HJT measurements. HJW and HJT were measured in both legs using a pachymeter. The same turkeys were after evaluated for AD in both HJ (right and left) following the criteria: 0 – normal; 1 – volume increase with no color change; 2 – volume increase and focal color change; 3 – volume increase and extensive area of color change; then, these turkeys were evaluated for SCD and JST in both HJ. Statistical analysis was performed using ANOVA and means were significant when $P < 0.05$ according to t-test, using SPSS. Lowers GS and AD were associate to lower BW and lower HJW and HJT (in both legs). Turkeys with lowers BW, HJW, and HJT had as well as lower AO. Turkeys classified as AD 0 and 1 in the right leg also presented lower SCD than turkeys with AD 2 and 3. In conclusion, higher BW and leg measures (HJW and HJT) seems to be related to occurrence and severity of arthritis in heavy turkey males, as well as with reduced walking ability.

Key Words: turkey, gait score, hock joints, arthritis, locomotor problems

Processing and Products

P205 The influence of genetics, housing system and storage time on changes to breast meat color Anthony Pescatore*, Jacqueline Jacob, Gregg Rentfrow, Michael Ford, Tatijana Fisher, Sunday Adedokun, Tuoying Ao *University of Kentucky*

This study compared the change in meat color for breast meat from four different sources (commercial broiler strain (BR), Barred Plymouth Rock (BPR), Rhode Island Red (RIR) and Black Australorp (BA)) finished on the floor or on pasture and stored for up to 7 days at 4°C. The values of L* (lightness), a* (redness) and b* (yellowness) for meat color were determined at 0, 3, and 7 days of storage with a Hunter Miniscan Colorimeter set to D65/10°. After 3 days of storage, the breast meat from the 3 alternative breeds became significantly lighter. The BR breast meat, however, became darker. There was no breed effect from 3-7 days of storage. Overall, the change in the L* values was significantly less for the BR than for the alternative breeds. Similarly, after 3 days of storage the breast meat of the 3 alternative breeds became less red, while that of the BR became redder. There were no significant differences among the among the breeds in the change in the a* value from 3-7 days of storage. Overall, the decrease in the a* values was significantly less for the BR breast meat than of the other 3 breeds. After 3 days of storage, the breast meat from the alternative breeds increased in yellowness more than that of the meat from the BR. Over the 3-7 days, there was no change in the yellowness of the BR meat, while it continued to increase for the alternative breeds. Overall, the increase in yellowness was double for the alternative breeds compared to the BR. After 3 days, there was no effect of finishing location on the lightness or redness values of the breast meat. The breast meat of the chickens finished on the floor, however, became more yellow than for the breast meat of those finished on pasture. Over the 3-7 days, there was no effect on yellowness or redness, but the decrease in the lightness of the color of the breast meat from the chickens raised on the floor was less than that of the chickens finished on pasture. There was no effect of sex on the changes in the lightness, redness, or yellowness throughout the 7 days of storage. The fact that poultry meat color changes during storage is well established in the literature. There was considerable variation in the change in L*, a* and b* values of the breast meat depending on the breed and location.

Key Words: Colr, Pasture, Breed

P206 Meat color of breast meat from broilers and alternative breeds finished on the floor or on pasture Jacqueline Jacob*, Anthony Pescatore, Gregg Rentfrow, Michael Ford, Tatijana Fisher, Sunday Adedokun, Touying Ao *University of Kentucky*

This study looked at the color of breast meat from broilers (BR) and three alternative breeds (Rhode Island Red (RIR), Barred Plymouth Rock (BPR), and Black Australorp (BA)) raised in floor pens or finished on pasture. All chicks received the same diet. At the end of the trial (7 wk for BR and 14 wk for alternative breeds) breast meat from 3 males and 3 females per treatment were sampled. Fillet color (lightness (L*), redness (a*), and yellowness (b*)) was determined after 0, 3, and 7 d of storage at 4°C. Prior to storage, there were significant differences in L*. BR breast meat was significantly lighter, followed by RIR, BPR and BA. There was a significant breed x sex interaction for L* after 3 d, but not for 7. At 3 d, males had lighter meat than the females for the BPR and BR, with the reverse for the other 2 breeds, but the differences were not significant. Breast meat from BR and RIR had similar lightness and were significantly different from that of BA, with BPR being intermediary. There was a significant breed x sex interaction effecting a* prior to cold storage, but by 3 d the interaction was no longer significant. The breast meat from BR and BA chickens was redder than that of RIR, with BPR being intermediary. By 7 d, the meat from BR and BA was significantly redder than that of RIR and BPR. There were significant breed x sex interactions on b* at all 3 times. For the meat from RIR and BA, there was more yellow color in the females than in the males, but there were no differences between the sexes for the other 2 breeds. This was consistent through the 3 time periods. There was a significant location x sex effect on L* values at 0 d and 3 d, but with no significant differences among the means. After 7 d, the males on the floor had lighter meat than the males on pasture with the females being intermediary. There was a significant location x sex interaction on a* at 0 d, but by 3 d the meat from those on the floor had significantly more red color than from those on pasture. There was a significant location x sex interaction effecting b* values at all 3 times. Females on pasture had significantly higher b* values than all the other chickens. Meat color varies with breed, sex and location and may affect consumer acceptance

Key Words: Color, Pasture, Breeds

P207 Broiler carcass aging, an effective method to improve tenderness of breast fillets from fast-growing big broilers Meredith Johnson*, Avery Smith, Laura Bauermeister, Allan Pinto, Ada Madrid, Jasmine Kataria, Amit Morey *Auburn University*

Big broilers are affected with wooden breast leading to quality issues such as tough meat texture. Toughness is possibly enhanced due to deboning broilers at 2-3 h post-mortem with incomplete resolution of rigor mortis. In a two-part study, we investigated the effects of delayed deboning times on meat quality and optimized them to obtain tender fillets. Breast fillets deboned at 2, 16, 20 and 24 h from big broilers (n = 90) were compared to normal, severely woody, and medium sized fillets. Data indicated that the texture of extended deboned fillets was tender (significantly low toughness values) when compared to woody fillets. There was no statistical difference between cook loss, color, and Blunt MORS (BMORS) ($p<0.05$) when compared to each other. Another study was conducted to evaluate if less than 16 h deboning times can be used to improve meat quality. Toughness values significantly reduced ($p<0.05$) with an increase in deboning times from, 2 to 26 h. Peak force and shear energy values at 0h decreased ($p<0.05$) correspondingly from 21.86 N and 264.15 N.mm to 16.16 N and 205.17 N.mm after 10 h post-mortem. In conclusion, extended deboning time of from 6-26 h can significantly help improve texture of breast fillets from fast-growing broilers.

Key Words: Wooden Breast, texture, debone, aging

P208 Synergistic effect of dietary inclusion of organic trace minerals and synthetic antioxidants in reducing wooden breast in 41d old broilers fed diets containing oxidized fat Vivek Kuttappan*, Megharaja Manangi, Juxing Chen, Mercedes Vazquez-Anon, Kim Walter, Alex Corzo, Bryan Fancher *Novus International Inc.*

Wooden breast (WB) is a degenerative myopathy of unclear etiology causing increased hardness on raw breast fillets leading to economic loss to the modern broiler industry. Our previous study showed that feeding oxidized fat increased incidence of severe degree of WB in broiler birds and dietary inclusion of antioxidant (SANTOQUIN®M6 with 66.6% Ethoxyquin (ETX) at 125ppm) reduced oxidative stress and tissue damage in muscle leading to reduced incidence of severe WB. The objective of the present study is to evaluate the effect of dietary inclusion of combination of organic trace minerals and synthetic antioxidant on the incidence of WB in broilers fed diets containing oxidized fat. Day old YPM x Ross-708 male chicks (n=1080; 15 chicks/pen; 18pens/treatment) were randomly assigned to 4 treatments: inorganic trace minerals (sulfates of Zn, Cu, and Mn at 110, 20, and 120ppm, respectively) without ETX (T1); T1 with ETX (T2), organic (HMTBA-chelated) trace minerals (MINTREX® Zn, Cu and Mn at 40, 25, and 40 ppm, respectively) without ETX (T3), and T3 with ETX (T4). Other than the treatments, the basal diets were nutritionally equivalent across the trial and contained oxidized soy oil with around 5meq peroxide/kg in the starter, and 7meq peroxide/kg in the grower and finisher diets. On 41d, 2 birds/pen were processed to score (0 to 3) the severity incidence of WB and muscle samples were collected to determine

Thiobarbituric acid reactive substances (TBARS) levels. Performance and TBARS data were analyzed using ANOVA and means were separated with Fisher's protected LSD test, while the proportion of individual WB scores were analyzed as binomial random variables using the SAS GLIMMIX procedure. At 41d, there were no differences (P>0.05) in body weight and cumulative feed intake between the treatments, however T2, T3, and T4 showed improved (P<0.05) cumulative feed conversion ratio and performance index compared to T1. Percentage of birds without WB were higher (P<0.05) and muscle tissue TBARS levels were lower (P<0.05) in T4 compared to T1. The results from the trial suggest that there is a synergistic effect between chelated organic trace minerals and synthetic antioxidants in reducing the oxidative stress in muscle tissue and increasing percentage of birds without WB.

Key Words: Myopathy, minerals, wooden-breast, antioxidants, broilers

P209 Image analysis as a potential tool for objective identification of Woody Breast characteristics in 8 week old commercial broiler carcasses Juan Caldas-Cueva*, Barbara Mallmann, Casey Owens *University of Arkansas*

Image analysis could be an objective, rapid and reliable tool to identify woody breast (WB) myopathy and reduce the significant economic loss to poultry producers. The aim of this study was to evaluate the relationships of broiler carcass and breast fillet measurements: carcass image dimensions, instrumental texture measurement, and palpation scores. Images of 8-wk male broiler carcasses (n=156) of high breast yielding commercial strain were captured prior to evisceration. Whole breast fillets were scored for WB severity based on tactile evaluation (0 or 0.5 as normal; 1 or 1.5 as mild, and 2, 2.5 or 3 as severe), and the instrumental texture of these were measured using the compression force method. Broiler carcass images were processed and analyzed using ImageJ software. Parameters for carcass conformation were M1: breast width in the cranial region; M2: a vertical line from the tip of keel to 1/5th of breast length; M3: breast width at the end of M2; M4: angle formed at the tip of keel and extending to outer points of M3; M5: area of the triangle formed by M3 and lines generated by M4; M6: area of the breast above M3; M7: M6 minus M5. Spearman correlation coefficients were estimated for WB severity scores, compression force and image measurements. ANOVA of broiler carcass dimensions across WB categories were also carried out. M4 (angle at keel) and M3 (caudal width) had the highest correlation to WB score (r=0.78 and 0.72, respectively; P<0.01) and compression force (r=0.71 and 0.65, respectively; P<0.01) followed by measurements M6, M7, M5 and M1, respectively (r=0.47 to 0.69, P<0.01), whereas M2, showing the lowest coefficients, was inversely correlated with WB score (r=-0.27, P<0.01) and compression force (r=-0.19, P<0.05). With exception to M2, measurements increased (P<0.05) as WB severity increased. These data support the feasibility of image analysis method to predict WB condition in broiler carcasses. The potential integration of these image measurements into current in-line vision grading technology would allow processors to identify and sort broiler carcasses by WB category. Nevertheless, further study is required to validate relationships when broilers from other strains and gender are included.

Key Words: wooden breast, image analysis, processing, in-line carcass control, meat quality

P210 Effect of Acid-Pak 4-Way® Liquid Hard Water Formula as a water acidifier for use in Canada Nicole Weidner, Kayla Price* *University of Guelph*

Water quality has an impact on gut health and is an often-overlooked aspect of livestock production. Low pH levels in the water have been shown to help monogastric animals maintain homeostatic intestinal pH levels. The efficacy of Acid-Pak 4-Way Liquid Hard Water Formula (AP-4WHW) was tested as a water acidifier for use in Canada. Water sample samples were collected from 3 geographic locations in Canada (Eastern, Central and Western). The water samples were obtained directly from water wells with no additional acidifier in the wells located on farms. The treatments were: 1) control with no supplementation; and 2) control water with AP4WHW (added at 2mL/L). There were 5 replicates of each treatment group per geographic location for a total of 30 samples. Samples were analyzed for pH levels according to standard laboratory procedures. Data were analyzed for statistical significance (p<0.05) using ANCOVA (SPSS). There was a significant effect of treatment (p=0.029) and location (p<0.001) on the pH of samples. The mean pH values by treatment are presented for each location. The mean control pH from Eastern, Central and Western Canada were 8.55, 7.90 and 7.93, respectively. The mean pH of the groups treated with AP4WHW from Eastern, Central and Western Canada were 5.61, 4.61 and 6.41, respectively. The combined pH values pre- and post- treatment for all the samples was 8.13 and 5.54, respectively (p<0.001). The addition of AP4WHW at 2mL/L in Canadian livestock drinking water has been demonstrated to effectively reduce the pH of water in Eastern, Central and Western Canadian locations.

Key Words: acidification, water, monogastric

P211 A protein and microbiological survey comparing organic and traditional eggs Linda Purvis*, M Miller, Swapna Bhat *University of North Georgia*

This undergraduate research study aimed to look at clearing the confusion surrounding egg labeling. Consumers can be confused by the cornucopia of options and variety of labels on egg cartons in grocery stores. One of the most common concerns is the difference between organic and traditionally produced eggs. Consumers have been encouraged by advertisers to equate an organic product to superior quality, higher nutritional content, and, therefore, a healthier option for consumption. In addition, organic products tend to be more expensive than traditionally produced foods. Is this perception based on fact? Are there nutritional differences between organic and traditionally produced products? If nutritional differences exist, then to what extent? Do these differences justify the extra expense? To begin to answer these questions we looked at microbial differences and compared protein content between two locally purchased egg products. "Store brand" eggs and organic eggs were purchased from a local grocer. We then used standard microbiological and biochemical analytical methods to compare these products. First, a microbiological survey was performed to see if there were any differences between the egg types. Second, the protein content of the eggs was compared by UV-vis spectroscopy, SDS-PAGE electrophoresis, and Bradford Protein Assay to determine if there are any quantifiable differences in protein content or the type of protein in each kind of egg. After collecting data, and running statistical analysis our findings indicated that there are no statistical differences in the microbial environment or protein content of the eggs. Future work will involve looking at fatty acid content as well as expanding our egg types to free-range and cage-free marketed eggs.

Key Words: Eggs, Microbiology, Protein

P212 Nutritional and physical characteristics of eggs from indigenous chicken breeds Madalena Lordelo*[1], Joana Cid[2], Rui Bessa[3], Inês Carolino[4] *[1]Instituto Superior de Agronomia, Universidade de Lisboa; [2]Universidade de Lisboa; [3]Faculdade de Medicina Veterinária, Universidade de Lisboa; [4]Instituto Nacional de Investigação Agrária e Veterinária*

There is an increased interest in animal products from more sustainable farming practices, which may include using local breeds. Eggs and meat produced by indigenous breeds of poultry is a compelling option for the European consumer. In addition, maintaining biodiversity is important and naturally, native breeds of chickens are well adapted to the local environmental conditions. However, little research attention has been given to these breeds and their products. In the current study, 286 eggs, from four Portuguese breeds of chickens (Branca, Amarela, Pedrês Portuguesa and Preta Lusitânica) and, for comparative purposes, from a commercial hybrid laying hen, were used. The chemical and physical characteristics of the eggs and the egg components were analyzed such as weights,

Haugh units, yolk color, albumen protein content, yolk fatty acid content, and mineral content in the albumen and yolk. It was found little variation between the parameters measured amongst the four different chicken breeds. However, the Branca breed produced eggs that were heavier, with a lighter brown shell color and lower Haugh unit values in comparison to the remaining native breeds ($P < 0.05$). The commercial hens produced eggs that were found to be more rounded shape than the ideal and with a darker colored shell and yolk in comparison to eggs from the four native breeds. In addition, the commercial hens also produced heavier eggs and with lower Haugh units in comparison to the Amarela, Pedrês Portuguesa and Preta Lusitânica breeds ($P < 0.05$). No differences were found in the mineral, protein, and fatty acid content of eggs from the different origins. The overall chemical and physical analysis indicated that eggs from these native breeds match the quality of a commercial product in many characteristics. Within specialized market niches where this type of eggs are available, consumers are purchasing a high quality product while investing in local farmers and maintaining biodiversity.

Key Words: Egg, Hen, Breeds, Quality, Biodiversity

P213 Application of Functional Ice to Prevent Spoilage of Raw Poultry during Storage and Transportation Jasmine Kataria*, Ada Sandoval, Meredith Johnson, Avery Smith, Laura Bauermeister, Amit Morey *Auburn University*

Existing antimicrobial intervention in the poultry industry include spray or dip treatments of mostly peracetic acid (PAA) which does not possess residual antimicrobial effect to reduce spoilage and improve food safety during storage and transportation (ST). Hence, there is a need to develop novel "sustained antimicrobial release mechanisms" which can suppress the growth of spoilage microorganisms as well as further reduce pathogen load during storage and transportation. We propose to develop, "Functional Ice" (FICE) which will not only provide a cooling effect as it melts but also will sustainably release antimicrobials resulting in suppression of spoilage microorganisms and reduce pathogens during ST. A proof-of-concept experiment was conducted to determine the efficacy of FICE to suppress spoilage microorganisms in fresh poultry.

Ice manufactured with PAA (75 and 150 ppm) with or without adjusting pH to 7.5 (using NaOH) was applied (1:1 ratio) on freshly deboned breast fillets obtained from a local processor. Samples were stored for 9 h and analyzed for color (L*a*b*), aerobic plate count, coliform and lactic acid bacteria. Statistical differences were determined using ANOVA ($p<0.05$).

Data indicated that FICE prepared using PAA at 75 and 150 ppm with or without pH adjustment did not affect ($p>0.05$) color, aerobic plate counts and coliform. However, FICE manufactured with PAA (150 ppm) and pH adjusted PAA treatments (75 ppm and 150 ppm) significantly ($p<0.05$) suppressed lactic acid bacteria, a specific spoilage microorganism.

The FICE concept can be further improved to reduce spoilage, improve shelf-life and food safety during storage and transportation.

Key Words: Functional ice, Spoilage, poultry, storage, transportation

P214 Investigation of novel Yersinia enterocolitica-specific bacteriophage isolated from poultry farm as a biocontrol agent Kyoung Gwak*, Om Choi, Hae-Yeong Lee, Mi-Kyung Park *School of Food Science and Technology Kyungpook National University*

Recently, bacteriophages have gained more attentions as a biocontrol agent due to their high level of specificity against target pathogens. In previous studies, *Yersinia enterocolitica*-specific bacteriophages (referred to as KFS-YE) were isolated and purified from a poultry farm. The purpose of this study was to identify and investigate the purified KFS-YE as a new biocontrol agent. The morphological characteristics were observed by using TME. DNA of KFS-YE was extracted using a NORGEN® Phage DNA isolation kit and digested with various restriction enzymes (*Eco*RI, *Hind*III, and *Xba*I) prior to performing 0.4% agarose gel-electrophoresis. Structural proteins of KFS-YE were analysed by SDS-polyacrylamide gel electrophoresis (PAGE). The stability of KFS-YE was investigated by exposing it (10^8 PFU/mL) to various pHs (3, 5, 7, 9, and 11) and temperatures (4, 22, 37, 50, 60 and 70 ºC). An aliquot of 100 µL of KFS-YE (10^8 PFU/mL) was then exposed to 900 µL of various organic solvents including ethanol and isopropanol. The purified KFS-YE consisted of icosahedral head (diameter of 102.4 ± 6.1 nm and length of 118.4 ± 11.6 nm) and a contractile tail (125.7 ± 15.8 nm), classified into *Myoviridae*. Genomic DNA of KFS-YE was digested by *Eco*RI treated restriction enzymes, however it was not digested by *Hind*III and *Xba*I. Three major protein bands and eleven minor protein bands were observed with molecular sizes of 36 to 170 kDa. The KFS-YE was stable over a range of pH and temperature of 4-11 and 4-50 ºC, respectively. KFS-YE was stable in ethanol and isopropanol but not chloroform. This study demonstrated the novelty and potential of KFS-YE as an efficient biocontrol agent against *Y. enterocolitica*.

Key Words: biocontrol, bacteriophage, Yersinia enterocolitica, poultry, novel

P215 Formulation of a selective medium for aerobic growth of Campylobacter Arthur Hinton Jr*, Nelson Cox *U. S. National Poultry Research Center, Agricultural Research Service, USDA*

A medium that can be used to aerobically culture *Campylobacter* has recently been described. However, since the medium is non-selective, it cannot be used to isolate this pathogen from samples containing other bacteria. Therefore, the objective of this study was to examine the effect of supplementing the medium with antibiotic mixtures on the growth of *Campylobacter* and other bacteria.

Basal medium containing beef extract, 50 g; tryptose, 10 g; soluble starch, 10 g; sodium lactate, 3.0 g; and agar, 0.5 g in 900 ml of distilled water was dispensed in 9 ml aliquots in screw capped test tubes and autoclaved. One ml of 1.5% sterile sodium bicarbonate was added to the cooled medium. The basal medium was supplemented with Bolton, Campy-Cefex, or Skirrow antibiotic mixtures.

The ability of *Campylobacter fetus*, *Campylobacter coli*, and *Campylobacter jejuni* to grow aerobically in basal medium and in supplemented medium was determined. Separate 10 ml aliquots of media in culture flasks were inoculated with each isolate, incubated aerobically at 37C for 48 h, then *Campylobacter* were enumerated on blood agar with Blaser-Wang antibiotic mixture with microaerobic incubation. Next, the ability of *Escherichia coli*, *Enterococcus faecalis*, *Listeria monocytogenes*, and *Salmonella* Kentucky to grow in basal and supplemented media was determined. After incubation, *E. faecalis* was enumerated on m-Enterococcus agar, *E. coli* on Levine EMB agar, *L. monocytogenes* on *Listeria* Selective Agar, and *Salmonella* Kentucky on XLT4 agar.

Results indicated that after aerobic incubation there was significant ($p < 0.05$) growth of all *Campylobacter* isolates in basal media and in media supplemented with antibiotic mixtures, except for *C. coli* in the Skirrow supplement. There was also significant growth of all other bacterial isolates in basal medium, significant growth of *E. coli* and *Salmonella* Kentucky in the Skirrow supplemented medium and growth of *E. faecalis* in the Cefex supplemented medium. There was no significant growth of any of these bacteria in Bolton supplemented medium.

Findings indicate that basal medium supplemented with the Bolton mixture is the best formulation for a selective medium for using aerobic incubation to isolate *Campylobacter* from samples containing other bacteria.

Key Words: Campylobacter, aerobic, incubation, selective, medium

P216 Evaluation of a high pressure wash cabinet to reduce bacterial load on broiler carcasses pre-scald, post-scald and post pick Douglas Cosby*, Joshua DeVoll, Nelson Cox, Mike McIntyre, Mark Berrang, Arthur Hinton, Jr. *U.S. Dept. of Agriculture, U.S. National Poultry Research Center*

Reduction of bacterial load on broiler carcasses being transferred from the slaughter line to the evisceration line is often overlooked as an intervention strategy to reduce or eliminate foodborne pathogens. The objective of this study was to evaluate a wash cabinet with high pressure, low volume, fluidic nozzles to reduce carcass bacterial load at three points, pre-scald, post-scald and post-pick. Fifteen carcasses (n=5 per sample site) were obtained from a processing plant, individually bagged, placed in a cooler and transported warm to the laboratory (USNPRC). Carcasses were placed in shackles, moistened pre-wash breast swabs were collected, carcasses were washed in the cabinet at 150 psi and 7.95 gpm following which post-wash breast swabs were collected. Swabs were placed on ice, transported to the laboratory where 10 mL of buffered peptone water was added; swabs were stomached for 60 s, and serially diluted. Appropriate dilutions were plated onto Petri-Film total aerobic (TAC), *Enterobacteriaceae* (Ent) and *E. coli* (TEC) plates and Campy-Cefex agar (CCA) plates for enumeration. Additionally, 1.0 mL of the sponge rinsate was transferred to 9.0 mL of BPW to pre-enrich for *Salmonella.* All PetriFilm® were incubated at 37°C for 24 h, CCA plates were incubated in a microaerobic environment at 42°C for 48 h and *Salmonella* pre-enrichment was incubated at 37°C for 24 h. After incubation, characteristic colonies on PetriFilm® and CCA plates were counted and recorded. The *Salmonella* pre-enrichments were analyzed by standard *Salmonella* recovery procedures and the results reported as present or absent. Typical colonies on CCA were confirmed as *Campylobacter* by latex agglutination and wet-mount microscopy. Paired t-tests were conducted and significant differences ($p<0.05$) were noted between the pre-wash and post-wash counts on the TAC, ENT, TEC and CCA for the post scald carcasses with reductions of 1.4, 0.9, 1.0 and 0.7 $\log_{10}$, respectively. On CCA, a reduction of 1.1 $\log_{10}$ was observed for post-pick carcasses which was significant ($p<0.05$). No *Salmonella* was recovered from any post-scald swabs but all other swabs were positive. This cabinet design has the potential to reduce the overall bacterial load entering the evisceration line.

Key Words: Bacteria, Salmonella, Campylobacter, Carcasses, Broiler

P217 Pulsed UV light as a Salmonella reduction intervention for boneless/skinless chicken thigh meat Joshua Cassar*[GS], Ali Demirci, Edward Mills *The Pennsylvania State University*

Salmonella is a pathogen of concern in poultry processing. In 2012, 4.3% of all chicken carcasses sampled by USDA tested positive for *Salmonella* species. Pulsed Ultra Violet (PUV) light is an effective antimicrobial treatment with only limited use in the food industry. Research using PUV light has established that it can be a more effective antimicrobial treatment than conventional UV light. UV wavelengths include a spectrum of 100 – 400 nm. The germicidal, UV-C wavelengths fall between 100 – 280 nm with the optimum germicidal effect at 254 nm. PUV light includes a much broader spectrum, 100 – 1100 nm, with 50% of the energy deriving from the UV region. Unlike the continuous, low intensity output of conventional UV light, PUV light is emitted in short bursts of very high intensity light. Previous research has shown that PUV light treatment can produce measurable bacterial reductions in various food products. The current project has investigated application of PUV for destruction of *Salmonella* on chicken thigh meat. To validate the PUV light system, inactivation of *Salmonella* in peptone water was tested using treatment times of 5 to 15 seconds. Complete inactivation was observed for all treatment times ($p<0.05$). To evaluate PUV effectiveness in a more complex system, boneless and skinless chicken thighs were inoculated with 6-7 log/cm^2 *Salmonella* before exposure to PUV. Treatment variables included the distance from the quartz window of the PUV light, 8 and 13 cm, and application time, 5, 15, 30, and 45 seconds. Six thighs were used for each treatment. An overall 2-way ANOVA with predicator variables, distance and treatment time, showed a significant reduction in bacteria with average of 2.15 log reduction ($p<0.05$). The evaluation of each treatment variable, while holding the other variable constant, showed no significant difference due to distance. There was a significant difference due to treatment time ($p<0.05$). In a Tukey multiple comparison test a significantly greater reduction was observed at 45- second versus 5- second treatment time ($p<0.05$). In conclusion, this study clearly demonstrated the potential of using PUV light as a microbial intervention on chicken meat.

Key Words: chicken, decontamination, pulsed ultra-violet light, Salmonella

P218 Campylobacter prevalence in retail chicken liver Tori Thompson*[UG], Mark Berrang, Nelson Cox, Richard Meinersmann *USDA-ARS-US National Poultry Research Center*

f. campylobacteriosis has been linked to undercooked chicken liver. It is unknown how commonly chicken livers are contaminated with *Campylobacter*. The objective of this study was to determine the prevalence of *Campylobacter* on chicken livers available at retail. For each of five weeks, two to four packages of chicken livers (total of 15), each representing a unique combination of processing plant and sell by date, were purchased at local supermarkets. From each package, three separate, whole livers were selected for sampling (n=45). Each liver was sampled by three methods, first a swab was used to sample the outer surface. Then, the surface was seared followed by sampling of the inner tissue by a swab stabbed through the sterilized surface. Finally, each liver was placed in 50 mL of enrichment broth and blended in a paddle blender; blended whole liver was sampled by swab. All swabs were used to directly apply sample to the surface of campy-cefex agar (CCA). Each swab and blended liver was also enriched in 10 mL *Campylobacter* enrichment broth 24 h at 42°C; the enriched sample was plated on CCA. All CCA plates were incubated 48 h at 42°C in a micro-aerobic atmosphere. Plates were examined for characteristic *Campylobacter* colonies which were confirmed by observation of cellular morphology and motility under phase contrast microscopy and positive reaction to a *Campylobacter* specific latex agglutination test. *Campylobacter* was detected on chicken livers: 64% of outside samples, 31% of inner tissue samples, and 64% of whole liver samples were positive for *Campylobacter*. To prevent campylobacteriosis, chicken livers should be fully cooked before consumption.

Key Words: Campylobacter, liver

P219 Rapid determination of multiple quality parameters in rendered poultry fat using Fourier transform near infrared spectroscopy Jason Erickson* *Bruker Optics*

Rendered poultry fat is an important ingredient in the feed industry and quality control of poultry fat is critical. Increased free fatty acid (FFA) levels can reduce the digestibility and overall energy content of poultry fat and excess moisture can promote oxidation and rancidity.

The traditional wet chemical methods used for these analyses are labor intensive and time consuming. Fourier Transform Near Infrared (FT-NIR) spectroscopy, by comparison, offers a rapid method with minimal sample preparation required for analyzing multiple quality parameters in poultry fat.

Partial Least Squares (PLS) calibration models for FFA and MIU's (moisture, insoluble impurities and unsaponifiable matter) were developed for poultry fat. FT-NIR spectra of representative samples were measured and the corresponding reference values (as determined by official AOCS methods) for each parameter were obtained for model development.

Leave-one-out cross validation was used to create the models, yielding the following statistics:

FFA: RMSECV = 0.2, R^2 = 99.19, Range = 0.5 – 13.1

Moisture: RMSECV = 0.03, R^2 = 92.16, Range = 0.01 – 0.53

Insoluble Impurities: RMSECV = 0.03, R^2 = 93.73, Range = 0.06 – 0.76

Unsaponifiable Matter: RMSECV = 0.09, R^2 = 83.75, Range = 0.32 – 1.24

The data set was also divided into *calibration* and *test* samples and calibration models for FFA and moisture were created from the calibration set and used to predict the validation set, yielding the following statistics:

FFA: RMSEP = 0.2, R^2 = 99.60.

Moisture: RMSEP = 0.03, R^2 = 91.43.

Overall, FT-NIR spectroscopy was shown to provide accurate results for samples with values within the range of suggested quality control specifications for poultry fat. The robustness of the FFA and moisture models was demonstrated by the ability to remove half of the samples and predict them as true unknowns with virtually the same statistics as the cross validated models. This technique could be used for routine analysis of multiple quality parameters of poultry fat.

Key Words: Rendering, Quality, FT-NIR, Ingredients, Analysis

Pathology

P220 Incidence and degree of Salmonella Heidelberg colonization of day old broiler chicks using several methods of inoculation Ade Oladeinde*[1], Kim Cook[1], Nelson Cox[1], Casey Ritz[2], Douglas Cosby[1], Jodie Plumblee Lawrence[1], Sandra House[1], Gregory Zock[1], Jeromey Jackson[2] *[1]U.S. National Poultry Research Center; [2]University of Georgia*

Before beginning a study that involves a large number of birds, it may be helpful to know what method of inoculation would be best for the experiment in question. The objective of this study was to compare several methods of *Salmonella* challenge (oral gavage, intracloacal inoculation and the seeder bird approach). Day-old broiler chicks (n=100) were obtained from a commercial hatchery and inoculated either orally, intracloacally or using seeder birds with 10^6 cells of a nalidixic acid resistant strain of *Salmonella* Heidelberg (SH). Chicks (n=25) inoculated by each route were placed in floor pens at a stocking density of 650.3 cm2/chick on fresh pine shavings litter. For the seeder batch, 5 colonized chicks were placed with 20 pen mates. All birds were given water and feed *ad libitum*. Two weeks after inoculation, 10 birds from each pen were euthanized, the abdominal cavity was sprayed with 70% alcohol and the ceca were aseptically removed, placed in a stomacher bag, put on ice and brought to the laboratory for analysis. Next the ceca were weighed and buffered peptone water was added 3X volume to weight and mashed with a rubber mallet. Serial dilutions were made and plated onto BG Sulfa plates containing 200 ppm nalidixic acid. The plates were incubated along with the smashed ceca and broth for 24 h at 37˚C. If no colonies appeared on the plates then an additional plate was streaked from the enriched broth bag and it was incubated for an additional 24 h at 37˚C. Number of SH positive birds out of 10 sampled in each group was 5, 8, 5 for oral gavage, intracloacal and seeder, respectively. Following 24 h enrichment it was 8, 10, 7. The level of SH per gram of ceca was log (standard error) 2.45 (0.42), 1.66 (0.22), 2.87 (0.28) for oral, intracloacal and seeder, respectively. Also, the level of the SH per gram of litter for the different groups was log 6 for oral and intracloacal and log 4.6 for the seeder bird group. In conclusion, this study suggests that intracloacal is the method to use if you want to make sure all of the challenged birds are colonized. However, if you prefer to have a smaller percentage of the birds colonized with higher levels, then oral or seeder bird challenge may be better.

Key Words: inoculation, Salmonella, Heidelberg, chicks

P221 Outbreaks of gangrenous dermatitis in poultry chickens in Argentina enzo redondo*[GS], leandro redondo, fernando delgado, mariano fernandez miyakawa *conicet*

Gangrenous dermatitis (GD) is a disease of turkeys and chickens caused by *Clostridium perfringens*, *C. septicum and/or Staphylococcus aureus*. Sudden and quick rise in deaths is often a first signal of a GD outbreak. The disease is characterized by necrosis of different skin areas and severe cellulitis of the subcutaneous tissue, in breast, abdomen, wing, or thigh. This work describes the pathological and bacteriological findings of chickens from 4 outbreaks from different farms. The disease occurred endemically with low morbidity and summer mortality peaks. Affected animals were 35 d or older, corresponding to the shift of feed wit no antibiotic. In each of these outbreaks, a complete necropsy of 3 healthy birds and 4 with clinical signs was performed. In all cases, sick animals showed gangrenous foci prone to crackling with dark skin in ventral region, which extended caudally. Subcutaneous tissue showed an extensive blood-tinged edema and emphysema. The muscle was observed discolored, with a grayish color that extended in depth and hemorrhagic stippling surface. Paired samples from each animal skin and subcutaneous tissue, pectoral muscles and different portions of the intestine were taken for bacteriological and histopathological studies. Bacteriological analysis resulted in colonies positives for *C. perfringens* and *C. septicum,* confirmed by biochemical tests and PCR. The duodenum of all sick animals presented congestive mucosa, with muco-hemorrhagic content in some cases. Microscopic changes were characterized by nonsuppurative lymphangitis and edema, nonsuppurative panniculitis with bleeding and presence of Gram positive bacilli in skin and subcutaneous. The underlying skeletal muscle presented predominantly interfascicular mononuclear infiltrate, muscle fibers showing different degrees of vacuolar degeneration, hyaline and even coagulation necrosis. In duodenal mucosa, villus atrophy and pseudomembranes was observed with thickening of lamina propia and mononuclear inflammatory infiltrate with few heterophils, and diffuse bleeding areas. A diagnosis of dermatitis/panniculitis gangrene of a co-infection of *C. perfringens* and *C. septicum* is suggested. Further work is necessary to test effective strategies to control GD in endemic areas of poultry production.

Key Words: gangrenous dermatitis, clostridium, poultry

P222 Erysipelothrix rhusiopathiae vegetative endocarditis in a backyard rooster: case report and discussion Fernando Ruiz Jiménez*, Luary Martínez Chavarría, Rigoberto Hernández Castro, Félix Sánchez Godoy *Department of Poultry Science; College of Veterinary Medicine; National Autonomous University of Mexico*

Erysipelas is a septicemic disease which generally occurs sporadically in various avian populations. It is worldwide in distribution and the infection and disease in birds have been described as an acute, fulminating disease, even though, the chronic form occasionally occurs after acute outbreaks. Erysipelas also affects a wide variety of mammalian species and has been reported in reptiles and amphibians. In the present report, we describe a chronic systemic infection by *Erysipelothrix rhusiopathiae* in a 2 years-old backyard rooster (*Gallus gallus).* The bird showed respiratory disorders and was emaciated. In the pathologic examination, we observed severe chronic diffuse airsacculitis, chronic vegetative valvular endocarditis with chronic epicarditis and pericarditis, mucoid degeneration of pericardial fat and severe acute extensive necrotic hepatitis. A copious amount of gram-positive bacilli in the heart tissue and in the blood vessels of the lungs, spleen, liver, and kidneys were found in the histopathologic examination. This allowed us to guide the diagnosis towards Erysipelas. We also found PAS-positive, elongated and ramified mycotic structures in the lungs tissue consistent with Aspergillosis. The diagnosis was confirmed

by a duplex PCR test that differentiates *Erysipelothrix rhusiopathiae* vaccine strains and wild-type strains, based on the DNA polymerase IV gene. The results of this case are described and discussed in the present report.

Key Words: backyard, poultry, endocarditis, erysipelas

P223 Evaluation of long term immunity and protection against Salmonella spp by orally administrated inactivated vaccine Emanuel Gumina*[UG], Gonzalo Almaraz, Ariel Sugezky, Sherryll Layton *University of Buenos Aires, Department of Veterinary Sciences*

Salmonella is the leading cause of foodborne infections and is a major public health concern worldwide. Poultry meat and eggs are a major reservoir of foodborne *Salmonella* serovars and vaccination against the pathogen is one of the important measures to curb infectious outbreaks and reduce antibiotic use, currently a major concern for consumers. In this study we evaluated the efficacy of a commercially available inactive, orally administered, subunit vaccine (Biotech Vac Salmonella) and determined if the immune response was protective and persistent (long-term immunity) against mobile *Salmonella* spp. in two separate commercial layer hen flocks (n=120,000 layers/lot) located in Pergamino, Buenos Aires, Argentina. Pullets were given 2 doses of Biotech Vac Salmonella administered at day 3 and 16 post hatch; the third dose was administered in Lot 1 at 91 days post-hatch and in Lot 2 at 84 days post-hatch. Intestinal mucosal scrapings and serum were collected from 15 birds at 6, 21, 33,67 and 89 weeks post hatch in Lot 1 and 5, 13, 22, 56 and 78 weeks post-hatch in Lot 2. Biotech Vac Salmonella specific mucosal immune response (sIgA) and systemic immune response (IgY/IgG) was evaluated by a proprietary antigen capture ELISA and S/P ratios calculated to determine vaccine specific antibody response. Results show that at all sampling time points after the administration of Biotech Vac Salmonella there was significant ($p < 0.05$) sIgA mucosal and IgY/IgG serum antibody production that persisted for the duration of the field trials; 89 and 78 weeks respectively. Mucosal immune responses were considered protective when S/P ratios were greater than 2.0. Presence or absence of *Salmonella* spp was evaluated directly in the bird or in the environment by traditional microbiological culture methods. Throughout the course of the field trial there were no samples (birds or environmental) positive for *Salmonella* spp. This study provides strong evidence that vaccination with Biotech Vac Salmonella induces a strong mucosal and systemic immune response and protects poultry from *Salmonella* spp infection. Furthermore, this protective response provides long-term immunity important for long life poultry (layers and breeders).

Key Words: salmonella, immunity, layers, Mucosa

P224 Erythroplastids and reticulocyte atypia of duck blood – indications of dyscrasia Paul Cotter*[1], Debbie Jeffrey[2] [1]*Cotter Laboratory;* [2]*Maple Leaf Farms*

A majority of avian erythrocytes retains a nucleus during their lifespan; however, anuclear forms, "erythroplastids" can occur in blood. Some authors indicate they are common in smears of circulating blood; others associate erythroplastids with dyscrasia. Erythroplastids and their nucleated companions, "pyrenocytes" were routinely found in duck blood at ages from late stage embryos through intermediate ages and adults. It is the purpose to illustrate the variation of types. Size ranges from ~ 1µm dwarfs to giants > 12µm. Erythroplastid formation occurs at all developmental stages ranging from reticulocytes through fully hemoglobinized (orthochromatic) types. They are common in smears with high frequencies of reticulocytes. It is likely that erythroplastids develop by several mechanisms. Classical cytokinesis begins with nuclear condensation (pyknosis) followed by displacement to the narrow end of the parent cell, and finally its expulsion. The result is two daughters, the anuclear fragment, erythroplastid, and a nucleated pyrenocyte. Alternative mechanisms of nucleolysis with production of Howell-Jolly like bodies have also been detected. The causes of erythroplastid production are unknown but toxicosis and vitamin deficiency are candidates. Erythroplastids and reticulocytes may be useful indicators in measuring stress or in determining poor incubation conditions, the existence of disease, and establishing welfare status.

Key Words: erythrocyte, erythroplastid, stress, disease, welfare

P225 Hematology variation of the commercial turkey - indicators of inflammation and immunosuppression Paul Cotter* *Cotter Laboratory*

The purpose is to describe atypical size variants of turkey lymphocytes and heterophils detected during standard differential counts (SDC) .Large lymphocytes have diameters of ~ 12µm and express plasma cell features of eccentric nuclei, condensed chromatin, and paranuclear Hof's. Heterophil atypia include defective granulation, giants, swollen cells (oncosis) and dwarf types. Cells as these among the circulation indicate complex hemograms and render H/L ratios moot. Collectively they are indications of simultaneous inflammation and immunosuppression.

Key Words: heterophil, lymphocyte, atypical, inflammation, immunosuppression

P226 Establishment of a Pathogenicity Index for Mice to Pasteurella multocida Strains Isolated from Poultry and Swine Brunna Emery*[GS], Hamilton Moraes, Vladimir Nascimento, Thales Furian, Gabriela Chitolina, Carlos Salle *Universidade Federal do Rio Grande do Sul*

Fowl cholera is a contagious disease that results from infection by the bacterium Pasteurella multocida. This microorganism is extensively distributed among animal species, but little is known regarding its pathogenesis and specificity to various hosts. Many studies using pathogenicity evaluation methods are subjective and difficult to quantify because they are often only involved the observation of the lethal capacity of the agent in experimental inoculation. Due to a lack of more consistent data, this study aimed to establish a classification model of P. multocida pathogenicity in mice using strains isolated from poultry and swine. A total of 94 strains of P. multocida isolated from clinical cases of FC and from lungs of swine were tested. A volume of 0.1 mL of bacterial suspension was obtained from the concentration of 106 CFU/mL and inoculated by an intraperitoneal route in five mice. The animals were observed every six hours over seven days. In addition to the mortality observed, the time of death and gross lesions were also analyzed. The Pathogenicity Indexes obtained showed significant differences ($p<0.05$) according to the origin of the strains. Likewise, the number of gross lesions and isolation percentages were also varied ($p<0.05$) among strains isolated from poultry and swine. From the observed ratios, the isolates were grouped into three pathogenicity classes: high, medium and low. This study proposed a consistent measurement and classification of P. multocida pathogenicity. The obtained results will be used to generate other adjusted models, as well as to form the basis for disease diagnosis.

Key Words: Mice, pasteurella, multocida, pathogenicity, index

P227 Evaluation of the biofilm formation capacity of Pasteurella multocida strains isolated from cases of fowl cholera and swine lungs and its relationship with pathogenicity Brunna Emery*, Thales Furian, Vladimir Nascimento, Gabriela Chitolina, Karen Borges *Universidade Federal do Rio Grande do Sul*

Pasteurella multocida is a Gram-negative bacillus that causes economic losses due to the development of respiratory diseases in several animal species. Among the mechanisms of virulence, the formation of biofilms is an important factor for bacterial survival in hostile environments. Studies of biofilm formation by P. multocida are needed because P. multocida is an important pathogen involved in respiratory infections. However, in contrast to other microorganisms, few studies of biofilm formation have examined P. multocida. Studies com-paring the pathogenicity of microbial strains as a function of their biofilm production capa-city are also rare. Consequently, the aim of this study was to evaluate the biofilm formation capacity of 94 P. multocida strains isolated from cases of fowl cholera and from swine lungs on polystyrene plates. The associations of the biofilm

formation capacity with the pathogenicity index (PI) in vivo and with the presence of four genes (screened by PCR) of the tad locus (tadB, tadD, tadE and tadG), described as adhesion markers, were also determined. Strains from both animal origins were able to form biofilms. However, most of the speci-mens (52.13%) were classified as weak producers, and more than 40% of the strains of P. multocida (40.42%) did not produce biofilms. There was no significant difference ($p>0.05$) in the degree of biofilm production between the two sources of isolation. Of the analyzed strains, 56.52% contained all four genes (tadB, tadD, tadE and tadG). The PI arithmetic mean of the strains classified as non-biofilm producers was significantly different ($p<0.05$) from the PI of moderate-producer strains. The PI of specimens classified as weak biofilm producers also differed significantly ($p<0.05$) from that of the moderate-producer strains. The results indicate that even though the P. multocida strains isolated from cases of fowl cholera and swine lungs formed biofilms on polystyrene surfaces, adhesion was usually weak. The genes tadB, tadD, tadE and tadG were not significantly associated ($p>0.05$) with the production of biofilms and with the origin of a given strain. Finally, low virulence strains may suggest a higher biofilm formation capacity on polystyrene plates.

Key Words: Pasteurella, multocida, tad, biofilm, pathogenicity

P228 Resistance profile of Enterococcus spp. recovered from healthy poultry against antimicrobial growth promoters. Leandro Redondo, Jessica Bucci, Johana Dominguez, Enzo Redondo, Mariano Fernandez-Miyakawa* *INTA-CONICET*

Antimicrobial agents have been extensively used in food animals for growth promotion, resulting in selective pressure for antimicrobial resistant bacteria which can spread to humans. For this reason, global concern about development of antimicrobial resistance and transference of resistance genes from animal to human is rising. To understand the antimicrobial resistance evolution in food-borne bacteria under commercial productive conditions, and as a technical service to poultry producers, we have been monitoring the susceptibility to antimicrobial agents using indicator bacteria isolated from intensive farming systems. Enterococci were selected as indicator microorganisms, because they are widely distributed in the environment, and they are opportunistic pathogens with the ability to acquire and transfer antimicrobial resistance. The aim of the present study was to investigate antimicrobial susceptibility profiles of Enterococcus spp. isolates collected from healthy broiler chickens in commercial farms. A total of 192 isolates of Enterococcus spp. were obtained from 53 broiler farms. The minimum inhibitory concentration (MICs) for antimicrobials commonly used in poultry commercial farms as growth promoters was calculated by broth microdilution method. To define MIC epidemiological cut-off, MIC from poultry isolates were compared with MIC values from E. fecalis ATCC 29212 and Enterococci strains from our bacteria collection. Poultry strains show high rates of resistance to avilamycin (93%), bacitracin (94%), flavomycin (99%), josamycin (81%) and lincomycin (86%). No clear reduction in susceptibility, but elevated MIC90 for enramycin and virginiamycin, were found in these chicken isolates in comparison to reference strains. In this study, we provide an estimation of antimicrobial resistance predominance in Enterococci isolates present in the environment of intensive production system. A wide range of resistance was identified against a number of commonly applied antimicrobial growth promoters in poultry farms. Works like this are the first step towards a better understanding of the impact of antimicrobial livestock use in the generation and spread of antimicrobial resistance.

Key Words: Enterococcus, Antimicrobial, Growth promoters, Resistance

P229 Yeast cell wall mannan-rich fraction decreases the prevalence of antibiotic resistant bacteria in broiler chickens Helen Smith, Kate Jacques*, Richard Murphy *Alltech*

Antibiotic resistance is a major global health concern affecting both animal and human health, as once treatable diseases are becoming incurable. There is political pressure worldwide to restrict the use of antibiotics in animals to therapeutic use only, leading to an increased pressure on livestock producers to reduce their antibiotic load. Alternative strategies that promote animal health without negatively affecting the world's food supply are required. The focus of this research is to assess the role of mannan rich fraction (MRF) from the yeast cell wall of *Saccharomyces cerevisiae* in mitigating antibiotic resistance. We examined the abundance of antibiotic resistant bacteria in the caecal contents of broiler chickens whose diet was supplemented with MRF or control broilers not supplemented with MRF. Supplement inclusion levels within the diet were as follows; starter 1.3 kg/t (day 0 – 10), grower 1 kg/t (day 11 – 25), and finisher 0.6 kg/t (day 26 – 35). Caecal samples were taken from 12 birds on day 35 post hatch. The effect of MRF on the prevalence of resistant bacteria was examined using replica plating following enrichment. Resistance to antibiotics commonly used in veterinary practice such as co-trimazine, amoxinsol and lincospectin were assessed. Broilers which contained MRF in their diet demonstrated a significant reduction ($p \leq 0.05$) in the number of resistant colonies compared to diets not supplemented with MRF. In broiler chickens whose diet was supplemented with MRF the number of ampicillin, tetracycline, amoxicillin and doxycycline resistant colonies isolated reduced by 60 – 79 % compared with control broilers not supplemented with MRF. Additionally, the number of isolated colonies resistant to common veterinary antibiotics co-trimazine and amoxinsol was reduced by 83 and 78%, respectively. The results demonstrate the ability of MRF to reduce growth, prevalence and transmission of multi-drug resistant bacteria populations in the gastro-intestinal microbiome of broiler chickens. These results may be beneficial in the search of alternate strategies to promote animal health without contributing to the growing issue of antimicrobial resistance, in a market which is under increased pressure to reduce antibiotic use.

Key Words: Antibiotics, Resistance, Broiler, Yeast

P230 Effect of yeast cell wall mannan-rich fraction on antibiotic resistant Enterobacteriaceae Helen Smith, Kate Jacques*, Richard Murphy *Alltech*

Antibiotic resistance has the potential to become one of the greatest problems of our generation. Issues related to antimicrobial resistant bacteria in the United States cost the health-care system in excess of $20 billion annually, as once treatable diseases are becoming incurable. Alternative strategies that promote animal health without negatively affecting the world's food supply are required. The focus of this research is to assess the role of mannan rich fraction (MRF) from the yeast cell wall of *Saccharomyces cerevisiae* in mitigating antibiotic resistance in drug resistant Enterobacteriaceae. The effect of MRF on resistant *E. coli* and *Salmonella* strains was assessed by monitoring microbial growth, in the presence and absence of MRF and antibiotics. Each resistant species of *E. coli* and *Salmonella* demonstrated a statistically significant increase in sensitivity to antibiotics in the presence of MRF (0.5%, w/v). Growth of extended-spectrum beta-lactamase (ESBL) producing *E. coli* was reduced by 21% ($p \leq 0.05$) in the presence of cefotaxime (4.5 μgmL^{-1}). Growth of ampicillin resistant transformed *E. coli* in the presence of ampicillin (45 μgmL^{-1}) was reduced by 43% ($p \leq 0.05$). A statistically significant increase in antibiotic sensitivity, up to 25%, was revealed in the presence of MRF (0.5% w/v) and ciprofloxacin (450 $\mu g/mL^{-1}$) ($p \leq 0.05$). MRF was also observed to induce an increase in sensitivity of multi-drug resistant *Salmonella enterica* serovar Dublin to ticarcillin and piperacillin (45 μgmL^{-1}), by 19% and 21% respectively ($p \leq 0.05$). Thereby, MRF was noted to enhance the sensitivity of the resistant strains to the relevant antibiotic. In an industry which is under increased pressure to restrict the use of antibiotics

in livestock production, increasing the sensitivity of infectious resistant pathogens to antimicrobials could potentially result in a reduction in usage and relieve pressure on producers to decrease their antibiotic load. Given the effects of MRF it may be an ideal alternative to the use of AGPs in livestock. These results support the search for alternative strategies to promote animal health without contributing to the growing issue of antimicrobial resistance.

Key Words: Antibiotics, Resistance, Yeast, Enterobacteriaceae

P231 Antimicrobial resistance in Salmonella enterica serovar Heidelberg isolates from poultry sources in 2006 and 2016. Anabele Santos*, Juliana Bassani, Daiane Wilsmann, Mariana Paravisi, Daiane Carvalho, Carlos Tadeu Salle, Vladimir Do Nascimento *Federal University of Rio Grande do Sul*

Brazil is the world's second largest chicken meat producer and the first exporting country, which means that poultry farms need a strict biosecurity program to prevent the emergence of pathogens that can cause damage to the animals and to the final product. In this scenario, Salmonella *enterica* serovar Heidelberg plays a fundamental role because of its high prevalence and significance in public health, especially regarding to antimicrobial resistance. Motivated by the difficulty in eliminating this pathogen from the poultry chain, its zoonotic potential and increased antimicrobial resistance, this study aimed at analyzing *Salmonella* Heidelberg isolates for antimicrobial susceptibility through the use of Minimum Inhibitory Concentration (MIC) Test. A total of 40 *Salmonella* Heidelberg isolates from chicken sources were recovered, 20 from the year 2006, and 20 from 2016. The antimicrobials tested were gentamicin, tetracycline and chloramphenicol. Two interpretive criteria were used to evaluate the MIC test, the clinical breakpoint values and the epidemiological cut-off values (ECOFF). For breakpoint values, no evidence of chloramphenicol resistance was observed for any of the isolates of 2006, while only 1 (5%) isolate demonstrated resistance to gentamicin and 1 (5%) to tetracycline. Among the isolates from 2006, 18 (90%) were susceptible to all antimicrobials tested. In 2016, 6 (30%) and 15 (75%) of the isolates showed resistance to gentamicin and tetracycline, while no isolates were resistant to chloramphenicol. In addition, 5 (25%) isolates from 2016 were sensitive to all classes of antimicrobials tested. For ECOFF values in 2006, chloramphenicol was the only antimicrobial that did not present nWT (Non-wild Type) isolates, and the frequencies for gentamicin and tetracycline were 10% and 5% of nWT isolates, respectively. For 2016 samples, chloramphenicol also did not present nWT isolates, whereas gentamicin and tetracycline presented frequencies of 30% and 75% of nWT isolates, respectively. The results show the increasing trend of S. Heidelberg's resistance to antimicrobials over time. Therefore, actions should be taken in order to encourage the judicious use of these drugs in veterinary as well as human medicine.

Key Words: Salmonella, poultry, antimicrobials, resistance

P232 Are chicken embryos sterile? An investigation through both culture-dependent and independent methods. Bishnu Adhikari*[GS], Guillermo Tellez-Isaias, Kyle Teague, Young Kwon *University of Arkansas*

Traditionally it has been assumed that the acquisition of gut microbiota starts after the hatch of chicks, and thus the embryo was considered as sterile. On the contrary, recent studies using the culture-independent method have suggested the existence of microbiomes in chick embryos as well as fetus of human and other animals. However, these studies lacked appropriate controls to exclude any false positives due to potential contamination and failed to correlate the findings through culture-based methods. In this study, we studied the potential microbiomes in chicken embryos through both culture-dependent and -independent methods with appropriate controls. For this purpose, gastrointestinal tract (GIT) and yolk sac (YS) were aseptically collected from 10 chicken embryos from the same hen at each embryonic age of 14, 17, and 20 days. GIT from 3 day-old-chicks of the same hen were also collected. All GIT and YS samples were homogenized and enriched in selective media (TSB, MRS, and TSB with thioglycollate), and plated on respective selective agar media, which were incubated under aerobic or anaerobic conditions at 37°C for 24 hr. The remaining homogenized samples were used for genomic DNA extraction with a negative control included in each batch of extraction. PCR was performed to amplify V1-V3 region of 16S rRNA gene from each sample using barcoded universal primers. A decontamination kit containing DNase was used before adding template DNA in PCR reaction to remove any double stranded DNA contaminant from PCR reagents. Two positive controls (a mock community sample and a chicken ileal sample) as well as two negative controls (a control from DNA extraction and a control with no template DNA) were included in each batch of PCR. All culture results from embryonic samples were negative, however, all 3 post-hatch GIT samples showed positive growth of bacteria on tryptic soy agar (TSA) at aerobic condition. All PCR results from embryonic samples were negative except one YS sample from 20 day old, which will be further characterized by MiSeq. All 3 post-hatch GIT samples showed expected PCR products. Overall, the present findings suggested that either the chicken embryos may be completely sterile or may contain microbiome biomass under the detection limit.

Key Words: Chicken-embryo, Microbiome, GIT, Yolk-sac

SCAD

P233 Effects of a dry hydrogen peroxide (DHP) air sanitation system used in an egg cooler on hatchability and chick quality Érica Melo*[GS], Jeanna Wilson, Brian Jordan, Julia McElreath *Universidade Federal de Minas Gerais*

In commercial poultry production, hatcheries are a source of continual contamination. Sanitation in the hatchery is a constant process, where minimal beneficial results are seen if done correctly, but drastic negative impacts are felt when done improperly. A sanitation method that could continually clean and disinfect a hatchery in support of daily cleaning procedures would be valuable in commercial hatcheries. For this reason, a commercially available gaseous dry hydrogen peroxide (DHP) system has been introduced into hatcheries to continually combat microbes in the air and on surfaces. Preliminary studies have shown that this sytem can indeed reduce the microbial population in hatcheries and on the surface of eggs however, the effects of this new system on hatchability and chick quality need to be evaluated. A total of 3,960 fertile eggs were collected from an approximately 40 week old Ross 308 broiler breeder flock maintained at the Poultry Science research farm and distributed in 2 treatments: control (no disinfection) and treated. For the treated group, one DHP air sanitizer was placed inside an egg cooler at the Poultry Science research farm and two other machines were placed in the common area outside. Both areas were treated for 7 days prior to placement of eggs, and then eggs were collected and placed inside the cooler over a four day period. Eggs were stored for 3 days after the last collection prior to placement in the incubator. During pre-treatment and egg storage, DHP levels were measured inside the cooler and air samples were taken to evaluate environmental microbial load. After storage, eggs were placed into a single stage Natureform incubator. For the control group, all DHP machines were removed from the cooler and external room five days before placing the eggs and the egg cooler was cleaned. During the treated phase of the trial, a daily increasing level of DHP was measured in the egg cooler, with an average level of 12 ppb, and a concomitant reduction in microbial load from air samples was seen, confirming that the room was treated and the system was killing microbes as expected. The hatch and chick quality data

from this project will provide valuable knowledge for hatcheries when deciding the merits of this sanitation system.

Key Words: biosafety, incubation, omphalitis

P234 Reducing microbial load on hatching eggs using a dry hydrogen peroxide gas system Julia McElreath*[GS], Eric Shepard, Russ Stephens, Brian Jordan *University of Georgia*

It is widely known that eggs, even those free of organic material, have a microbiological population on the shell in high numbers. For this reason, the microbiology of the egg can significantly impact hatchery cleanliness as well as chick health at hatch. A sanitation method that reduces bacterial load is essential, but washing hatching eggs removes the protective cuticle layer and is not practiced in the US. The objective of this study was to determine the ability of a novel dry hydrogen peroxide (DHP) gas to reduce the microbial load on hatching eggs, which has the advantage of prolonged exposure in gas form without removing the protective cuticle layer. This experiment was divided into two trials. In trial 1, non-treated eggs were placed in a closed biosecurity cabinet and treated eggs were placed in an office with DHP machines. Trial 2 took place in a hatchery egg cooler where machines were turned on for the treated group and kept off for the non-treated group. Microbial load in both trials was evaluated by washing eggs in tryptic soy broth after 0, 24, 72, and 120 hours of exposure. Samples were then serial diluted 1:10 into TSB in triplicate and incubated for 24 hours before being transferred onto selective MacConkey and tryptic soy agar (TSA). Inoculated media was incubated for 24 hours, bacterial growth was counted, and the Most Probable Number of bacterial colonies was calculated. Trial 1 results showed that the treated group had significantly less growth on TSA plates at 72 and 120 hours ($p=0.05$) of exposure, while the non-treated group had consistent growth on TSA. Growth on MacConkey agar remained low and not statistically different throughout the trial in both groups. In trial 2, the treated group showed more growth on TSA at 120 hours ($p=0.0001$) but a reduction in growth on MacConkey media at 72 and 120 hours ($p=0.001$). The non-treated group showed increased growth on TSA at 72 and 120 hours where MacConkey agar had increased growth at 24 hours but reduced growth at 72 hours ($p=0.05$). This data indicates that the DHP system can prevent expansion or reduce microbial load on hatching eggs after short-term exposure.

Key Words: sanitation, peroxide, hatchery, microbe

P235 The effect of Akkemansia muciniphila on gut microbiota for the control of necrotic enteritis in broilers Yue-Jia Lee, Wen-Yuan Yang, Hsin-Yi Lu*, Chinling Wang *Mississippi state university*

Akkemansia muciniphila (AM), a mucin-degrading anaerobe and a probiotic supplement, has anti-inflammatory response and the improvement of gut integrity effects. Some studies suggest that the accumulation of mucin facilitates the growth of *Clostridium perfringens* in chicken intestines. Therefore, the objective of this study was to investigate the effects of *A. muciniphila* on intestinal health and gut microbiota against *C. perfringens*-challenged chickens. Commercial broiler chicks were divided into five groups and challenged with *Eimeria*, *Eimeria* + CP, *Eimeria* + CP + AM, *Eimeria*+ AM, or a placebo. Jejunum samples were collected, prepared and analyzed using 16S rRNA sequencing. The taxonomic composition and the abundance of selective genera of gut microbiome were diverse among all five groups based on the principle coordination analysis and heat map analysis. Firmicutes, Proteobacteria, Actinobacteria were the most abundant phyla in the jejunum. When birds were challenged with *C. perfringens* or administered with *A. muciniphila,* the relative abundance of Firmicutes and Proteobacteria increased, and Actinobacteria decreased. The gut microbial diversity at species level was decreased, *Escherichia* was increased, and *Bifidobacterium* and *Lactobacillus* were decreased. In conclusion, *A. muciniphila* did not protect against *Clostridium perfringens*-challenged chickens against NE. The abundance of *Bifidobacterium* and *Lactobacillus* in the jejunum seem to protect chickens against NE.

Key Words: Microbiota, Necrotic-enteritis, Clostridium_perfringens, Akkermansia_muciniphila

P236 Correlation between necrotic enteritis and an imbalance of Clostridium perfringens in the gut of broilers. Nathaniel Ollis*, Charles Hofacre, Virginia Baxter, Greg Mathis, Brett Lumpkins, Roy Berghaus *Southern Poultry Research Group*

Statement: Necrotic enteritis (NE) is a disease caused by an imbalance of *Clostridium perfringens* in the gut flora of chickens, which is becoming a growing problem due to the trend of the poultry industry moving toward an antibiotic free (ABF) system pushed by the food industry recognizing the strong marketability of ABF meats. This has led to the rise of other alternative treatments to decrease NE mortality, increase bird performance, and keep production costs down. Design: In a comparison of four similar studies, when birds in battery cages were given *Eimeria maxima* (~5000 oocysts) at ~2 weeks of age then dosed with *C. perfringens* (~$1x10^8$ cfu/ml) on days 19, 20, and 21. Then birds were lesion scored on a scale of 0-3 on day 22, ileal samples were taken from two of the three lesion scored birds for the enumeration of C. *perfringens*. The method for enumeration was a modified MPN method of Berghaus et al. where thioglycollate was added to each sample and 10-fold dilutions performed from $1x10^3$ to $1x10^8$, then plated to PEA agar and anaerobically incubated for 24 hours at 37°C. Results: In all four studies the challenge controls showed the highest mean lesion scores and NE mortality which were significantly different from the negative controls. Using a GEE linear model adjusted for clustering of birds in cages, challenge controls showed in all four studies a numerically higher enumeration of *C. perfringens* with means ranging from $\log_{10}$ cfu/g 4.33 to 8.34 and negative controls means ranging from 3.12 to 7.92, although only showing a statistically significant difference in two of the studies. The mortality across the four studies caused by NE ranged from 21.8 – 54% in the challenge controls, while the negative controls had no NE caused mortality. The challenge controls had lesion score means that ranged from 0.9-1.25, while the negative controls all scored 0. Bird performance was statistically different between the challenge and negative controls in all four studies with the challenge controls being impaired on both feed conversion and weight gained. Enumerating *C. perfringens* from the small intestine appears to correlate well with lesions and performance in a NE challenge model. Future work may look at enumeration of *C. perfringens* in a natural outbreak.

Key Words: Clostridium, Broilers

P237 Protective effect for broiler chickens of Valerins and the antibiotic BMD in necrotic enteritis challenge model Charles Hofacre*, G. Mathis, Richard Sygall *Southern Poultry Research*

The broiler chicken disease Necrotic Enteritis (N. E.) is most often a multifactorial disease of

intestinal insult and *Clostridium perfringens (C.* P.). Short chain fatty acids have been effective for both clinical and subclinical N. E. However, these compounds can have a strong odor and be quickly reabsorbed from the upper intestine before reaching mid gut where N. E. occurs. Valerins, being glycerolesters of valeric acid, may provide benefits of triggering the physiological responses associated with improving intestinal health by the short chain fatty acid minus any negative effects associated with non-esterified short chain fatty acids. A 32 cage study evaluated four in-feed treatments with 8 replicates of day old Cobb male broiler chickens: No additive/No C. P. challenge; No additive C. P challenge; BMD 50g/ton/C. P. challenge; Valerins 1.5 kg/mt/C. P. challenge. The N. E. challenge model used was *E. maxima* gavage (~5000 oocysts/chick) at 14 days, C. P. (~10^8 cfu/chick) gavage on days 19, 20, and 21. Intestine lesion scores (LS) were performed per Hofacre et al. 1998 on Day 21. Feed consumption and body weight (BW) were evaluated on days 14 and 28. Clinical N.

E. results were the no additive/no C. P. had 0%^{c} N. E. mortality and L. S. were 0.0$^{c;}$ no additive/C. P. 43.8%^{a} N. E. mortality and L. S. 1.3^{a}; BMD N. E. mortality 7.8%^{c} and L. S. 0.8^{b}; Valerins N. E. mortality 17.2%^{b} and L. S. 0.9^{b}. Subclinical N. E. results Day 0-28 were no additives/no C. P. BW 1.015kg^{a} and mortality adjusted (adj.) FCR 1.526^{c}; no additive/C. P. BW 0.845kg^{b} and adj FCR 1.966^{a}; BMD BW1.044kg^{a} and adj FCR 1.548^{bc}; Monovalerin BW 1.064kg^{a} and adj FCR 1.675^{b}. In conclusion, this study demonstrated, as expected, antibiotic BMD had the greatest impact in reducing effects of *C. perfringens* for both clinical and subclinical N. E. In a program without antibiotics, Valerins alone in feed significantly prevented both clinical and subclinical N. E

Key Words: necrotic, enter

P238 Descriptive analysis of affected Focal Duodenal Necrosis flocks based on a questionnaire survey. Ana Villegas*^{GS}, Roy Berghaus, Guillermo Zavala, Monique França *University of Georgia*

Focal duodenal necrosis (FDN) is an intestinal disease of egg laying chickens, characterized by multifocal mucosal erosions in the duodenal loop and proximal jejunum. It is currently considered by the American Association of Veterinarians in Egg Production and the United States Animal Health Association as one of the top 5 disease concerns of the table egg industry in the US. The purpose of this study was to investigate the epidemiological characteristics of table egg layer flocks affected with FDN. An online questionnaire was distributed to commercial layer operations in 7 different states in the USA. Layer farms that had diagnosed FDN within the past 12 months were surveyed. The questionnaire had 45 questions about management, nutrition, housing and methods for disease prevention and control. Thirty-seven surveys were sent, 21 were completed which represents a response rate of 56.7%. The survey results showed presence of FDN in 5 egg layer genetic lines of different ages, with most cases reported between 30-39 weeks of age. The pullets were cage-reared in all affected flocks and the majority of flocks in production were housed in traditional cages. Most of FDN-affected flocks received more than 12 different feed formulations from pre-lay to 60 weeks of age. Distiller's dried grains with solubles was a common ingredient added to the feed in the majority of affected flocks and all flocks were provided with limestone as a calcium source for egg production. Most surveys reported that coccidiosis and roundworm parasitism were not problems in affected flocks in production; however, pests such as flies and rodents were reported as problems in most affected flocks. Additionally, for most affected flocks reported suboptimal cleaning and disinfection procedures were reported during downtime, which may not be sufficient to prevent the persistency and transmission of the causative agent of FDN. In conclusion, several management practices that have been associated with enteric disease including Clostridial-associated enteritis were described by the majority of FDN affected flocks. Additional studies are needed to determine if management and health practices identified in this survey represent risk factors for FDN.

Key Words: Focal Duodenal Necrosis, Commercial layer farms, Epidemiological profile, FDN affected flocks, Online questionnaire

P239 In vitro anaerobic incubation of Salmonella enteritidis and poultry feed substrates with cecal samples from in ovo lactobacillus injected broiler chicks Lizza Macalintal*, Phyllis Glenney, Anthony Pescatore, Tuoying Ao, Michael Ford, Karl Dawson *Alltech-University of Kentucky Poultry Nutrition Research Alliance*

Lactic acid bacteria (LAB)-based probiotic as feed additive are believed to exert their effects by suppressing the growth of pathogenic bacteria in the intestinal tract. In this study, the effects of lactic acid bacteria injected *in ovo* into fertile eggs were evaluated for their ability to suppress *Salmonella enteritidis* (SE) populations using an *in vitro* anaerobic culture system. After 18 days of incubation, embryos were injected intra-amnion with 10^6 colony forming units (cfu) of *Lactobacillus acidophilus* (LA; B-23431, USDA), *Lactobacillus platarun* (LP; B-4496, USDA) or *Pediococcus acidilactici* (PA; ATCC 8042), (Alltech, Inc), suspended in 100ul MRS (de Man, Rogosa and Sharpe) broth. Non-injected eggs were included as control treatment group. At day of hatch and 3-days post hatch, composite bacterial suspensions from each of four treatment groups were aseptically prepared in triplicate from the ceca of 3 birds in McDougall's buffer (10 % cecal contents by volume) under anaerobic conditions. Ten milliliters of each cell suspension was added to separate serum bottles containing 1 g of layer feed as a substrate under an oxygen-free gas phase. To provide the pathogen challenge, each serum bottle was inoculated with a SE broth culture containing approximately 5.5 x10^5 cfu/ml. The SE in each bottle was enumerated after serial dilution on Brilliant Green Agar spread plates after 24h at 37C. Cultured cecal samples collected at day of hatch harvested from birds receiving *in ovo* LP and LA showed significant reduction in the total salmonella count compared to PA and non-injected control ($P<0.05$). The log concentration of SE in the LA and LP cecal samples were reduced to 8.35 and 7.60 compared to 8.44 and 8.45 for PA and control, respectively. At D3, average log SE concentration from cecal samples taken from control group were 7.79 while LP, LA, PA. had no SE content (< 2.00 log10 CFU). However, the total lactobacillus bacterial concentration enumerated on MRS agar plate were significantly higher for control and PA compared to LP and LA. In these studies, cecal bacterial suspensions from birds that had received *in ovo* inoculation with live lactobacillus significantly suppressed the challenge SE.

Key Words: inovo, probiotic, lactobacillus, salmonella, invitro

P240 Protective effects of direct fed microbials in combination with the live vaccine MeganVac 1 against Salmonella Heidelberg in broilers Virginia Baxter*, Charles Hofacre, Chris Tate, Roy Berghaus *Southern Poultry Research Group*

The Centers for Disease Control and Prevention estimates that nontyphoidal *Salmonella* species are second, only to norovirus, as a leading cause of foodborne illness in the United States, causing approximately 11% of all domestically-acquired foodborne illnesses. While field evidence suggests that vaccination with a live *Salmonella* typhimurium vaccine can cross protect against other serovars and reduce loads and/or prevalence, foodborne illness statistics suggest that considerable room for improvement remains. Other strategies that reduce colonization of broilers are needed to augment vaccination and management practices to achieve further reductions of *Salmonella* in poultry meat at processing. Various direct fed microbials (DFMs) have been shown to inhibit colonization of *Salmonella* in poultry and may be a useful adjunct to other current strategies. Three floor pen studies with broilers were done to ensure various DFMs do not inhibit protection provided by live S.T. vaccine. All three studies were given MeganVac 1 at day of age by coarse spray, and boosted at 14 days in one study. Liver/spleen pools and ceca were collected at ~3 days of age for vaccine isolation to indicate the vaccine was present in the internal organs and not inhibited by the DFMs. Birds were orally gavaged with ~1x10^8 *Salmonella* heidelberg (25 ug nalidixic acid resistant) at ~3 days of age. Cloaca swabs (10) were taken at 28 days of age, and ceca (10-15) were collected at 42 days of age. Prevalence of S.H. was determined by culture in tetrathionate broth (42^{o} C) and struck to XLT-4 (25 ug/ml nalidixic acid). Enumeration of the ceca and cloaca swabs were done by the Most Probable Number (MPN) method of Berghaus and Thayer (2013). Statistical analysis was done using Fisher's exact test and one-way analysis of variance. Although there were not many significant reductions in S.H. prevalence in these studies, there was a consistent numerical reduction in the load (MPN) of S.H. in the ceca and cloaca swabs. Application of the vaccine alone or with DFM resulted in lower MPN values than the untreated groups. There was also no inhibition of colonization of the live S.T. vaccine in organs and no significant effect on either the live vaccine or the DFMs ability to inhibit the S.H. colonization.

Key Words: Salmonella, Broilers, Vaccination

P241 Comparison of various live salmonella vaccination programs on protection against a Salmonella heidelberg challenge in SPF leghorns Kalen Cookson*, Manuel Da Costa, John Dickson, Jon Schaeffer *Zoetis*

Live *salmonella typhimurium* (ST) vaccination of young pullets helps reduce salmonella colonization and shedding prior to giving inactivated vaccines. There are currently three live ST vaccines available on the market. The purpose of this study was to compare the three most common programs for their ability to reduce infections/loads after challenge with a virulent *Salmonella heidelberg* (SH). Study Design: 280 SPF leghorns were placed into 8 different isolators (35 per) at day of age. Two isolators were randomly selected to house one of 4 different ST vaccine treatments based on the following (day spray) primer/booster (14 day) programs: 1) Vaccines A/A, 2) Vaccines B/B, 3) Vaccines B/C and 4) No Vaccine. At 4 days of age, several chicks were removed from each isolator to culture spleens and cecas for vaccine recovery. At 50 days of age, all birds were challenged orally with SH at a target dose of 10^6 CFU per bird. One week later, birds were necropsied and spleens and cecas collected. The spleen and one cecal pouch were cultured by enrichment and the other cecal pouch was cultured using the MPN enumeration method. Body weights were recorded at 14, 21, 43 and 57 days of age. Results: Both hatchery vaccines, A and B, were recovered from 100% of the cecal samples at 4 days of age while spleen recovery was 94.4% and 87.5%, respectively. Both males and females receiving Vaccine B at hatch had significantly lower body weights at 14 days of age while the same was seen in B/C males and females at 21 days. However, by 43 and 57 days of age there were no longer significant differences between vaccine treatments. There were no significant differences in any groups on salmonella incidence in either spleens or cecas as 29/30 challenge controls were positive on both organs. However, Programs A/A and B/C had the lowest geometric mean cecal counts (78 and 21) compared to B/B and Controls (1293 and 2322). Program B/C was significantly lower than B/B in cecal counts while A/A was nearly significantly lower (P=0.066) than B/B on super shedders (MPN>100) at 23% vs. 73%. Discussion: Vaccines A and B both gave 100% "takes" after day of age vaccination but Program B/B did not reduce cecal loads of SH or the percentage of super shedders like Programs A/A and B/C.

Key Words: salmonella, vaccination, pullets, challenge, protection

P242 Local innate immune responses after ocular or oral inoculation with virulent strain of infectious laryngotracheitis virus (ILTV). Gabriela Beltrán*[GS], Sylva Riblet, Leah Read, Shayan Sharif, Maricarmen García *University of Georgia*

Infectious laryngotracheitis (ILT) is a highly contagious acute respiratory disease of chickens caused by the avian alphaherpesvirus Gallid herpesvirus 1 (GaHV-1), commonly known as infectious laryngotracheitis virus (ILTV). During natural infection ILTV enters its host via the respiratory and ocular routes getting first into contact with cells of the conjunctiva, nasal cavity and Harderian gland. These structures harbour associated lymphoid tissues that play essential roles in the induction of local immune responses against viral invasion. The interferon system is considered the first line of defense against pathogen invasion. Type I and II interferons (IFN a,b and g) are essential for the expression of interferon-stimulated genes (ISGs) such as 2′–5′ oligoadenylate synthetase (OAS), and the interferon-induced transmembrane (IFITMs) proteins. These ISGs induce the cells to an intrinsic antiviral state restricting viral replication during the early stages of infection. In a previous study, we demonstrated that independently of the route of ILTV entry (ocular or oral) the signaling pathway downstream TLR activation was negatively affected diminishing the expression of pro-inflammatory cytokines and type I and II IFNs. In the present study gene expression of ISGs such as OAS, IFITM3, 5, and MHCI and II will be quantified at 6, 12 and 24 hours post-inoculation.

Key Words: ILTV, Immunology

P243 Evaluation of the effects of a Turkey Cellulitis/Dermatitis oil emulsion vaccine on immune response and mortality under commercial conditions Brittany Graham*[GS], Kabel Robbins, Lucas Graham, Kyle Teague, Amanda Wolfenden, Guillermo Tellez, Billy Hargis *University of Arkansas*

Alpha-toxigenic *Clostridium septicum* (CS) is the cause of Turkey Cellulitis and tends to cause the most detriment in turkey flocks around 16 weeks of age. It results in devastating mortality and high costs for the industry. Various vaccinations have been utilized to try to combat this disease with little success. Oil emulsion vaccines induce robust immunity due to the continuous stimulation of the immune system. For two field trials, a water-in-oil (W/O) emulsion vaccine was prepared using a CS bacterin-toxoid with Seppic Montanide 71 R VG adjuvant. Production procedures for the vaccine included determining droplet size of emulsion, duration and speed of homogenization, and stability after storage. In Exp 1 and 2, treatments included a control group and a vaccinated group boosted around 8 weeks. Exp 1 consisted of 3 different houses: House 1 (HS1), House 2 (HS2), and House 3 (HS3). Antibody levels were determined by ELISA and mortality associated with Cellulitis was recorded once the first case was observed. Blood samples were obtained from 20 turkeys/group from each house at 8, 12, and 16 weeks. Antibody levels (S/P ratio) in vaccinated groups for weeks 12 and 16 for all three houses were significantly higher ($P<0.05$) than the control groups. In HS1 and HS2, there was no significant difference in mortality/total (%) between the control and vaccinated group. In HS3, control mortality/total (%) vs vaccinated mortality/total (%) was significantly different (<0.001), possibly indicating this house was presented with a higher challenge. Exp 2 consisted of 6 farms with 1-4 houses/farm. Vaccinated mortality/total (%) was significantly lower (<0.05) than control mortality/total (%) in 5 out of the 6 farms. In Exp 2, antibody levels in vaccinated groups were significantly higher ($P<0.05$) than the control groups at 12 and 16 weeks for all 6 farms. Based on these results, W/O emulsion vaccines, such as this alpha-toxin bacterin-toxiod with Montanide 71 R VG adjuvant, can be used to increase antibody levels for Turkey Cellulitis and may have the ability to reduce Cellulitis related mortalities in the field.

Key Words: Cellulitis, oil-emulsion, vaccine, alpha-toxin

Environment, Management and Animal Well-Being, Behavior

P244 Environmental factors influence dust-bathing in commercially housed broiler chickens Douglas Aldridge*[1], Chad Hayes[1], Colin Scanes[1], Karen Christensen[2] *[1]University of Arkansas; [2]Tyson*

The study reports on the location of dust-bathing within broiler houses and differences between two lighting systems, conventional LED (Overdrive®, Global Consumer Products Inc. Clifton, NJ) and feeder lights (AviLighting™ AHPharma Inc. Hebron, MD). Dust-bathing is generally viewed as one of the essentially expressed behaviors of chickens and has been extensively examined in laying hens but also occurs in broilers. Dust-bathing and evidence of dustbathing was methodically observed under both aforementioned lighting conditions from day 15 to 39. Data collection took place under commercial conditions on used and new bedding. Data was collected from two flocks with placement densities of .017kg/m^2 and .018kg/m^2. Average densities at the conclusion of data collection for 'Trial 1' were 9.708kg/m^2 and 8.621kg/m^2; for 'Trial 2', 9.411kg/m^2 and 10.098kg/m^2. Data were combined for the 19 time points and statistically analyzed by repeated measures ANOVA with means separated by Tukey's range test and by regression analysis. Birds were rarely observed to dust-bathe within .914m of feeders. In contrast, there was substantial dustbathing distant to the feeders usually occurring between inside waterlines. There was both a greater number of birds actively dust-bathing ($p<0.01$) and evidence of dust-bathing ($p<0.01$) in the presence of feeder lights than

in the conventional lit house. Unexpectedly, there was also a marked graduation of both the number of birds dust-bathing ($p<0.001$) and evidence of dust-bathing ($p<0.001$) nearer to natural light leakage via active tunnel ventilation fans within each house. It is concluded that broiler chickens exhibit marked preferences in location for dust-bathing preferring areas with low levels of disturbance. It was hypothesized that increased dust-bathing in the presence of feeder lights was associated with high light intensity near the feeders (>20lux) and/or the greater range in light intensity present within the house; the latter is supported by the higher incidence of dust-bathing toward the fan end of the house.

Key Words: Dust-bathing, broilers, lighting

P245 Effects of functional sensory molecules on the behavior of broiler chicks under two different stocking density Jean Francois GABARROU*[1], Seon RYU[2] *[1]Laboratoires PHODE; [2]Chon Buk National University*

The objective of the study was to measure the effect of a sensory feed additive based on orange essential oil rich in D-Limonene (VéO,Premium - 250g/t which is expected to reduce stress perception, provided by Laboratoires PHODE) on broilers behavior in a situation of density stress. It was compared to a control feed without sensory molecules on 1572 Ross broiler chicks during 5 weeks in the CHONBUK experimental station. They were randomly distributed into 12 flocks of 51 and 12 of 80 birds each (14 birds/m^2 as the low stocking density (LSD) and 22 birds/m^2 as the high stoking density (HSD)). Half birds (6 flocks) of each density were feed with VeO, others are controls flocks. Birds were reared in floor pens in a controlled housing system. Behavior was measured using cameras daily for 4 hours during 5 days per week and recorded as frequency. Data were analyzed using two way ANOVA (Density X Feed additive).

During the period from 8 to 21 days of age, VeO significantly decreased the walking activity in the LSD (49.75 vs. 26.75 ±2.49) although it significantly increased it in the HSD (26.25 vs. 37.25 ± 2.49). HSD also significantly increased drinking time (71.88 vs. 99.38 ± 5.74) and flapping behavior (1.75 vs. 3.88 ± 0.53) and decreased preening behavior (26.00 vs. 11.88 ± 3.16). A trend ($p<0.10$) to reduce pecking in HSD when VeO is used was observed (4.50 vs. 2.25 ± 0.69).

During the rearing period from 22 to 35 days of age, birds reared in HSD spent significantly more time standing when treated with VéO (20.75 vs. 39.75 ± 2.24). Indeed, the preening behavior was drastically reduced in HSD condition compared to LSD condition (4.00 vs. 19.25 ± 2.22), but this behavior was maintained when using VeO (12.25 ± 2.22).VeO decreased preening behavior more drastically in HSD. HSD increased behaviors that could be interpreted as stress indicators like flapping and decreased indicator of welfare like preening. These behaviors were less exhibited in the VeO group. Veo has also reduced pecking.

Sensory feed additives could modulate the broilers behavior in stressful situations like high stocking density.

Key Words: density, sensory, behavior, broiler

P246 Effects of layer strains and storage time on the dimension of eggshell translucence Xueting Huo*[UG], Qiugang Ma, Lihong Zhao, Jianyun Zhang, Cheng Ji *China Agricultural University*

Eggshell translucence is caused by the translocation and accumulation of water from the egg's contents to the eggshell, which negatively affects the appearance of eggs and reduces their commercial value. This study was conducted to introduce a five-score grading system for evaluating the effect of layer strains and storage time on the dimension of eggshell translucence. The five-score grading system was defined according to translucent spots area in the vision under LED light (0 to 20 percent ranking score 1; 20 to 40 percent ranking score 2; 40 to 60 percent ranking score 3; 60 to 80 percent ranking score 4; 80 to 100 percent ranking score 5). Six hundred eggs from four layer strains (Jingfen No.1、Jinghong No.1、DaWu Jinfeng and Hyline brown; 160 eggs for each strain) were collected, stored in the same environment (Temperature 20 to 25 °C; Relative humidity 40 to 50 %), and subjected to eggshell translucence ranking at 0 , 24 , 48 , 72 and 168 h. Results showed that eggshell translucence increased with the storage time ($P<0.01$); Eggshell translucence grading levels showed rapid increase during the first 24 h, while it reached a plateau at 72 h (1.72 for Jingfen No.1; 1.85 for Jinghong No.1; 1.44 for DaWu Jinfeng and 1.57 for Hyline brown). Regardless of storage time, eggshell translucence grading levels were different among strains of laying hens; The eggshell translucence of Jingfen No.1 and Jinghong No.1 were significantly higher than that of DaWu Jinfeng and Hyline brown($P<0.01$). In conclusion, eggshell translucence varied among laying hen strains and increased with storage time.

Key Words: Eggshelltranslucence, Storagetime, Layinghens

P247 Resolving the broiler breeder paradox with N-acylethanolamines (NAEs), an unexplored family of neuroactive lipids with demonstrable benefits to gut health, behavior welfare, satiety and adiposity. Kristin Moncada*, Sohini Bhatia, Paige Spencer, Andrew Feigley, Noura Dosoky, Sean Davies, Suresh Pillai, Rosemary Walzem *Department of Poultry Science, Texas A&M University*

Feed restrictions used during the breeder pullet phase improve livability and egg production but result in chronic hunger that is increasingly viewed as a welfare issue. N-acylethanolamines (NAE) comprise a neuroactive lipid family active in appetite control, behavior and immunity. Grossly underexplored in poultry, suitable modification of NAE signaling could provide novel inroads to improved welfare. Gene expression studies from this laboratory showed tissue specific expression of key NAE metabolizing proteins to confirm that this system is present and responsive to feed intake in breeder hens. N-acyl-phosphatidylethanolamines (NAPEs) are the immediate precursors of NAE and can be produced by probiotic e. coli Nissle 1917 carrying expression plasmid for the A. thaliana NAPE acyltransferase (At1g78690p, pNAPE-EcN). We sought to establish culture and induction conditions for pNAPE-EcN and empty vector control bacteria (pEcN) to allow for bulk probiotic production and live bird trials.

Liquid cultures were obtained from the Davies laboratory and stored overnight at 4°C until plated on LB-Amp selection plates. Individual colonies were picked the next day and suspended in 20 mL of LB-broth at 37°C under microaerophilic conditions with orbital shaking at 250rpm overnight to an OD_{600} of > 0.79 for both. Three milliliters of culture was added to 150mL LB-broth and incubated for 4h with shaking at 37°C to establish initial growth rates. Cell numbers increased an average of 23.9 and 26.2 fold ($p<0.05$ by t-test) in pNAPE-EcN and pEcN cultures, respectively. Extracts from pooled cultures were analyzed for NAPE content using LC/MS as described (see https://doi.org/10.1172/JCI72517) alone or following induction with isopropyl-beta-D-thiogalactopyranoside, IPTG. Cultures of pNAPE-EcN had markedly increased concentrations of saturated and monounsaturated NAPE species as compared to pEcN cultures transduced with empty vector. Specifically NAPE species with (16:1, n9), (16:0), (18:1n9) fatty acid moieties were 15-fold, 5-fold and 10-fold enriched while (18:2, n6) and (18:0) fatty acid species were unchanged from pEcN cultures. Culture conditions are now established for bulk probiotic production. AgriLife Research project #8738

Key Words: Probiotic, N-acylethanolamines, Satiety

Environment, Management and Animal Well-Being, Incubation

P248 Seeder bird production following inoculation with Campylobacter and Salmonella through various body openings Nelson Cox*[1], Douglas Cosby[1], Mark Berrang[1], Arthur Hinton, Jr.[1], Mark Harrison[2] *[1]U.S. National Poultry Research Center; [2]University of Georgia*

Newly hatched chicks may be exposed to varying levels of *Campylobacter* and *Salmonella* from an assortment of sources including the hatching cabinet, hatchery environment, broiler house, etc. These bacteria can enter the chick through several body openings (mouth, cloaca, eye, nasal passage or wet navel) and upon reaching the ceca, may attach and multiply to relatively high numbers in a short period of time resulting in a seeder bird. Seeder birds can spread bacterial contamination to other birds in the grow-out house. The objective of this study was to determine which of these entry routes would lead to the establishment of seeder birds. A nalidixic acid resistant strain of *Salmonella* Typhimurium (ST^{NR}) or a gentamicin resistant *Campylobacter coli* (CC^{GR}) was administered to the day-of-hatch chicks. Inoculum levels ranged from 10^1 to 10^4 cfu/mL and were inoculated into the various openings. The birds were housed in wire floor isolation units according to inoculation routes under a 24 h light regimen and *ad libitum* access to food and water. At 7 days of age, birds were humanely sacrificed and the ceca removed and examined for the presence and level of the target organism. With ST^{NR} and CC^{GR}, the production of seeder birds readily occurred when either organism was introduced via mouth, cloaca, eye or nasal passage. This study suggests that both *Campylobacter* and *Salmonella* seeder birds can result from bacteria entering an assortment of body openings in very young chicks. It will be essential for any strategy aimed at controlling contamination of breeder and/or broiler chickens by these human foodborne enteropathogens to apply effective sanitation systems designed to disinfect hatching eggs and cabinet air to prevent early exposure in a grow house. Multiple intervention strategies may be required.

Key Words: colonization, Campylobacter, Salmonella, broilers

P249 Effects of in ovo injection of L-ascorbic acid on early growth performance and systemic antioxidant capacity in broiler chickens Seyed Fatemi*, Haijun Zhang, Katie Elliott, Oluwaseun Durojaye, E.David Peebles *Department of Poultry Science, Mississippi State University, Mississippi State*

The effects of *in ovo* injection of various concentrations of L-ascorbic acid (AA) on early post-hatch broiler performance and antioxidant capacity were investigated. A total of 2,200 17 d of incubation (DOI) Ross 708 broiler embryos were randomly assigned to 6 treatments: non-injected control, saline-injected control, or saline containing 3, 6 ,12 or 36 mg AA per egg. At 17 DOI, a 100 µL volume of sterile 0.85% saline alone or containing different levels of AA were injected by an Inovoject m semi-automatic multi-egg injector. At hatch, 140 male hatchlings from each treatment group were randomly placed in 10 floor pens (replicates). Growth performances were determined from 0 to 7, 7 to 14, and 0 to 14 d of age (DOA). Plasma at hatch and 7 and 14 DOA were determined for total superoxide dismutase (T-SOD) activity and malondialdehyde (MDA) content by colorimetric assay. Experiment was conducted as completely randomized block design and data were analyzed using SAS 9.4. Birds hatched from 3 mg AA/egg had higher BW at 7 DOA (179.8 g, P=0.046) and higher ADG from 0 to 7 DOA (19.1 g, P=0.039) compared with non-injected birds (169.1 g and 17.6 g respectively), and also, higher ADG from 7 to 14 DOA (42.9 g, P=0.037) relative to saline-injected controls (41.4 g). Chicks that received 3 or 36 mg AA/egg had a reduced plasma MDA content at hatch by 39 and 31% respectively, and at 7 DOA by 19 and 13% respectively compared to the non-injected control (P<0.05). Plasma T-SOD activities increased in birds that received 3 or 6 mg AA/egg compared to non-injected or saline-injected controls by 34 to 62% at 7 DOA (P<0.05). The overall results suggested that the *in ovo* injection of AA (3 to 6 mg/egg) had the potential to promote the growth and ameliorate the systemic antioxidant capacity during early post-hatch phase.

Key Words: L-ascorbic acid, in ovo injection, growth performance, antioxidant capacity

P250 Novel method for sanitation of hatching eggs during egg storage and incubation. Wallace Berry*, Joe Hess, Ken Macklin *Auburn University*

Conventional methods for sanitizing hatching eggs each have shortcomings including toxicity to embryos and hatchery personnel, difficult application, wetting of the eggs with subsequent concentration of pathogens, and poor efficacy due to neutralization by organic material. The objective of the present study was to test a novel submicron mist “dry fog” technology for sanitizing hatching eggs. Specific aims were to determine effects of the technology on hatchability of commercial broiler breeder eggs and effects on first week mortality of chicks from treated eggs. Two replicate studies were conducted. Aviagen 708 hatching eggs were sourced from commercial hatcheries. The eggs had been stored for 2 to 3 days prior to delivery. Eggs were placed into incubator racks and in separate egg storage rooms and held at 65-67°F and 70% RH to simulate commercial storage conditions. Eggs were then stored for two days. In Trial 1, Eggs in room 1 were designated as “Treated”. Eggs in room 2 were “Control”. In Trial 2, the treatment rooms were reversed. Treated eggs were exposed to the dry fog for three 15-minute periods each storage day. All eggs were then incubated at 99.5°F and 65% relative humidity until simulated *in-ovo* inoculation at 18 days of incubation. The dry fog treatment did not change egg storage room temperature or RH and did not wet the eggs. Treated eggs were fogged as before for 15 minutes prior to inoculation and post-inoculation. All eggs were then placed into the incubators and allowed to hatch. In both trials, 400 chicks from each treatment were placed 25/pen in pine shaving floored pens in a single room broiler house under typical broiler chick management and feeding for one week. In both trials, hatch of fertile eggs was increased in the Treated groups (Trial 1: Treated = 95, Control = 92; Trial 2: Treated = 91, Control = 88). Treated chicks had lower 7-day % mortality in both trials (Trial 1: Treated = 1.5%, Control= 22.8%; Trial 2: Treated=0.8%, Control = 3.0%).

Key Words: hatching eggs, egg sanitation, egg holding, hatchability, mortality

P251 Identification, selection and weighting of food safety risk factors to be considered for their inclusion in the Canadian Food Inspection Agency’s Hatchery Risk Assessment model Marie-Lou Gaucher*[1], Alexandre Tremblay[1], Alexandre Leroux[2], Sylvain Quessy[1], Sunny Ng[2], Geneviève Comeau[2], Pascal Moreau[2], Teresa Cereno[2], Manon Racicot[2] *[1]Université de Montréal; [2]CFIA*

Controlling pathogens of public health significance in poultry production is a core challenge. In the transmission pathway of many pathogens of poultry, such as *Salmonella*, the hatchery represents a critical juncture. Various factors can influence the risk of disseminating pathogens for a hatchery facility. As part of the continuous improvement of its inspection system, the Canadian Food Inspection Agency (CFIA) is currently developing a risk assessment-based model for hatcheries in which the relative contribution of those factors will guide CFIA in properly allocating inspection resources based on the food safety risk of hatcheries under CFIA’s jurisdiction.

The objective of the current study was to identify, select and weight the critical food safety-related risk factors that should be included in a hatchery foodborne risk assessment-based model. A literature review along with advices from an expert panel were used to identify risk factors. Among the 33 risk factors selected, 9, 10 and 14 were clustered as inherent, mitigation and compliance factors. Criteria for assessing individual risk factors

were defined for the two-round Delphi approach. The Delphi's objective, involving 11 Canadian hatchery experts was to assess the relative contribution of each assessment criteria on the human food safety burden attributed to hatcheries. When experts were asked to provide weightings representing the relative risk to human health for each assessment criterion, 64% (63/99 scores) of them were assigned a value of less than 2. Thirty-one percent (31/99) were assigned a value between 2 and 3 (e.g. Hatching multiple species in the hatchery; Requiring information on the foodborne pathogens status of supply flocks) and 5% (5/99) were assigned a value of more than 3 (e.g. Fluff samples tested positive for *Salmonella spp.*; being subject to enforcement actions such as prosecution and license or registration suspension/cancellation).

There was broad consensus among participating experts regarding the various assessment criteria and clusters weighted, regardless of the respondent professional profile. The median values assigned to each assessment criterion and cluster will be used in the new CFIA's Establishment-based Risk Assessment model for Hatcheries.

Key Words: poultry, hatchery, pathogen, risk, inspection

Environment, Management and Animal Well-Being, Photoperiod and Lighting Programs

P252 Main effects of light sources, photoperiod, and strain on growth performance and carcass characteristics of broilers grown to heavy weights. Hammed Olanrewaju*, Joseph Purswell, Stephanie Collier, Scott Branton *USDA-ARS, Poultry Research Unit, Mississippi State*

Effects of light sources, photoperiod, and strain on growth performance and carcass characteristics of broilers grown to heavy weights (>3 kg) were evaluated. The experimental design was a 4 × 2 × 2 factorial treatments consisted of 4 light sources [incandescent (ICD, standard), compact fluorescent (CFL), neutral light emitting diode (Neutral-LED), and cool poultry specific filtered LED (Cool-PSF-LED)], 2 photoperiods (Regular/intermittent [2L:2D], and Short [8L:16D], and 2 strain sources (Ross × Ross 708, Cobb 700). In each trial, chicks of 2 different strains from different commercial hatcheries were equally and randomly distributed into environmentally-controlled rooms at 1 d of age. Each room was randomly assigned one of sixteen treatments from d 1 to 56 d of age. Feed and water were provided *ad libitum*. Birds were provided a four phase-feeding program (starter, grower, finisher, withdrawal). Birds and feed were weighed on 0, 14, 28, 42, and 56 d of age for growth performance. On d 56, 20 (10 males and 10 females) birds from each room were processed to determine weights and yields. The BW, BW gain, live weight and carcass weights of birds reared under PSF-LED were different ($P \leq 0.05$) in comparison with birds reared under ICD, but FI, FCR, mortality, and carcass characteristics were not affected by treatments. Also, broilers subjected to short/non-intermittent photoperiod had the lowest ($P \leq 0.05$) growth performance and carcass characteristics compared with values obtained for long and regular/intermittent photoperiods. Feed conversion, mortality, fat and yield were not affected by treatments. In addition, genetic strain was significant ($P \leq 0.05$) for most of the examined variables. There was no effect of strain, photoperiod, light sources, or their interactions on mortality and plasma corticosterone levels. This study shows positive impacts on alternative light sources for ICD along with regular/intermittent photoperiod in commercial poultry facilities, thereby reducing energy cost and optimizing production efficiency without inducing physiological stress on broilers grown to heavy weights. It also provides more economical availability of poultry meat without compromising the welfare of broilers grown to heavy weights.

Key Words: light-sources, photoperiod, strain, welfare, broiler

P253 Optimization of age in Japanese quail at photo stimulation for better egg quality and hatching results Syed Ali Raza Haider*, Athar Mahmud, Jibran Hussain, Muhammad Usman, Muhammad Waqas, Sohail Ahmad *University of Veterinary and Animal Sciences*

Light plays an important role in controlling the age at sexual maturity in birds; attaining sexual maturity at an appropriate age and weight is the key to reproductive success and the present study was an effort in the same direction. To develop specific practice for quail production and to optimize the best age for photo-stimulation present experiment was conducted. In total 225 females and 75 males of 5 week old were studied. A completely randomized design with three treatment of 5 replicates with 15 female and 5 male each, was applied. Treatment consisted 3 different ages of photo-stimulation (30, 35 and 40 days). Reproduction performance (fertile egg, hatchability, dead germ, dead in shell percent and hatchling body weight) and egg characteristics (egg weight, shell thickness, yolk index and Haugh unit) were evaluated. Study revealed that the photo-stimulating the birds at 35 days improve egg weight and hatchling weight. Birds stimulated at 40 days of age showed improved yolk index and Haugh unit score. Photo-stimulation at 40 days of age significantly influence the infertile, hatchability, dead germ, dead in shell %. It was concluded that photo-stimulation at 40 days of age not only improves reproductive and egg quality traits but also gives the consistency in all respects of these traits.

Key Words: Photo-stimulation, age, egg-quality, hatching-trait, Japanese-quail

Environment, Management and Animal Well-Being, Stress Responses

P254 The effect of stocking density on the stress response in turkeys Katie Hackney*[UG], Edgar Oviedo-Rondón, Kenneth Anderson, Jesse Grimes, Kimberly Livingston *North Carolina State University*

Stocking density is important to the turkey industry. However, there is no agreement on which density is most appropriate for animal welfare. Consequently, understanding how density influences the bird's overall stress physiology is paramount. This study was conducted to evaluate the effects of stocking density on blood corticosterone (CORT) level, heterophil:lymphocyte ratios (H:L), and antibody production. Stocking densities were determine by altering the pen space which provided 25.41, 23.30, 21.18, and 19.06 m^2 with 4 replicate pens per density. At hatch, 960 male poults were obtained from a Butterball hatchery (Goldsboro, NC. Poults were randomly assigned to pens representing each treatments with 60 poults/pen. From 0-5 wk of age, all birds were reared at the same density. Density treatments began at 5 wk of age and were obtained by changing pen size and holding the pen population constant. This resulted in the following stocking densities: 0.423 (T1), 0.388 (T2), 0.353 (T3), 0.318 (T4) m^2/bird All pens were provided with the same typical commercial feed rations and had the same feeder and drinker space per bird. At 8, 14, 15, and 19 weeks of age, blood was collected from 3 randomly selected poults per pen and put in lithium heparin tubes. Three µl of blood was placed on a glass slide for blood smears and fixed with methanol and stained with Wright's stain. The remaining blood was centrifuged at 1200 x g for 30 minutes to collect plasma for CORT analysis. At 14 weeks of age, 3 birds per pen were challenged with 1 ml of 7% sheep red blood cells (SRBC) via the brachial vein. At 14, 15, and 19 weeks of age, 3 mls of blood was collected to determine antibody titers against SRBC. Data were analyzed as a 2 way ANOVA where density and age were the independent variables using the GLM procedures of SAS®. At 8 weeks of age, poults with the least amount of space (T4) had greater CORT ($P<0.05$). However, this effect disappeared as the poults aged. No differences were observed among

the density treatments and H:L or SRBC titers. In conclusion, stocking density did initially affect the CORT levels early in the study, the poults appear to adapt to the stress caused by the density as no differences in CORT, H:L, or SRBC antibody titers are observed at 19 weeks of age.

Key Words: stocking density, turkey, stress

P255 The effect of stocking density on the stress response in broilers Xin Chuang*[UG], Jesse Grimes, Kenneth Anderson, Edgar Oviedo-Rondón, Kimberly Livingston *North Carolina State University*

Suboptimal stocking density can cause undue stress which can lead to reduced performance and aggression. Therefore, this study was conducted to evaluate the effects of stocking density on blood corticosterone (CORT) level, heterophil:lymphocyte ratios (H:L), and antibody production. Stocking densities were determine by altering the pen space which provided 8.12, 7.55, 6.96, and 6.37 m^2 with 4 replicate pens per density. There were 9 nipple drinkers and one 20-inch diameter tube feeder in each pen. At hatch, 1,376 Ross 708 male chicks were placed in 16 pens with new wood shavings with 86 chicks/pen. The resulting stocking densities were 10.59, 11.39, 12.36 and 13.50 chicks/m^2. At 12, 33, and 47 days of age, blood was collected from 3 randomly selected chicks per pen and put in lithium heparin tubes. Three µl of blood was placed on a glass slide for blood smears and fixed with methanol and stained with Wright's stain. The remaining blood was centrifuged at 1200 x g for 30 minutes to collect plasma for CORT analysis. At 26 days of age, 3 birds per pen were challenged with 0.1 ml of 7% sheep red blood cells (SRBC) via the brachial vein. At 12, 33, and 47 days of age, 3 mls of blood was collected to determine antibody titers against SRBC. Data were analyzed as a 2 way ANOVA where density and age were the independent variables using the GLM procedures of SAS®. Stocking density did appear to affect the H:L whereby a density of 10.59 chicks/m^2 resulted in the lowest H:L and 12.36 chicks/m^2 had the highest (P=0.007). However, this effect did not transfer to CORT or SRBC antibody titers (P>0.05). In conclusion, stocking density does not appear to adversely affect various measures of stress including CORT, H:L, and antibody titers.

Key Words: stocking density, broiler, stress

P256 The effect of reduced nocturnal temperatures on performance of broiler chicks from 0 to 21 days of age. David Clizer*[GS], Jeffre Firman *University of Missouri*

Heating costs make up most of production's utility costs, especially in the winter months of more northern poultry operations. Reductions in temperature of poultry barns should yield cost savings. Previous research in pigs, where temperature was dropped 8.3 degrees Celsius during the nocturnal period (1900 to 0700 h) had no effect on pig performance or health. This drop in nocturnal temperature represented a 30% reduction in heating fuel and 20% reduction in electrical use in pigs. This study was conducted to evaluate the effect of reduced nocturnal temperature on the performance of broiler chicks from 0 to 21 days of age. We hypothesized that broilers exposed to a reduction in nocturnal temperature would perform similarly to broilers not exposed to a reduction in nocturnal temperature. Two experiments were conducted at the University of Missouri, where 420 broiler chicks were received at hatch and placed in 3 thermally controlled rooms which each contained 4 pens. Treatments for experiment 1 were comprised of nocturnal temperature drops of 0, 2.8, and 5.6 degrees Celsius. There was no statistical difference in body weight, feed conversion or mortality between treatments. In a second experiment, temperatures were dropped further with nocturnal temperature drops of 0, 4.2 and 8.3 degrees Celsius. There was no statistical difference in body weight, feed conversion or mortality between treatments in this experiment either. This preliminary research suggests that a reduction in nocturnal temperatures up to 8.3 degrees Celsius have the potential to save producers utility costs without sacrificing broiler performance or health. More research is still needed to be done in a larger setting, but reductions in nocturnal temperatures of broiler chicks may be a viable option for reducing energy usage, production costs, and greenhouse gas emissions in the poultry industry.

Key Words: Broilers, Reduced Nocturnal Temperature, Energy, Feed Conversion

P257 Differential expression of water-channel and noncoding RNA biogenesis-related genes in three chicken lines under short-term water restriction Sara Orlowski*[GS], Joshua Flees, Nicholas Anthony, Sami Dridi *University of Arkansas*

Genetic selection for broiler growth rate has resulted in correlated changes in feed efficiency and carcass yield. Water consumption has also changed but it is unclear if the change is due to a modification of water homeostasis. Therefore a study was conducted to determine the effect of short-term water restriction on the expression of water channel and noncoding RNA biogenesis related genes. Kidney and whole blood samples were collected from three random bred populations; Jungle Fowl (JF), a broiler population from the 1990s (RB1990), and a modern broiler population (RB2015). Body weight matched birds from all lines were either subjected to water restriction (WR) for 3 h or ad libitum water access (control). The expression of target genes was estimated through real time quantitative-PCR. In the kidney, WR up-regulated the expression of AQP2 in all chicken populations, AQP3 in the RB1995, and ATP1B1 in JF and RB2015. AQP4 was downregulated in RB2015 with no effect on AVP expression. The expression of RNase III family enzymes was altered by WR in a population-dependent manner, with DICER1 being down-regulated in JF and RB1995, Drosha was decreased in RB1995, and ARG2 was up-regulated in RB2015. The expression of DGCR8 and TRBP1 was not affected by WR; however, DGCR8 mRNA levels were lower in RB1995 and RB2015 compared to JF under both conditions. TRBP1 gene expression was lower in RB1995 and RB2015 compared to JF under WR conditions. In the blood, gene expression was altered by WR, but with different patterns than the kidney. The mRNA abundances of AQP, AVP, DICER1, DGCR8, AGO2, and TRBP1 were decreased by WR in RB1995. However, the expression of AQP2, AVP, DGCR8, and TRBP1 was increased in WR-RB2015 compared to the control. In the JF, there was no difference in the expression of these genes except for a significant up-regulation of TRBP1 in WR compared to the control group. It appears that water channels and the RNase III enzymes are differentially regulated by WR in a population-dependent manner. Their expression in circulation could open new avenues for identification of new molecular signatures involved in adaptation to water-deprivation stress typical of areas were water is scarce.

Key Words: Broiler, Water Channel, Gene Expression, noncoding-RNA

P258 In-vitro and in-vivo comparison of the effect of several plant extracts against two protozoa genus, respectively Trypanosoma and Eimeria Sylvain Kerros*[1], Sébastien Pomel[2], Philippe Loiseau[2], Thibaut Chabrillat[1], Isabelle Deuve-Riou[1] *[1]Phytosynthese; [2]Université Paris Sud*

Demedication and natural healthy alternatives are clearly identified as long-term societal trends. One consequence in animal nutrition is to reduce the use of chemical coccidiostats. Phytogenics have already be proven to be efficient to prevent coccidiosis but their mode of action remain unclear. The objective of this work is to investigate and to compare the *in-vitro* and *in-vivo* antiprotozoal action of plant extracts used to replace chemical coccidiostats.

For the *in vitro* experience*, Trypanosoma brucei gambianse* reference strain was exposed to 6 different plant extracts in triplicate to determine antiprotozoal activity according 2 parameters: MEC (minimal concentration to kill 100% of trypanosomes) and IC50 (concentration inhibiting 50% of parasite growth).

The *in vivo* experience consisted in 2 consecutive coccidiosis challenged trials where male chicks Ross 308 were reared in cages from 8 to 35 days. Each trial was composed of 2 replicates of 8 birds, 1 negative control and 3 experimental groups. A 1mL inoculum/bird (*E.tenella* 200 000 ookysts;

E.acervulina 10 000 ookysts; *E.maxima* 10 000 ookysts) was nebulized at day 14.

For 3 plant extract families (Acanthaceae, Allioideae and Lamiaceae), we observed simultaneously a strong *in-vitro* trypanostatic activity (IC50 values between 1 and 10 mg/mL), a statistically significant *in-vivo* reduction of ookysts at D26 ($p<0.0001$) and improved lesion index (0.33 vs. 2.64) and FCR (-8%, -6%, -11% respectively).

2 plants extracts do not show correlation between the *in vitro* and *in vivo* experimentations. When Myrtaceae (genus *Psidium*) extract showed also a strong *in vitro* activity (IC50: 6 mg/mL), *in vivo* results such as lesion index (3.33 vs. 3.00) and FCR (-2%) were not different from control. On the opposite, Urticaceae extract showed very low *in vitro* trypanostatic activity (IC50: 506 mg/mL), all in-vivo parameters (Lesion: 0,67; FCR -8%) were strongly improved compared to control.

In this study, we demonstrate selected plant extracts can help to prevent coccidiosis. The direct antiprotozoal action of most of extracts as Acanthaceae, Allioideae or Lamiaceae seems to explain the improved performances; others secondary effects of plants must be investigated to explain the Myrtaceae and Urticaceae performances on broilers.

Key Words: Herbs, Extracts, Screening, Antiprotozoal, Coccidiosis

P259 Evaluation of the effect of formaldehyde or heat stress on body weights and body weight gain in neonatal broiler chickens Paula Johnson*[GS], Jacob Lum, Lucas Graham, Kyle Teague, Brittany Mahaffey-Graham, Mikayla Baxter, Juan David Latorre, Cheryl Lester, Guillermo Tellez-Isaias, Karen Christensen, Billy Hargis *University of Arkansas*

Formaldehyde has been used as a disinfectant in poultry hatching cabinets to aid in controlling key pathogenic organisms, such as *Salmonella* and *E.coli*. Post-hatch environmental heat stress has been shown to negatively impact performance in poultry. The objective of these experiments was to evaluate the effect of formaldehyde treatment or heat stress in the hatch cabinet on body weights (BWs) and body weight gain (BWG). In Exp 1, 18 day embryos (N=100 candled embryos/hatch cabinet – Exp 1 and 2) were placed in either a control, non-treated hatcher (37.2˚ C), or a formaldehyde-treated hatcher, where formaldehyde was maintained between 1-2 ppm. Chicks were co-mingled post-hatch (N=100/group) and individual BW were recorded on day 0, 7, and 10. At day 7 and day 10, the formaldehyde group had significantly lower ($p<0.05$) BWs than the control, non-treated group although there was no difference in BWG. Exp 2 evaluated and compared the effects of formaldehyde treatment or heat stress in the hatching environment. In Exp 2, 18 day embryos were placed into a control (37.2˚ C), non-treated hatcher, formaldehyde-treated hatcher (maintained at 1-2 ppm), or heat stress- (37.8˚C) treated hatcher. At day 10 post-hatch, both the formaldehyde-treated group (BW and BWG) and heat stress treated group (BWG) were significantly lower ($p<0.05$) than the control, non-treated group. Based on these results, 1-2 ppm formaldehyde treatment may have a negative impact on neonatal chick performance when small (N=100) groups of candled eggs are transferred to small cabinets. However, in large commercial hatchers with the potential for large bacterial contamination blooms, formaldehyde may offer a benefit. Heat stress of embryos/chicks during the hatching period appears to have an effect when temperatures are elevated to 37.8˚C. Temperature fluctuations may occasionally exceed this temperature in commercial hatchers.

Key Words: Hatching Cabinet, Formaldehyde, Heat Stress, Chick Performance

P260 Effects of Heat Stress on Tight Junction Protein Integrity in Broiler Chickens given a Noni-supplement Diet Guillermo Tellez*[UG], Elizabeth Greene, Joshua Flees, Anamika Gupta, Narayan Rath, Walter Bottje, Sami Dridi *University of Arkansas*

Heat stress has a detrimental effect on gut health in Broilers by impeding their ability to absorb nutrients which leads to a lower feed efficiency, a decrease in growth, and overall negatively affects their wellbeing. Therefore, there is a critical need to identify mechanism-based strategies to alleviate these effects. The purpose of the present study was to evaluate the effect of *Morinda Citrifolia* (Noni)-supplementation on the expression of tight junction proteins. Male Broilers (Cobb 500, 3 wks, n=480) were subjected to 2 environmental conditions (TN, 24°C *vs*. HS, 35°C), and fed two diets (control *vs*. 0.2% Noni) in a 2x2 factorial design. Chickens received water and feed *ad libitum.* Functional *in vitro* studies were conducted using primary chicken gut epithelial cells, and IPEC-J2 cell lines. At 80% confluence, cells were exposed to HS (45°C) for 2h and 30 min. Control cells were maintained at 37°C. The expression of target genes and proteins were determined by quantitative real-time PCR using $2^{-\Delta\Delta CT}$ method and Western blot, respectively. A significant increase in the expression of heat-shock protein (HSP70 and HSP90) were observed in the gut of control and noni feed chickens that were exposed to HS, as well as in cell cultures indicating a stress status. Furthermore, in primary chicken gut epithelial cells, the expression of ZO-2 gene was downregulated. In IPEC-J2 cells, Occludin and TAZ expression were altered. In the 1-week HS chicken gut, there was upregulation of claudin 5 protein and a downregulation of ZO-1, ZO-2 and Occludin. In Noni-fed chickens, ZO-1 and ZO-2 proteins were downregulated in both control and heat stressed birds while claudin 5 was upregulated. Together, our results indicate that HS dysregulates the expression of certain tight junction proteins which may explain the alteration of intestinal barrier integrity and leaky gut. The Mechanistic understanding of how heat stress regulates tight junction protein and affects gut integrity merits further in depth investigation.

Key Words: Heat Stress, Noni, Tight Junction Proteins, Intestinal Epithelial Cells

P261 Dry and heat stress affects H2S production of Salmonella on selective plating media Kurt Richardson*, Nelson Cox, Doug Cosby, Mark Berrang *Anitox Corp*

It has been shown that the pH of *Salmonella* pre-enrichment media becomes acidic (pH 4.0 to 5.0) when feeds and ingredients are incubated for 24 hrs. *Salmonella* in poultry feed have been stressed by heat and desiccation and exhibit different tolerances to the lower pH's than non-stressed cultures. .Acidic conditions affect the biochemical pathways and injure or kill *Salmonella*. In this study, eight serotypes, four from feed (*S*. Montevideo (SMo), *S*. Senftenberg (SS), *S*. Tennessee (STn) and *S*. Schwarzengrund (SSc)) and four from the processing plant (*S.* Typhimurium (STy), *S.* Enteritidis (SE), *S.* Infantis (SI), and *S*. Heidelberg (SH)), were grown in sterile meat and bone meal for 48hrs at 35°C, centrifuged and the sediment subjected to desiccation and heat exposure (37°C for 36-48 hrs under partial vacuum) to stress them. Isolates were subsequently exposed to acidic pH from 4.0 to 7.0 in 0.5 pH increments (3 replicates/pH increment) in citrate buffer. At 6 and 24 hrs, serial dilutions were plated in duplicate on xylose lysine tergitol 4 (XLT4) agar. Four serotypes (SE, SI, SM and SSc) showed an impaired ability to decarboxylate lysine on XLT4. At a pH of 6, 56.61 - 57.04% of the colonies of unstressed cultures of SI and SSc were H_2S negative within 6 hrs. This percentage significantly ($P<0.05$) increased for SSc at 24 hrs. Stressed SE and SI resulted in the greatest overall change in the ability of the isolate to decarboxylate lysine on XLT4. At a pH of 6, 92.51% of the SE colonies were H_2S positive at 6 hrs, however, at 24 hrs, 87.54% of the colonies were H_2S negative. In the case of SI, 90.24% of the colonies were H_2S negative at 6hrs. 24 Hr results were not significantly different ($P<0.05$). At a pH of 6, stressed cultures of SM and SSc produced an approximate 50:50 ratio of H_2S positive: H_2S negative colonies at both 6 and 24 hrs. When further examined using the API20 biochemical test, with the exception of SI, cultures were still able to decarboxylate lysine. This suggests that XLT4 agar contains a biochemical stressor(s) which affects the rate of decarboxylation by Salmonella instead of the isolate losing the ability to decarboxylate lysine. These results suggest that the acidic conditions may influence the detection and confirmation of *Salmonella* in feed.

Key Words: Salmonella, feed, injury, detection, confirmation

P262 Effect of cold stress on corticosterone and femur development in SPF chicks. Anderlise Borsoi*, Antônio Piantino-Ferreira, Caludete Astolfi-Ferreira, João Palermo-Neto *Tuiuti University of Paraná*

The present study aimed to investigate the effect of cold stress in chick's bone development. Specific pathogen free (SPF) day-old White Leghorn chicks were housed at the Experimental Center of Avian Pathology, University of São Paulo (USP) in two equal isolators equipped with HEPA filters operating under negative pressure. Water and feed, free from antimicrobials and animal protein, were provided ad libitum. The birds were used in accordance with USP Ethics Committee. On experimental Day 1 (ED1), 30 chicks were allocated into two groups: control group (Group C; n=15 birds) and cold stressed group (Group CS; n=15 birds). From ED1 to ED21, the chickens from the C group were kept under thermal comfort. Birds of the CS group were subjected to a temperature of 19±1°C for 6 h/day from ED1 to ED7; from ED8 to ED21 the chickens of these groups were kept under thermal comfort. At ED21, the birds were weighed euthanized. For histological examination, the right femur of all birds was prepared and stained using hematoxylin and eosin. The proliferative zone was measured in three locations of each section. The lengths of proliferating zone of the growth plate were captured analyzed by Motic Image Plus 2.0 software. Eight birds per group were selected, and blood samples were collected from their hearts. To avoid circadian rhythm influences, blood was taken from the birds between 08:00 am and 10:00 am at ED21. The corticosterone (CORT) concentrations were determined in the plasma using an Enzyme Immunoassay Kit (Arbor Assays). The results showed statistical difference: the CS birds analyzed had increased concentrations of corticosterone in the plasma, 29,92±3,6 ng/mL, compared to those of the C group, 4,70 ± 1,8 ng/mL. The femur proliferative zone measurements to the C group were 6,8 ± 0,69 mm and to CS birds were 6,30 ± 080. No difference in body weight and feed intake was found. Our results were in accordance to previous researches, when the CORT injected in broilers retarded the longitudinal growth of the long bones by inhibiting the proliferation and differentiation of chondrocytes in growth plate in broilers. The cold stress applied in the present work in the first week of chick's life had effects on CORT concentrations and femur development verified at 21 days old.

Key Words: stress, chicks, bone, corticosterone

P263 Effect of cold stress on corticosterone and Campylobacter jejuni isolation in SPF chicks. Anderlise Borsoi*, Atilio Calefi, Antônio Piantino-Ferreira, João Palermo-Neto *Tuiuti University of Paraná*

The present study aimed to investigate the effect of cold stress and *Capylobacter jejuni* (CJ) infection in chick in cecum bacterial isolation. Specific pathogen free (SPF) day-old White Leghorn chicks were housed at the Experimental Center of Avian Pathology, University of São Paulo (USP) in four isolators operating under negative pressure. Water and feed, free from antimicrobials and animal protein, were provided ad libitum. The birds were used in accordance with USP Ethics Committee. On experimental Day 1 (ED1), the chicks were allocated into four distinct groups (n=30 each): control group (C); cold stressed group (CS), infected with CJ (CJ), infected with CJ and cold stressed group (CJS). Infected groups received 0,5 ml of BHI broth containing 3.5 $\times 10^4$ cfu/ml of CJ. Eight birds per group were selected, and blood samples were collected from their hearts. To avoid circadian rhythm influences, blood was taken from the birds between 08:00 am and 10:00 am at ED7 and ED21. From euthanized birds, the cecum was collected. The corticosterone (CORT) concentrations were determined in the plasma using an Enzyme Immunoassay Kit (Arbor Assays). To campylobacters isolation. it was used Bolton broth and MCCDA agar (Oxoid). The cecum isolation at ED7 was high in the thermoneutral group (CJ) 100% and in the CJS group, 50% of the birds were positive. At ED21, the CJ group had 80% positives birds and the stressed CJS had an increase in positive birds 100%. The CORT measurements (ng/mL±sd) at ED7 were CJ= 42,2±10.14, and statistically high to CJS= 109,95±27,57. To ED 21 the CORT were C= 4,70 ±1,8; CS= 29,92±3,6; CJ= 55,27±8,8 and CJS= 59,54±8,4; data statistically high to infected groups compared to non-infected. It was possible to note that the initial infection was high in the thermoneutral group CJ compared to CJS, However, at ED21, CJ infected and stressed birds increase the corticosterone level and the cecum bacterial positivity. Recent research has shown that immune clearance following campylobacters infection takes many weeks, allowing long-term persistence in the ceca of birds beyond slaughter. Within this context, it seems that stress prevention is a relevant factor to achieve not only chicken welfare but also safe poultry production.

Key Words: stress, chicks, campylobacter

P264 Efficacy of plant extracts (CLEANACTIV and AEN) on the performances of broiler chickens reared in experimental conditions compared to conventional ionophore program. Thibaut Chabrillat*, Isabelle Deuve-Riou, Georgina Farré, Marçal Verdù, Francesc Cots, Sylvain Kerros *Phytosynthese*

Due to the growing pressure of governments and consumers to reduce the use of antibiotics and coccidiostats in animal feed, new alternatives to prevent coccidiosis are now available beyond the use of vaccines. Plant extracts are commonly used in Europe to reduce incidence of coccidiosis. This trial was implemented to compare a plant extract digestive program (AEN and CleanActiv) with conventional coccidiostat program and an untreated group. 4920 broilers, mixed males and females ROSS were reared in a private experimental farm until slaughtering (37 days old). The trial was composed of 3 groups with 8 pens of 205 broilers for each group. All animal received the same feeding program; control group (C-) did not receive any supplementation. The coccidiostat group (C+) was supplemented with Nicarbazin + Narasin (50 ppm) from D0 to D21 then with Monensin (120 ppm) from D21 to D30 then Narasin from D30 to Day37 (70 ppm). Plant extract group (PE) received a feed containing AEN at 150 g/ton and CleanActiv at 500 g/ton from D0 to D21 then AEN at 300 g/ton and CleanActiv at 500 g/ton from D21 to D37. Feed intake, live weight and mortality were measured at Day 7, 21, 28 and 35 for each pen. One bird per pen was necropsied at day 19, 28 and 35 to determine coccidiosis lesion scores. At D28 and D35 live weights were higher for PE than C- (35 days live weight: 2.077kg vs. 2.134 kg; p< 0, 0001). The global average daily gain (ADG) was higher for PE than C- (58.255 g/d vs. 59.860 g; p< 0.0001). The Feed Conversion Ratio (FCR) was reduced for PE group compared to C- (1.595 vs 1.584; p=0,037). No significant differences were observed on LW, ADG or FCR between PE and C+ at any period. Performances were improved in treated groups from 21 days, when the daily growth and risk of coccidiosis and enteritis becoming higher. Regarding necropsies, only *Eimeria maxima* and *Eimeria tenella* lesions were observed; due to experimental conditions and no artificial challenge un-significant differences were obtained. In conclusion this type of program with plants extracts is very promising for the future to reduce antibiotics and coccidiostats uses.

Key Words: herbs, extracts, coccidiosis, ionophores, broilers

P265 Identifying new antimicrobials that may improve broiler litter quality Abdulmohsen Alqhtani*[GS], Tomi Obe, Christopher McDaniel, Aaron Kiess *Mississippi State University*

Pathogens and high ammonia are often associated with poor litter quality which can create serious consequences for farm workers and broilers. Currently, commercially available litter amendments are used to help reduce broiler litter pH, ammonia and pathogens, but there effectiveness is time sensitive. Therefore, the objective of the current study was to investigate 2 new antimicrobials, a mineral compound (MC) and an organic acid (OA), for their ability, *in vitro*, to inhibit or eliminate pathogens and their feasibility for use as new litter amendments. To obtain the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the MC and OA against *Salmonella* Typhimurium (*ST*) and *Escherichia coli* (*EC*), the Clinical Laboratory Standards Institute guidelines were used. This was completed by exposing 10^6 cfu/ml of each pathogen to

multiple concentrations of MC or OA in 2 different media, buffered peptone water (BPW) or Tryptic Soy Broth (TSB) using 96 well microtiter plates. The plates were incubated for 24h at 37ºC; the first well that demonstrated no visual turbidity after incubation was considered the MIC. The MBC was obtained by plating 50 µL of content on tryptic soy agar plates from all visually clear wells and incubating them for an additional 24h at 37°C. The well with the lowest concentration of MC or OA that demonstrated no bacterial growth was considered the MBC. The data were analyzed using a randomized complete block design and differences were separated using Fisher's protected LSD (P≤0.05). For *ST* and *EC*, an antimicrobial by media interaction was detected for both the MIC (P<0.0001; P=0.003) and MBC (P<0.0001; P<0.0001). For *ST*, the MIC and MBC of both MC and OA were higher in TSB than BPW. With *EC*, TSB increased the MIC and MBC of OA compared to BPW. However, the MIC and MBC of MC was not affected by the media. It was also discovered, that for both *ST* and *EC,* MC lowered the MIC and MBC when compared to OA for both media types. In conclusion the results suggest that both antimicrobials are capable of eliminating pathogens, *in vitro*, but nutrients can impact the effect of the antimicrobial, and MC eliminates pathogens at lower concentrations possibly making it a more suitable candidate as a litter amendment.

Key Words: mineral compound, organic acid, pathogens, broilers, litter

P266 Turkey litter mass, nutrients, and nutrients per bird from different management styles in Pennsylvania Erica Rogers, R. Michael Hulet, Douglas Beegle, Paul Patterson* *The Pennsylvania State University*

Turkey production in the mid-Atlantic region has evolved considerably along with the poultry industry as a whole. Consumers and the EPA are concerned about environmental health and water quality including that in the Chesapeake Bay (CB) watershed. A nutrient "diet" (total maximum daily load= TMDL) has been established for the CB allowing for a discrete number of nutrients to be released into the watershed (Nitrogen (N): 84.30 million kg, Phosphorus (P): 5.67 million kg, and Sediment: 2.90 billion kg). Unfortunately, the model numbers utilized in the Chesapeake Bay TMDL do not represent current poultry industry standards and management techniques. In order to address this issue, a field study was performed in which turkey litter samples were collected from different turkey farms across Pennsylvania to measure litter production and nutrient concentration. Management styles including All-In-All Out Hens (AIAO Hens), All-In-All Out Toms (AIAO Toms), Two-Stage Light Hens (TSL Hens), Two-Stage Heavy Hens (TSH Hens) and Two-Stage Toms (TS Toms) were compared with two or three flocks per management style. No significant differences were observed between management styles for total-N, P_2O_5, or K_2O. Litter production values currently being used in the models are 1,724 kg of as-excreted manure per 100 birds for hens and 3,538 kg for toms with an average 74% moisture. The current study shows that the highest litter production from the hens was 792 kg/100 birds and 1,811 kg/100 birds for toms with a range of 35 to 46% moisture. The results showed that management styles did not influence the amount of litter or its nutrient concentration (P > 0.05). However, the commercial turkeys herein produce less litter, with lower moisture and P_2O_5 than previous studies would suggest, and indicate models used in establishing the TMDL should reflect the nutrient efficiency of modern turkey production practices.

Key Words: Turkey, Litter, Nutrients, Chesapeake, Bay

P267 Relationships between deoxynivalenol and heavy metal concentrations of corn grains Ahmet Pekel*, Ali Çalık, Mustafa Alataş, Eren Kuter, Özcan Cengiz, Gülden Omurtag *Istanbul University*

A study was carried out to determine the deoxynivalenol (DON), Ca, P, and heavy metal (Cd, Cr, Cu, Ni, Pb, and Zn) concentrations of corn grains (n = 54) collected from different feed mills located in Turkey during the summer of 2016. The aim of the study was to determine whether the heavy metals and DON concentrations were correlated. Deoxynivalenol was extracted with water and the extract then was passed through an immunoaffinity column followed by HPLC analysis. The heavy metal concentrations were determined by an ICP/Mass spectrometry. Proc corr procedure in SAS was used to determine the correlations. All the heavy metal concentrations were lower than certain limits posted by regulation agencies for animals. Occurrence of DON was 53%, with the highest level of 0.725 ppm. Although the DON occurrence was relatively high, none of the DON positive samples had concentrations above maximum tolerable levels set by FDA and EU for animals. Although there was a significant correlation between Cr and DON concentration, it lacked the practical importance. There were 7 highly significant ($P < 0.01$) correlations between heavy metals. However, only the significant correlations between Zn−Ca, Pb−Cu, and Pb−Cd produced the highest R (0.64 to 0.67) and R^2 (0.41 to 0.45) values that would be of practical importance. In conclusion, the significant correlations found between heavy metals would be of practical importance to feed and animal producers

Key Words: Corn, Correlations, Deoxynivalenol, Metals

P268 Evaluation of shell quality of hens receiving reduced calcium diets Lauren Nolan*[GS], Anthony Pescatore, Michael Ford *University of Kentucky*

This study was conducted to determine the effectiveness of standard egg quality measurements and scanning electron microscopy for determining changes in shell structure. Twelve hens were divided into two groups and were fed either a diet sufficient in calcium (3.90% of the diet) or a calcium deficient diet (0.80% of the diet). Hens were housed individually in cages and eggs were collected daily beginning at the hen's sixty-sixth week of lay (82 weeks of age) for 20d. Eggs were weighed and the following parameters were measured: specific gravity, shell breaking strength, haugh unit, dry shell weight (g), and percent shell. Shells from the first and last egg collected from each hen were soaked in a 6% sodium hypochlorite, 4.12% sodium chloride, and 0.15% sodium hydroxide solution overnight, rinsed and dried for 48 hrs, to remove shell membranes. Membrane free shells were prepared for scanning electron microscopy (SEM). SEM images were used to determine 'a' and 'b' bodies for each egg within a $32.35in^2$ image. Using a univariate approach, data were analyzed looking at egg quality parameters for each treatment. There were no significant differences in egg weight between treatments 1 and 2 (64.17g and 64.06g respectively). The eggs from hens receiving 3.90% Ca (treatment 1) had a significantly higher shell breaking strength (2.67kgf) compared to eggs from hens receiving 0.80% Ca in the diet (1.68 kgf, P<0.0001). Treatment 1 had a significantly higher shell weight (P<0.0001) (4.92g) compared to treatment 2 (3.71g). There was a significant difference in percent shell for treatment 1 (7.63%) compared to treatment 2 (5.82%). This is to be expected when reduced calcium diets are provided to laying hens, especially at late stages of the lay cycle. Hens in treatment 2 had a greater haugh unit (63.1HU) while hens in treatment 1, which had 58.4HU (P = 0.001). Specific gravity between treatments were not biologically different, therefore, it was not a useful parameter in determining shell quality. Using SEM imaging, the ratio of A:B bodies were higher for treatment 1 (40.04) compared to treatment 2 (14.94). This change in A:B ratio is an area that needs further research in order to understand changes in egg structure and strength over the hen's productive life.

Key Words: egg quality, calcium, electron scanning microscopy

P269 Effect of body weight, sex, age and handling procedures on tonic immobility time and leucocyte profile in commercial broilers. Diego Martinez*, Jorge Tay, Fabiola Caqui, Cristian Uculmana, Mayra Calagua *LIAN Development & Service*

In order to determine the effect of body weight (BW), sex, age and testing procedures on stress indicators, 341,131 Cobb-500 day-old broiler chicks assigned to 24 commercial sex-segregated broiler houses with 14,214 chicks each (SD: 4,494) were raised, within the same geographical area, under standard conditions: feeding, management, facilities, equip-

ment and housing densities (11-12 chicks/m^2 and 34-30 kg/m^2 for males-females, respectively). To test tonic immobility time (TIT) at 31 or 42 days of age, a 50 m^2 bird-free working area was established inside the house. 10 birds were weighted and placed face up on the litter, restrained for 20 seconds (s) while making eye-to-eye contact. TIT was the time elapsed until the bird righted itself. The procedure restarted up to 2 times if it was ≤10 s. 5 of these were selected for individual blood sampling (BS) before or after TIT evaluation to apply BS as a stressing factor for TIT and vice versa. Heterophil to lymphocyte (HLR), heterophil to basophil (HBR) and lymphocyte to monocyte (LMR) ratios were determined on Wright-stained slide smears. TIT values and all ratios were natural log-transformed to achieve normality. To determine the effect of sex/age and test order, two Completely Randomized Unbalanced Designs under 2×2 (sexes, ages) and 2x2x2 (also testing orders) factorial arrangements were applied having the house or the bird as the experimental unit, respectively. TIT was correlated to BW within each age and sex. All the ratios and TIT values were correlated to each other. Variances were analyzed with GLM procedure of SAS and differences compared using t-tests by LSMEANS option. Spearman's correlation coefficients are shown. TIT was not correlated to age, BW or BW gain (P>0.50). HLR and HBR were higher in older birds (P<0.05) and females (P<0.02), respectively. TIT procedure increased HLR (P<0.03) but not HBR or LMR (P>0.50). BS increased TIT (P<0.04) only in females. TIT was not correlated to HLR (P>0.24) but with LMR (0.29; P<0.02). HLR was correlated with HBR (0.45; P<0.01) and LMR (-0.28; P<0.01). In conclusion, TIT was not affected by age or BW gain, the evaluated variables were more sensitive in females than males, and HBR and LMR may be used as stress indicators in addition to HLR and TIT.

Key Words: tonic-immobility, growth-rate, leucocyte-count, heterophil-to-lymphocyte-ratio, commercial-evaluation

Metabolism and Nutrition, Feed Additives

P270 Applying meta-analysis to improve the value of commercial tests: a phytogenic feed additive case study. Diego Martinez*, Carlos Vilchez *La Molina National Agrarian University*

The objective was to demonstrate the capability of meta-analysis (MA) to improve the value of commercial tests (CT) conducted under a wide variety of conditions. Feed conversion ratio (FCR) data from 9 CT with a total of 622,496 broilers up to 42 days, were the treatment was the presence of a specific phytogenic feed additive based on essential oils, was used to determine the overall effect of the feed additive and the heterogeneity among the different CT. Only those CT including a non-supplemented control group and at least 2 replications per treatment were used. The nature of the 9 selected CT were: as part of buying decision-making processes (BDMP) (5 in commercial farms; 1 in an experimental facility), to document results (2 on commercial farms), and as a project at a local university (1). The number of replications per evaluation ranged from 2 (BDMP) to 6 (university). Data from each independent CT was analyzed under a Completely Randomized Design with the GLM procedure of SAS 9.4. The MA was performed with the Metafor package in *R* version 3.4.2 and heterogeneity was quantified using a random-effects model by the test of homogeneity, the between-study variance (τ^2), and the H^2 statistic. After individually analyzing the data from the 9 CT, the relative difference in FCR between treated and control broilers varied from -4.5% to +1.2% but with no statistical significance (P>0.14) in any of them. As expected, P values were high (P>0.60) in low-replicated CT, but also in the CT conducted at a university (P>0.14), therefore no evaluation offered conclusive outcomes. The results of the MA showed no significant heterogeneity among the different CT (P>0.87; τ^2=0±0.0013; H^2=1.0) and an overall significative effect of the feed additive on FCR of -0.0346 points (P=0.0460) with a confidence interval (95%) of -0.0686 to -0.0006. The obtained results showed that the pool of CT with no statistical effects of the feed additive may even produce an overall statistically significant effect. From the MA results, it may be claimed that the feed additive produced an overall effect of -0.0346 points in FCR ranging within the rank of -0.0686 to -0.0006 with a confidence of 95%. In conclusion, MA is a useful technique to improve the value of CT.

Key Words: meta-analysis, commercial-tests, field-tests, statistical-significance, feed-conversion-ratio

P271 Characterisation of a novel fungal muramidase Mikkel Klausen, Zoltán Pragai, Rual Lopez-Ulibarri, Marianne Cohn* *Novozymes A/S*

A novel muramidase has been developed by DSM and Novozymes. The muramidase is a microbial enzyme that hydrolyses the peptidoglycan polymer layer of bacterial cell wall fragments. In nature, all muramidases have a potential anti-microbial activity that could be regarded as a risk for possible increased selection of cross-resistant bacteria in the environment, including the gut of domesticated animals, and is therefore a risk at feed-inclusion level. To assess this risk, it is recommended by regulatory bodies (i.e. European Food Safety Authority (EFSA)) to perform a Minimal Inhibitory Concentration (MIC) assay.

In this study, the anti-microbial potency of the muramidase was evaluated according to EFSA guidelines, testing the potency of the muramidase against five bacterial strains known to be susceptible to a large number of antibiotics, and in addition, against 27 additional bacterial strains representing potential pathogens and commensals isolated from poultry. In conclusion, no anti-microbial potency of the novel muramidase was detected at a broad concentration range for all bacteria in the test panel.

Key Words: Muramidase, Antimicrobial, MIC, Peptidoglycans, Safety

P272 Evaluation of the effects of supplementation of a novel muramidase in poultry feed Mikkel Klausen[1], Marianne Thorup Cohn[1], Estefania Pérez Calvo[2], Pietro Celi[2], Rual Lopez-Ulibarri*[2] [1]*Novozymes A/S;* [2]*DSM Nutritional Products*

Muramidases (EC 3.2.1.17) belong to the family of glycosyl hydrolytic enzymes, as do glucanases and hemi-cellulases. Muramidase cleaves the β-1,4 glycosidic linkages between N-acetylmuramic acid and N-acetyl-glucosamine in the carbohydrate backbone of peptidoglycans, which are the major structural component of bacterial cell walls. Bacterial cell wall debris is produced when the microbiota in the digestive tract of animals is replicating or dying. Muramidases, naturally present in the gut, act locally as a digestive aid by hydrolyzing peptidoglycans in cell wall fragments, optimizing availability of nutrients and supporting gastrointestinal functionality. Novozymes and DSM Nutritional products have developed a new enzyme product: a muramidase from *Acremonium alcalophilum* based on state-of-the-art enzyme technology used to produce feed enzymes. The addition of this dietary muramidase to poultry feed has resulted in significant improvements in performance, nutrient absorption and digestibility under a variety of test conditions. Although muramidases are known to have anti-microbial activity with different potencies, dedicated *in vitro* assays done in accordance with EFSA guidelines showed that the muramidase has no antimicrobial potency at the recommended dose. Moreover, total microbial counts (CFU) of intestinal samples from broilers supplemented with this muramidase compared to non-supplemented birds did not show a decrease in total CFU values, suggesting the absence of significant effect on the gut microbiota by the muramidase. In addition, no direct notable microbiota modulation effect has been detected by using the muramidase in *in vivo* trials. Furthermore, *in vitro* studies have shown that the muramidase preferentially hydrolyzes peptidoglycans from bacterial cell wall fragments rather than peptidoglycans from live bacteria. In summary, these studies suggest that addition of A. *alcalophilum* muramidase in poultry feed facilitates the utilization of bacterial cell debris in the gut

by hydrolyzing peptidoglycans, thus optimizing gastrointestinal functionality and resulting in improved nutrient utilization.

Key Words: Muramidase, peptidoglycans, gastrointestinal-functionality, bacterial-cell-debris, broiler-chickens

P273 Effects of a malabsorptive rye diet on growth and adipose tissue in commercial broilers Lemuel Reber, Mikayla Baxter, Billy Hargis, Guillermo Tellez*, Dawn Koltes *University of Arkansas*

Diets high in non-starch polysaccharides such as rye are known to reduce nutrient absorption resulting decreased performance. Upon refeeding with, birds increase weight gain to rates similar to non-restricted cohorts. Historically this weight gain would be expected to be partitioned toward adipose. However, with modern selection strategies it is unclear if this traditional view still holds true. To determine if adipose tissue accretion was similar following a 10 d malabsorptive diet modern broilers (n=36) were assigned to 1 of 4 dietary treatments which consisted of a control corn based diet (CD); an early phase malabsorptive diet where birds were on a rye diet from day 0 to 10 then switched to a corn diet until day 20 (EMD); a late phase malabsorptive diet where birds were on a corn diet from day 0 to 10 then a rye diet until day 20 (LMD); and a malabsorptive (rye) diet throughout (MD). Birds were weighed on day 9 and 19 and sampled on either day 10 or 20. From day 0 until day 10, only the CD or MD diets were applied. At day 9, body weight was not reduced with the rye diet ($P = 0.25$); however, birds on the MD diet were numerically lighter. At day 10, the CD fed birds had greater percentage of adipose tissue per amount of body weight compared to MD ($P < 0.01$). This was accompanied by an increase in adipocyte area ($P < 0.01$) indicating increased lipid fill. By day 19, body weight, percent of adipose tissue per unit of body weight, and adipocytes area were significantly different across the four dietary treatments ($P < 0.01$). Birds fed CD or EMD were the heaviest and the highest percent of adipose tissue; with birds on a rye diet during the late phase (LMD) an intermediate to the MD and CD or EMD ($P < 0.05$). Adipocytes area was greatest in CD birds compared to MD ($P < 0.01$) with the EMD and CD being similar and EMD and LMD being similar ($P > 0.5$). In conclusion, recovery from a malabsorptive diet did not significantly increase the amount of adipose or lipid fill of adipocytes compared to standard diets; however, there was a 7% increase in adipose tissue in those birds that underwent nutrient restriction compared to those that did not undergo nutrient restriction suggesting that extra nutrient may be diverted to adipose accretion.

Key Words: compensatory, fat, undernutrition

P274 Dietary microalgae and zn proteinate improves hatch of broiler breeder offspring during post-peak lay Marquisha Paul*[GS], Lizza Macalintal, Lauren Nolan, Anthony Pescatore, Tuoying Ao, Michael Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

The purpose of this study was to evaluate the effect of supplementing microalgae (FORPLUS™, Alltech, Inc.) and two sources of Zn (ZnO or Bioplex® Zn, Alltech, Inc.) in broiler breeder diets on fertility, embryo mortality, and hatch of offspring during post-peak lay. An RCB design with a 2x2 factorial trt structure was used for this study. A total of 240 Cobb500™ broiler breeder hens and 20 Cobb500™ broiler breeder roosters were randomly assigned to a corn-soybean meal diet containing either 0% or 2% microalgae and an additional 40 mg Zn/kg diet as ZnO or Bioplex® Zn at hatch. Broiler breeders were raised in floor pens with *ad libitum* access to water and nest boxes, 15L: 9D lighting, and controlled access to feed. Each dietary trt consisted of 5 rep floor pens (12 hens + 1 rooster/pen). During post-peak lay (28-45 wk of lay), 60 eggs/pen were collected at 30-31, 35-37, and 41-43 wk of lay and set for incubation. Eggs were candled to determine fertility and embryo mortality before transfer to a hatcher. Hatchability, hatch of fertile eggs (HOF), and % pip were determined after 21 d of incubation. A main effect of dietary microalgae level was observed on hatchability at 30-31 wk of lay. Hatchability increased (P=0.01) when breeders were fed 2% FORPLUS™ vs 0% FORPLUS™ (74.0% vs 66.1%). At 35-37 wk of lay, an interaction of microalgae level and Zn source was observed for embryo mortality, HOF, and % no pip. Embryo mortality was reduced (P<0.05) when breeders were fed 2% FORPLUS™ + ZnO vs. 0% FORPLUS™ + ZnO (2.98% vs 7.53%), however embryo mortality was not significantly different when compared to the diets with Bioplex® Zn. HOF decreased (P=0.01) and % no pip increased (P=0.01) when breeders were fed 0% FORPLUS™ + ZnO compared to all other diets. At 41-43 wk of lay, an interaction of microalgae level and Zn source was observed for HOF and % no pip. When either 2% FORPLUS™ or Bioplex® Zn were added singularly to the breeder diets, HOF and % no pip were improved. Fertility was not affected by dietary trt for this study. In conclusion, dietary supplementation of FORPLUS™ or Bioplex® Zn improved the hatch of broiler breeder offspring throughout post-peak lay.

Key Words: Broiler-breeder, Postpeak-lay, Microalgae, Zinc, Hatch

P275 Dietary supplementation with fish oil or a combination of linseed oil and microalgae improves performance, serum composition, meat quality, antioxidant status and fatty acid profile of broilers Shenfei Long*[GS], Li Liu, Sheng Kang, Xiaokang Ma, Qianqian Wang, Gang Lin, Anne Koontz, Xiangshu Piao *State Key Laboratory of Animal Nutrition, Ministry of Agriculture Feed Industry Centre, China Agricultural University*

The study was conducted to determine the effects of dietary supplementation of fish oil (FO) or a combination of linseed oil (LO) and microalgae (MA) on performance, serum composition, meat quality, antioxidant status and fatty acid profile of broilers. As-hatched Arbor Acres chicks (n = 126; 1-d-old; weighing 45.5 ± 0.72 g) were randomly allocated to 1 of 3 treatments with 7 replicate pens per treatment (6 birds per pen). The dietary treatments included a corn-soybean basal diet supplemented with 3% soybean oil (SO) (CON), basal diet supplemented with 1% MA, 1% LO and 1% SO (ML), and basal diet supplemented with 2% FO and 1% SO (FO). Data was subjected to ANOVA using the GLM procedure of SAS (SAS Institute, 1996). Pen was the experimental unit. Differences among treatments were separated by Duncan's multiple range test. Significance was designated at $P < 0.05$. Compared with CON, birds supplemented with ML or FO had greater ($P < 0.05$) body weight and average daily gain in phase 1 (d 1 to 21), phase 2 (d 22 to 42) and overall (d 1 to 42), as well as higher ($P < 0.05$) feed efficiency in phase 1 and overall. Birds fed with FO showed lower ($P \leq 0.05$) lightness in thigh muscle on d 21 and higher ($P \leq 0.05$) liver index, pancreas index and redness in breast and thigh muscle on d 42 compared with CON. Birds fed with ML or FO showed lower ($P < 0.05$) abdominal fat index and concentration of total serum cholesterol in phase 1, while these birds had higher ($P < 0.05$) content of serum glucose in phase 2 compared with CON. Birds fed with ML or FO also had improved ($P < 0.05$) concentration of eicosapentaenoic acid, docosahexaenoic acid and n-3 polyunsaturated fatty acid (PUFA), as well as decreased ($P < 0.05$) content of n-6 PUFA, n-6 / n-3 ratio in breast and thigh muscle compared with CON. Birds supplemented with ML or FO both showed greater ($P < 0.01$) concentration of total antioxidant capacity, superoxide dismutase and glutathione peroxidase, and lower ($P < 0.05$) concentration of malondialdehyde in breast and thigh muscle compared with CON. In conclusion, 2% fish oil or a combination of 1% linseed oil and 1% microalgae both showed positive effects on performance, serum composition, meat quality, antioxidant status and fatty acid profile in birds.

Key Words: broiler, DHA, algae

P276 Effects of oregano extract oil as an alternative to antibiotics on growth performance, immunity, antioxidant activity and cecum microbiota in broilers Liyuan Zhang*[GS], Qiugang Ma, Lihong Zhao, Jianyun zhang, Cheng Ji *china agricultural university*

The aim of this experiment was to study the alternative effect of oregano extract oil (OEO) to antibiotics by comparing the effects of oregano extract oil (OEO) and antibiotics on growth performance, immunity, antioxidant activity, and gut microbiota in broilers. A total of 576 (half male and half female) AA broilers were randomly allocated to 6 treatments with 6

replicates of 16 broilers each (8 male and 8 female). The negative control group (A) was fed with basal diet; positive groups (B, C and D) were fed with different antibiotics based on basal diet, virginiamycin, enramycin, avilamycin combination with colistin sulfate, respectively; group E fed diet with 200 mg/kg natural oregano extract oil (NOEO); group F fed diet with 200 mg/kg mixture of thymol and carvacrol (MTC) which has the same active components and ratio as NOEO. The experiment last for 42 days. The results showed that comparing to negative control, diets supplemented with antibiotics, NOEO and MTC could increase average daily gain (ADG) of 21d broilers, decrease feed growth ratio (F:G) of 1~21d and 1~42d broilers (P<0.05), antibiotics, NOEO and MTC showed great enhance effects on B-lymphocyte transformation rate of 21d and 42d broilers as well as T-lymphocyte transformation rate of 42d birds, concentrations of serum immunoglobulins A, G, M and complement 3 (C3), complement 4 (C4) of 21d broilers were dramatic increased, along with the concentrations of serum immunoglobulin M and C4 of 42d birds(P<0.05); diets supplemented with antibiotics, NOEO and MTC significantly increased the activities of glutathione peoxidase (GSH-Px) and superoxie dismutase (MTCD) in serum and duodenum tissue of 21d broilers (P<0.05); antibiotics, NOEO and MTC could alMTC distinctly increase *Lactobacillus spp.* counts, restrain *Escherichia. coli*. counts in ileum and cecum, still salmonella counts in ileum and cecum, *salmonella* counts in ileum and cecum of 21d birds. Conclusion: oregano extract oil (OEO) had similar effects on growth performance, immunity, antioxidant activity, cecum microbiota in broilers with antibiotics, meanwhile, natural oregano extract oil had relative better effects than mixtur of thymol and carvacrol, showing a promising alternative effects to antibiotics.

Key Words: oreganoextractoil, antibiotics, broiler, growthperformance, immunity

P277 Effects of dietary supplementation with an antimicrobial peptide-Pulete on growth performance and cecum morphology in broilers Jinglin Ma*[UG], Lihong Zhao, Qiugang Ma, Jianyun Zhang, Cheng Ji, Yongpeng Guo *china agricultural university*

The aim of this experiment is to study the effect of antimicrobial peptide on growth performance and ileum morphology in broilers. Two hundred eighty eight 1d old male broilers (AA) were randomly allotted to 4 dietary treatments, each consisting of 6 pens as replicates, with 12 chicks in each pen. The dietary treatments were: NC (negative control; basal diet), PC (positive control; basal diet supplemented with 10 mg enramycin/kg diet) and two treatments of antimicrobial peptide (AMP) (basal diet supplemented with 100 and 200 mg AMP /kg diets). The chickens were fed experimental diets in 2 phases, starter (d 0–21) and finisher (d 22–42). The average daily body weight gain (ADG) and feed conversion ratio (FCR) were greater (P<0.05) in chicks fed the PC diet than birds fed the NC diet in starter, finisher and overall period. There were no significant effects of dietary supplement AMP on ADG during starter, but the ADG in finisher and overall) and FCR in starter, finisher and overall were greater ((P<0.05) in chicks fed the PLT diet than chicks fed the NC diet. Moreover, increasing levels of dietary AMP linearly (P<0.05) improved ADG in finisher and overall. The crypt depth of ileum and villus height :crypt depth (VH:CD) of the ileum were greater (P<0.05) in the birds fed the PC and 200 mg AMP /kg diets than birds fed the NC diet during finisher. Moreover, increasing levels of dietary AMP linearly increased (P<0.05) villus height and VH:CD of the ileum.

Key Words: antimicrobialpeptide, broiler, growthperformance, ileummorphology

P278 Evaluation of multiple levels of phosphatidic acid on growth performance and breast meat yield in broilers Jason Lee*, Scott Hagerman, Gregory Archer *Texas A&M University*

Improving feed conversion while increasing growth is a goal of any broiler nutrition program. The use of feed additives to obtain this goal has increased in recent years. Therefore, it is important to evaluate potential feed additives for not only increased performance but for the pitfalls that may accompany it. A study was conducted to evaluate the effects of feeding phosphatidic acid (PA) in the feed at differing levels on the growth and meat yield of broilers. Dietary treatments included: T1, control (CON), T2, 5 mg/bird/day of PA (LowPA), T3, 10 mg/bird/day of PA (MidPA), and T4, 15 mg/bird/day of PA (HighPA). All birds were weighed on d 14, 28, 42 and 49 to obtain average pen weights and feed conversion ratios. On day 49, eight birds per pen were processed and carcass and breast yield were determined. Breast filets were evaluated for the presence and severity of woody breast and white striping. Differences ($P < 0.05$) in live bird weights between the control birds (1.659kg) and all PA treatments (pooled mean: 1.731kg) began at 28 days; however, only the LowPA carried that effect ($P = 0.05$) through the conclusion of the trial (3.553 vs 3.818 kg). Overall, LowPA (1.649) and MidPA (1.694) had lower ($P < 0.05$) FCR than the CON treatment (1.741). Increased growth observed in live bird weights in the LowPA translated to increased ($P < 0.05$) overall carcass weights (2.783 vs 2.991 kg) and specifically breast filet weights (0.693 vs 0.769 kg). Yields did not differ ($P > 0.05$) but with the increased weight feeding LowPA resulted in more total breast meat. None of the doses of PA affected ($P > 0.05$) woody breast (pooled mean: 1.23) or white stripping scores (pooled mean: 1.06). In conclusion utilizing dietary PA increased live bird weights, improved FCR, and increased breast filet weight. This improvement in BW and breast weight was obtained without increasing woody breast, or white stripping. These data indicate that dietary PA may increase production efficiency in broilers.

Key Words: broiler, phosphatidicacid, growth, Breastyield

P279 Supplementation of broiler diets with All-G-Rich™: Effects on productivity, blood parameters and meat DHA content Colm Moran*, Jason Keegan, Doug Currie, Anne Knox *Alltech SARL*

The nutritional value of chicken meat and eggs can be improved through dietary supplementation with omega-3 fatty acid (n-3 FA) rich microalgae. *Aurantiochytrium limacinum* (CCAP 4087/2, All-G-Rich™, Alltech Inc) is a docosahexaenoic acid (DHA) rich microalgae that can be produced heterotrophically in a sustainable manner. The purpose of this experiment was to determine the effect of dietary supplementation of broilers with *A. limacinum* over a 21 day period, on productivity, blood parameters and meat DHA content. Healthy day-old male Ross 308 chicks (n=2,240) were randomly allocated to one of four treatment groups. From day 0-21 all chicks were provided with a starter diet containing no algae. The experimental diets were provided *ad libitum* from day 21. Algae was added as a percentage of the diet to provide a no algae control (T1=0%), and algae included at a level of 0.5 (T2=0.25%), 1 (T3=0.5%) and 5 (T4=1%) times the recommended intake. The study was conducted using 64 pens, providing 16 replicates for each treatment with 35 birds per pen. On days 0, 21 and 42, the birds were weighed and the amount of feed provided/removed was recorded per pen. On day 42, blood was taken from 1 bird per pen for haematological and biochemical analysis. One bird from each pen was sacrificed and breast and thigh samples were taken for DHA content analysis. No differences in average weight gain, average feed intake or feed conversion ratio were observed between the control and supplemented groups during any period of the trial. Overall, mortality was low (1.74%), and no differences between the groups in terms of mortality or blood haematology and biochemistry were observed, indicating that the algae was well tolerated. Each increase in dose corresponded to a significantly higher DHA content for both the thigh and breast tissue (thigh: 4.6, 19.0, 32.7 and 50.7 mg /100 g; breast tissue: 4.4, 17.6, 31.7 and 47.9 mg DHA /100 g tissue for T1, T2, T3 and T4 respectively, P<0.001). These results indicate that supplementation with All-G-Rich™ for 21 days under floor pen conditions can enrich chicken breast and thigh meat with DHA in proportion to the level of supplementation, without negatively impacting productivity.

Key Words: Algae, Omega-3, DHA, Broiler

P280 Tolerance of broilers to diets supplemented with All-G-Rich™ Colm Moran*[1], Jason Keegan[2], Doug Currie[3], Anne Knox[3] *[1]Alltech SARL; [2]Alltech Inc; [3]Roslin Nutrition Ltd*

Omega-3 Fatty Acid (n-3 FA) rich microalgae can be sustainably produced heterotrophically and added to chicken diets to increase the n-3 FA content of chicken meat and eggs, improving the nutritional value of these products. The purpose of this study was to investigate the tolerance of broilers to supplementation with a docosahexaenoic acid (DHA) rich microalgae (*Aurantiochytrium limacinum*, CCAP 4087/2, All-G-Rich™, Alltech Inc) to ensure its safe use. Healthy day-old male Ross 308 chicks (n=1,120) were randomly allocated to one of four diets which were provided *ad libitum*. Algae was included as a percentage of the diet to provide a no algae control (T1=0%), algae at the authorised maximum level (T2=0.5%), and algae at 5 (T3=2.5%) and 10 times (T4=5%) the maximum authorised intake. The study was conducted in a house of 32 pens, providing 8 replicates for each treatment with 35 birds per pen. Body weight and the amount of feed consumed was measured per pen on days 0, 21 and 42. Mortality was recorded daily. On day 42, blood was taken from 1 bird per pen for haematological and biochemical analysis. Two birds from each pen were sacrificed and breast and thigh samples were taken for DHA content analysis. No significant differences were observed in terms of performance between the control and treatment groups. The results indicated that supplementation with algae was well tolerated by all treatment groups. Mortality was less than 5% and no significant differences in mortality were observed between the 4 groups (P = 0.15). In addition, no significant differences were observed between the groups in terms of blood haematology and biochemistry with the exception of cholesterol which was lower in the T4 than the T1 group (2.93 vs 3.69 nmol/l, P = 0.02), and glutathione peroxidase which was higher in the T4 than the T1 group (119.96 vs 95.33 u/ml RBC, P = 0.02). Each increase in dose corresponded to a significantly higher DHA content for both the thigh and breast tissue (thigh: 12.1, 34.4, 89.0 and 139.5 mg /100 g; breast: 14.1, 42.5, 114.5 and 179.8 mg DHA /100 g tissue for T1, T2, T3 and T4 respectively, P<0.001). These results indicate that supplementation with up to 10 times the maximum recommended dose of All-G-Rich™ is well tolerated by broilers.

Key Words: Algae, Omega-3, DHA, Broiler

P281 Effect of protected sodium butyrate in pullet performance Oscar Vazquez*, Monica Puyalto, Cinta Sol, Juan Mallo, Paulina Vazquez *NOREL MÉXICO S.A. DE C.V.*

The effect of protected sodium butyrate was evaluated in a pullet rearing trial under field conditions. A total of 110,027 one-day-old pullets (Lohmann lsl-lite) were alloted in four poultry yards and randomly distributed to two treatments: standard diet with antibiotic growth promoters (AGPs: Bacitracin Methylene Disalicylate and Colistin at 55 and 125 g/t of feed respectively) (Control), and the Control diet with AGPs plus protected sodium butyrate (Gustor N´RGY) at 1 kg/t of feed (PSB). Pullets were fed with starter and grower diets based on corn-soybean meal and nutrient levels according to Lohmann manual recommendations. Enzymes and coccidiostats were included in both treatments. All data were analyzed by GLM procedure (SAS 9). Body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), flock uniformity (FU) and mortality were recorded at the end of the trial (15 weeks). There were no differences between treatments for BWG, FI and FCR at seven weeks. However, at 15 weeks PSB group tended to increase BWG (1060 vs 1080 g; P=0.106) by 20 g. Mortality was not affected (P=0.406) by butyrate inclusion in the diet. Nevertheless, FU was higher in PSB treatment (83.5% vs 90%; P=0.049). We can conclude that the inclusion of protected sodium butyrate at the doses used in this test allowed to achieve a better flock uniformity and tended to improve growth performance, even if AGPs were used in the diet.

Key Words: protected, sodium, butyrate, flock, uniformity

P282 Comparison of a feed or drinking water application of butyric acid in broilers challenged with necrotic enteritis Haci Bayir*[GS], Jundi Liu, Greg Mathis, Brett Lumpkins, Justin Fowler *University of Georgia*

Butyric acid has been identified as a potential feeding strategy for managing necrotic enteritis (NE) in broilers raised without antibiotics. This study was conducted to evaluate the effect of butyric acid (SmartFeedsUSA) when added in either the feed or the water on growth performance and the alleviation of NE. A basal diet was formulated and fed to a challenged and non-challenged control treatment. Two additional treatments received butyrate either in the feed or the drinking water. A total of 160 Cobb-Cobb male chicks were obtained on the day of hatch and randomly distributed among treatment pens (8 birds/pen). Birds from challenged treatments were orally gavaged with ~5000 *Eimeria maxima* oocysts on day 14. On day 19, 20 and 21, the challenged birds received 10^8 cfu/ml *Clostridium perfingens*. Body weight and feed intake were recorded on day 14, 21 and 28, and mortality was recorded daily. On day 21, 3 birds per pen were randomly selected and scored for the incidence and severity of intestinal lesions. Data were analyzed using one-way ANOVA via SPSS, with significant differences ($P \leq 0.05$) separated by Duncan's Multiple Range Tests. Results showed no significant differences in BW among treatments prior to the challenge on day 14. On day 21, all challenged treatments showed lower BW and higher FCR compared to the non-challenged control. For BW gain post-challenge, the water-added butyrate was higher than the challenged control, and both forms of butyrate showed improved FCR. When butyrate was added in the water, birds ended with BW and FCR comparable to the non-challenged treatment on day 28. On both day 21 and 28, in treatments with butyrate in either the feed or water, mortality was lower than the challenged control. In this study, butyrate offered benefits to growth and gut health in NE-challenged broilers over the control. Further, a drinking water application showed a greater effect than when added in the feed.

Key Words: butyric acid, necrotic enteritis, broilers, gut health

P283 Use of protected sodium butyrate in broilers under antibiotic free production system, a field trial Oscar Vazquez*, David Arteaga, Monica Puyalto, Cinta Sol, Juan Mallo *NOREL MÉXICO S.A. DE C.V.*

The current need to produce chickens without antibiotic growth promoters (AGPs) is a major challenge for producers; organic acids have been considered a viable alternative for several years. The aim of this trial was to compare the effect of protected sodium butyrate (Gustor N'RGY) and AGPs on productive parameters in broilers, under field conditions. Ten thousand one-day-old male broilers Ross were divided in two pens and randomly distributed in two treatments: Control treatment (C), a standard diet with AGP combination (Bacitracin Methylene Disalicylate, Colistin, Halquinol) and experimental treatment (PSB), the same standard diet without AGP and protected sodium butyrate (Gustor N'RGY) added at 2 kg/t. Live body weight (BW), feed intake (FI), feed conversion ratio (FCR), flock uniformity (FU) and mortality were recorded at the end of the trial. The BW was 21 g higher at the end of the trial for PSB (2.955 kg vs 2.976 kg), while fed intake was not different (5.44 kg), and as a result the FCR was 0.6% lower in PSB (1.84 vs 1.83). The FU was not different between treatments (9.25%), although the mortality was 1.2% lower in C group (5.5 vs 6.7%). Finally, the productivity index was similar between treatments (320 vs 325). It can be concluded that under the conditions of this trial the use of protected sodium butyrate in broiler diets allows to get similar productive performance than AGPs.

Key Words: sodium, butyrate, antibiotic, growth, promotors

P284 Cecal digesta microbial activity in broiler chickens fed corn-soybean meal diets without or with yeast nucleotides upon challenge with Eimeria Haley Leung*, Alexander Yitbarek, Rob Patterson, John Barta, Elijah Kiarie *University of Guelph*

There is limited data on yeast nucleotides (YN) effects on poultry cecal microbial activity in the context of enteric disease challenge. 360 d old

male broiler chicks (Ross 708) were used to evaluate effects of YN on cecal digesta short chain fatty acids (SCFA) concentration and microbial communities upon *Eimeria* challenge. Birds were housed in floor pens and provided a corn-soybean meal based diet without or with YN (500 g/t) (n=12). On d 10, birds in six pens/ diet were orally given 1-mL of *Eimeria* culture (*E. acervulina* and *E. maxima* sporulated oocysts) and the rest given 1-mL of distilled water. On d 15, five birds/pen were euthanized for cecal digesta. Genomic DNA was extracted, and the V3-V4 regions of the 16S rRNA gene was sequenced using the Illumina Miseq® platform, and sequences were curated using Mothur as described in the MiSeq SOP. An interaction *(P* = 0.04) was observed for cecal pH such that YN increased pH in challenged birds (6.21 *vs.* 6.85) only. There was an interaction between YN and *Eimeria* on citric acid (*P* = 0.001), propionic acid (*P* = 0.001) and total SCFA (*P* = 0.006) such that YN increased total SCFA in the absence of *Eimeria* and decreased SCFA in the presence of *Eimeria*. There was no interaction (*P* > 0.10) between YN and *Eimeria* on the cecal digesta microbial community. Feeding YN tended (*P* = 0.07) to decrease microbial community alpha diversity. *Eimeria* challenge tended (*P* < 0.06) to decrease abundance of phylum *Firmicutes* (83.8 *vs.* 88.7%) and reduced (*P* < 0.05) genus *Clostridium XlVa* (5.4 *vs.* 9.9%) and *Oscillibacter* (1.1 *vs.* 1.7%). Relative to birds not fed YN, YN fed birds had greater abundance of the genus *Anaerostipes* (1.2 *vs.* 0.3 %; *P* = 0.01) and tendency for greater abundance for genus *Oscillibacter* (1.7 *vs.* 1.1%; *P* = 0.06). YN fed birds had a tendency for lower abundance of *Clostridium XlVa* (6.1 *vs.* 9.6%; *P = 0.10*) relative to birds not fed YN. In summary, effects of YN and *Eimeria* were interactive on fermentation metabolites but independent of the microbial community. *Eimeria* tended to reduce *Firmicutes,* which accounts for >85% of the gut bacteria population whereas YN tended to reduce microbial diversity commensurate to antimicrobials. *Eimeria* and YN reduced abundance of genus *Clostridium XIVa* linked to butyrate production.

Key Words: Eimeria, nucleotide, cecal, microbial, activity

P285 Extended heat treatment of a broiler starter diet containing formaldehyde does not impact the overall amino acid digestibility or TMEn content of the diet Matthew Jones*, Kurt Richardson, Charles Starkey, Nick Dale, Adam Davis *University of Georgia*

Pathogens can infiltrate food animal production systems at several points including through the diet the animals are fed. Dietary introduction of pathogens is also a serious issue because the animal production units all serviced by the same feed mill can be affected. In broiler production, the elimination of pathogens such as *Salmonella* that can cause food borne illness in humans remains a major goal. *Salmonella* contamination of diets can be mitigated by chemical measures such as the addition of formaldehyde and/or prolonged heat treatment during production. However, there is concern that such treatments can reduce the nutrient availability of the treated diet. Therefore, the goal of the present research was to determine if the application of a specific formaldehyde feed additive, Termin-8, in combination with extended heat treatment, reduces the TMEn and available amino acid content of a broiler starter diet. Cecectomized and intact adult Single Comb White Leghorn roosters were used to determine amino acid digestibility and TMEn, respectively, of a broiler starter diet treated with 0 or 0.3% formaldehyde solution prior to heat treatment at 82°C for 4.5 minutes. The digestibility coefficients of the amino acids of the diets were equal except for arginine. The arginine digestibility coefficient in the control diet was 94.17% and this was reduced (P < 0.05) to 93.39% in the formaldehyde containing diet. The addition of formaldehyde did not affect the TMEn content of the diet. The results indicate that the addition of a formaldehyde solution prior to extended heat treatment to control *Salmonella* contamination does not impact the metabolizable energy and digestible protein content of poultry diets.

Key Words: formaldehyde, Termin-8, Salmonella, poultry

P286 Dose responses in live performance to a new dietary direct feed microbial (GalliPro Fit) in broilers under Clostridium perfringens challenge Alfred Blanch*, Dorthe Sandvang, Florence Rudeaux, Michael Sims *Chr Hansen AS*

The aim of this study was to determine the responses in body weight gain, feed efficiency, and mortality of broiler chickens supplemented with different dosages of a new direct feed microbial (DFM, GalliPro Fit), when exposed to Clostridium perfringens during a 42-d floor pen evaluation. A total of 1,080 day-old Cobb male chicks were distributed in 36 floor pens (30 birds/pen), allocated to 3 dietary treatments (12 pens / group): control group without DFM (T1), 500 ppm of GalliPro Fit (T2) and 1,000 ppm of GalliPro Fit (T3). Continuous lighting was provided. Each feed type (starter, grower, and finisher) was prepared from a single basal diet by blending in the corresponding DFM dosage for each treatment. Lower calculated protein (19.5, 18.4, and 17.8%, respectively) commercial-type diets were provided by age of bird (starter, 0-14 d; grower, 15 – 28 d; and finisher 29 – 42 d). The calculated ME values by phases were 3,044, 3,086, and 3,142 kcal/kg, respectively. On d 17, 18 and 19, a one-hour feed and water fast was observed before infecting birds with a Clostridium perfringens isolate by oral gavage (~10^9 cfu/mL). Body weight gain, feed intake, feed conversion (FCR), and mortality adjusted FCR were assessed at d 21 and 42. Total mortality was documented throughout the study. Results were tested for dietary treatment effect in a Randomized Complete Block Design. If the effect was significant (P < 0.05), means were separated using Tukey's test. No significant differences between experimental groups were observed from 0 to 21 d. However, when considering the whole experimental period (0-42 d), T3 resulted in significantly higher body weight gain (kg) than T1, whereas T2 showed an intermediate value (T1 1.539b, T2 1.728ab, T3, 1.860a; p < 0.05). Furthermore, the diets with GalliPro Fit at 500 ppm (T2) or 1,000 ppm (T3) resulted in broilers with reduced FCR by 18.9% and 24.5% compared to T1, respectively (T1 2.537a, T2 2.057ab, T3 1.915b; p < 0.05). Likewise, mortality adjusted FCR was improved when the diets included the DFM. There were no significant differences in mortality between experimental groups (T1 8.67%, T2 5.33%, T3 5.33%, p = 0.44).

Key Words: DFM, Bacillus, chickens, NE

P287 In vivo antimicrobial efficacy of silver acetate against Clostridium perfringens-induced necrotic enteritis in broiler chicken Akhil Alsadwi*[GS1], Yansoon Al-Jumaa[1], Raghad Abdaljaleel[1], Elise Voltura[1], Kimberly Gardner[1], Hector Leyva-Jimenez[1], Morouj Al-Ajeeli[1], Carolyn Cannon[1], James Byrd[2], Christopher Bailey[1] *[1]Texas A&M University; [2]USDA Southern Plains Agriculture Research Center*

The efficacy of silver acetate (SA) delivered either by adding into the feed or via drinking water was evaluated across a wide range of doses for in vivo antimicrobial activity against experimentally-induced necrotic enteritis (NE). For the feed-addition, 240 one-day old (Cobb-500) broiler chickens were placed into 48 battery pens (5 birds by pen, 2 sq. ft./cage), located in an environmentally controlled rearing room. Birds were blocked in 8 dietary treatment groups (6 replicates/5 birds) as follows: Positive Control (no challenge and no SA), Negative Control (challenged only), and 6 dietary treatments challenged and fed 10, 20, 40, 60, 80, 100 mg/kg feed SA for 21 d rearing period. Necrotic enteritis was experimentally induced using an oral administration of *Clostridium perfrringens* (CP) (3 ml per bird, 10^7 CFU/mL) on days 16 and 17 following a Bursa-Vac at 10X the recommended dose on day 10. Secondly, the same protocol was followed except the birds (Ross-308) received SA concentrations via drinking water for the last 4 days of the 21 day rearing period. Treated groups received SA fresh daily or as needed using a jack-waterer. Body weight and feed intake were recorded by pen on days 10, 16 and 21 for both the feed and water application trials. All birds were necropsied on day 21 to measure intestinal lesions associated with NE, and 12 samples/treatment of mid-ileum contents were collected for CP enumeration. All data were analyzed as One–Way ANOVA using the JMP Pro 12 with means separated using

Tukey's HSD test at P <0.05. Whether SA was added into the feed or in the water, there were no differences seen in body weight, and feed conversion ratio on day 21. Mortality was only observed among the challenged treatments, however, the addition of SA in either the feed or water did not have any significant effect on this variable. The broilers receiving the water-applied SA had lower (P < 0.001) lesion scores compared with the feed-treated group at all concentrations tested, although there were no significant differences in CP enumeration in both groups. It is possible that the more readily released silver ions from SA interacted with biologically-relevant compounds (such as organic material or chloride) in the drinking water, preventing their availability.

Key Words: Silver, Clostridium, enteritis, necrotic, antimicrobial

P288 Reduced survival of Campylobacter in in vitro mixed cultures containing Diamond V Original XPC™. Peter Rubinelli, Hilary Pavlidis, Donald McIntyre, Steven Ricke* *University of Arkansas*

Campylobacter infections are a major cause of food-borne gastroenteritis and are sometimes associated with chronic auto-immune reactions such as Guillain-Barré syndrome, reactive arthritis, and inflammatory bowel disease. Genetic typing of *Campylobacter* strains from human patients has demonstrated that the *Campylobacter* often originates from the consumption of chicken meat. Our previous experiments indicated that Diamond V Original XPC™ (hereafter XPC) reduced the *Salmonella* population both in *in vitro* mixed culture and *in vivo* as a feed component, presumably through a prebiotic-like mechanism. We therefore hypothesized that *Campylobacter* numbers might be similarly reduced in the presence of XPC in the intestinal tract of chickens. To test this concept, we cultured a chloramphenicol resistant *Campylobacter jejuni* marker strain in Bolton broth in the presence of cecal contents with and without XPC in a microaerobic atmosphere. *C. jejuni* was enumerated in the cultures by dilution plating of the cultures onto Cefex + 10 mg/ml chloramphenicol agar plates at 0, 24, and 48 h after addition of the *C. jejuni* marker strain. Enumeration was also conducted by Petroff counter in one experiment. Four independent experiments all showed significant reduction in the *C. jejuni* marker strain after 24 and/or 48 h growth in the presence of XPC and cecal contents compared to the numbers of *C. jejuni* marker strain in the presence of cecal contents alone ($P < 0.05$, two-tailed t-test). Reduction was typically a 1-2 log reduction. These results suggest that XPC could potentially reduce *Campylobacter* in a mixed microbial environment such as the chicken cecum to a significant extent.

Key Words: Additive, Prebiotic, Microbiota, Campylobacter, Broiler

P289 Performance and relative weight of organs of broilers fed with different levels of passion fruit seed oil Leonardo Zanetti*, José Sartoti, Juliana Denadai, Laura Granero, Mariana Poletto, Guilherme Aguiar Pasquali, Everton Muro, Tatiane Santos, Daniele Souza, Robert Guaracy Araujo, Livia Dornelas, Raimundo Ferreira Netto, Armando Contin Neto, Julianna Batistioli, Gustavo Barbosa *São Paulo State University (UNESP), College of Veterinary Medicine and Animal Science*

Passion fruit (*Passiflora edulis*) is a tropical fruit whose production is concentrated primarily in South America and main producer and consumer is Brazil. The by-products from the pulp and seeds can be used as an alternative food ingredient and, its oil is obtained by processing the seed. The passion fruit seed oil is rich in polyunsaturated fatty acids, vitamins, minerals, and antioxidants. This study was conducted to determine the effect of passion fruit seed oil in broiler performance during the starter phase (1 to 21-d old) and total phase (1 to 42-d old). A total of 1,680 1-d-old male Cobb chicks were placed in 2m² pens with reused wood-shaving litter. The experimental design was completely randomized with 7 treatments: PC (positive control, with antibiotic inclusion); NC (negative control, without antibiotic inclusion) and 5 levels of passion fruit seed oil (0.1, 0.2, 0.3, 0.4, and 0.5% inclusion in the diet), and 8 replications of 30 birds per pen. Diets were isoenergetic and isonitrogenous. Water and feed were provided *ad libitum* during the experimental period. Birds and experimental diets were weighed at 1, 21 and 42-d old to determine feed intake (obtained through the difference between total feed provided and collected at the end of each period), feed conversion (calculated as the ratio between total feed intake and weight gain and corrected by the weight of dead birds), and weight gain. In this study, passion fruit seed oil supplementation in the diets affected (P<0.05) the weight gain (g) and feed conversion at 1 to 42-d old. These treatments with passion fruit seed oil did not differ from the PC. This fact is interesting because it shows that passion fruit seed oil can be a potential substitute for the antibiotic use in feeding broilers. The relative weights of the immune system organs (spleen, thymus, liver, and bursa of *Fabricius*) obtained on 21 and 42-d old were not affected (P>0.05) by the treatments with passion fruit seed oil. Thus, the results of this study indicate that the addition of passion fruit seed oil can be used to improve performance, but not the relative weights of immune system organs.

Key Words: by-product, phytogenic, Fruits

P290 Performance of slow-growing broiler chickens is positively influenced by isoquinoline alkaloids Anja Pastor*, Hervé Bezille, Christophe Alleno *Phytobiotics*

To improve animal welfare in broiler chickens is the aim of many producers. Consequently, slow-growing birds gain the attention of many producers. The aim of the study was to evaluate the effect of a standardized blend of plant-derived isoquinoline alkaloids (IQs, Sangrovit® Extra) in slow-growing broiler chickens with a target body weight around 1,500 g. A two-factorial design was applied: sex (male/female) x feed additive (1) Control (CON), no feed additive 2) IQ: IQs (60 ppm, day (d) 1-32). 888-day-old male and female broiler chicks (JV breed, Hubbard) were randomly distributed to the trial groups. Each group consisted of 6 replicates, with 37 birds/replicate. Birds had *ad libitum* access to feed supplemented with a coccidiostat. IQs improved averaged final body weight of male and female significantly ($p \leq 0.05$) (1,499 and 1,525 g, respectively). No difference was observed for feed intake. Consequently, FCR was improved on average by 3 units, if IQs were applied ($p \leq 0.05$) (1.59 and 1.56, respectively). No differences were found for mortality. Higher European Production Index was observed in birds supplemented with IQs (275 and 286, respectively). Male birds showed a significantly higher incidence of severe pododermatatis lesions (score 2; score from 0-2) than female birds ($p \leq 0.0001$). Addition of IQs tended to decrease severe lesions in male and female broiler chickens ($p \geq 0.05$). IQs improved performance in slow-growing broiler chickens and therefore support an economical production in concepts with increased animal welfare by choosing slow-growing genetics.

Key Words: alkaloids, slow-growing, welfare

P291 Supplementation of maternal microbiota at day-of-hatch improves mortality and enteric health of young broilers. Evan Hutchison*, Josh Rehberger, Xandra Smith, Tom Rehberger *Arm & Hammer Animal Nutrition*

Early colonizing microbiota play a critical role in developing and maintaining immune function, bacterial homeostasis and nutrient acquisition for the duration of the bird's life. The sanitation-driven design of modern hatchery systems subjects newly-hatched birds to passive inoculation by organisms in their immediate hatching environment that have survived the sanitation process. A large survey investigating the incidence of vertical transmission of bacteria among broilers shows that the gastrointestinal tracts of day-of-hatch (DOH) broilers have very low microbial diversity, being composed primarily of *Enterococcus faecalis* and avian pathogenic *Escherichia coli* (APEC), and possess very few beneficial microbiota from their breeder hens. It is likely that this combination of a heavy pathogen load and a lack of maternal microbiota is the underpinning of common problems among young broilers, including 7 day mortality and subclinical APEC challenges. In the current study, we hypothesized that supplementing DOH chicks with the missing beneficial bacterial strains found in their hens can improve performance and reduce enteric challenges of the young

bird. To test this, DOH birds were fed a gel-based direct fed microbial (DFM) product, which was formulated with specific beneficial microbiota that were identified as being present in breeder hens, but missing in DOH chicks along with APEC-reducing *Bacillus* strains. This DFM product was applied to DOH chicks at a large (1 million birds/week) US broiler hatchery in alternating weeks over a 12 week period. Performance metrics were tracked for flocks from both treated and untreated weeks. Enteric health was also measured at DOH and 7 days of age by enumerating gastrointestinal APEC levels from representative birds. Results showed that treatment with the DFM led to a numerical reduction of 7 day mortality and significantly lower APEC levels at 7 days of age, despite starting with a higher average APEC load at DOH ($p>0.05$). Treated birds also saw a significant reduction in the coefficient of variation (CV) of flock weights at harvest. These findings demonstrate that replenishing DOH chicks with maternal microbiota confers health and performance improvements to young broilers.

Key Words: Lactobacillus, Microbiota, Bacillus, Broiler, Hatchery

P292 Performance and apparent retention of nutrients in Shaver White pullets and laying hens in response to dietary supplementation of graded levels of a single strain Bacillus probiotic Mohamed Neijat*, Rob Shirley, Elijah Kiarie *Department of Animal Biosciences, University of Guelph*

Administered in adequate amounts, probiotics can be an alternative to antibiotics in poultry production. This study evaluated the dose response of a single strain of *Bacillus subtilis* (SSB, Alterion-NE50®, strain DSM*29784*, Adisseo, USA) on hen performance at different stages of growth and production. The design consisted of a total of 720, 4 week-old Shaver White chicks (commercial stock) that were allotted across 48 cages, with 12 replicate cages per treatment. After placement, the chicks were given *ad libitum* access to one of four corn-soybean meal based diets: 0 (control; Treatment 1), 1.1E+08 (low; Treatment 2), 2.2E+08 (intermediate; Treatment 3) or 1.1E+09 (high; Treatment 4) cfu of SSB/kg of diet. Performance indices and nutrient digestibilities were assessed in the grower, developer and layer phases; these phases corresponded to 5-10, 11-16 and 19-28 weeks-of-age. Compared to the control group, supplementation of the probiotic improved body weight (BW; $P<0.0001$) and reduced feed intake (FI) across the pullet phases ($P<0.0001$). These improvements included a 3% lower FCR with SSB supplementation vs. the control during the grower phase ($P<0.001$). In the layer phase, BW and FI were not influenced by the SSB; however, egg mass was improved (EM, $P < 0.001$) and FCR reduced ($P<0.0001$). The latter may relate to early initiation of egg production in week-19, on average 30% ($P<0.0001$) higher egg-lay with the intermediate level of SSB compared with the control. Dry matter (DM) digestibility and apparent metabolizable energy (AME) were improved throughout the pullet (grower, $P<0.05$; developer, $P<0.0001$) and layer ($P<0.0001$) phases. The addition of SSB resulted in a linear reduction ($P<0.0001$) in the excreta moisture content during the grower phase. While these results demonstrate the effect of Alterion-NE50® in enhancing nutrient utilization at all stages of growth and production, the distinct attributes of the SSB were evident mainly in the grower phase.

Key Words: Bacillus subtilis, pullet, laying hen, performance, nutrient digestibility

P293 Dose response evaluation of a single strain Bacillus subtilis probiotic in the first 28 days of poults life when fed antibiotic free corn-soybean meal diet Mohsen Mohammadigheisar*, Robert Shirely, Elijah Kiarie *University of Guelph*

In light of the restricted use of antibiotic growth promoters in turkey production, the adaptation of the gastrointestinal (GI) tract to environmental stress is a critical characteristic for optimal turkey performance. This 28-d study evaluated the dose response of a single strain of *Bacillus subtilis* (SSB, Alterion-NE50®, Adisseo, USA) on growth performance, GI development and excreta moisture in poults. The design consisted of a total of 960 d-old Hybrid male turkeys that were weighed (average initial body weight (BW) = 69 g), placed in groups of 20 poults/cage, and allocated to one of four dietary treatments to give 12 replicate cages/treatment. The birds were housed in an environmentally-controlled room (32-24°C; 60% relative humidity) and given free access to one of four corn-soybean meal based diets: Control (0; Treatment 1), low (1.1E+08; Treatment 2), intermediate (2.2E+08; Treatment 3) or high (1.1E+09; Treatment 4) cfu of SSB/kg of diet. The birds and feeders were weighed on a weekly basis to monitor body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Excreta samples were collected and pooled on days 25, 26, and 27. On d 28, 5 birds were euthanized by cervical dislocation to measure gizzard, small intestine and ceca weights. Supplemental SSB increased BWG in a linear ($P = 0.002$) and quadratic (P=0.004) manner; specifically, the respective BWG responses were 1,181, 1,235, 1,241 and 1,230 g/bird for Treatments 1-4. The FI also exhibited linear ($P = 0.005$) and quadratic ($P = 0.010$) responses to increasing doses of SSB, where the respective FI for Treatments 1-4 were 1,449, 1,484, 1,518 and 1,490 g/bird. There was no effect of diet on FCR ($P > 0.10$), as values were 1.23, 1.20, 1.22 and 1.21 g/ for Treatments 1-4. Supplemental SSB did not have an effect on gizzard, small intestine or ceca weights ($P > 0.05$). The moisture content of the excreta linearly increased as the level of SSB increased ($P = 0.043$); the respective excreta moisture for Treatments 1-4 were 75.46, 73.65, 75.98, and 76.90%. In conclusion, feeding a single strain of *Bacillus subtilis* (Alterion-NE50®) in a corn-soybean meal starter diet can promote poult growth through the stimulation of feed intake and reduce the moisture content in the excreta at the lower level.

Key Words: Digestive, excreta, probiotics, Bacillus, turkey

P294 TLR-Mediated Immune Modulation of Chicken Macrophages by Saccharomyces boulardii Altaf Hussain*, Bai Wang, Li Gong, Wei Li *Chinese academy of agriculture science, Harbin veterinary research institute*

Macrophages are professional phagocytic cells that play critical roles in activating immune responses of the host. It is possible that macrophages are targeted by microbes to modulate these responses. Here we demonstrate that lipopolysaccharide (LPS), *Saccharomyces boulardii* (Sb) altered the phenotype and biological functions of avian macrophages. Using scanning electron microscopy, we showed that the altered morphology was due to the direct attachment of Sb on the surface of chicken macrophages. Additionally, transmission electron microscopy revealed the efficient engulfing and degradation of Sb. In an effort to demonstrate immunological changes in responses to these stimuli, we observed that LPS could induce higher gene expression levels of surface markers (CD40, CD80 and CD83), cytokines (IL-6 and iNOS), TLRS (TLR1, TLR4 and TLR15) and associated factors (MyD88, TRAF6, NF-κBp65 and JNK) in chicken macrophage compared to Sb. On the other hands, Sb could decrease the response of macrophage to LPS by TLR signaling pathway. Conclusively, these results emphasize a potentially important role of *Saccharomyces boulardii* in modulating immunological functions of chicken macrophages by TLR signaling pathway.

Key Words: LPS, HD11 cell, Saccharomyces boulardii, macrophages

P295 Effects of Bacillus subtilis, Enterococcus faecium, citric acid, fructooligosaccharides and Macleaya cordata extract on laying performance and microbial safety in eggs and feces of laying hens Wenxiang Li*, Huanlei Qin, Qiugang Ma, Lihong Zhao, Jianyun Zhang, Cheng Ji *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University*

Escherichia coli (*E. coli*) and *Salmonella* can be transmitted to human through consumption of contaminated eggs owing to the special structure of the cloaca in laying hens. Moreover, due to the prohibition of the use of antibiotics in hens' diets, many antibiotic substitutes are added in feeds because of their functions of reducing harmful bacteria in eggs and promoting nutrient absorption. To reduce the effects of harmful bacteria

in eggs and feces, the effects of *Bacillus subtilis, Enterococcus faecium*, citric acid, fructooligosaccharides and *Macleaya cordata* extract on laying performance and microbial safety in eggs and feces of laying hens during the late laying period were investigated. A total of 1296 52-week-old Hy-Line Brown commercial laying hens were randomly allotted by laying rate (80.4 ± 0.77%) and body weight (1.71 ± 0.04 kg) to 1 of 6 treatments with 6 replicates of 36 birds per replicate for each treatment in a completely randomized design. Laying hens were fed a corn-soybean meal basal diet containing no antibiotics (CK) or the basal diet supplemented with 0.05% *Bacillus subtilis* (BS), 0.01% *Enterococcus faecium* (EF), 0.1% citric acid (CA), 0.05% fructooligosaccharides (FOS), and 0.004% *Macleaya cordata* extract (MCE) for 5 weeks. Dietary supplemental antibiotic substitutes had no effect ($P > 0.05$) on daily feed intake. The MCE group increased ($P < 0.05$) the egg yeild and energy efficiency, and decreased ($P < 0.05$) the egg weight and feed:egg ratio as compared with CK group, while no differences were observed among EF, CA, and FOS groups. Compared with hens in CK group, the numbers of microorganisms ($P < 0.05$) and *E. coli* ($P < 0.05$) in feces of hens from BS group were decreased, while there were no differences among CK, EF, CA, and MCE groups. Moreover, the numbers of *Salmonella* ($P < 0.05$) in feces and microorganisms ($P < 0.05$) in eggshells of hens from all 5 antibiotics substitutes groups were decreased. Microorganisms, *E. coli* and *Salmonella* were not detected in egg albumen and yolk of all hens. In conclusion, the antibiotics substitutes could improve the laying performance and microbial safety in eggs and feces of laying hens.

Key Words: Laying hens, Macleaya cordata extract, Bacillus subtilis, Enterococcus faecium, Fructooligosaccharides

P296 Is meat antioxidants, chemical composition and performance of commercial broilers affected by Moringa oleifera pods supplemented as feed additive? Shakeel Ahmad*, Anjum Khalique, Talat Pasha, Shahid Mahmood, Sohail Ahmad *University of Veterinary and Animal Sciences*

This study was conducted to explore the effect of Moringa oleifera pods meal (MPM) on growth performance, meat quality and immunity of broilers. For the purpose, two hundred broiler birds were assigned to four treatments with five replicates and ten birds per replicate in a completely randomized design. Starter and finisher diets were added with four levels (0, 0.5, 1.0 and 1.5% of MPM) over and above. Results of this study showed that growth performance was improved as feed conversion ratio (FCR) and feed intake were decreased with the increase in supplementation level ($P \leq 0.05$). Dressing percentage was lowered, whereas giblet weights were improved with the MPM supplementation levels ($P \leq 0.05$). β-carotene, Quercetin and Selenium content of breast meat was linearly increased resulting in higher values of DPPH radical scavenging and improved shelf life. Serum biochemical compounds like SGPT, Creatinine, Glucose and meat cholesterol level was significantly decreased and was recorded lowest in 1.5% MPM supplemented diet ($P \leq 0.05$). At the end of the trial it was concluded that Moringa pods may positively affect the growth and chemical composition of broiler meat.

Key Words: Moringa, Broiler, β-carotene, DPPH, Selenium

P297 Dietary prebiotics and probiotics to decrease manure moisture and ammonia emissions from broilers Kurtis Miller*[UG], Lisa Kitto, Amy Barkly, Denver Greenawalt, Paul Patterson *The Pennsylvania State University*

The increased interest in poultry produced through perceived natural methods (organic, antibiotic-free, and free-range) has led to new products being developed and licensed for these production systems. Certain dietary probiotics and prebiotics used to improve gut health have been purported to improve welfare and manure quality in broiler houses through reduced moisture and ammonia emissions. Similarly, probiotics in both human and animal health have long promised improved gut health and overall performance. The objective of this trial was to determine if dietary pre and probiotics can decrease manure moisture and ammonia levels. Hubbard Redbro (256) chicks were divided into four groups of 64 chicks each, with 6 replicate pens per group. The groups were each fed a mash diet, with inclusion of one of three additives (Nutri-Core® Feather Fresh ES, Diamond V® Original XPC™, a commercially available *Lactobacillus* or a negative control). A dynamic flux chamber and photoacoustic gas monitor were used to measure ammonia flux at 12d and 28d. Body weight, feed intake, manure wet weight, and manure water activity were measured, and feed conversion and manure moisture were calculated. All data was analyzed with a one-way ANOVA using the MIXED procedure of SAS (9.4) with differences between the means being deemed significant at $P \leq 0.05$ and Tukey's range test was used for means comparisons as needed. At 12d, the ammonia flux was shown to be reduced by the Diamond V® product, compared to the *Lactobacillus* (-0.54 and 1.88 respectively). The *Lactobacillus* and Nutri-Core® diets significantly increased the proportion of manure DM at 28d, compared to the control diet (12.84 and 11.21, vs 7.87 respectively). The control diet also showed an increase in percent moisture at 28d, compared to the *Lactobacillus* and Nutri-Core® diets (58.12% vs 49.80% and 51.67%, respectively). The *Lactobacillus* diet also showed a greater manure moisture compared to Diamond V® (57.12%). In conclusion, probiotics showed promising results in the control of manure moisture and ammonia. More research is needed to support these findings.

Key Words: broiler, ammonia, moisture, manure

P298 Immunomodulatory and histological effects of saponin supplementation during a mixed coccidian challenge in broilers Maci Oelschlager*[GS], Muhammed Shameer Abdul Rasheed, Brooke Smith, Mike Rincker, Ryan Dilger *University of Illinois*

Coccidiosis is caused by the genus *Eimeria* and is characterized by destruction of intestinal epithelia and decreased absorption of nutrients. We sought to determine if dietary supplementation with Micro-Aid an extract from *Yucca schidigera* (Micro-Aid® Feed Grade Concentrate; DPI Global, Porterville, CA), could ameliorate the immune and growth responses of broilers during a mixed coccidian challenge. A total of 576 two-day-old male Ross 308 broiler chicks were housed in galvanized starter batteries and randomly assigned to 1 of 4 dietary treatment groups (12 replicate cages of 7 birds). Diets were corn-soybean meal-based and treatments included: 1) uninfected/no Micro-Aid, 2) infected/no Micro-Aid, 3) infected/250mg Micro-Aid/kg diet, and 4) infected/500 mg Micro-Aid/kg diet. On study d 14, birds were orally inoculated with 1.5 ml of tap water containing *E. acervulina, E. maxima, and E. tenella* (100,000, 40,000, 30,000 oocysts/dose, respectively), or sham-inoculated with 1.5 ml of tap water. Individual bird and feeder weights were recorded on study d 0, 14, 21, and 28. Total and differential blood cell counts were measured at 0, 7, and 14 days post inoculation (DPI), and sections of the ileum, jejunum, duodenum, and cecum were collected at 14 DPI for histological analysis. *Eimeria*-challenged birds exhibited a reduction in growth compared with uninfected birds ($P < 0.001$), but there was no detectable differences due to dietary treatment within *Eimeria*- challenged groups. Lymphocyte counts were increased ($P < 0.014$) in all *Eimeria*- challenged groups at 7 DPI compared with uninfected birds, but birds fed Micro-Aid at 250 mg/kg were not significantly different than uninfected birds. Mucosal thickness in the jejunum was increased in all infected groups, but there was no difference between infected groups; however, Micro-Aid included at 250 mg/kg elicited similar results compared to uninfected birds. Birds receiving 250 mg Micro-Aid/kg diet exhibited similar lymphocyte proportions at 7 DPI and mucosal thickness in the jejunum at 14 DPI compared with uninfected birds. These results suggest that Micro-Aid may possess some measurable immunomodulatory effects during infection due to its effects on lymphocyte responses and changes in intestinal structure.

Key Words: coccidiosis, broiler, immunomodulatory, histology, saponin

P299 Effect of the inclusion of a Bacillus Direct-fed microbial on growth performance, lesion score and intestinal barrier integrity in broiler chickens using a necrotic enteritis model Juan Latorre*, Daniel Hernandez-Patlán, Bruno Solis-Cruz, Karine Pontin, Lisa Bielke, Billy Hargis, Guillermo Tellez *University of Arkansas*

Reductions in the use of antibiotic growth promoters (AGP) and anticoccidials in poultry diets have caused an increase in the presentation of enteric diseases such as necrotic enteritis (NE). However, the incorporation of selected *Bacillus* direct-fed microbials (DFM) has been proposed as an alternative. Presently, the NE model included a challenge with *Salmonella* Typhimurium (day 1) followed by administration of *Eimeria maxima*-M6 (day 13) and *Clostridium perfringens* (day 18). At day-of-hatch, chicks were randomly assigned to three different groups: 1) Negative control; 2) Positive control; 3) *Bacillus*-DFM. Four pens of 10 chicks were used per group (n=40). *Bacillus*-DFM spores were included to reach a concentration of 10^6 spores/g of feed. Performance parameters such as body weight (BW), and body weight gain (BWG) were evaluated weekly, meanwhile, feed intake (FI) and feed conversion ratio (FCR) were measured at day 21. Additionally, liver samples were collected to assess bacterial translocation (BT) and blood samples were used to measure the activity of superoxide dismutase (SOD) and leakage of fluorescein isothiocyanate-dextran (FITC-d). Intestinal contents were also obtained for determination of total IgA. All broilers were NE lesion scored at the end of the study. Chickens consuming the DFM improved BWG in comparison to the positive control after challenge with *S.* Typhimurium and *E. maxima* ($P < 0.05$). Additionally, DFM supplemented chickens have numerically better BW, FI, and FCR compared with the positive control at the end of the trial ($P > 0.05$). On the other hand, the *Bacillus*-DFM group showed a reduction in NE lesion scores and IgA levels, as well as improvements in the intestinal barrier integrity parameters ($P < 0.05$). No differences were observed in SOD levels ($P > 0.05$). Results of this study suggest that the dietary inclusion of a previously selected *Bacillus*-DFM could contribute to mitigate the negative impact of NE on intestinal permeability and inflammation, therefore alleviating detrimental effects on growth performance. However, consideration of the probable synergetic effects of the blend of *Bacillus*-DFM with other feed additives may be important in future NE studies. Continued research is needed to confirm and extend these findings.

Key Words: Bacillus-DFM, Necrotic-Enteritis, Coccidiosis, Gut-Health, Performance

P300 Toxicological effects and tissue distribution of methylsulfonylmethane (MSM) after acute and sub chronic oral gavage in broilers. Muhammed Shameer Abdul Rasheed*[GS], Maci Oelschlager, Brooke Smith, Rose Whelan, Behnam Saremi, Ryan Dilger *University of Illinois*

MSM is reported be beneficial in human health and disease, but data is lacking on its effects in agricultural animals. In this study, we investigated the toxicological effects and tissue distribution of MSM following oral gavage in broilers. 2 studies were conducted using Ross 308 male broiler chicks. In study 1 (acute dosing), chicks (406 ± 12.4 g initial BW; 6 replicates of 15 birds) were allotted to 6 treatments at 15 d post-hatch and administered a single oral dose of MSM at 0, 50, 100, 300, 1,000, or 2,000 mg/kg BW as mixed in water. In study 2 (sub-chronic dosing), 3-d-old chicks (51 ± 0.5 g initial BW; 6 replicates of 14 birds) were subjected to daily oral gavage of either 0, or 1,500 mg/kg BW of MSM for 21 d. Samples of blood, liver, spleen, heart, kidney, brain, cecal tonsils, hock joint, and abdominal skin were collected at various time-points and analyzed for MSM concentrations. Toxicological effects were assessed through changes in clinical blood parameters. A one-way ANOVA was performed to separate treatment means, and all MSM data were log transformed prior to analysis; statistical significance was accepted at $P < 0.05$. In study 1, plasma MSM concentrations were below 167 µg/ml at all time-points in birds receiving up to 300 mg/kg BW, and while MSM concentrations at these time-points neither differed between groups or from zero, all values were lower ($p < 0.05$) than in birds receiving 1,000 or, 2,000 mg/kg BW. Similarly, only the highest 2 MSM dosages elicited increased lymphocyte and deceased heterophil counts at 8 h ($P < 0.05$). Growth performance and clinical parameters were not affected by oral MSM in study 2, and plasma and tissue MSM concentration were highest on study d 21, with MSM-dosed birds always have higher ($P < 0.03$) concentrations compared with the control. Importantly, MSM was detected in plasma and all tissues of control groups, confirming that MSM is synthesized de novo in chickens, but mean values were often not statistically different from zero. In conclusion, oral administration of MSM at either acute (> 1,000mg/kg BW) or sub-chronic (1,500 mg/kg BW daily for 21 days) concentrations did not cause any adverse effect on growth performance or clinical parameters and appeared to be absorbed and distributed throughout the body.

Key Words: Methylsulfonylmethane, boilers, toxicity, tissue-distribution

P301 Mycotoxin Prevalence in the 2017 Corn Crop Eduardo Vicuna*, Timothy Jenkins *BIOMIN*

Commercial harvests may be contaminated by mycotoxins, these metabolites are produced by different fungal species. Fungus can be classified into two species, the ones producing metabolites on the field (e.g. Fusarium spp.) and the ones on storage (e.g. Aspergillus and Penicilium spp.). Mycotoxins content on animal feed is an important measurement in order to determine the risk on animal health and performance. BIOMIN has worked on mycotoxins contamination determination through its survey since 2004 and in America since 2012.

3053 corn samples were collected and analyzed from January to September 2017, the method used was LC-MS/MS. The six major mycotoxin groups analyzed comprised of aflatoxins, type A trichothecenes such as T-2 toxin, type B trichothecenes such as deoxynivalenol (DON; vomitoxin), ochratoxin-A, fumonisins (FUM), and zearalenone (ZEN) derivatives.. However, there was a 20% increase in the number of samples contaminated with multiple mycotoxins (≥2 mycotoxins, 2017: 77%; 2016: 57%). This is primarily due to increased prevalence of DON in the 2017 crop (DON: 81) compared to the 2016 crop (DON: 53%). In addition to the increased DON prevalence, the concentrations of FUM in positive samples tended to be higher in 2017 than 2016. In contrast, the most prevalent mycotoxin FUM had relatively unchanged prevalence (2017: 90% prevalence, 2016: 94%).

The preliminary results from the 2017 corn harvest suggest an occurrence of co-occurrence with mycotoxins produced by *Fusarium* fungal species. This includes contamination with FUM, DON, and ZEN. Because of the high frequency of multi-mycotoxin contamination in samples thus far, multiple strategies of mitigating risk are needed beyond adsorption, including biotransformation and providing support to immune and liver function.

Key Words: Deoxynivalenol, Fumonisin, Zearalenone, Co-occurrence, Biotransformation

P302 Prevalence of mycotoxins in the 2017 corn crop G. Raj Murugesan*, Paige Gott, Erika Hendel, Chasity Pender, Ursula Hofstetter-Schahs *BIOMIN America Inc.*

Mycotoxin contamination of feed materials is a global concern, as exposure to mycotoxins significantly impacts animal health and productivity. Mycotoxins also predispose livestock and poultry for pathogenic bacterial and viral diseases as well as shown to compromise vaccine efficiency, leading to loss of productivity. BIOMIN has been conducting global mycotoxin surveys on commodity crops and complete feeds annually since 2004, including annual corn surveys in the United States since 2012. A total of 99 corn and corn product samples collected from mid-Aug to Oct 2017 were analyzed utilizing the LC-MS/MS method. The mycotoxin groups analyzed comprised of aflatoxins, type A trichothecenes such as T-2 toxin, type B trichothecenes such as deoxynivalenol (DON; vomitoxin), ochratoxin A, fumonisins (FUM), and zearalenone (ZEN) and their deriva-

tives. Preliminary results suggest almost all surveyed samples contained at least one mycotoxin type (96%), similar to 2016 (96%) but increase from 2015 (85%). The co-occurrence of more than one mycotoxin in the same sample (43%) was brought back to similar levels seen in 2015 (77% in 2016; 46% in 2015). Deoxynivalenol continues to be the most prevalent mycotoxin at 87%, continuing the overall increasing trend (87%, 72%, 62%, 27% in 2016, 2015, 2014, and 2013, respectively). Mean contamination levels of DON (1,519 ppb) were similar to 2016 (1,687 ppb), a 2X increase from 2015 (691 ppb), while the maximum contamination showed a nearly 2X increase from 2016 (54,149 ppb in 2017 vs. 30,440 ppb in 2016). Mean contamination level of FUM (4,097 ppb) was similar to 2016 (4,424 ppb), over 3X increase from 2015 (1,190 ppb). Samples with FUM over 1,000 ppb (57%) also were similar to 2016 (57%) but increased from 2015 (30%). Co-contamination of ZEN and DON remains 3X the level of 2015 corn, but appears improved compared to 2016 corn. Preliminary results indicate co-occurrence with *Fusarium* mycotoxins DON, ZEN, and FUM at 16% to be lower than 2016, but still elevated compared to prior years (2016: 38%, 2015: 11%). Because of the high frequency of multi-mycotoxin contamination in samples thus far, multiple strategies of mitigating risk are needed beyond adsorption, including biotransformation and providing support to immune and liver function.

Key Words: Deoxynivalenol, Fumonisin, Zearalenone, Co-occurrence, Biotransformation

P303 Effects of a specific blend of essential oils and oleoresins of spices compared to an antibiotic growth promoter program on the broiler performances Ivan GIRARD, Jean Francois GABARROU* *Probiotech International*

Considering the simple removal of antibiotic growth promoters might have a negative economic impact, search for alternative additives has been incentivised. The main goal of this experiment is to evaluate the efficiency of a blend of essential oils and oleoresins of spices on performance of broiler between 0 to 36 days compared to usual conditions of production based on antibiotic growth promoter (AGP).

288 one day chicken (Ross-308) were randomly distributed into two groups with 6 replicates (6x24 birds) in each one. They received Coxivac-B at 5 times the normal dose of vaccine at the time of their arrival. Positive control feed was supplemented with Coyden (0-21 days), Salinomycin (22-36 days) and 55 ppm of Bacitracin Methylene Disalicylate (BMD) like growth factors (0 - 36 days of age). In the tested group, BMD was substituted by a phytogenic feed additive (Oleobiotec®, Laboratoires PHODE - France) at 100 g/MT. *Statistical analysis* - Statistical analysis was carried out using the Mixed procedure of SAS® software (SAS v. 9.3 Cary, N.C.).

Both groups (phytogenic *vs* BMD) presented the same performance on mortality rate, growth performance and feed efficiency. The final liveweight (LW) at 36 days of age showed a significant improvement in the antibiotic free group (Oleobiotec®): + 68.9 g/bird, i.e. +2.77%.

The phytogenic feed additive Oleobiotec® (Laboratoire PHODE), formulated as a specific blend of essential oils and oleoresins could be proposed as an efficient alternative to substitute antibiotic growth promoter (BMD in this case). In economical point of view, a net profit was calculated over the trial and appeared to be in favour of the natural alternative : 0.558 *vs* 0.528 CA$ (+5.68 %) per kg of produced LW, for the Oleobiotec® and positive control groups, respectively.

Key Words: essential-oil, oleoresin, broiler, growth-promotor

Metabolism and Nutrition, Vitamins and Minerals

P304 Effect of chelated copper on growth performance and meat quality in broilers Juxing Chen*, Frances Yan, Karen Wedekind, Ashley LaMontagne, Mercedes Vazquez-Anon *Novus International Inc*

Copper (Cu) has been widely used at high levels as growth promoter in poultry. The role of Cu in woody breast (WB) and white striping (WS) has not been widely studied. There are different forms of chelated and inorganic Cu sources available for the poultry producers to use at different doses. A floor pen study was conducted with 896 day-old male broilers to evaluate the effects of Cu methionine hydroxy-analogue chelate (Cu-MHAC) (MINTREX® Cu, Novus International, Inc.) vs CuSO4 on growth performance and incidence of WB and WS in broilers. The study consisted of 7 dietary treatments: 0, 30, 60 and 120 ppm Cu-MHAC or $CuSO_4$, each with 8 replicate pens of 16 birds. The levels of other minerals from inorganic sources were equal among all treatments. Nutritionally complete corn soybean meal based diets were formulated for starter (0-14 d), grower (15-27 d), and finisher (28-41 d) phases. All diets were pelleted, and starter diet was crumbled after pelleting. All birds were orally gavaged with a coccidiosis vaccine at 10× the recommended vaccination dose on d14. Breast fillets in broilers at 42d of age were scored to 4 categories (normal, mild, moderate, severe) for WB and WS. Performance, WB and WS scores were analyzed by one-way ANOVA and source*dose factorial, means were separated by Fisher's protected LSD test. Incidence of WB and WS was analyzed by Chi-square. A P-Value ≤ 0.05 was considered statistically different. Compared to 0 ppm Cu, all doses of Cu-MHAC significantly improved performance index (Perfidx), only 60 and 120 ppm CuSO4 significantly improved Perfidx on d14 and d28; Cu-MHAC significantly improved FCR on d14 (60 and 120 ppm) and d28 (120 ppm), but not CuSO4 at any doses. There was significant 1) source effect with Cu-MHAC improving FCR on d14, reducing WS score and incidence of moderate WS, and increasing normal fillets; 2) dose effect with 120 ppm Cu increasing the incidence of moderate WB vs 30 ppm Cu, suggesting high levels of Cu may work as prooxidant to cause oxidative stress therefore exacerbating WB. In summary, Cu could improve FCR and Perfidx in broilers of 14 and 28 d of age with better effect of Cu-MHAC vs CuSO4 on d14, Cu-MHAC improved meat quality by reducing WS score and incidence of moderate WS in broiler of 42 d of age.

Key Words: Chelated copper, CuSO4, woody breast, white striping, broiler

P305 Effects of 25-Hydroxycholecalciferol with two D3 vitamin levels on Production And Immunity Parameters In Broiler Chickens Jacqueline Vázquez Reyes, Gabriela Gómez Verduzco*, Carlos López Coello, Arturo Cortés Cuevas, Sergio Fernández Tinoco, Ezequiel Rosales Martínez, Ernesto Ávila González. Ávila González. *Poultry Science Department, National Autonomous University of Mexico City, Mexico*

This study was performed in Ross 308 chickens aged 1–21 days and aimed to evaluate whether the addition of 25-hydroxycholecalciferol ($25(OH)D_3$) to broiler chicken diets affects their growth performance and immunity. A completely random 2×2 factorial arrangement was used with two levels of vitamin D_3 and the absence or presence of $25(OH)D_3$, corresponding to four treatments based on sorghum + soybean diets: 1) 200 IU of vitamin D_3/kg of feed (Diet 1) (NRC, 1994), 2) Diet 1 + 69 µg of $25(OH)D_3$/kg of feed (Diet 2), 3) 5,000 IU of vitamin D_3/kg of feed (Diet 3), and 4) Diet 3 + 69 µg of $25(OH)D_3$/kg of feed (Diet 4). Each treatment was conducted with six replicates of 10 chickens each. Water and food was supplied *ad libitum.* The results showed significantly increased growth and tibia ash ($P<0.05$) in the birds fed 5 000, IU of vitamin D_3/kg + $25(OH)D_3$. Additionally, the cellular immune response increased significantly ($P<0.05$) in both treatments with added $25(OH)D_3$. Based on the results obtained under the current test conditions, the addition of 25(OH)

D_3 at a rate of 69 µg /kg to diets containing vitamin D_3 improved the cellular immune response and mineral deposition in the bones of broilers aged 1–21 days. Because these parameters are very important in modern poultry farming, these results indicate that supplementation with 25(OH) D3 should improve broiler production

Key Words: Broiler chicken, 25-Hydroxycholecalciferol, Production parameters.

P306 Effects of in ovo-injected vitamin D3 sources on eggshell temperature and the early post-hatch performance of Ross 708 broilers Saman Fatemi*[GS], Katie Collins Elliott, Abiodun Bello, Oluwaseun Durojaye, Haijun Zhang, Bradley Turner, E. David Peebles *Mississippi State University*

Effects of *in ovo* injected-vitamin D_3 sources on eggshell temperature (ET) and broiler performance through 14 d of age (doa) were investigated. In a preliminary trial, serum 25- hydroxylcholecalciferol ($25OHD_3$) levels were determined at 19.5 d of incubation (doi) after injecting diluent or diluent containing 1.2 µg of either vitamin D_3 (D_3) or $25OHD_3$. In a main trial, 1,440 Ross 708 broiler hatching eggs were set on 12 replicate tray levels in a single-stage incubator with all treatments represented on each tray. From 15 to 18 doi, ET of eggs on each tray was recorded using an infrared thermometer at 7 AM and 7 PM. At 18 doi, eggs received one of the following treatments: 1) non-injected; 2) diluent; or diluent containing 2.4 µg either of 3) D_3 or 4) $25OHD_3$. After injection, consecutive ET readings were recorded at 18 (1 PM and 7 PM) and 19 (7 AM, 1 PM, and7 PM) doi. Hatchability and hatching BW were determined at 21 doi. Equal numbers of male and female chicks were placed in each pen and grown out for 14 d. Pen weight BW was recorded on 7 and 14 doa and BW gain, feed intake (FI), and feed conversion ratio (FCR) were calculated from 0 to 14 doa. Embryos that received either vitamin D_3 source had higher ($P<0.001$) serum $25OHD_3$ levels compared to diluent-injected controls. The ET from 15 to 19 doi did not differ between treatments ($P=0.307$), but after injection, ET was highest at 7 PM on 18 doi (38.15 °C; $P\leq0.001$). Across treatments, ET at 1 PM on 18 doi (38.01 °C) and at 7 PM on 19 doi (38.02 °C) was higher ($P\leq0.001$) compared to that at 7 AM on 19 doi (37.89 °C) or at 1 PM on 19 doi (37.99 °C). Hatching BW ($P=0.397$), hatchability ($P=0.291$), and BW ($P=0.969$) at 14 doa did not differ between treatment. The *in ovo* injection of $25OHD_3$ resulted in significantly a lower FCR (1.28g ± 0.015; $P=0.004$) and a higher FI (35.58g ± 0.422; $P= 0.008$) as compared to the other treatment groups. It was concluded that ET differed between time periods after d of injection, but had no clear pattern until hatch. Although the type of vitamin D_3 source injected did not affect ET, the *in ovo* injection of $25OHD_3$ displayed a potential to improve early post-hatch broiler performance.

Key Words: Eggshell, Vitamin, inovo, 25-hydroxylcholecalciferol, broiler

P307 Effects of different levels of calcium and 25-hydroxy vitamin D3 on egg production and shell quality in 74 weeks old Lohmann LSL-lite layers Reza Akbari Moghaddam Kakhki*[GS], Thomas Heuthorst, Alisha Wornath-Vanhumbeck, Mohamed Neijat, Elijah Kiarie *University of Guelph*

Poor egg shell quality is often a concern in later stages of lay cycle. We investigated the effects of different levels of Ca and top dressing 25-hydroxy vitamin D3 ($25OHD_3$) on egg production (EP) and weight (EW) and shell quality (thickness, EST and breaking strength, ESBS) in Lohmann LSL-lite layers from 74 to 81 wk of age. Four levels of Ca (3.0, 3.5, 4.0 and 4.5%) and three levels of $25OHD_3$ (0, 69 and 138 µg/kg) were tested. All diets had a basal level of 330 KIU of vitamin D3/kg. Calcium was supplied by coarse (≥2 mm) and fine (<2 mm) limestone included at a ratio of 5.7: 1 wt/wt. A total of 84 individually housed hens were allocated to the diets based on prior two-wk EP data. Production was monitored daily, and shell quality was assessed once per wk with EST measured by gauge and ESBS by force reader (ORKA Food Technology). Data were reported on two 28-d periods. Based on feed intake and assayed Ca, birds consumed 3.4, 4.1, 4.3 and 4.7 g/b/d in the first and 3.4, 4.2, 4.2 and 4.8 g/b/d in the second period. Feed intake and EP was not influenced ($P > 0.05$) by treatments. The EP was 86 and 83% in the 1st and 2nd period, respectively. The EW linearly ($P < 0.001$) decreased by 2.3% and 4.4% in response to Ca intake from 3.4 to 4.3 g/b/d in the 1st period and 3.4 to 4.2 g/b/d in the 2nd period, respectively. An increase in 25OHD3 from 0 to 69 µg/kg improved EW by 1.9 and 4.4% in 1st and 2nd periods, respectively. Moreover, an interaction ($P< 0.001$) between Ca and $25OHD_3$ was observed for EW in the 1st period such that 25OHD3 linearly increased EW at 3 Ca intake levels. Dietary Ca intake affected EST quadratically ($P<0.05$), increasing with Ca intake 3.4 to 4.2 g/b/d and decreasing with further Ca intake. Similar quadratic response, to Ca intake ($P<0.01$) was observed for ESBS. Lowest and highest Ca intake in the 1st (3.4 and 4.7 g/b/d) and 2nd period (3.6 and 4.8 g/b/d) had poor ESBS relative to intermediate Ca intakes. There was no ($P>0.05$) 25OHD3 or interaction with Ca effects on EST and ESBS. Number of cracked and shell less eggs were not affected ($P>0.05$) by treatments. In conclusion, the data revealed that Ca intake of 4.1 to 4.2 g/b/d could improve egg shell quality without any adverse in egg production and top dressing with 25OHD3 could improve egg weight.

Key Words: Layers, Calcium, 25-hydroxyvitamin-D, Egg, Shell-quality

P308 Impact of replacing inorganic trace minerals with reduced levels of proteinates in laying hen diets with or without phytase on the performance and egg shell quality of layers in a single lay cycle Tuoying Ao*, Anthony Pescatore, Marquisha Paul, Lauren Nolan, Lizza Macalintal, Mike Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

A study was conducted to investigate the effect of replacing an inorganic trace mineral premix with reduced levels of proteinates (Bioplex®, Alltech Inc.) in laying hen diets with or without phytase on performance and egg shell quality in a single lay cycle. A 2 x 2 factorial treatment structure with two different trace mineral premixes (inorganic or Bioplex®) and two levels of phytase (0 or 600 PU/kg) in a corn-soy based diet was used. Four hundred eighty Hy-Line Brown layers at 16 wk of age were randomly assigned to each of four dietary treatments with 10 replicate units of 12 layers. The layers were raised in layer cages using two birds per cage (25 x 41 cm) and photo-stimulated with 16L: 8D. Feed and water were provided ad libitum. Dietary treatments continued through 96 wk of age. Bad shell eggs characterized as soft-shelled, cracked, corrugated and wrinkled etc. were counted from wk 60 to 80 of production and the percentage was calculated. In entire 80 wk of production, dietary Bioplex® improved ($P<0.05$) feed conversion ratio (1.67 vs. 1.62 kg/dz). Dietary phytase increased ($P<0.05$) average egg weight (61.3 vs. 62.2 g), but negatively impacted ($p<0.05$) feed conversion ratio (1.62 vs. 1.67 kg/dz). The percentage of bad shell eggs was reduced ($P<0.05$) by dietary Bioplex® with or without phytase. The values were 1.95, 1.05, 1.45 and 1.38% for hens fed corn-soy diet with: inorganic mineral premix, Bioplex® mineral premix, inorganic mineral premix + phytase and Bioplex® mineral premix + phytase respectively. The results indicate that the total replacement of inorganic trace mineral premix with reduced level of Bioplex® mineral premix in the diets with or without phytase can improve feed conversion ratio and egg shell quality of layers in a single lay cycle.

Key Words: layer, performance, shell, minerals

P309 Effects of dietary inorganic trace minerals replaced by organically bound trace minerals on tissue mineral deposition and fecal excretion in broiler breeders Geng Wang*[GS], Lujie Liu, Bojing Liu, Minqi Wang, Gang Lin, Yan Xue, Tuoying Ao *College of Animal Science, Zhejiang University*

This study investigated effects of inorganic trace mineral (ITMs) replacement with organic trace minerals (BIOPLEX PP) on tissue mineral retention and fecal excretion in ZhenNing yellow feather broiler breeders. Six hundred hens, age 40 wks (1.70±0.07 kg BW) were randomly divided

into 5 dietary treatments and fed for 10 wks (including 2 wks adaptation), with 4 replicates of 30 broiler breeder hens per treatment. Dietary treatments were: (G1) basal + ITMs at commercial level; (G2) basal + ITMs at low level (equivalent to 1.0 kg BIOPLEX PP/Ton, about 50% commercial level or less except Se); (G3), (G4), (G5): basal + 0.75, 1.0, 1.25kg BIOPLEX PP/Ton respectively. After the feeding trial, 3 birds from each replicate were sampled to collect serum, muscle and selected organ samples. All studied parameters were analyzed with one-way ANOVA with separated means considered significant at P<0.05. In serum, there was no difference for Cu concentration among the treatment groups. Zn, Fe, Mn and Se concentration of G5 was higher than that of G2 (P<0.05). Mn and Se concentration of G4 was higher than that of G2 (P<0.01). Mn and Se concentration of G1 was higher than that of G2 (P<0.01). In pectoral muscle, Cu and Fe concentration of G3 was lower than that of G1 and G5 (P<0.01), Zn and Se concentration of G3 was lower than that of G5 (P<0.01). Zn and Fe concentration of G4 was higher than that of G2 (P<0.05). In liver, Se concentration of G5 was markedly higher than that of G2 and G3 (P<0.01), and Se concentration of G4 was higher than that of G3 (P<0.05). In kidney, Se concentration of G5 was higher than that of G2 and G3 (P<0.01). In heart, Fe concentration of G5 was higher than that of G2 and G3 (P<0.05). Se concentration of G3 was higher than that of G2 (P<0.01). In pancreas, Cu concentration of G2 and G3 was lower than that of G1 and G5 (P<0.05). The concentration of all minerals (Cu, Zn, Fe, Mn, Se) of G3 was lower than that of G5 (P<0.05). Fecal Cu, Zn, and Mn concentration of G3 was lower than that of G1 and Mn concentration of G4 is lower than that of G2 (P<0.01). This study indicated that dietary ITM replacement with BIOPLEX PP resulted in better tissue mineral deposition and less fecal mineral excretion in broiler breeders.

Key Words: minerals, retention, excretion, breeders

P310 Effects of Amino Acid Complexed Trace Minerals on Performance and Bone Measurements of Male Turkeys from 0 to 7 Weeks of Age. Colwayne Morris*[GS], Leonardo Linares, Jeffre Firman, Thim Cheng, Macro Rebollo *University of Missouri*

Dietary supplementation of amino acid complexed (AAC) trace minerals may improve performance, decrease incidence of leg abnormalities, and increase bone strength. Improving general health conditions in the first 7 weeks of production presents a major opportunity for the turkey industry. Leg problems, which often results in mortality, can exceed 1% per week in turkey production. The objectives of this study were to evaluate the effects of supplemental Availa®Zn, Availa®Z/M, and Availa®ZMC (Zinpro Corporation) on performance and bone measurements of male turkeys from hatch to 7 weeks. A total of 1,200-day-old Hybrid Converter male poults were randomly assigned to 1 of 4 dietary treatments, with 12 replicate pens and 25 birds/replicate. Corn and SBM-based diets were formulated to be nutritionally equal and birds were allowed *ad libitum* access to feed in two phases: Starter (d 1 to 19; crumble) and Grower (d 19 to 50; pellet), including phytase, xylanase, and a coccidiostat throughout the study. Treatments included: 1) Sulfates; 2) Zn-AAC; 3) Zn/Mn-AAC; 4) Zn/Mn/Cu-AAC and were formulated to obtain final dietary concentrations of 125 mg/kg Zn, 125 mg/kg Mn, and 7 mg/kg Cu. Data were analyzed by one-way ANOVA to determine statistical differences among treatments. No treatment effect was observed for performance parameters in either phase (P > 0.05), except for feed conversion (P = 0.05) during days 19 to 50, where birds fed Zn/Mn-AAC and Zn/Mn/Cu-AAC were 7 and 9 points more efficient, respectively, than those fed Sulfates. Feed conversion, from d 0 to 50, was lower (P = 0.06) for birds fed Zn/Mn-AAC and Zn/Mn/Cu-AAC versus Sulfates, at 6 and 8 points, respectively. No difference (P > 0.05) was observed for tibia weight (% BW). Tibia mid-section measurements were not different (P > 0.05) among treatments. Percent tibia ash of birds fed Sulfates and Zn/Mn-AAC was greater (P = 0.04) than those consuming Zn/Mn/Cu-AAC, whereas tibia ash as a percent of BW was not different (P > 0.2). In conclusion, birds consuming supplemental amino acid complexed trace minerals were observed to have improved feed conversion, both statistically in the Grower phase, and numerically throughout the total experimental period, compared to birds consuming sulfate minerals.

Key Words: Ash, copper, manganese, turkey, tibia zinc

P311 Choline digestibility of organic feed ingredients in broiler chicks Darlene Bloxham*, Ali Boone, Mary Formo, Gene Pesti *University of Georgia*

Organic poultry production is increasing in recent years. Chickens raised organically must be grown under different management practices than conventionally raised poultry. Different feed ingredients are utilized between the two management systems to maximize both systems. Organic poultry production may incorporate feedstuffs that have not been extensively fed in conventional production systems, and therefore there is limited research on how to feed these novel feed ingredients. Choline digestibility is especially important because of its ability to lower the methionine requirement. The availability of nutrients in novel feedstuffs needs to be studied. This study was performed to evaluate the digestibility of choline of organic feed ingredients in broiler chickens. Six hundred and forty Cobb 500 male broilers were fed nine organic feed ingredients in two trials. The ingredients were oats, flaxseed, field peas, millet, alfalfa meal, canola meal, sunflower meal, sorghum, and barley. The choline contents (mg/kg) of the individual feed ingredients are: 1112±122 (oats), 1074±43 (flaxseed), 1165±77 (field peas), 1248±95(millet), 1201±63 (alfalfa meal), 3208±156 (canola meal), 2531±74 (sunflower meal), 531±26 (sorghum), and 1565±88 (barley). There was also a basal diet to determine endogenous choline losses. Test diets were fed between 10 and 14 days of age. Total ileal contents were collected at fourteen days of age. Canola meal and flaxseed have the lowest choline digestibility with digestibilities of 36.7±4.3 and 50.8±16.1%, respectively. Sunflower meal had intermediate choline digestibility of 61.8±13.4%. Oats, field peas, alfalfa meal, sorghum, millet, and barley had statistically similar choline digestibilities to each other of 88.8±7.3, 87.7±2.5, 94.0±1.9, 94.8±2.5, 98.4±0.9, 84.5±3.9 % respectively. In conclusion, choline digestibility in grains appear to be higher choline availability than oilseed meals and flaxseed. Alfalfa meal and field peas have high digestibility as well.

Key Words: Choline, Digestibility, Broiler, Organic

P312 Assessment of organic mineral bond stability using computational and experimental approaches Laurann Byrne, Kate Jacques*, Richard Murphy *European Bioscience Centre, Alltech Inc.*

Trace minerals are an essential addition to poultry diets and are involved in many physiological processes. However, a number of dietary components can act as antagonists, rendering the minerals less effective from a nutritional perspective. Organically-bound mineral supplements vary in composition and chemical characteristics. One means of useful comparison among them is in terms of the strength of bond between the mineral and organic component, which is expressed as a stability constant. Knowledge of stability constants enables the behaviour of a mineral ion with one or more ligands to be modelled as a function of pH and reactant concentration. However, difficulties arise in determining the stability constants for such organo-mineral products when protonation data are unavailable for particular bonding groups. In this work, complementary approaches were taken to understand how ligand type influences organic mineral bond strength with a view towards development of a general model allowing comparison of organically-complexed trace minerals. Copper(II) complexes of amino acid and peptide ligands (proteinates) with previously published protonation constants were investigated with a modified version of the HYPERQUAD computer program to assess the effect of ligand conformation on bond strength. Additionally, ion-selective electrode (ISE) potentiometry was used to assess the relative pH-dependant stabilities of commercially available copper(II) complexes by measuring free Cu^{2+} under pH conditions reflective of gastrointestinal conditions. The complementary approaches indicated, that in the case of copper(II) amino acid and peptide complexes, not only the type but also the conformation of

ligand played a significant role in the stability of organic mineral sources. Of the products tested, proteinates were inherently more stable across a wider pH range and in particular at acidic pH levels.

Key Words: Trace minerals, Bond strength, Stability

Metabolism and Nutrition, Enzymes

P313 The effect of mineral premix source on phytase function. Mark Gaffney, Richard Murphy, Kate Jacques*, Sean Doyle *Alltech*

Unlike ruminants, monogastrics have low levels of intrinsic phytase activity, thus the inclusion of exogenous phytase enzymes to respective diets is common practice. Exogenous phytases are typically added via premixes containing concentrated forms of inorganic trace minerals (ITM). Certain trace minerals have been shown to negatively affect phytase function. The replacement of ITM with organic trace minerals (OTM) may help to reduce potential interactions between phytases and premix components.

The present study investigated the influence of sulphate (ITM) and Bioplex® (OTM) forms of mineral mixes (containing Fe, Cu, Zn, and Mn) on the activity of two individual commercial phytases (Phy 1 and 2). The effect of a simulated gastric environment (pH and temperature) on the activity of phytases exposed to mineral mixes was also assessed by incubation at pH 5, reflective of the crop, followed by increasing to pH 2.5 of the proventriculus at 40 ºC. All mineral concentrations were equal or relative to inclusion levels typically found in monogastric premix formulations. Means and SD are based on the readings of 9 replicates, with significance determined relative to control with one-way ANOVA.

Results indicated that mineral source and concentration had a pronounced effect on phytase activity. Sulphate mixes strongly inhibited the activity of both phytase enzymes, with reductions of 80 – 90 % ($p \leq 0.05$). Bioplex® mixes caused considerably less inhibition, retaining 50 – 80 % activity. Gastric pH was influential to phytase function in the presence of mineral mixes for Phy 1. Sulphate mixes reduced activity of Phy 1 by 85 % and 90 % at pH 5 and pH 2.5, respectively. Bioplex® mixes reduced activity of Phy 1 by 50 % at pH 5, but a significant increase of 15 % was observed at pH 2.5 ($p \leq 0.05$). Conversely, gastric pH did not appear to influence the activity of Phy 2 in the presence of mineral mixes.

These *in vitro* results demonstrate that there is potential for negative interactions between high cost feed ingredients, such as enzymes and minerals. These potential interactions carry real and often underappreciated financial costs and may also go towards explaining the varied animal responses observed *in vivo* following phytase supplementation.

Key Words: Phytase, Proteinate, Monogastric, Poultry

P314 Influence of age and length of adaptation to low phosphorus diet on phytase efficacy during the starter phase of broiler chickens Olufemi Babatunde*[GS1], Aaron Cowieson[2], Jonathan Wilson[2], Olayiwola Adeola[1] [1]*Purdue University;* [2]*DSM Nutritional Products*

Phosphorus (P) is bound in phytin in most plant sources and require phytase to increase P availability in poultry fed diets rich in plant materials. A total of 1,408 male Cobb 500 broiler chickens were used to evaluate the impact of age and length of dietary adaptation on the efficacy of phytase using growth performance, nutrient utilization, tibia ash and digestibility of myo-inositol as criteria for evaluation. Four dietary treatments with 8 replicates each were fed for 5 adaptation periods which were from 6 to 8 d (12 birds per replicate), 6 to 22 d, 9 to 14 d, 12 to 14 d and 20 to 22 d (8 birds per replicate), parameters were measured on the last day of each period. Diets were formulated with 2 non-phytate P (nPP) concentrations (0.20 and 0.40%) and two phytase concentrations (1000 and 2000 FTU/kg) added to the 0.20% nPP diet. Adaptation effect was observed by comparing responses of birds at 14 d and birds at 22 d while age effect was determined by comparing responses from birds with 2 d adaptation at age 8, 14 and 22 d post hatch. Body weight gain and gain to feed ratio were improved ($P < 0.01$) in birds supplemented with phytase irrespective of age or adaptation period, however, an increase in dietary adaptation length improved ($P < 0.01$) gain to feed ratio with birds at 16 d adaptation performing better than birds at 5 d and 2 d adaptation. In addition, increased age and adaptation length improved ($P < 0.01$) apparent P and Calcium (Ca) digestibility and retention with phytase supplementation. The effect of phytase on mineral utilization was lower ($P < 0.01$) as dietary adaptation increased above 5 d and as birds grew older than 14 d. Tibia ash increased ($P < 0.01$) with age from 44% in younger birds to 49% in older birds with phytase addition and reduced ($P < 0.01$) with increasing dietary adaptation as birds at 2 d adaptation had more tibia ash than birds at 5 d and 16 d adaptation. The effect of phytase on tibia ash increased ($P < 0.01$) as adaptation period increased. There was a linear increase ($P < 0.01$) in plasma myo-inositol across all age and adaptation periods with phytase addition. In conclusion, phytase efficacy can be affected by age and adaptation period with a potential reduction in effectiveness as age and adaptation length increases.

Key Words: Adaptation, Age, Calcium, Phosphorus, Phytase

P315 Comparison of two sources of microbial phytases on performance and bone ash of broiler chicks fed non-phytate phosphorus deficient corn-soybean meal based diets Megharaja Manangi*, Jenea Wright, Juxing Chen, Mercedes Vazquez-Anon *Novus International, Inc.*

A 17d experiment was conducted to evaluate the source and dose effects of supplementation of a next generation microbial phytase (CIBENZA® PHYTAVERSE®; phytase-A) and a commercial 6-phytase (phytase-B) in broiler chicks fed non-phytate phosphorus (npp) deficient corn-SBM based diets. A total of 560 Ross-308 male broiler chicks were assigned to 10 treatments with 7 cages/treatment and 8 chicks/cage. Treatments consisted of T1 with 0.21% npp (Negative Control, NC); T2 to T5 with 250, 500, 750, and 1500u of phytase-A/kg diet added to T1, respectively; T6 to T9 with 250, 500, 750, and 1500u of phytase-B/kg diet added to T1, respectively; T10 with 0.45%npp (Positive Control, PC). Diets were formulated to have 0.93% Ca. All the diets were pelleted at 80^0 C and crumbled. The study was carried out as RCBD and the data were analyzed using 1-way ANOVA and 2-way ANOVA (factorial for 2 phytase sources and 4 doses). Significance was tested at $P \leq 0.05$. Orthogonal polynomial contrasts were used to test the linear and quadratic effects of phytases' doses. Overall, significant treatment effects were observed for gain, FCR adjusted for mortality/culls (FCR), feed intake (FI), and g of ash/bone. Significant differences ($P<0.05$) between NC and PC were observed for both performance and bone ash. Significant ($P<0.05$) quadratic effects were observed for both phytases for performance and ash. Factorial analysis indicated only significant interaction ($P<0.05$) for FCR adjusted for mortality/culls (FCR) suggesting no differences between phytase-A and B at 250 and 500u. At 750 and 1500u, phytase-A improved FCR by 2.2 and 2.4 points, respectively, over phytase-B. Significant source and dose effects ($P<0.05$) were observed for gain, and g of ash/bone suggesting phytase-A improved gain by 2.3% and g of ash/bone by 8% over phytase-B. Dose effect for gain indicates no response after 500u irrespective of source, and for g of ash/bone a significant increase at each tested dose was observed irrespective of source. There was a trend ($P=0.057$) for source effect on FI. In summary, main effects outcome indicated that the phytase-A significantly improved gain and g of ash/bone over phytase B irrespective of dose.

Key Words: Phytase, Broiler

P316 Parameters of bone quality of broilers fed diet with low available phosphorus level and bacterial phytases Tatiane Santos, José Sartori*, Maria Sartori, Juliana Denadai, Mayara Santana-Eich, Paola Serpa, Camila Sarkis, Luiz Vulcano, Eduardo Piber Neto *São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science*

The use of phytase associated with low available phosphorus level in broiler diets reduces the antinutritional effect of phytate and improves the utilization of phytic phosphorus. This study aimed to evaluate the effect of three commercial bacterial phytases derived from *Escherichia coli* on the bone quality of broilers at 35 d old. A total of 2,100 one-day-old male Cobb chicks were housed in 70 pens in a completely randomized design in a factorial arrangement. Positive control* (PC*) and negative control* (NC*) diets had nutritional value of phytase recommended by the manufacturer (0.12% available phosphorus) in relation to positive control (PC) and negative control (NC) diets. The factorial design was 2x4+2: two levels of available phosphorus (aP) (PC* = 0.33% (1 to 21d) and 0.28 % (22 to 35d) aP) and (NC* = 0.23% (1 to 21d) and 0.18% (22 to 35d) aP) × phytases (A, B, C, none) + positive control, PC (0.45% (1 to 21d) and 0.40% (22 to 35d) aP no phytases) and negative control, NC (0.35% (1 to 21d) and 0.30% (22 to 35d) aP no phytases) totalizing 10 treatments with seven replicate. At 35 d old, two broilers with average weight per pen were selected and sacrificied by cervical dislocation, and the left femur were removed for evaluation of index Seedor, bone resistance and bone ash. Data were analyzed by Minitab 16 and significant means of the factorial were compared by Tukey Test 5%. There was interaction between the factors ($P < 0.05$) for index Seedor, bone resistance and bone ash, showing that the PC* diets with the inclusion of phytase were more effective for bone quality than the NC* diets. The index Seedor is the ratio between bone weight and length, and this value indicates the density, and the higher the density, the higher the resistance. The results showed that for bone quality, broiler fed PC* diets with phytase had better index Seedor, bone resistance and bone ash. They also indicated that the three phytases had the same efficiency, but NC* diets impaired bone quality. Acknowledgments: BrNova for donating the premix used in the experiment and FAPESP (Process: 2014/27175-8).

Key Words: enzyme, mineral, femur

P317 Effect of a new 6-phytase, Natuphos E®, on ileal digestibility of sodium and broiler performance at variable sodium levels in corn/soy diets Mike Coelho* *BASF Corporation*

The objective of this study was to evaluate the efficacy of a 6-novel phytase, Natuphos E, on ileal digestibility of sodium and broiler performance in standard industry corn/soy diets through day 14. A total of 1449 Male Cobb 500 broilers were used in a randomized complete block design pen trial (23 birds/pen x 7 phytase doses x 9 replicates). Phytase dose rates were 0 (NC), 500, 1000, 1500, 2000, 2500 and 3000 FTU/kg with a 0.15% P and 0.19% Ca inorganic reduction. Apparent distal ileal sodium digestibility (IDS) and bird performance at 14 days were measured. TiO2 was used as an indigestible marker. Data were subjected to ANOVA and orthogonal contrasts were used to determine linear and quadratic responses to phytase doses. Birds fed 500, 1000, 1500, 2000, 2500 and 3000 FTU/kg Natuphos E had higher ($P<0.05$) IDS versus NC (-0.49, -0.38, -0.33, -0.32, -0.30 and -0.27 versus -0.78%), increased ($P<0.05$) 14d body weights versus NC (422, 438, 440, 457, 459, 467g versus 366g, decreased ($P<0.05$) 0-14d FCR (1.220, 1.209, 1.204, 1.195, 1.192 and 1.189 versus 1.254). Body weight gain was correlated with increased in IDS with a R^2 of 0.95. A subsequent trial investigated the effect of variable sodium levels, while maintaining electrolyte balance, on broiler performance in a standard industry corn/soy diets in the presence of 1000 FTU/kg Natuphos E, while removing 0.184% nPP and 0.220% Ca. A total of 1242 Male Cobb 500 broilers were used in a randomized complete block design pen trial (25 birds/pen x 6 sodium levels x 9 replicates). Sodium (Na) levels in starter were 0.13, 0.14, 0.15, 0.16, 0.17 and 0.18%, grower 0.14, 0.15, 0.16, 0.17, 0.18 and 0.19%, and finisher 0.15, 0.16, 0.17, 0.18, 0.19 and 0.20%. Body weights (BW) at d14 with 0.15% Na (587g) was higher ($P<0.05$) than 0.13, 0.17% and 0.18% Na (473, 476 and 465 g), BW at d28 with 0.16% Na (1448g) was higher ($P<0.05$) than 0.14, 0.18 and 0.19% (1,362, 1,265 and 1,097g), and BW at 42d with 0.17% Na (2465g) was higher ($P<0.05$) than 0.15, and 0.19% Na (2,345 and 2,214g). In summary, Natuphos E increased sodium digestibility, increase in IDS was correlated with bird weight gain and FCR. Best broiler performance was with 0.15%, 0.16% and 0.17% sodium in starter/grower/finisher.

Key Words: Phytase, Broiler, Performance, Sodium, Digestibility

P318 Effect of a NSPase enzyme, Natugrain TS®, on feed passage rate, jejunum viscosity, energy release and performance on broilers fed mash and pelleted corn/soy diets processed at variable conditions mike coelho* *BASF Corporation*

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

Key Words: NSPase, broiler, performance, viscosity, passage

P319 High doses of phytase, different water pH and acclimatization system on broiler performance Mariane Fernandes*, Alexandre Rosa, José Sorbara, Leila da Silva, Natália Pedroso, Janaina de Moura, Julia Silva, Catarina Stefanello, Natiele Santos *Federal University of Santa Maria*

The experiment was carried out at Poultry Science Laboratory at the Federal University of Santa Maria in the summer season. A total of 3000 males one-day-old Cobb-500 broilers were used. Birds were distributed in a completely randomized design with a 3x2x2 factorial arrangement. Three phytase levels (Negative control, NC - reduction of 0.15% Ca and 0.15% P), NC + 100 ppm and NC + 250 ppm HiPhos GT), two water pH (6.2 and 8.2), two acclimatization system (conventional and acclimatized poultry house), totaling 12 treatments with 7 replicates (50 birds) in the acclimatized poultry house and 6 replicates (25 birds) in the conventional poultry house. Pelleting diets based on corn and soybean meal were used. A four phase feeding program composed by pre-starter (1 to 7d), starter

(8 to 21d), grower (22 to 35d) and finisher (36 to 42 days) was used. A concentrated solution of sodium bicarbonate ($NaHCO_3$) was included to adjust the water pH. The parameters studied were: body weight (BW), feed consumption (FC), body weight gain (BWG) and feed conversion rate (FCR). Data were submitted to analysis of variance, and the Tukey test was used to detect significant differences at 5%. Birds fed with negative control diets had the worst performance in all parameters evaluated ($P<0.05$). The NC supplemented with 100 ppm of phytase was enough to increase broiler performance ($P<0.05$). The effect of addition of 250 ppm HiPhos in the diet was superior up to 21 days of age for BW, FC, BWG and FCR. It was observed the significant interaction pH X poultry house ($P<0.05$), where birds in acclimatized poultry house had better performance than conventional. The worst performance was in the conventional poultry house with pH 6.2, in the same poultry facilities birds submitted to pH 8.2 had similar results to the acclimatized poultry house. In conclusion, high doses of phytase can improve the productive performance of the birds in the initial phase. Broiler chickens housed in the conventional poultry house when fed diets with high doses of phytase (250 ppm of HiPhos) had better performance.

Key Words: enzyme, alkalinity, basic, acid

P320 Variation of non-cellulosic polysaccharides in different genetic lines of whole soybeans and SBM Pramir Maharjan*, Katie Hilton, Michael Schlumbohm Schlumbohm, Antonio Guerra, Garrett Mullenix, Jordan Weil, Judith England, Craig Coon *U of Arkansas*

Non starch polysaccharides (NSP) in feed are considered as anti-nutritional factors. This study was conducted to understand variation in non -starch polysaccharide (NSP) levels in whole soybean samples from nine genetic lines and seven different SBM samples (unknown lines) collected from a feed mill. NSP was measured as soluble and insoluble non-cellulosic polysaccharide (NCP) at individual monosaccharide levels in their alditol acetate form using gas chromatography-flame ionization detector (GC-FID) as described by Englyst et al. (1994). Pectin levels were measured as uronic acid in the form of galacturonic acid using colorimetric assay. Samples were run in triplicate. Results (DM basis) showed that insoluble NSP content ranged from 7.51 % to 9.01 % and soluble NSP content from 0.2 to 0.4 % for whole soybean samples from different lines ($P < 0.05$). NSP content in SBM samples from unknown lines varied from 8.21 % to 11.38 %, whereas soluble NSP content measured 0.31 % to 0.72 percent. All the samples analyzed exhibited similar pattern for major monosaccharides detected in NSP: galactose was predominant hexose (as high as 5 %), followed by arabinose (2-3 %), xylose (1-2 %); and mannose, glucose, rhamnose and fucose being less than 1 percent. Pectin levels in whole soybean samples for genetic lines ranged from 2. 17 % through 4.98 % for insoluble NSP fraction and 0.7 % to 0.11 % for soluble NSP fraction. Findings demonstrate that NSP levels in SBM and whole soybeans can vary with different samples and between genetic lines. Profiling NSP levels may be beneficial when using SBM in poultry feed.

Key Words: soybean, non-starch, polysaccharides, variation

P321 Importance of dietary mineral source on retained xylanase function Mark Gaffney[1], Mark Furlong[1], Rachel O'Rourke[1], Kate Jacques*[2], Richard Murphy[1] [1]*Alltech European Bioscience Centre;* [2]*Alltech Inc*

Anti-nutritional factors such as non-starch polysaccharides (NSPs) are highly abundant in feed-grade cereals, resulting in poor nutrient availability and decreased metabolisable energy. To alleviate this, exogenous xylanases are routinely added to monogastric feed to degrade NSPs, helping to reduce intestinal viscosity and increase nutrient availability. Given the benefits of exogenous xylanase, and in appreciation of the complexity of modern day feed formulations, it has become increasingly necessary to identify and avoid any potential negative interactions between supplemented feed ingredients.

The present study assessed the antagonistic effects of dietary trace minerals on xylanase activity *in vitro*, focusing on the differences between organic and inorganic minerals. Inorganic trace minerals (ITMs) are often supplemented to monogastric feeds at levels exceeding those recommended by the National Research Council (NRC). Comparatively, organic trace minerals (OTMs), such as those in the Bioplex® range, can be used at lower inclusion rates due to their greater bioavailability.

Results indicated that supplemental iron had a particularly negative effect on xylanase function. At pHs reflective of the lower intestinal tract (pH5.3), xylanase activity was relatively unaffected by Bioplex-Fe®, at either reduced rates (97.6% activity retained) or at NRC recommended levels (103.8%). Comparatively, when iron was added as an ITM at NRC rates, activity significantly reduced 43.8% ($p\leq0.05$). Given that mineral bonding strength can change with pH, these interactions were also assessed at more acidic pHs, reflective of the upper intestinal tract (pH3.5). Under these conditions, xylanase activity dropped a further 19.3% when inorganic iron was used. Interestingly, Bioplex-Fe® at recommended inclusion levels only reduced activity a further 4.1%. This difference was most likely due to the extra stability associated with organic minerals at acidic pHs, a trait not observed in inorganic minerals which readily dissociate in such conditions.

This study emphasises the potential antagonisms that may occur between feed components in changing pH conditions, as well as the importance of mineral source in retaining enzyme function and overall feed ration value.

Key Words: Xylanase, Inhibition, Mineral, Organic, Inorganic

P322 Effects of dietary xylanase supplementation on the growth performance and processing characteristics of pekin ducks Zachary Lowman, Jimmy Jurgielewicz, Miguel Barrios*, Anthony Quant *Joe Jurgielewicz and Son ltd.*

An experiment was conducted to evaluate the growth performance and processing characteristics of white pekin ducks fed reduced energy diets that were supplemented with a xylanase enzyme (Belfeed B110MP, Jefo Nutrition, Saint-Hyacinthe, QC). The experiment was a 42 d trial that utilized 1,080 broiler-type white-Pekin ducks, that were randomly allotted to 3 dietary treatments with 36 pens of 30 ducks/pen (12 replicate pens/treatment). The dietary treatments consisted of a positive control (PC), a negative control (NC) with a 132 kcal/kg AME reduction throughout the experiment, and the NC supplemented with the xylanase enzyme (XYL). Ducks were administered diets in 2 phases; a starter diet through 14 d of age and a grower diet from 15-42 d of age. The experimental diets were pelleted and consisted of corn, soybean meal, wheat middling's, and corn distiller's dried grains with solubles as the primary feedstuffs. Feed consumption (FC) and body weight (BW) data were collected weekly to calculate feed conversion ratio (FCR), and carcass data was collected at the time of processing. The data from this experiment was analyzed using the one-way ANOVA procedure of JMP 12. Average BW was not significantly different between dietary treatments ($P>0.05$) through 14, 21, 28, 35, and 42 d of age. The energy reduction in the NC negatively impacted feed conversion compared to the PC and XYL treatments at 42 d of age ($P<0.01$). The XYL treatment displayed significant improvements in FCR over the NC at 35 d ($P<0.05$) and 42 d ($P<0.01$), and was not different from the PC treatment at these times. Furthermore, xylanase supplementation improved boneless breast weight by 10.3% compared to the PC, and 7.9% compared the NC ($P<0.05$). These results demonstrate that the inclusion of this xylanase enzyme in reduced energy diets fed to ducks can produce equivalent growth performance and FCR to that of the PC, while also displaying improvements in processing yield.

Key Words: Duck, Xylanase, Enzyme, Growth Performance

P323 Phytase and a combination of xylanase-glucanase increased apparent metabolizable energy value of barley in 21-day old broiler chicken Brian Bryson*[GS], Anthony Pescatore, Merlin Lindemann, Tayo Adedokun *University of Kentucky*

The effects of supplemental phytase (Natuphos®E, 500 FTU) and xylanase-glucanase (X-G; Natugrain®TS, 100 g/ton) individually or in combination on the AME and AMEn of corn-SBM (reference diets) or corn-SBM-barley diets were evaluated. A total of 280 14-d-old male broilers (Cobb 500) were assigned to 8 dietary treatments in a completely randomized design with 7 replicate cages/diet and 5 birds/ cage. Excreta samples were quantitatively collected on d 19, 20, and 21. Barley's AME and AMEn were determined using the difference method. Data were analyzed using the Proc GLM procedure of SAS with mean comparison using orthogonal contrasts. Feed intake increased, while final BW, gain/bird, and feed efficiency decreased ($P < 0.05$) with barley inclusion (30%). Dry matter retention was higher ($P < 0.05$) in the C-SBM-barley diets compared to the reference diets. N retention improved ($P = 0.012$) with enzyme supplementation of diets containing barley compared to the reference diets. Phytase supplementation to the reference diet resulted in higher ($P< 0.05$) N retention compared to X-G supplementation (76.5 vs. 73.9%). The combination of the enzymes improved N retention by 2% compared to the same diet supplemented with only phytase (reference diets). A combination of phytase and X-G decreased ($P < 0.05$) N retention by 1.5% (76.3 vs. 75.2%) compared to X-G supplemented C-SBM-barley diets. AME and AMEn of the diets were lower ($P < 0.05$) in C-SBM-barley diets compared to the reference diets irrespective of enzyme supplementation. AMEn values for the C-SBM-barley diets improved by 58 (phytase), 71 (X-G), and 89 (phytase and X-G) kcal/kg of diet when compared to the unsupplemented C-SBM-barley diet. Values for the reference diets were 37 (phytase), 26 (X-G), and 28 (phytase and X-G) kcal/kg of diet. The AMEn of barley was higher ($P < 0.05$) with X-G supplementation (3,292 kcal/kg) compared to the control (no supplementation) diet (3,156 kcal/kg). Exogenous enzyme supplementation increased AMEn of barley by 62 (phytase), 137 (X-G), and 95 (phytase and X-G) kcal/kg. The corresponding values for AME were 53, 94, and 57 kcal/kg. Results showed that phytase and X-G improved energy utilization of a complete diet and barley in broiler chickens but the combined effects were not additive.

Key Words: Apparent metabolizable energy, barley, broiler, nitrogen retention

P324 Effects of multi-enzyme composite on energy utilization in full fat soybean processed by roasting or extrusion and fed to broiler chickens Aizwarya Thanabalan*, Mohsen Mohammadigheisar, Nelson Ward, Aaron Cowieson, Elijah Kiarie *University of Guelph*

Heat treated full fat soybean (FFSB) can be a good source of amino acids and energy for poultry. However, FFSB is rich in indigestible fiber-protein-phytate complexes which may have negative effects on nutrient utilization. We evaluated the effects of a multi-enzyme supplement (Victus™ Broiler) on apparent retention (AR) and AME content in roasted and extruded FFSB. Roasted FFSB was processed in a direct-fired roaster at 118-120°C for 75 min and extruded FFSB at 135-140°C for 30 s. The FFSB samples were hammer milled prior to feed manufacture. The diets were: 1) N-free corn starch basal diet; 2) basal plus 55% roasted FFSB; 3) 2 + enzyme; 4) basal plus 55% extruded FFSB and 5) 4 + enzyme. All diets had TiO_2 indigestible marker. The enzyme supplement supplied phytase, protease, xylanase, β-glucanase at 1,500, 5,625, 270 and 70 U/kg of feed, respectively. A total of 400 d-old Ross 708 male chicks were used in a 21-d trial. All the birds were fed a commercial diet to d 13. On d 14, birds were weighed individually and allocated to cages (10 birds/cage; n=8). The birds had free access to feed and water. Excreta samples were collected on d 18-21. The AME content of FFSB was calculated using difference method. There was no processing and enzyme interaction ($P > 0.05$) on AR of CP, crude fat and NDF. The main effects of processing ($P < 0.05$) were such that roasted FFSB had higher AR of CP (59.6 *vs.* 53.3%) but lower AR of crude fat (63.1 *vs.* 90.8%) and NDF (56.0 *vs*. 63.1%) than extruded FFSB. Birds fed enzyme had higher ($P<0.05$) AR of CP (57.6 *vs*. 55.3%) and organic matter (74.1 *vs.* 72.8%). There was an interaction ($P = 0.30$) between processing and enzyme on AR of gross energy, AME and AMEn. In this context, enzyme improved energy utilization in roasted and not in extruded FFSB. The AMEn values for extruded FFSB without and with enzyme were 4,180 and 4,159 kcal/kg DM and corresponding values for roasted FFSB were 3,284 *vs.* 3,545 kcal/kg DM. Birds fed extruded FFSB exhibited higher energy utilization ($P < 0.01$) than birds fed roasted FFSB. In conclusion, lower utilization of energy in roasted FFSB relative to extruded was indicative of inferior processing. Supplemental enzyme improvement on energy utilization in roasted FFSB suggested value in heat processed feedstuffs.

Key Words: Soybean, Broiler, Multi-enzyme, AME

P325 Development of a more sensitive protease model for apparent ileal amino acid digestibility in broilers Karen Wedekind*, Frances Yan, Mercedes Vazquez-Anon *Novus International, Inc.*

Soybean products are the most important source of dietary protein for poultry in the US and much of the world. Variation in protein quality among soy ingredients can occur due to processing. The use of exogenous protease in poultry diets may improve amino acid digestibility, but it is difficult to demonstrate efficacy of protease in diets containing highly digestible ingredients such as SBM with low trypsin inhibitor (TI). The purpose of this study was to develop a more sensitive protease model. This was achieved by increasing SBM, thereby increasing TI concentrations and decreasing apparent ileal amino acid digestibility (AIAAD). 256 Ross 308 male broilers were allocated to 4 dietary treatments with 8 replicates pens of 8 birds in a 2 x 2 factorial arrangement of treatments in a randomized complete block design. The dietary factors were SBM inclusion (42 or 96%; SBM TI = 3.85 mg/g), or protease inclusion (0 or 300 U/g diet, CIBENZA® DP100 protease, Novus International). A common corn-SBM starter diet was fed to all chicks from d 0 to 16. Experimental test diets were fed from d 22 to 30, ileal digesta was collected on d 30. Performance and ileal AA digestibility were analyzed by one-way ANOVA. Fisher's protected LSD multiple pair-wise comparison procedures were used to compare treatment means. With increasing inclusion of SBM, weight gain, FI, FCR and performance index decreased ($P<0.05$) and apparent ileal AA digestibility (AIAAD) was decreased 6-8% ($P<0.05$). There was an uplift with protease in AIAAD in diets containing 96% SBM (3.6%; $P<0.05$), but not in diets containing 42% SBM (0.3%). Although 96% inclusion of SBM does not represent a commercially relevant diet, it does simulate diets containing lower AA digestibility such as alternative protein ingredients and/or diets containing higher TI levels. In conclusion, CIBENZA® DP100 could be effective in mitigating poor quality raw materials by increasing AIAAD digestibility.

Key Words: broiler, protease, SBM, trypsin inhibitor, digestibility

P326 Evaluating the effects of a novel protease on the growth performance and processing characteristics of Pekin Ducks Mitchell Hoysock*[UG], Zachary Lowman, Miguel Barrios, Anthony Quant, Jimmy Jurgielewicz, Meghan Schwartz *The Pennsylvania State University*

The use of protease enzymes in poultry diets has been shown to be beneficial in commercial chicken and turkey production; however, limited research exists on the effects of proteases on ducks. Two experiments were conducted to evaluate the effects of feeding a novel protease enzyme (Jefo Protease, Jefo Nutrition, Saint-Hyacinthe, QC, Canada) to growing ducks and the impact on growth performance and processing characteristics. Experiment 1 (Exp. 1) utilized 1,680 broiler-type Pekin ducks allotted to 4 dietary treatments with 48 pens of 35 birds/pen (12 replicates/treatment). The dietary treatments for Exp. 1 were: positive control (PC), negative control (NC1; reduced crude protein/amino acids), NC1 + Protease (NP1; amino acid matrix applied), and NC1 + Protease (PP1; no matrix applied). Experiment 2 (Exp. 2) utilized 2,880 broiler-type Pekin ducks that were

allotted to 3 dietary treatments with 24 pens of 120 birds/pen (8 replicates/treatment). The dietary treatments for Exp. 2 were: positive control (PC2), negative control (NC2; 5% crude protein/amino acid reduction), and NC2 + Protease (PP2; no matrix applied). The data from these experiments was analyzed using the one-way ANOVA procedure of JMP 12. In Exp. 1, final body weight (BW) at 42 d was not different among treatments (P=0.73). In addition, feed conversion ratio (FCR) at 42d in Exp. 1 was not different among treatments (P=0.47). There was a trend for improved boneless breast weight due to dietary protease supplementation in Exp. 1 (P=0.06). In Exp. 2, there were no differences among dietary treatments for BW at 42 d; however, there was a trend for improvement in feed conversion ratio for PC2 (P=0.09) at 42 d. Weight without giblets (WOG) in Exp. 2 was significantly lower (P<0.01) for NC2 compared to PC2 and PP2. These results indicate that supplementation of the protease enzyme to a reduced crude protein/amino acid diet yielded similar growth performance to the positive control groups, while displaying improvements in the processing characteristics of Pekin ducks. Furthermore, application of an amino acid matrix to the protease enzyme when used in practical diet formulation for Pekin ducks may represent considerable finished feed cost savings, without sacrificing growth performance.

Key Words: Duck, Protease, Enzyme, Growth Performance

P327 Energy utilization in a mixture of flaxseed and pulses (LinPRO) fed without or with multi-carbohydrases supplement to broiler breeder hens Aizwarya Thanabalan*[GS], Elijah Kiarie *University of Guelph*

Flaxseed products have been proposed as a potential strategy to enrich broiler breeder eggs with omega-3 fatty acids to benefit chicks during embryogenesis. However, flaxseed is also rich in fibre and it is necessary to determine potential of fiber degrading enzymes to improve energy utilization. We evaluated apparent retention (AR) of components and AME in a dry extruded flaxseed product (LinPRO, O&T Farms, Saskatoon, Canada): a mixture of full-fat flaxseed and ground pulses (1:1 wt/wt) fed to broiler breeders without or with 0.05% multi-carbohydrase (MC) supplement. The MC supplied 1,200 U of xylanase, 600 U of β-glucanase, 2,800 U of cellulase, 400 U of mannanase, 2,500 U of amylase and other minor activities per g. The diets were a corn–soybean meal–based basal diet formulated to meet breeder specifications, the basal diet with energy-, and AA-yielding ingredients replaced by 18% of LinPRO without or with MC. All the diets contained TiO_2 (0.50%) as indigestible marker and were fed as mash. A total of 60, thirty-wk old cobb 500 broiler breeder hens were allocated to 30 identical cages (2 birds/cage) and allowed 1-wk for adaptation on basal diet. The 3 diets were randomly assigned to 10 replicate cages based on BW and fed in accord to breeder curve for 30 d. Excreta samples were collected between d 28 and 30. The AME of LinPRO was determined by the difference method. Birds fed LinPRO diet without or with MC showed (P < 0.01) reduced AR of DM, OM and CP. The AR of OM for the control, LinPRO, and LinPRO plus MC was 77.7, 66.6 and 64.1%, respectively. Diets did not affect (P > 0.05) AR of crude fat and gross energy. The AR of crude fat in the control, LinPRO, and LinPRO plus MC was 79.0, 82.5 and 77.0%, respectively and corresponding values for gross energy were 79.4, 76.3 and 74.3, respectively. The diet AME was higher (P < 0.05) for LinPRO diets compared with the control. Birds fed LinPRO diets showed increased (P = 0.01) flow of NDF compared with the control. The AME values for LinPRO without and with MC was similar (P > 0.05) and were 3,533 and 2,975 kcal/kg DM, respectively. In conclusion, the data provided AME value for LinPRO in broiler breeder females. Supplemental multi-carbohydrases did not improve utilization of energy in LinPRO.

Key Words: Breeders, LinPRO, AME, Multi-carbohydrases

P328 Effects of Multiple Enzymes on Male Broiler Performance in a Reduced Energy Diet Mallori Williams*, Hunter Walters, Craig Coufal, Alfredo Manon, Roshan Adhikari, Jason Lee *Texas A&M AgriLife Research*

The objective of the current study was to evaluate the effects of a xylanase and alpha-galactosidase in reduced energy broiler diets on performance and processing parameters. The experimental design consisted of four dietary treatments including a positive control (PC), negative control (NC) with a reduction of 110 kcal/kg ME compared to the PC, the NC supplemented with xylanase, and the NC supplemented with alpha-galactosidase. Each treatment included ten replicates with 47 Ross 708 male broilers placed per replicate (1880 broilers total). The dietary program consisted of four dietary phases including a starter through d 13, grower from d 14 to 29, finisher from d 30 to 39, and withdrawal from d 40 to 48. Broilers were weighed and feed consumption determined on days of dietary changes (13, 29, 39, and 48). On d 49 following an 8 hour feed withdrawal period, seven birds per replicate were removed and processed for carcass, breast and tender weights and yield. Throughout the trial the inclusion of xylanase or alpha-galactosidase did not impact body weight compared to the control diets. During the grower phase, the reduction of dietary energy in the NC increased (P<0.05) FCR compared to the PC. A reduction (P<0.05) in FCR during the finisher phase was observed with the inclusion of alpha-galactosidase compared to the NC. Xylanase reduced finisher FCR to levels comparable to the PC, however was not statistically different from the NC. At the conclusion of the trial, the inclusion of xylanase and alpha-galactosidase reduced (P<0.05) total FCR compared to the NC to levels that were comparable to the PC diet. Cumulatively for the starter and grower phases, the inclusion of alpha-galactosidase reduced feed consumption (FC) compared to the NC to levels that were similar to the PC. The lack of separation of in body weight resulted in similar processing weights and yields with the exception of tenderloin. The inclusion of xylanase and alpha-galactosidase increased tenderloin weight compared to the PC. The results of this study confirm the ability of alpha-galactosidase and xylanase to reduce the performance losses associated with reductions in dietary energy.

Key Words: energy, broiler, xylanase, alpha-galactosidase

Metabolism and Nutrition, Amino Acids

P329 Standardized ileal digestible lysine requirements of male broilers from 0 to 10 days Youn Kyoung Sung*, Jinyoung Lee, Su Hyun An, Changsu Kong *Easy Bio, Inc.*

Two experiments were conducted to determine the standardized ileal digestible (SID) lysine requirement for 10-day-old male broilers. In experiment 1, to investigate standardized ileal digestibility of amino acid in corn and soybean meal for 11-day-old broilers, three experimental diets consisting of corn or soybean meal as the sole source of amino acid and an N-free diet were used. A total of 480 birds were allocated to 3 treatments with a randomized complete block design and each treatment included 8 cages per treatment and 20 birds per cage. In experiment 2, a total of 720 one-day-old birds were used for 10 days. Birds were allocated to six diets and eight blocks with a randomized complete block design to estimate the SID lysine requirements. Six experimental diets were based on corn-soybean meal and the concentrations of SID lysine in the diets equally increased from 0.98 to 1.48% with consistent ratio of other indispensable amino acids. As the SID lysine increased, the average daily gain and gain :feed ratio increased linearly and quadratically (P < 0.05). The SID lysine requirements for average daily gain and gain:feed ratio were 1.33 and 1.29% for one-slope broken-line, 1.53 and 1.40% for quadratic broken-line, 1.46 and 1.32% for 95% of upper asymptote of quadratic model, and 1.42 and 1.36% for the first intercept between the plateau of one-slope broken-line and quadratic model, respectively.

Key Words: broiler, digestibility, lysine, requirement

P330 Determining the digestible lysine requirement of Cobb MV × Cobb 500 male broilers during the first fourteen days of age Rosana Hirai*, Leonel Mejia, Cesar Coto, Justina Caldas, Christopher McDaniel, Kelley Wamsley *Mississippi State University*

In order to optimize performance of a new commercial strain, Cobb MV × Cobb 500 broilers, determining amino acid (AA) requirements is warranted. Among AA, lysine is used as a basis to relate all other AA when calculating an ideal balance. Previous research has suggested that the digestible lysine (dLys) requirement for Cobb MV × Cobb 500 broilers from 0-7 and 0-11 d was between 1.2 and 1.4%. However, in some cases, it was likely the requirement was not met and >1.4% dLys should be evaluated. Therefore, the objective of this study was to evaluate the dLys requirement of male Cobb MV × Cobb 500 male broilers from 0-14 days of age. A total of 1,344 male chicks were obtained from a commercial hatchery. Two basal diets comprised of mostly corn and soybean meal were formulated: LLys (Treatment 1)- 0.88% dLys and HLys (Treatment 8)- 1.44% dLys. The other 6 experimental diets ranged between 0.96 to 1.44% dLys in increments of 0.08% which were obtained by blending in different proportions the LLys and HLys diets; and a positive control diet containing 1.28% dLys. Dietary treatments were provided to the birds from 0-14 d. The study utilized a RCBD with 9 treatments being represented by Trt 3-8 (11 replicate floor pens) and Trt 1, 2, and 9 (10 replicate floor pens) with 14 chicks/pen (0.08 sq m/bird). All dLys requirements were estimated using quadratic broken line methodology. Measured variables were BW, BW gain, FCR, and % mortality. Results suggests that for BW and BW gain, the dLys requirement for Cobb MV × Cobb 500 male broilers from 0-14 d is approximately 1.28%. The dLys requirement for FCR was not met in the levels evaluated and could only be calculated to be approximately 1.48%. These results suggest that dLys requirements are higher than previously reported. Future research should evaluate higher dLys levels than those used in this study.

Key Words: new commercial strain, requirement, amino acid

P331 Standardized ileal digestible methionine, threonine, and tryptophan requirements of male broilers from 0 to 10 days Su Hyun An*, Jinyoung Lee, Youn Kyoung Sung, Changsu Kong *Kyungpook National University*

The objective of this study was to develop the amino acid (AA) ratio based on the standardized ileal digestible (SID) lysine, methionine, threonine, and tryptophan requirements for 10-day-old male broiler chickens. Three experiments were conducted with Ross 308 male broiler chickens fed diets based on corn, soybean meal, and additional graded concentration of crystalline AA with 22.8% of crude protein in the diet. A total of 720 birds were grouped by weight in 8 blocks and allocated to 6 treatments with 15 birds per cage with a randomized complete block design and measured body weight and group feed intake on day 10. The SID methionine, threonine, and tryptophan concentrations in the experimental diets in the respective experiment ranged from 0.29 to 0.69% with increment of 0.08%, from 0.45 to 0.85% with increment of 0.08%, and from 0.11 to 0.21% with increment of 0.02%. Average daily gain and gain:feed ratio were linearly and quadratically affected by increasing levels of SID methionine, threonine, and tryptophan, respectively ($P < 0.01$). Standardized ileal digestible AA requirements for 10-day-old broiler chickens were conducted with 1.24% of SID lysine concentration in the treatments and the requirement for average daily gain and gain:feed ratio were 0.35 and 0.40% for SID methionine, 0.66 and 0.76% for SID threonine, and 0.15 and 0.14% for SID tryptophan, respectively. By using the greater estimates of the broken-line requirement, the ideal AA ratios relative to lysine (expressed as a percentage) for 10-day-old broiler chickens was calculated to be 30.6% methionine, 59.7% threonine, and 12.9% tryptophan on SID basis.

Key Words: broiler, digestibility, protein, requirement

P332 Evaluation of DL -methionine, L -methionine and hydroxy-4-(methylthio) butanoic acid in male broilers through 17 days of age Corey Johnson*, Chris Eagleson, Keith Haydon, Jason Lee *Poultry Science, Texas A&M University*

An experiment was conducted to evaluate the inclusion of three forms of methionine when included at different levels of inclusion in battery reared male broilers to 17 days of age. The three forms of methionine included DL-methionine (DL-Met), L-methionine (L-Met), and 2-Hydroxy-4-(Methylthio) Butanoic acid (HMTBa). Each form was supplemented into a basal diet with a calculated dig methionine level of 0.312 at 0.10%, 0.20%, and 0.30%. Inclusion rates were corrected based on labeled concentration level of each product using a 100% bioavailable value. All other essential AA ratios, except dSAA, were formulated above their requirements. Thus a 3 (form) x 3 (level) factorial design with a basal reference (no supplemented methionine) was used for a total of 10 experimental treatment groups. Each treatment group contained 14 replicate pens of seven male broilers per replicate for a total placement of 980 broilers placed for a 17 day assay period. Broilers and feed were weighed on day 12 and day 17 of age. Data was analyzed using a 3 x 3 factorial to determine potential separation of form and level. Regression analysis was conducted on each methionine source using the reference diet as the fourth level of inclusion for all methionine forms. The evaluated parameters were mortality-corrected feed conversion ratio (FCR) and body weight (BW). As expected, increasing levels of methionine increased BW as the 0.10% added methionine level had significantly lower BW as compared to 0.20 and 0.30%. Body weight gain from 12 to 17 days of age was elevated in the L-meth birds as compared to the HMTBa fed broilers, however no differences in BW at the conclusion of the experiment were observed. Feed conversion ratio was not impacted by the form of methionine included. Although similar to BW, FCR was elevated in the broilers fed the lowest concentration of methionine compared to the two higher levels. Quadratic regression equations were used to calculate the maximum observed performance for DL-Met. Comparisons indicated relative values for d 17 BW and FCR for L-Meth of 102.3 and 101.5 and HMTBa of 100.5 and 100.6.

Key Words: methionine, broiler, performance

P333 Egg production and quality responses of adding up to 7.5% black soldier fly larva meal in a corn-soybean meal diet fed to pullets from week 19 to 27 of age Zipporah Mwaniki*[GS], Mohamed Neijat, Elijah Kiarie *University of Guelph, Department of Animal Biosciences*

Insect meal has been proposed as an alternative and sustainable feedstuff. We examined egg production and quality responses of up to 7.5% inclusion of black soldier fly larvae meal (BSFLM) in a corn-soybean meal diet fed to laying pullets (19 to 27 wk of age). The analyzed concentration of CP, fat and starch in BSFLM sample was 59.3, 7.0 and 6.1%, respectively. Analyzed proximate values were used for AME estimation and books values were used for digestible AA. A control corn-soybean meal and two additional diets containing either 5.0 or 7.5% of BSFLM were formulated to meet specifications. All diets were prepared in crumble form. A total of 108,19-wk old shaver white pullets were placed in conventional cages (6 birds/cage) and allocated to diets (n=6). The birds were allowed free access to feed and water to wk 27. Hen day egg production (EP) and weight (EW) on cage basis was monitored daily. Feed intake was monitored on weekly basis for calculation of FCR. Egg quality (EQ) parameters: individual EW (IEW), albumen height in haugh units (HU), yolk color (YC), egg shell breaking strength (SBF) and thickness (ST) were assessed on individual eggs collected on 5th d of wk 22, 24 and 26. Body weight was monitored on bi-weekly basis. A quadratic response ($P<0.02$) was observed for EP, EW and egg mass. Specifically, birds fed control and 7.5% BSFLM diets had similar ($P >0.05$) values for these parameters with birds fed 5.0% BSFLM showing lower EP than control or 7.5% BSFLM fed birds. The EP was 89.4, 84.8 and 87.8 for control, 5.0 and 7.5% BSFLM, respectively. Feeding BSFLM linearly ($P < 0.01$) increased feed intake and FCR. There was no diet effect ($P > 0.05$) on IEW and HU, however, BSFLM

linearly ($P = 0.02$) reduced CV of IEW. The mean IEW values were 53.7, 52.3 and 53.0 g for control, 5.0 and 7.5% BSFLM, respectively and corresponding CV values were 7.9, 5.2 and 5.1%. Feeding BSFLM linearly ($P < 0.01$) increased yolk color, shell breaking strength and shell thickness. In conclusions, feeding up to 7.5% BSFLM had similar performance to corn-soy bean meal diet in egg production. However, the quadratic response on egg production warrant further investigations. Stronger egg shell might be indicative of improved Ca metabolism in birds fed BSFLM.

Key Words: Insect, Eggs, Layers, Quality, Performance

P334 Wheat cultivar choice modulates broiler chicken jejunal gene expression and wheat feeding value Muhammad Azhar*, Stephen Mansbridge, Stephen Rose, Michael Bedford, Vasil Pirgozliev *Harper Adams University*

Wheat is the main cereal used in broiler feed formulations in the UK and many European countries. Understanding the variation in nutritional value of wheat is commercially important and the subject of ongoing research. The aim of this study was to examine the differences in the nutritional value of 17 different wheat cultivars for broiler chickens and their effect on gut physiology. Six-hundred and eighty Ross 308 male chicks were allocated to 136 floor pens, 5 birds in each pen. The birds were placed on clean wood shavings. Birds were fed *ad libitum* one of the seventeen experimental mash diets from 0 to 7d and pelleted diets from 7 to 21d of age. The diets were formulated to be iso-nitrogenous containing CP 125 g/kg. The diets contained 670 g/kg of one of 17 wheat cultivar samples and 330 g/kg of a balancer. The diets did not contain any coccidiostats or antimicrobial growth promoters, other similar additives. Feed conversion ratio (FCR) and dietary apparent metabolisable energy (AME) were determined. At 21d of age one bird from each pen, with a body weight nearest to the pen average weight, was killed by cervical dislocation and a 1 cm segment from the jejunal section of the small intestine was excised for determination of gene expression. The expression (copies/reaction) of sodium glucose transporter 1 (SLC5A1), occludin (OCLN), mucin2 (MUC2) and peptide YY (PYY) genes was assessed. There was a negative relationship ($P < 0.05$; $r = -0.611$)) between the cultivar mean average Log^{10} expression of OCLN and AME. There was also a tendency ($P = 0.1$) for a negative correlation between Log^{10} SLC5A1 and AME. There was a tendency ($P < 0.1$; $r = 0.470$)) for a positive correlation between Log^{10} PYY and FCR. There was a significant correlation ($P < 0.001$; $r = 0.829$) between Log^{10} SLC5A1 and Log^{10} OCLN. There was also a significant correlation ($P < 0.001$; $r = 0.869$) between OCLN and MUC2. The studied genes are implicated in energy (glucose) transport, maintenance of tight junctions, development of intestinal mucosal cells, tissues formation and appetite regulation.

Key Words: Broilers, Wheat, Gene expression, gut

P335 Evaluating the effect of replacing fish meal with soybean meal and poultry by-product meal on broiler performance and cost of production Nana Frempong*, Chad Paulk, Charles Stark *Kansas State University*

Fish meal has been traditionally used in Ghana as a protein source in poultry diets. However, the limited availability and increased price of fish meal has forced poultry farmers to look for alternative protein sources for diets. The objective of this study was to evaluate the effects of replacing fish meal with either soybean meal or poultry by-product meal on broiler performance. A total of 900 male-day-old broiler chicks were randomly assigned to floor pens with 25 broilers per pen. Pens were randomly allocated to 3 dietary treatments (soybean meal (SBM), fish meal (FM) and poultry-by-product meal (PBM)) within location block. Dietary treatments were balanced based on lysine and metabolizable energy (ME). There were 12 pens per treatment in a randomized complete block design. Broiler body weights (BW), feed intake and FCR were determined at 14, 28 and 42 d of age. For 0-14 d, broilers fed FM had poorer ($P<0.05$) BW, ADFI, and FCR compared to those fed SBM and PBM. The FCR of birds fed the SBM, PBM, and FM were 1.25, 1.27, and 1.36, respectively. For 0-28 d, broilers fed FM had decreased ($P<0.05$) BW and ADFI compared to those fed SBM and PBM. There were no differences in FCR. For 0-42 d, broilers fed FM had decreased BW ($P<0.05$) compared to those fed SBM and PBM. Broilers fed SBM had increased ($P<0.05$) ADFI compared to that fed FM with broilers fed PBM being intermediate. There were no differences in FCR. The results of this study indicated that SBM or PBM diets yielded improved performance compared to FM when fed to broilers.

Key Words: Soybean meal, Poultry by-product meal, Fish meal, Average Daily Gain, Feed Conversion Ration

P336 Growth performance and amino acid digestibility coefficients of broilers fed vegetarian versus conventional diets reared in a research and commercial setting during a 62 day grow-out Courtney Ennis*[GS], Kelley Wamsley *Mississippi State University*

Three experiments were simultaneously conducted with the objective to determine the growth performance (in a research and commercial setting), as well as amino acid digestibility (AAD) coefficients of broilers fed vegetarian (VD) or conventional diets (CD). Both VD and CD were corn/soy based diets with similar AAD metrics. However, VD was antibiotic-free, all vegetable, while CD was conventional with antibiotics, meat and bone meal blend, and animal fat. Experiment (EX) 1 investigated the differences in AAD coefficients. Randomly chosen birds were moved to battery cages to which treatments were assigned in a randomized complete block design. Ground diets containing TiO_2 (3.0 g/kg) were fed 48 h prior to digesta collection on d 17, 34, 45, and 64. Ileal digesta was collected, lyophilized, and sent to a laboratory for AA analysis (Lys, Met, Thr, Phe, Cys, Phe, Trp, Val, Ile, Leu, His, Arg, Asp, Ser, Glu, Ala, Pro, and Tyr) and TiO_2 analysis. In EX 2 performance was compared of birds fed VD or CD. On d 0, chicks (Ross x Ross 708) were weighed by pen and randomly placed into floor pens. Pen weights were recorded at the end of each feeding phase on d 19, 34, 48, and 62. Performance from EX 3 was compared between commercial flocks fed VD or CD using a crossover design with 3 replications of 2 commercial houses. For EX 1, AAD coefficients during the starter and withdrawal 2 (WD2) phases demonstrating improvements in birds fed CD ($P\leq0.05$). However, AAD coefficients were not affected during the grower and withdrawal (WD) ($P>0.05$). For EX 2, BW was increased in birds fed VD during the starter and grower phase ($P<0.01$). Trends for improvements in feed conversion ratio (FCR) for birds fed VD were observed in the grower ($P=0.082$), whereas birds fed CD had improved FCR during the WD2 phase ($P=0.076$). Birds fed VD exhibited higher feed intake (FI) during starter and grower ($P<0.01$). During WD1, a trend was found for FI to be higher in birds fed CD ($P=0.076$). In EX 3, a trend was demonstrated for birds fed VD being heavier in BW than those fed CD ($P=0.1349$). No differences were found in FCR, product produced, mortality, water intake, paw scores and grower payout ($P>0.05$). Results suggests that similar d 0-62 BW can be achieved using VD or CD, despite variances in AAD.

Key Words: performance, digestibility, conventional, vegetarian

P337 Three major phases of sigmoid growth curve can be identified by ontogenetic growth force Manoel Garcia-Neto*, Sílvia Perri, Thainá Barros, Rodrigo Cassiano, Max Faria-Junior, Marcos Pinto *Unesp – Univ Estadual Paulista*

Ontogenetic growth process is defined as the development of an animal from birth mass to mature mass. Thus, the catabolism (maintenance) and the anabolism (new biomass) provide a general view of mass increasing as a function of time. However, time passing, more and more energy goes to sustain the organism. Therefore, the gain is the net result of metabolic forces. In order to better understanding about the mechanistic aspects of body increase, the freely available Practical Program for Modeling -PPM (https://goo.gl/5je0GV) is an appropriate instrument for interpreting the trajectory of ontogenetic metabolic force and, in addition, propound three

particular timescale for growth in the sigmoidal curves function (Logistic, Gompertz, Richards and Weibull). All of these stages fall along the growth curve, and they are the key to the relationship between mechanistic viewpoints to the analysis of growth process (metabolic forces) (Shimojo et al., 2006/http://catalog.lib.kyushu-u.ac.jp/handle/2324/9243/p285.pdf), thereby allowing new aspects to production analysis and providing a fundamental concept for study of animal growth (or any biological system). The PPM program also shows that is possible to identify the energy required for gain and maintenance. Then, the value offering to ontogenetic growth force for rise will be approaching zero, close to mature body size. Likewise, the PPM demonstrates four critic points where in the growth curve the driving energy allocation to length of the: 1-exponential growth phase, 2-quasi-linear phase, 3-exponential decay phase. Finally, in the saturation of the growth, the energy requirements for maintenance the asymptotic maximus body size makes impossible the synthesis the new cell mass, reflecting cessation of animal growth. Thereby, the PPM can be predicts the wave-shape the metabolic forces trajectory based on energy allocation to maintenance and to produce a new tissue. Thus, the modeling improvement that can be carried out with respect to metabolic processes, mechanistically based derivatives of well-known growth function, relating to metabolic forces and ontogenetic growth with flexibility, accuracy and meaningful analytical properties.

Key Words: bioenergetics, energy, metabolism, modeling

P338 Optimum dietary level of inclusion of a detoxified castor bean meal (Ricinus comunnis L.) in broiler chickens Maria de Lourdes Angeles*, Sergio Gomez Rosales, Luis Humberto Lopez-Hernandez, Rafael Jimenez-Ocamop *CENID-Fisiologia.INIFAP.*

The objective of the study was to evaluate the apparent metabolizable energy (AMEn), the apparent ileal digestibility of amino acids (AIDAA), growth performance, the antioxidant status of the meat of broiler fed increasing levels of a detoxified castor bean meal (DCSM) in microwave. In Exp. 1, 60 broiler chickens from 35-49 d of age were allocated in individual crates and assigned to four increasing levels of DCSM (0, 10, 20 and 30%) using the substitution methodology. Diets were added with chromium oxide as a internal marker. Broilers had a 9 day adaptation period; then the total excreta was collected during 4 consecutive days and at the end all birds were killed to obtain the ileal digesta. In Exp. 2, 144 broilers from 25-45 d of age were allocated in individual crates and assigned to 8 dietary treatments, in a factorial arrangement of 2 cereal grains (Corn or sorghum) and 4 increasing levels of DCSM (0, 5, 10 and 15%). The inclusion of DCSM was base on the AMEn and the AIDAA from Exp. 1. There were 18 replication per treatment; the results of both experiments were subjected to ANOVA and linear regression analysis. In Exp. 1, the CP, AMEn and AID of Lys, Thr, Arg, Ile and Val were 35.32%, 1767 Kcal/kg, 64.6%, 58.6%, 77.0%, 58.6% and 81.0%. In Exp. 2, most of the productive responses were similar between the 0 and 5% dietary inclusion of DCSM, but there was a fall on these variables at levels of 10-15% of DCSM (Quadratic responses, $P < 0.05$). Regression equations were developed and were derived to obtain the optimum inclusion dietary level of DCSM for daily weight gain (2.37%), feed conversion ratio (2.07%) and breast weight (6.1%). Quadratic effects were also observed in the percentage of water loss by compression and dripping ($P < 0.01$) in the breast meat, being similar in broilers fed 0 and 5% DCSM, but had higher values at 10-15% dietary DCSM. The water holding capacity ($P < 0.07$) and the ferric reducing antioxidant potential ($P < 0.01$) were similar in broilers fed 0 and 5% DCSM, but had lower values at 10-15% dietary DCSM. In summary, the concentration of AMEn and average AIDAA of the DCSM used in this study was 1767 Kcal/kg and 68.8%; the optimum level of dietary DCSM to prevent negative effects in the growth performance of broiler chickens was 2.89%.

Key Words: Castor seed meal, Metabolizable energy, Amino acid digestibility, Production, Antioxidant status

P339 Protein degradation of broiler breeders from the onset of lay through peak production Garrett Mullenix*, Xuemei Ding, Jinrong Wang, Judith England, Katie Hilton, Michael Schlumbohm, Craig Coon *University of Arkansas*

The objective of the study was to see how protein degradation effects the onset and peak of lay in broiler breeders. Excreta was collected from hens at 28, 31, 35, 37, 39, 41 and 44 wks of age. 3-methylhistidine (3-mh) was extracted from a 24 hr excreta collection and measured on an Agilent 7890A gas chromatographer/5975C mass spectrometer. 648 broilerized pureline pullets were obtained at 21 wks of age and placed in individual layer coupes. At 28 wks of age 90 birds (n=30 birds/weight group) were allocated into low, medium and high weight range groups. All birds were restrictively fed a generic corn/soybean breeder diet throughout the trial. Although the different weight range birds showed no statistical difference ($p=0.4191$) in degradation of 3-mh between the high ≈4188.06g (426.09 ± 37.13 µmol/kg), medium ≈3668.93 (386.18 ± 41.8µmol/kg) and low ≈3043.1 (361.77 ± 31.6 µmol/kg) ranges, there was a linear relationship ($R^2 = 0.9942$). Protein degradation did significantly increase for all birds from 28 wks (446 ± 53 µmol/kg) to 31 wks (780.72 ± 53 µmol/kg) of age ($p<.0001$), before decreasing to 296.58 ± 53 µmol/kg at 44 wks. Total eggs produced per week by hen ($p<.0001$) and eggs produced per day by hen ($p<.0001$) increased significantly from 28 wk to 31 wk of age; and from 31 wk to peak production at 37 wk of age ($p<.0001$). Excreta 3-methylhistindine didn't show a correlation to eggs in a week; however when a hen laid more than 1 egg in a 24 hr period their 3-methylhistidine increased substantially (^280µmol/kg of BW). This data indicates that broiler breeders are maximizing their skeletal protein degradation during the onset of lay to bring themselves into production and there is a linear relationship of 3-mh and BW.

Key Words: Protein Degradation, Broiler Breeders, 3-methylhistidine

P340 Determination of nitrogen corrected true metabolizable energy by near infrared reflectance spectroscopy Matthew Jones*, Lauren Reid, Adam Davis *University of Georgia*

Although dietary energy is not a nutrient, it provides the fuel for maintenance, growth and production. One of the best measures of the available energy for feed ingredients and diets for poultry is nitrogen corrected true metabolizable energy (TME_N). Unfortunately, the amount of time and resources that are required to perform this analysis through an animal bioassay and subsequent laboratory wet chemistry analyses are significant. Near infrared reflectance spectroscopy (NIRS) is a rapid analysis method that enables a Multi-Purpose Analyzer to be calibrated to predict nutritional component values after analyzing the reflectance of a feed ingredient in the near infrared spectrum. In the feed industry, NIRS calibrations are successfully used for determining parameters such as protein, fat, vitamin, mineral, amino acid and dry matter content in diets and feed ingredients. Once a NIRS calibration has been developed and validated, determinations of the parameter of interest in subsequent samples is fast, involves no chemicals and creates no waste. Therefore, the goal of the current research was to create and validate a NIRS calibration for TME_N. A calibration was made for TME_N (Kcal/kg) using 53 feed ingredient samples which resulted in prediction equation with a correlation coefficient of 0.96. This prediction equation was then validated with 48 new feed ingredient samples and the correlation coefficient between the NIRS and bioassay TME_N values was 0.92 indicating a highly successful NIRS calibration was achieved with a relatively small number of varied feed ingredient samples. The results suggest that NIRS could reduce the need for time consuming animal bioassays for determining TME_N values of feed ingredients, and provide nutritionists the ability to formulate diets based on TME_N values of the ingredients actually delivered to the feed mill.

Key Words: diet formulation, poultry, feed ingredients

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