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ABSTRACTS
2020 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 27-28, 2020

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ABSTRACTS

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Dendy Keynote Lecture

KEYNOTE, **The future of plant-based meat alternatives (from a chef's perspective)** Maynard Kolskog *NAIT Industry Solutions*

Plant-based meat alternatives are front and centre today due to changing perspectives on healthy eating and concerns about environmental sustainability. This has resulted in an explosion of plant-based food offerings from food companies as they seek to meet consumer demands and expectations. Northern Alberta Institute of Technology's Centre for Culinary Innovation (Centre) in Edmonton Alberta, Canada has established specialized expertise in the area of plant-based foods because our research partners (food companies) want to develop new products and evaluate the functionality of plant-based ingredients in culinary applications.

Creating convincing meat, dairy, and egg alternatives is challenging; however, the increased availability of plant-based ingredient options with better flavour, colour, and functionality has supported the development of new products that are closer to the foods they seek to mimic, and not only please those that have chosen a vegan lifestyle, but also those who are looking to increase plant-based food consumption for health and/or environmental reasons. In the arena of plant-based foods and especially meat alternatives, it is essential that the product compares favourably to the traditional product in the aspects of flavour, texture, and aroma which will deliver an enhanced eating experience. The toughest challenge and the holy grail for the Centre is a successful meat alternative. The challenge? Texture. Flavour, aroma, colour and cooking characteristics are on point, but the bite and chewiness are not convincing.

Although plant-based foods are predicted to have unprecedented growth in the coming years there are still some aspects of the food category that are under debate. Are plant-based foods better for you? Are they better for the planet? Why are plant-based meat-alternatives generally more expensive? Will plant-based meat alternatives reduce the consumption of animal protein?

Key Words: plant-based, meat alternatives

***Author presenting paper**

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Physiology/Endocrinology

M1, **Liquid storage capability of tris-coconut-water orange juice extender on different strains of cocks semen** Adedeji Balogun*, Raman Narang, Ranjna Cheema, Dubey Prem Prakash *Guru Angad Dev Veterinary and Animal Science University*

Liquid storage of cock semen remains the only easy and efficient close substitute to cryopreservation for *in-situ* conservation and accelerated genetic improvement in poultry breeding. The experiment was conducted to evaluate the preservation efficacy of formulated Tris Coconut-Water Orange Juice organic based extender on seven different Indigenous strains of cock semen in Guru Angad Dev Veterinary and Animal Science University poultry farm, Ludhiana, Punjab, India. Preparation of TCWOE was done, and maintained at 37°C. Seven different strains of cock within 40-45 weeks of age from the Directorate of Livestock Farms, comprising of Punjab brown 2(PB2), Desi, Punjab red, Desi Cross, Kadaknath, Rhode Island red and Assel were used. Ejaculated Semen from each strain were diluted and preserved with TCW-O extender to evaluate strains variability response to the standardized organic based extender. Semen was collected from two cocks per strain separately in 5 ml centrifuge tubes, diluted in ratio (1:2) semen : extender, kept for period of 72hrs at 4°C and assessed at 0hr, 4hrs, 24hrs, 48hrs and 72hrs for motility, vaibility, membrane and acrosome integrity respectively. Dilution and preservation of semen from seven strains of cock with TCW-O organic based extender exhibited statistically similarity (P>0.05) which ranged; motility (85±2.8 - 90±0.0 %), viability (87.90±2.6 – 94.63±1.9 %) and membrane integrity (81.23 – 89.47 %). Motility, viability and membrane integrity remained non-significantly higher in PB-2 (75.0±2.8, 87.50±0.2, 85.10±0.3 %) than other breeds (60.0±0.0 - 68.33±1.6 %, 75.80±0.9 – 85.27±0.5 %, 75.90±0.1 – 83.43±0.3 %) after 24 hrs of preservation at 4°C till 72hrs. Conclusively, Tris coconut water Orange Juice Organic based developed extender is considered to have an important electrolyte consisting of essential organic compounds that maintain integrity of the spermatozoa via *in-vitro* storage for up to 72hrs and likely extend its preservation ability *in-vivo* in sperm nest of the hens till fertilization takes place.

Key Words: Liquid Storage, Cock, Semen, Extender, Strains

M2, **Comparison of vapour freezing and automated freezing methods on PB2 rooster semen extended with tris coconut-water extender** Adedeji Balogun*, Raman Narang, Ranjna Cheema, Ajeet Kumar, Narinder Singh, Malik Singh *Guru Angad Dev Veterinary and Animal Science University*

This study was conducted to investigate the effects of four different freezing protocols (FP) on cryopreservation of roosters semen extended with Tris coconut-water extender (TCWE). A total of 20 roosters was used and trained for semen collection. TCWE was prepared by adding coconut water to tris buffer and kept at 37°C. Pooled semen was collected from the roosters. The pooled semen was evaluated for motility before dilution procedure. Three different concentrations (8, 10 and 15%) of two intracellular cryoprotectants (Glycerol and DMSO) where supplemented in TCWE. Pooled semen was divided into six equal fractions and TCWE containing cryoprotectants in different concentrations were diluted with the semen in ratio 1:2(semen: extender). Diluted semen was manually filled in 0.25ml straws and sealed. Semen straws were equilibrated for 4hrs at 4°C. Each straws fractions was further divided into four parts, and subjected to four FP (1, 2, 3 and 4). Each FP was done on samples containing 8, 10 & 15% glycerol 8, 10 & 15% DMSO. After each protocol, semen straws were finally deep into liquid Nitrogen -197°C. After 48hrs the straws were removed, and thawed individually to evaluate post/thawed motility, viability and membtrane integrity. TCWE 8% glycerol extended Rooster semen revealed that FP1 is most desirable and has significant (P>0.05) highest percentage motility (73.33%) compared to FPs 2, 3, and 4 (56.68, 50.00 and 23.33%). At 10% glycerol, FP2 had the highest motility (48.33%) and HOST (64.00%). At 15% glycerol, FP4 (Fast freezing) had the highest perecntage motility (71.67%), viability (76.33%) and HOST (71.67%). TCWE with DMSO revealed that FP4 had highest significant (P>0.05) motility, viability and HOST, 10% and 15% DMSO revealed little or no significant (P>0.05) in all FPs. Conclusively, fast freezing performed better with 15% concentration of glycerol, while slow and fast freezing performed better with 10%and 15% DMSO concentrations.

Key Words: PB2 rooster, Sperm, Cryoprotectants, freezing protocols, Cryopreservation

M3, **Effect of development and delayed access to feed post-hatch on luminal and mucosal bacterial populations in the gastrointestinal tract of broiler chickens** Monika Proszkowiec-Weglarz*, Lori Schreier, Katarzyna Miska, Stanislaw Kahl, Beverly Russell, Theodore Elsasser *USDA-ARS, ABBL*

The first two weeks of post-hatch (PH) development in broilers are critical for gut development and microbiota colonization. In the current broiler system, chicks may not receive feed and water for 24 to 72 h due to variation in hatching time and hatchery treatments. PH feed delay affects BW, FCR, mortality, and gut development. The goal of this study was to investigate changes in microbiome in broiler chickens early PH and the effect of delayed access to feed on the microbiome. Chicks either received feed and water immediately after hatch or feed access was delayed for 48 h to feed to mimic commercial hatchery settings (treatment, trt). Both groups were sampled (n=6) at -48, 0, 4, 24, 48, 72, 96, 144, 192, 240, 288 and 336 h PH. Ileal (IL) and cecal (CE) epithelial scrapings and digesta were collected for microbiome analysis. Microbiota was determined by sequencing the V3-V4 region of bacterial 16S rRNA and analyzed using Qiime2. During development, β-diversity analysis showed clear separation between IL and CE mucosal (M) and luminal (L) bacterial communities. Microbial α-diversity analysis revealed that IL-M were characterized by the highest richness while CE-L were characterized by the highest evenness. PERMANOVA analysis showed significant (P<0.05) differences among all four specimens during development. Taxonomic analysis indicated that relative abundance of bacteria was affected by age of the birds in all four specimens. A high level (P<0.05) of unclassified bacterial species were observed from -48 to 4 h PH in IL and CE. When access to feed was delayed a-diversity was affected (P<0.05) by trt in CE-M, but in CE-L, CE-M and IL-L this effect depended on the age. Beta-diversity analysis (Unweighted UniFrac) showed separation of bacterial communities in CE-M, CE-L and IL-L due to trt. Significant differences in microbial communities due to trt were also detected by PERMANOVA. Taxonomic composition was affected (P<0.05) by trt in CE-L, CE-S, IL-L and IL-M at every taxonomic level and this effect depended on the age. These results show transient

changes in chicken microbiome biodiversity during first two weeks PH and indicate that delayed access to feed affects microbiome development.

Key Words: Delayed feeding, microbiome, development, gastrointestinal tract, 16S

M4, **Advanced digestive enhancing protein plus technology (ADEPPT) inhibits coccidiosis and increases growth performance in Broiler chickens** Jamil Talukder*[1], Dan DuBourdieu[1,2], Hasanuzzaman Talukder[1], Ajay Srivastava[1], Rajiv Lall[1], Darwin Rajamanickam[1] [1] *Bangladesh Agricultural University,* [2] *Vets Plus Inc*

Background: Protozoa *Eimeria* species cause malabsorption of nutrients, reduces production, and increased mortality in chickens. An efficacious, natural alternative to pharmaceutical coccidiostats is yet to be discovered. The ADEPPT composed of naturally egg derived polypeptide (O-22) and polysaccharide (CH-22), has been developed and has shown antimicrobial efficacy against several pathogenic organisms. We hypothesized that ADEPPT would benefit chickens to control coccidiosis. Aim: The objectives of this investigation was to evaluate the effects of ADEPPT on growth performance, including feed conversion rate and inhibition of *E. tenella* infection. Methods: Day-old chicks (n=125) were housed under standard conditions and randomly divided into groups. Commercially available feeds and water were given as basal diets (BD) to all birds. Different percentages of O-22 and CH-22 alone or combined, were mixed with feed and given from day-7 through -21 to the treated groups. The control group received starch and positive control group received starch and sporulated oocysts. Approximately 10^6 numbers of sporulated oocysts were administered orally at day-14 to all groups except control. Body weight and feed intake were measured, oocysts in the feces were counted. Birds were sacrificed at the end of the experiments; cecum samples were collected, and histopathology was performed. Results: Body weight was increased by both O-22 (46%) and CH-22 (40%) treatments as compared to controls. Both O-22 and CH-22 independently or combinedly inhibited *E. tenella* infection while body weight continued to increase. Feed conversion rate was higher in both treated groups. Oocyst counts in fecal samples were significantly lower in O-22 and CH-22 treated groups as compared with positive control. Histopathology also showed normal structures in treated groups for cecal epithelium, but not in the positive control groups. The positive control (*E. tenella)* group showed a significant lower body weight gain. Conclusions: Data demonstrated that ADEPPT could be used either independently or in combination with feed additives as a natural coccidiostat, thereby increasing production in poultry.

Key Words: Coccidiosis, Chicken, ADEPPT, Transferrin, Polysaccharide

M5, **The effect of systemic infusion of active and non-active ghrelin on feed intake, weight gain, and corticosterone concentrations in male broiler chickens.** Nurudeen Taofeek*[GS], Nigel Chimbetete, Natalia Ceron-Romero, Martha Verghese, Jorge Vizcarra *Alabama A & M University*

Chicken ghrelin is a 26-amino acid peptide that is involved in feed intake and energy homeostasis. There are two circulating forms of ghrelin, acyl- and des-acyl-ghrelin (active and non-active, respectively). In mammals, exogenous infusion of ghrelin increases feed intake (FI). However, the effect of ghrelin isoforms on FI and BW gain in chickens has not been reported. Therefore, we evaluated the effect of systemic infusion of acyl- and des-acyl-ghrelin on FI, BW gain and concentrations of corticosterone and glucose in male broilers. Forty one-day-old chicks were reared as recommended by the industry. At 3 weeks of age (WOA), birds were randomly assigned into a 2 X 5 factorial arrangement of treatments (FAT). The first factor in the FAT included the two forms of ghrelin (acyl- and des-acyl ghrelin; FORM) and the second factor included different doses (0, 0.5, 1, 4 nm/100g BW/day of ghrelin in pulse every 2 hours) and a continuous infusion of 1 nm/100g BW/day (DOSEFREQ). Treatments were applied for eight consecutive days (DAY). To facilitate treatment delivery and blood sampling, birds were catheterized in the jugular vein on experimental day-1 (D-1) at a body weight of 1.5 ± 0.1 kg. From the first day of infusion (D0) to the last day of the experiment (D8), treatments were delivered in a saline citrate gentamycin solution via a programmable pump. Daily feed intake was recorded, and weekly BW and blood samples were obtained. The effect of treatments on feed intake, BW gain, glucose, and corticosterone concentrations were analyzed using repeated measurements over time (GLIMMIX, SAS). There was a qualitative FORM x DOSEFREQ interaction on feed intake ($P = 0.006$). Infusion of acyl-ghrelin decreased feed intake as the DOSEFREQ increased, whereas infusion of des-acyl-ghrelin increased feed intake as the DOSEFREQ increased. Consequently, birds infused with des-acyl-ghrelin gained more weight than birds infused with acyl-ghrelin (FORM effect; $P = 0.006$). Concentrations of glucose were not affected by treatments or days; however, corticosterone concentrations were increased on D8 as compared to D0 (DAY effect; $P = 0.005$). We conclude that exogenous infusion of acyl- and des-acyl-ghrelin had a differential effect on feed intake and BW gain in broiler chickens.

Key Words: Active ghrelin, Non active ghrelin, Feed intake, Broilers, Infusion

M6, **The effect of a ghrelin receptor agonist (Capromorelin) on feed intake and body weight gain in broiler chickens (Gallus gallus domesticus).** Natalia Ceron-Romero*[1GS], Nurudeen Taofeek[1], Ethan Vroonland[1], Aulisea Thomas[1], Hailey Chance[1], Tameka Smith[1], Martha Verghese[1], Ernst Heinen[2], Jorge Vizcarra[1] [1] *Alabama A&M University,* [2] *Aratana Therapeutics, Inc*

Ghrelin is a neuropeptide hormone that induces orexigenic effects in all mammals studied up to date. However, in avian species, there is scant and conflictive results on the effect of ghrelin on feed intake. Therefore, we evaluated the effect of a ghrelin receptor agonist (capromorelin; Entyce®) on feed intake, body weight gain, water intake, ghrelin, glucose, and insulin concentrations in broiler chickens. One-day-old male broiler chickens (n=24) were reared as recommended by the poultry industry. At four weeks of age (experimental day 0; D0), birds were blocked by BW, individually caged and randomly assigned to three treatments (0, 6 or 12 mg/kgBW/day) of capromorelin in drinking water. Feed intake (FI) and water intake (WI) were measured three times a day at 0700h (Period 1; P1), 1200h (P2) and 1700h (P3), whereas BW was recorded daily during five experimental days (D0-D5). Blood samples were collected on D-1 (one day before treatments) and D5 (end of the experiment). Bird behavior associated with feeding (number of pecks/h), seating (min) and standing (min) was evaluated for nine consecutive hours on D2 (n = 2/trt). Data was analyzed using a completely randomized block design with repeated measures over time. When appropriate orthogonal polynomial contrasts were used to determine linear and quadratic effects of increasing levels of capromorelin. There was a significant treatment effect on FI and ADG. Polynomial contrasts showed that higher doses of capromorelin increased ($P < 0.01$) FI and ADG. Concentrations of glucose and growth hormone were not significantly different between treatments. However, there was a treatment x day interaction ($P = 0.04$) on insulin concentrations. Concentrations of insulin were higher on D5 for the 0 and 12 treatments as compared with D-1. Ghrelin concentrations were not significantly different between treatments (103.9±10.0 pg/mL). There was a significant increase in the number of pecks/h in birds on treatment 12 as compared with 6 and 0 treatments ($P = 0.04$). Per hour FI and WI was higher during P1 (i.e. 0700 to 1200) as compared to P2 and P3 ($P < 0.001$). We concluded that capromorelin doses increased feed intake, number of pecks/h and body weight gain in male broilers chickens.

Key Words: Ghrelin, Capromorelin, Feed Intake, Broilers, Behavior

M7, **Evaluation of the impact of 25-hydroxycholecalciferol supplementation on performance, intestinal integrity and vitamin D3 status in broilers subjected to coccidiosis vaccine** Juan Suarez[*1GS], John Carey[1], Koyle Knape[1], Omar Gutierrez[2], Nasser Al Haj Ali[1] [1] *Texas A&M University,* [2] *Huvepharma*

Previous research indicates the use of 25-hydroxycholecalciferol (25-OH-D3) as replacement or partial replacement of cholecalciferol (D3) in broiler rations can result in significant differences in performance and bone mineralization. The objective of this study was to compare the effect of D3 and 25-OH-D3 on performance, intestinal integrity, and vitamin D status of broilers subjected to a 2x dose coccidiosis vaccine. Broiler chicks were placed in floor pens for a 21-day trial period. Experimental treatments consisted of a basal corn-soy broiler starter diet formulated using a mineral-vitamin premix devoid of D3 and supplemented with 25-OH-D3 by Bio-D™500 (BioD) from Huevepharma (Peachtree, GA) and/or vitamin D3 from a commercially available D3 source (CS), AHI, (Nacogdoches, Texas). Birds were randomly assigned to a vaccinated or not vaccinated group for each of the four dietary treatments. Replicates were arranged in a completely randomized block design with twenty-five male Cobb broilers per replicate and six replicates per treatment, a 4x2 factorial arrangement. The treatments consisted of (Control) 2750 IU/Kg of vitamin D3 from CS, 2750 IU/Kg of vitamin D3 from Bio-D, 1375 IU/Kg vitamin D3 each from CS and Bio-D, lastly 1375 IU/Kg vitamin D3 from Bio-D. Feed consumption (FC), body weights (BW), and mortality-corrected feed conversion (FCR) were calculated at day 7, 14, and 21. Serum concentration of 25-OH-D and tibia breaking strength (TBS) were measured on day 10, and 21 as well as lesion scores. There was a significant difference ($p>0.05$) in BW, FCR, and TBS between one or more BioD dietary treatments and Control on all evaluated days. Cumulative FCR was significantly lower ($p>0.05$) in one or more Bio-D treatments compared to Control at day 21. TBS at day 10, and 21 was significantly higher ($p>0.05$) between one or more BioD treatments compared to Control. Lesion scores were significantly higher ($p>0.05$) in vaccinated groups compared to not vaccinated groups, indicative of a good vaccine challenge. Overall, the results indicate that the use Bio-D positively modulates levels of 25-OH-D in serum, TBS, and performance, both in vaccinated and not vaccinated groups compared to Control, with more noticeable differences in vaccinated groups.

Key Words: cholecalciferol, coccidiosis, 25-hydroxycholecalciferol, broiler, performance

M8, **Effect of dietary protein source on broiler chicken growth performance, myogenic stem cell activity and heterogeneity, and muscle fiber morphometrics** Samuel Leiva[*GS], Jorge Sandoval, A. Jake Keel, Oscar Tejeda, Charles Starkey, Jessica Starkey *Auburn University Department of Poultry Science*

Dietary protein source has been shown to affect broiler chicken growth performance and muscle accretion. Myogenic stem cells (MSC), also called satellite cells, play an essential role in post-hatch skeletal muscle growth. A randomized complete block design experiment was conducted to assess the effect of dietary protein source on broiler growth performance, *Pectoralis major* (PM) MSC mitotic activity and heterogeneity, and muscle fiber cross-sectional area (CSA). On d of hatch, female Yield Plus × Ross 708 broilers (n = 360) were placed in raised floor pens with new litter and randomly allotted to 1 of 3 dietary treatments. The 3 corn and soybean meal-based crumbled diets fed from d 0 to 20 included: 1) soybean meal as the sole protein source (SBM), 2) soybean meal + a 50% poultry by-product meal and 50% poultry feather meal blend (PBM), or 3) soybean meal + porcine meat and bone meal (MBM). From d 0 to 8, birds were housed with 4 birds per pen (0.05 m^2 per bird) and individually (0.21 m^2 per bird) from d 9 to 20. On d 20, 1 h prior to PM muscle sample collection, birds ($n \geq 2$ per diet; total n = 12) were intraperitoneally injected with 5'-bromo-2'-deoxyuridine (BrdU) to label mitotically active cells. Muscle samples were analyzed using cryohistology followed by immunofluorescence staining to facilitate enumeration of the mitotically active (BrdU+), MSC populations expressing the myogenic regulatory factors (MRF) and common MSC markers, MyoD and Pax7, by digital fluorescence microscopy. Muscle fiber density on a mm^2 basis and fiber CSA were determined for each bird. Data were analyzed with SAS (V9.4) PROC GLIMMIX and means were separated with PDIFF at $P \leq 0.05$. Broiler feed intake, BW gain, and FCR from d 0 to 20 were not altered by dietary protein source ($P \geq 0.1925$). The PM muscle MSC mitotic activity and MRF heterogeneity as well as fiber CSA and density were similar among broilers fed the 3 different dietary protein sources ($P \geq 0.1304$). However, birds fed SBM and PBM had a greater proportion of fibers with CSA from 1,500 to 1,999 μm^2 compared with MBM ($P = 0.0227$). Overall, PM fiber CSA distribution was altered by dietary protein source and may warrant further investigation into the mechanism behind previously observed changes in muscle accretion.

Key Words: dietary protein source, myogenic stem cells, satellite cells, muscle fiber morphometrics, broiler growth performance

M9, **Establishment of a model to evaluate the effects of antibiotic-free programs on intestinal development and local immune response in the ileum of young broilers** A. Jake Keel[*GS], Allan Calderon, Oscar Tejeda, Jessica Starkey, Charles Starkey *Auburn University Department of Poultry Science*

As the broiler industry continues to shift to antibiotic-free broiler production, it has become increasingly important to develop a better understanding of the immune system and health of the small intestine to minimize reductions in growth performance of broilers not fed antibiotic growth promoters. Mitotically active cells, the majority of which will become enterocytes, help to maintain the cell populations that make up the intestinal epithelial barrier and function to absorb nutrients. Macrophages that reside in the lamina propria help prevent pathogen invasion through direct activity (phagocytosis) or when they function as immune response amplifying cells to aid in the recruitment of additional immune cells or stimulate cytokine production in other adjacent cells. The objective of this experiment was to evaluate commonly used practical production practices on the intestinal cell mitotic activity and local intestinal immunological responses. A randomized complete block design experiment with a 3 × 2 factorial treatment structure was conducted. The 3 dietary protein sources were: soybean meal (SBM), a mix of 50% poultry by-product meal and 50% feather meal (PFM), and porcine meat and bone meal (MBM) and broilers were reared on either new litter (NL) or used litter (UL). On d 3, 8, 11, 15, and 21, 6 birds per treatment from 6 different blocks (total n = 36 per d) were randomly selected for sampling. Broilers were injected intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU) 1 h prior to sampling to label mitotically active cells. Samples were analyzed using cryohistology, immunofluorescence staining, and digital fluorescence microscopy to determine the density of mitotically active cells and macrophages. Data were analyzed using SAS V9.4 PROC GLIMMIX and treatment means were separated using PDIFF at $P \leq 0.05$. Neither dietary protein source nor litter condition affected mitotically active cell or macrophage densities in the ileum on d 3, 8, 11, or 15. However, on d 21, broilers reared on UL had greater mitotically active cell density than those reared on NL ($P = 0.0009$). These results indicate litter condition may impact the physiology of the broiler small intestine though additional work with this model is necessary to understand the underlying mechanisms.

Key Words: macrophage, local immune response, stem cell proliferation, immunofluorescence cryohistology, broiler chicken

M10, **Knowledge and use of broiler chicken euthanasia methods by animal caretakers** Ranjit Boyal*[1GS], R Buhr[2], Caitlin Harris[2,3], L Bartenfeld-Josselson[2], Leonie Jacobs[4], Dianna Bourassa[1] [1] *Auburn University,* [2] *US National Poultry Research Center, USDA-ARS,* [3] *University of Georgia,* [4] *Virginia Tech*

In both research and commercial broiler chicken production settings, birds must be euthanized for a variety of reasons including culling of unfit or injured animals and prior to use for research sample collection. Animal care personnel require training in order to have the skills necessary to properly perform euthanasia when needed. The objective was to train or retrain care takers how to perform the conventional cervical dislocation euthanasia method as well as four alternative methods including mechanical cervical dislocation, captive bolt, carbon dioxide (CO_2), and electrical euthanasia. Following training, participants were surveyed on their previous experience and their evaluation of conventional and alternative methods. Following demonstration and training of each method participants rated their perception of animal welfare acceptability and ease of use. Data were analyzed using the Wilcoxon Mann-Whiney U-test and Kruskal-Wallis test of SAS. Prior to euthanasia training, 91% of participants had previous experience performing euthanasia on broilers with 100% of those having performed cervical dislocation, 33% previously performed electrical euthanasia, and 17% previously performed either CO_2 euthanasia or mechanical cervical dislocation. Ease of performing euthanasia by cervical dislocation was rated at 3.4 on a scale from 1 to 5 with 1 defined as very easy and 5 defined as very difficult. Interest in learning alternative methods of euthanasia was rated at 4.3 on a 1 to 5 scale with 1 defined as no interest and 5 defined as very interested. Although training did not influence the perceived animal welfare of each method, mechanical cervical dislocation was not perceived to be more or less acceptable than captive bolt ($P=0.3373$), but was perceived to be less acceptable than CO_2, electrical euthanasia, and cervical dislocation ($P<0.0402$). Perceived ease of use did not differ among these euthanasia methods ($P=0.1724$). Upon completion of training, participants had attained the necessary knowledge to apply these methods of euthanasia. The selected method used in the future will ultimately depend on the animal care takers' available resources and production situation.

Key Words: euthanasia, survey, extension, welfare

Processing and Products I

M11, **Distribution and prevalence of Salmonella in conventional and no antibiotics ever broiler farms.** Estefania Novoa Rama*[GS], Matthew Bailey, Sanjay Kumar, Cortney Leone, Harshavardhan Thippareddi, Manpreet Singh *University of Georgia*

Salmonella is the second most common causative agent of foodborne illnesses in the United States. Despite regulatory efforts to reduce disease incidence, the number of salmonellosis cases linked to poultry have remained stagnant over the years. Nowadays, more than 20% of the market for poultry meat in the US is occupied with alternative poultry (e.g., organic, no antibiotics ever) due to consumer perceptions on antibiotic use. Yet, little is known about the incidence of foodborne pathogens in large-scale commercial broiler farms that follow such practices. The aim of this study was to compare the prevalence and distribution of *Salmonella* in conventional and no antibiotics ever broiler farms in the US. Cecal (n=420), ileal (n=420), litter (n=24), feed (n=24) and water (n=24) samples were collected from birds during the grow out cycle of one flock. Samples were collected at six different time intervals corresponding to every feed change and after transport to the processing plant. One house per farm (2 conventional and 2 no antibiotics ever) was sampled. Following USDA-FSIS methods, samples were enriched in buffered peptone water, and the BAX® Real-Time system was used to screen the samples for *Salmonella*. Cecal samples from broilers raised on conventional farms had the highest *Salmonella* prevalence, up to 73%, varying greatly throughout the growing cycle. *Salmonella* prevalence in no antibiotics ever broilers, however, remained low (7% to 27%), and only increased (40%) after transport to slaughter. Ileal *Salmonella* prevalence was also higher in conventional broilers (up to 40%) when compared to no antibiotics ever broilers (up to 20%). Farms with high *Salmonella* levels had the pathogen persist in the litter from day 20 until the end of the grow out period. No *Salmonella* was found in feed and water samples. Overall, no antibiotics ever farms had a more robust biosecurity system. These results suggest that gut prevalence of *Salmonella* was higher in broilers raised under conventional practices than in no antibiotics ever broilers and highlight the impact of farm management practices on *Salmonella* prevalence.

Key Words: Salmonella, no antibiotics ever, conventional, broiler, farm

M12, **Isolation and characterization of Salmonella sp. on broiler farms in southcentral Mississippi.** Hailey Quercia*[1], Adebayo Sokale[2], Martha Pulido-Landínez[1], Hayden Miller[3], Heather Perry[3] [1] *Mississippi State University College of Veterinary Medicine, Poultry Research and Diagnostic Laboratory,* [2] *Evonik Corporation,* [3] *Mississippi State University College of Veterinary Medicine, Veterinary Research and Diagnostic Laboratory*

Salmonella sp. in poultry presents a significant food safety risk, and as such management and control of this pathogen is an important focus for the broiler industry. The objective of this field study was to better characterize *Salmonella* sp. on broiler farms, based on different factors. The following were evaluated: longitudinal change in *Salmonella* sp. prevalence during grow-out, impact of sample type (environmental vs. organ samples) on detection rate, and impact of water acidification on prevalence. Environmental samples (pooled fecal samples and boot swab samples) and organ samples (pooled ceca and pooled crop from a subset of farms) were collected and analyzed using NPIP approved *Salmonella* isolation methods. All isolates were serotyped and the differences in detected serotypes during grow-out period and between environmental and organ samples were analyzed. Furthermore, the impact of enrichment method on overall detection rate and on the detection rate for specific serotypes was assessed. Three enrichment methods were used, consisting of Tetrathionate broth, Rappaport-Vassiliadis broth, and a secondary enrichment method. All isolates were serotyped using the Intergenic Sequence Ribotyping method. *Salmonella* serotypes isolated included Enteritidis, Kentucky, Berta, Bareilly, and Grumpensis. Different serotype profiles were noted when environmental and organ sample isolates were compared. No significant difference in Salmonella prevalence was found following use of the acidification product ($P > 0.05$), although issues with sample collection likely impacted this result. When comparing the enrichment methods used, the secondary enrichment method had a statistically significant impact on both overall *Salmonella* sp. detection and S. Enteritidis detection ($P \leq 0.05$). The findings of this study provide better understanding of the differences in *Salmonella* sp. prevalence and detection rates in broiler houses, different aged birds, and sample types, which has important implications for control. Given the significant differences in serotype detection based on enrichment method the potential to bias serotype detection must be considered in future studies.

Key Words: Salmonella sp., Salmonella serotypes, Enrichment media, Salmonella monitoring, Broilers

M13, **Mode of persistence and antibiotic resistance of Salmonella isolates from poultry processing equipment** Tomi Obe*[1GS], Rama Nannapaneni [2], Wes Schilling [2], Li Zhang [1], Aaron Kiess [1] [1] *Department of Poultry Science, Mississippi State University,* [2] *Department of Food Science, Nutrition and Health Promotion, Mississippi State University*

Foodborne pathogens, like *Salmonella*, are problematic in poultry processing environments. Therefore, it is critical to understand the means of persistence to develop effective control measures. The objective of this study was to determine the mode of persistence of *Salmonella* isolates in the processing environment and their resistance to clinically important antibiotics. Twenty-five *Salmonella* isolates from five serotype groups were previously recovered from the equipment surfaces in different poultry processing plants after sanitization. The minimum inhibitory concentration (MIC) of antimicrobials (chlorine and quaternary ammonium compounds, QAC) used for sanitization was determined for the isolates using the CLSI guideline. The biofilm forming ability was subsequently determined using the crystal violet assay, and antibiotic susceptibility profiles of the isolates were determined using the Sensititre protocol. A randomized complete block design was used to analyze results and means were separated using Fisher's protected LSD at $p \leq 0.05$. The MIC results indicated that 20% and 32% of the isolates had moderate and high tolerance to chlorine, respectively. Differences ($P<0.0001$) were observed for the tolerance of the isolates to QAC with 56% and 12% having moderate and high tolerance, respectively. The biofilm forming ability of the isolates differed ($p<0.0001$); where 24%, 48%, and 28% were strong, moderate, and weak biofilm formers, respectively. All the isolates are multidrug resistant with 64% exhibiting resistance to at least 6 antibiotics in two distinct antibiotic classes including aminoglycosides and B-lactams. The results suggest that *Salmonella* isolates within a serogroup that could be inactivated by the sanitizer, due to low tolerance, may remain persistent because of their strong biofilm forming ability. *Salmonella* persistence in the processing environment could have severe consequences on the poultry industry because contaminated poultry meat could cause food safety risks and antibiotic treatment could become even more challenging. Further research should focus on determining antimicrobials that are effective at controlling *Salmonella* biofilms in processing environments to prevent public health concerns associated with poultry meat.

Key Words: Salmonella, biofilm, antibiotic resistance, poultry processing, QAC

M14, **Effect of spraying sanitizer or cleaner at high pressure to remove bacteria from broiler carcasses: pre-scald vs. post-scald evaluation** Osman KOYUN*[1GS], Douglas Cosby [2], Susan Mize [2], Mohamad Mortada [3], Joshuah DeVoll [4], Mike McIntyre [4], Todd Callaway [1] [1] *University of Georgia, Department of Animal and Dairy Science,* [2] *USDA/ARS, U.S. National Poultry Research Center,* [3] *University of Georgia, Department of Poultry Science,* [4] *Spray Systems, Inc.*

Reducing the bacterial load on poultry carcasses in the slaughter area of processing plants can result in fewer bacteria entering the evisceration line. The present study evaluated the efficacy of a high pressure (approx. 175 psi), low volume, fluidic nozzle equipped wash cabinet spraying an ionized hypochlorous acid sanitizer (pH=5.21, temp=20.7°C, free Cl^-=209), or an ionized sodium hydroxide cleaner (pH=11.94, temp=19.7°C) to remove bacteria from broiler carcasses. Three replications (n=20/replication) were conducted on three separate dates. Carcasses (n=60 in total) were collected from either pre-scald or post-scald of a slaughter line of a local plant, individually bagged and transported back in a cooler to a pilot plant. Carcasses (n=5 for pre-scald and post-scald carcasses each date, respectively) were rehung in standard shackles and breast swab samples were collected (i.e. breast/keel bone area was swabbed using a pre-moistened cellulose sponge kept in Whirl-Pak® sampling bags) and subsequently washed with either the cleaner or sanitizer (treatments: pre-scald+cleaner=T1, post-scald+cleaner=T2, pre-scald+sanitizer=T3, and post-scald+sanitizer=T4). After wash, each carcass was sampled again in a same manner as above to provide paired pre- and post- wash samples, which were placed on ice and transported to the laboratory. Buffered peptone (10 mL) of was added to each swab sample, stomached for 60s, and then serially diluted and plated onto total aerobic bacteria (TAB), *Enterobacteriaceae* (EB), and *E. coli* (EC) PetriFilm® cards which were incubated at 37°C for 24 h. Paired sample *t*-test *($p<0.05$)* was used for statistical analysis. Reductions were generally < 1 $\log_{10}$ cfm/ml except in T3, which had a 1.15 $\log_{10}$ reduction *($p<0.05$)* in TAB counts. T2 and T4 lowered *($p<0.05$)* counts of TAB. The counts for EB were reduced *($p<0.05$)* only by the effects of T3 and T4. EC counts were decreased *($p<0.05$)* by all treatments compared to their corresponding pre-wash counts. Results indicate that using either the sanitizer or the cleaner solutions in a pressurized wash cabinet might be an effective method to remove bacteria from carcasses at pre- or post-scald lines of plants, leading to fewer bacteria entering the evisceration line.

Key Words: bacterial load, sanitizer, cleaner, broilers, carcasses

M15, **Effects of dietary fat source and embryonic thermal manipulation on muscle yield and meat quality of heat stressed birds** Brittany Wall*[1GS], Kelly Brannan [2], Matthew Livingston [1], Kimberly Livingston [1], Christine Jansen van Rensburg [2] [1] *North Carolina State University,* [2] *University of Pretoria*

In the broiler industry, heat stress continues to be an issue that hinders growth performance. Dietary supplemental fats have been shown to aid in the recovery of the birds under heat stress due to high temperatures. Another heat management strategy is the use of thermal manipulation (TM) during incubation. This study sought to investigate alternative dietary supplemental fats and TM during incubation on growth performance and meat quality characteristics, including wooden breast and white striping, in broilers exposed to heat stress during late rearing. All eggs were set in one incubator at 37.6C with a relative humidity (RH) of 56% for the first week. After E7, half of the eggs were incubated at 39.5C with a RH of 65% for 12h a day from E7 to E16. After E16, the eggs were returned to the initial setter at starting temperature until transfer to hatcher at 36.9C with a RH of 54.6%. After hatch, one experimental house was placed with 60 floor pens (18 chicks per pen) and split into a 2x3 factorial design of incubation x diet. Standard commercial starter and grower diets were fed from 0d to 14d and 14d to 28d, respectively. Dietary treatments consisted of poultry fat (4.6%), soy oil (4.6%), and olive oil (4.6%) fed from 28d to 49d. Optimal house temperatures were utilized until 43d, when heat stress was applied for 4h ranging from 34C to 36C. After heat stress, birds remained at optimal temperatures until processing at 49d. Yield cut-outs, cook loss, drip loss, meat color, and myopathy scores were obtained. Data were analyzed as two-way ANOVA using JMP Pro 14. TM resulted in decreased in broiler performance, reduced carcass weight, fat pads, and percent fat pad ($P<0.05$). Dietary soy oil resulted in reduced drip loss ($P<0.05$). TM and olive oil resulted in an increase in the b* value of breast muscle ($P<0.01$). TM resulted in reduced prevalence in muscle myopathies ($P<0.05$). To conclude, dietary fat changes did not affect broiler performance or meat yield, however there was an improvement in meat quality characteristics. TM resulted in reduced broiler performance when compared to birds under standard incubation temperatures, and improvements in meat quality characteristics. TM may decrease the prevalence of muscle myopathies due to a decrease in growth performance.

Key Words: Thermal manipulation, Dietary fat, Meat quality, Heat stress

Processing and Products II

M16, **Adding value to co-products of broiler chicken processing** Marc Presume*[1GS], Lindsey Spencer [1], Catherine Odom [1], Laura Garner [1], Amit Morey [1], Robert Mason [2], Eric Altom [2], Charles Starkey [1] [1] *Auburn University Department of Poultry Science,* [2] *Balchem Corporation Animal Nutrition and Health*

Adding value to co-products of broiler processing is important to achieve optimal economic return for the integrated broiler industry. The use of reduced value co-products of broiler processing like carcass frames and breast fillets severely affected with the Wooden Breast meat quality defect may provide integrators an opportunity to generate additional revenue on otherwise low value product. The objective of this experiment was to determine the optimal ratio of whole ground frames (GF) to severely affected Wooden Breast meat (WB) as well as the optimal inclusion of a sodium alginate + encapsulated calcium lactate forming product (PetShure® Structure & Forming Agents, Balchem Corp., New Hampton, NY; ALGIN) to manufacture a pet treat. Whole carcass frames and score 3 WB-affected breast fillets were obtained from broilers processed at the Auburn University poultry processing facility and ground to 10 mm. The resulting ground products were mixed to achieve the following 11 ratios of GF to WB: 100% GF:0% WB, 90% GF:10% WB, 80% GF:20% WB, 70% GF:30% WB, 60% GF:40% WB, 50% GF:50% WB, 40%:GF 60% WB, 30% GF:70% WB, 20% GF:80% WB, 10% GF:90% WB, 0% GF:100% WB. Subsamples of the 11 GF:WB mixtures were then mixed with 3 different concentrations of ALGIN (0.5, 1, and 2% sodium alginate + 0.425, 0.85, and 1.7% calcium lactate) to produce the final batches (n = 33), stuffed into 40-mm casings, and frozen at -20°C for 48 h prior to slicing with a commercial meat slicer. Twenty 10-mm slices were oven dehydrated at 107˚C for 2 h for determination of cook loss and water activity. Ten 20-mm samples were used to measure expressible moisture and pH. Water activity for both raw and cooked samples decreased as the percentage of GF decreased ($P \leq 0.0001$), while ALGIN inclusion had no effect ($P > 0.05$). Products with higher inclusions of WB meat had lower pH values regardless of ALGIN concentration ($P \leq 0.0001$). Cook loss was reduced as inclusion of WB increased for all ALGIN concentrations ($P \leq 0.0001$). As ALGIN concentrations increased, expressible moisture decreased ($P \leq 0.0001$). Reduced value broiler processing co-products were successfully repurposed and used to produce a value-added pet treat.

Key Words: broiler chicken processing, broiler carcass frames, wooden breast meat, alginate, pet food

M17, **Augmented Reality technology implementation to reduce downtime in poultry processing plants** Alisha Kerklin*[GS], Amit Morey *Auburn University*

Poultry processing plants are using advanced technologies and machines to increase productivity of their operations. However, repairing these advanced machines in the absence of skilled engineers leads to significant downtime and reduced productivity. Maintenance technicians should be equipped with technologies such as Augmented Reality (AR) to reduce the communication gap between them and expert engineers to aid faster repairs. The objective of this study was to determine if wearable AR technology will improve the speed of mechanical repair of meat processing equipment. Vuzix Blade Smart Glasses (model #447) used in the study were equipped with a front facing camera (8 MP), an embedded video screen, and headsets which were paired with an iPhone via Bluetooth to receive sound input, internet connection and make video call through the Zoom app. Ten participants with low equipment maintenance skillset were selected and each was required to repair three pieces of small-scale meat processing equipment: hand mixer, electric tabletop grinder, and 5-pound capacity sausage stuffer were taken completely a part with the various pieces displayed on a table. Five participants were given a list of instructions (Group A) that directed how to assemble the machines, complete with added safety instructions to mimic safety procedures that are required in a production setting (lock out tag out and cut resistant gloves). The other five participants used the AR glasses (Group B) to communicate via Zoom video conference with an expert who instructed them on proper assembly of the equipment. The amount of time required to complete the tasks was recorded and analyzed using t-test ($p<0.05$). Participant behavior was also observed. Group A completed all the three tasks in average 14.21±3.92 min which was significantly higher ($p<0.05$) than Group B who took an average of 5.60±0.63 min. Group B was able to put together the grinder ($p<0.05$), mixer ($p<0.05$), stuffer ($p<0.01$) more efficiently compared to Group A. Group A did not follow the provided instructions and safety guidelines. AR technology can effectively reduce the time required to repair processing equipment while bridging the communication gap between the expert engineers and in-plant maintenance engineers.

Key Words: Augmented Reality

M18, **Expressible fluid measurements of broiler breast meat affected by emerging muscle abnormalities** Xiang Yu*[1], Brian Bowker [2], Hong Zhuang [2] [1] *College of Life science, Hubei Normal University,* [2] *Quality&Safety Assessment Research Unit, U.S. National Poultry Research Center, USDA-ARS*

The present study was aimed at investigating the influences of emerging broiler muscle myopathies, spaghetti meat (SM), wooden breast (WB), and white striping/WB (WS/WB), on meat water-holding capacity (WHC) measured with an expressible fluid (EF). Boneless skinless breast meat (Pectoralis major) was collected from the deboning line of a commercial processing plant and sorted into NORM, SM, WB, and WS/WB categories. The filter paper method was used to measure EF values of fresh, frozen and cooked meat samples. The ratio of the fluid area over the meat area was used to estimate EF or WHC. There were no differences in EF ratio between normal meat and bone side of WB and WS/WB meat regardless of raw meat state and also between normal and SM meat regardless of SM location and meat state. There were no differences in EF ratio between normal, SM, WB, WS/WB for cooked meat except for the samples from discolored area of cooked WB and WS meat. The EF values (> 4.01) of meat from skin side of WB and WS/WB were greater ($P < 0.05$) than those of normal meat (< 3.30) and those (< 3.89) from discolored area of cooked WB and WS/WB lower than normal meat (5.01). Data demonstrate that the emerging muscle myopathies can also influence meat WHC measured with the EF method. The effects vary with the myopathy and location of breast muscle with myopathy.

Key Words: water-holding capacity, spaghetti meat, wooden breast, white striping, filter-paper method

M19, **An Evaluation of Sodium Ferrate as a Green Processing Chemistry** Michael Barnas* [1,2], Anuradha Jeewantha [1], James McNaughton [2], Steve Auman [2], Salina Parveen [1], Jurgen Schwarz [1], Mick Roberts [2] [1] *University of Maryland Eastern Shore,* [2] *AHPharma, Inc.*

Ferrate (VI) is an oxidized state of iron with oxidation-reduction capacity superior to all other commercial chemical oxidizers and disinfectants used in wastewater treatment. Ferrate can be produced from relatively inexpensive commercial chemicals: trivalent ferric chloride ($FeCl_3$), sodium hypochlorite (NaOCl) and sodium hydroxide (NaOH); with sodium ferrate (Na_2FeO_4), sodium chloride salt (NaCl), ferric hydrate ($Fe(OH)_3$) and water being the odorless reaction products. The efficacy of sodium ferrate (SF) was tested using a commercial electrostatic misting system and boneless, skinless chicken thighs. An inoculum was made to approximately 5 log CFU/ml of *Salmonella* Typhimurium (resistant to Rifampicin) in phosphate buffer saline (PBS). Thigh meat was aseptically cut into 100 g samples then spread on each side with 10^5 CFU per sample using a sterile cell spreader. Samples were aseptically stored for 45 min for *Salmonella* attachment. After 45 min, all samples (n = 32) were randomized and as-

signed to 4 treatments. Samples from three treatments passed through an electrostatic misting system treated with: deionized water (DI) positive control (PC), DI + 0.15% sodium ferrate, or DI + 0.30% sodium ferrate for 15 s. The fourth treatment received no electrostatic misting. After treatment, samples were stored in sterile bags at 4°C for 2 h then diluted in 100 ml of buffered peptone water (BPW) and stomached for 1 min. Next a 20 ml aliquot was removed and diluted in 80 ml of BPW. Duplicate 10^{-1} serial dilutions were made in PBS then 50 µl amounts were automatically spiral plated onto xylose lysine Tergitol 4 agar containing 20 µg/ml Rifampicin. Plates were incubated at 37°C for 24 h then colonies were counted and CFU/g calculated. Both 0.15% and 0.30% SF significantly ($P < 0.0001$) reduced *Salmonella* recovery by 0.65 and 0.89 log CFU/g, respectively as compared to NC. However, levels of *Salmonella* recovered between 0.15% and 0.30% treatments were not significantly different ($P = 0.4267$). It should be noted that use of electrostatic misting might have compromised the disinfection potential of SF by promoting rapid destabilization and degradation. Further investigation will elucidate the antimicrobial properties of SF using various application systems in food processing.

Key Words: disinfectant, salmonella, ferrate, green chemistry

M20, **Effects of reducing dietary amino acid density and stocking density on growth performance, carcass characteristics, meat quality, and occurrence of white striping in broiler chickens** Ahmet Pekel*[1], Özcan Cengi[2], Onur Tatli[2], Ömer Sevi[2], Eren Kuter[3], Bekir Köksa[2], Umair Ahsan[2], Ehsan Khamseh[2], Bülent Özsoy[2] [1] *Istanbul University-Cerrahpasa,* [2] *Adnan Menderes University,* [3] *Mehmet Akif University*

A 49-d trial was conducted to determine the impact of dietary amino acid (AA) density and stocking density (SD) on growth performance, carcass traits, meat quality, and white striping (WS) occurrence in broiler chickens. A total of two hundred eighty eight Ross 308 male broilers consisting of 6 replicate cages with 8 broilers per replicate were used. Treatments were arranged in a 3 × 2 factorial and consisted of 3 AA densities [normal (Norm), 10% lower than Norm (10%low), or 20% lower than Norm (20%low)] and 2 different SD (High; 35 kg/m^2 or Low; 26 kg/m^2). Data were analyzed as a completely randomized design using the GLM procedure of SAS. Decreasing AA density decreased overall growth performance, carcass and breast yields and breast fillet dimensions linearly, whereas leg and rib cage yields increased linearly ($P < 0.01$). High SD decreased hot carcass, breast, wings, and rib cage weights in birds fed on Norm AA diets ($P < 0.05$). High SD increased the length of breast fillet ($P < 0.05$). Cooking loss, breast lightness (L*) and redness (a*) at 48 h postmortem increased linearly with decreasing AA density ($P < 0.01$), whereas breast ultimate pH (pH_u) decreased linearly ($P < 0.001$). Low SD caused a decrease in breast yellowness (b*) at 24 post mortem in birds fed on norm AA diet ($P < 0.05$). Nitrogen content of breast fillets decreased linearly with decreasing dietary AA density ($P < 0.05$). Breast muscles having severe WS contained higher fat when compared with breast muscles with moderate or no WS ($P < 0.05$). As the dietary AA density decreased, occurrence of no WS breast fillets increased, whereas occurrence of moderate WS fillets decreased ($P < 0.005$). However, dietary AA density did not affect the occurrence of severe WS fillets. Stocking density did not affect the occurrence of WS. Breast fillets having severe WS had higher pH_u and less b* than normal breast fillets ($P < 0.05$). There were no differences for water holding capacity and cooking loss between different degrees of WS. Taken together, growth depression created by lowering dietary AA density regardless of SD resulted in a decrease in mean WS score. Severe WS fillets were heavier and had higher pH_u, fat, and lower b* but no difference in water holding capacity compared to no WS fillets.

Key Words: amino acid density, breast fillets, broiler chickens, stocking density, white striping

M21, **Biochemical and physicochemical changes in Spaghetti Meat chicken breast during refrigerated storage** GIULIA TASONIERO*, HONG ZHUANG, BRIAN BOWKER *USDA-ARS*

The aim of the study was to provide insight on the postmortem evolution of Spaghetti Meat (SM), as no data are currently available on the effect of SM on the proteolytic processes occurring during meat aging and their impact on myowater. During two trials, 30 severe SM and 30 unaffected (N) fillets were subjected to 7 days of refrigerated storage. At days 0, 3 and 7, NMR traits (*T2B*, *T21*, *T22*, *P2B*, *P21*, *P22*), myofibrillar proteins profiles, calpain activity, and free calcium concentration were assessed on 5 different breasts per group. A two-way ANOVA was carried out considering group (G: N and SM), time (T: 0, 3, 7) and their interaction G × T as fixed effects and trial as a random effect. In SM fillets, the intramyofibrillar water population exhibited longer relaxation times (*T21*; N = 45.9 vs SM = 46.9 ms; $P = 0.0172$) and a lower proportion compared to N (*P21*; N = 83.8 vs SM = 80.7%; $P = 0.0118$). Concurrently, SM was characterized by a greater proportion of extramyofibrillar water (*P21*; N = 15.5 vs SM = 18.8%; $P = 0.0080$) possessing a longer relaxation time (*T22*; N = 180 vs SM = 200 ms; $P = 0.0001$). Overall, the SM myopathy had only a minor impact on the myofibrillar proteins profiles and did not exert significant effects on free calcium concentration or the proportions of µ/m native and autolyzed calpain. Postmortem storage time exerted a strong effect on the measured traits. An increasing trend of the *P21* water compartment ($P = 0.0006$) coupled with a decrease of *P22* ($P = 0.0005$) revealed that a redistribution of myowater occurred over time in both N and SM fillets. Changes in NMR traits reflected the proteolytic processes taking place during meat aging. As the concentration of free calcium increased from day 0 to 7 ($P = 0.0392$), casein zymograms indicated a progressive decrease in native µ/m calpain activity concomitant with the accumulation of its autolyzed form ($P = 0.0031$). The electrophoretic profiles of myofibrillar proteins from both N and SM fillets exhibited a similar progression of protein degradation with postmortem storage time. Overall, the lack of significant interaction effects between muscle condition and storage period suggested that SM and N breast meat experience similar proteolytic and physical changes during the postmortem period.

Key Words: broiler chicken meat, Spaghetti Meat abnormality, postmortem proteolytic processes

M22, **Influence of Organic and Inorganic Acids as Used for Poultry Part Dips on the Emergence of Spoilage Organisms During a Shelf-Life Study** Kristina Feye*[1], Dana Dittoe[2], Christina Ovall[3], Carl Knueven[3], Steve Ricke[2] [1] *USDA-ARS,* [2] *University of Arkansas,* [3] *Jones Hamilton*

Due to concern over the use of peracetic acid (PAA) on its potential to select for lactic acid producing bacteria (LAB) commonly associated with spoilage, alternatives are being explored. The objective of the current study was to determine the potential of sodium bisulfate (SBS) as an alternative to PAA in poultry part dips during a 21 d shelf-life study. In total, 200 wings (5 treatments, 4 times, 10 replications) were obtained from an Arkansas poultry processor and subjected to the following treatments, which included not treated (NT) or treated with 350 mL of the following 15-sec dips: tap water (TW), TW + PAA (500 ppm), TW + SBS (3%), TW + PAA + SBS. Wings were stored at 4 °C until microbial analyses. On d 0, 7, 14, and 21, wings were rinsed in 150 mL of nBPW and subsequently plated on MRS and TSA to enumerate total LAB and aerobes. Colonies were $\log_{10}$ transformed and reported as CFU per gram of wing. Data were analyzed in JMP 14.0 as a Randomized Complete Block Design using simple linear regression and one-way ANOVA with means being separated using Tukey's HSD ($P \leq 0.05$). Over time, both LAB and aerobes increased with TW + SBS and TW + SBS + PAA having lower loads of aerobic bacteria and LAB on d 7 and TW + SBS having lower loads of both on d 14 ($P < 0.05$). By day, there was no difference between treatments and controls on d 0, but on d 7 and 14 the load of LAB was below 1 $\log_{10}$ (CFU/g) or below the limit of detection for wings treated with TW + SBS and TW + SBS + PAA compared to those treated with the controls

and TW + PAA ($P < 0.05$). The rinsates were sequenced with Illumina MiSeq 16S rDNA sequencing and processed through QIIME2.2019.8. Data indicates that SBS (3) + PAA induces a unique microbiome that may improve shelf life based on the microbial data as Lactic acid producing bacteria are reduced ($Q < 0.05$: ANCOM). Additionally, the stability of the microbiomes of each of the parts over time varies as indicated by MAZ (microbiota-by-age score; $P < 0.05$) Therefore, it can be concluded that the use of 3% SBS alone or in combination with PAA has the potential to mitigate the selection of LAB when applied a short duration part dip over a 14 d shelf life.

Key Words: PAA, SBS, Shelf-Life, Pathogens, Microbiome

Pathology

M23, **Onset of Immunity of A Recombinant HVT-ND against of a Velogenic NDV Challenge in SPF Birds** Kelly Turner-Alston, Megan Bosserd, Lauren Taylor, John Dickson, Jennifer Embrey, Sing Rong *Zoetis - VMRD*

A recombinant HVT-ND was developed as a bi-valent vaccine for protection against Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers. In this study, SPF leghorns were injected with recombinant HVT-ND *in ovo* on E18 at minimum protective dose. On Day 14, 16 and 19, 40 vaccinated birds of the challenge control and the HVT-ND vaccinated groups were challenged with a velogenic NDV, respectively. Protection of 93% (37/40) was observed for Day 19 challenge. For Day 16 challenge, 85% efficacy (34/40) was observed. For Day 14 challenge, 75% efficacy (30/40) was observed. The details of experimental design and study results will be further discussed.

Key Words: Vaccine, HVT vector, Bivalent vaccine, Onset of Immunity, HVT-ND

M24, **Compatibility of a Recominant HVT-ND Vaccine with Bursaplex to Provide Protection Against Velogenic NDV and Virulent Classical IBDV Challenge in SPF Birds** Megan Bosserd*, Kelly Turner-Alston, Lauren Taylor, John Dickson, Jennifer Embrey, Sing Rong *Zoetis - VMRD*

Combination of a recombinant HVT vectored ND vaccine with an immune complex IBD vaccine (Bursaplex) will provide protections against Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds and infectious bursal disease (IBD), an acute and highly contagious viral infection of young chickens that can cause immune suppression. In this study using SPF leghorns, HVT-ND was combined with Bursaplex, and the two vaccines were either administered *in ovo* at E18, or subcutaneously on day of hatch. Compatibility was demonstrated against a velogenic NDV challenge at Day 28 (93% protection for *in ovo* and 95% protection for subcutaneous injection), and against a classic virulent IBDV challenge at Day 34 (100% protection for both *in ovo* and subcutaneous injection). The sero-conversion to both IBDV and NDV was shown to correlate with efficacy.

Key Words: HVT-ND, HVT Vector Vaccine, NDV, IBDV, Bursaplex

M25, **In vitro inhibitory effect of a new three-strains selected probiotic on several serovars of Salmonella** Christophe Bostvironnois*[1], Line Skjot Rasmussen[1], John Schleifer[2] *[1] Chr Hansen A/S, [2] Chr Hansen*

Bacillus are known to be capable of producing antimicrobial substances including e.g. bacteriocins which may be able to inhibit Gram-negative bacteria such as *Salmonella* (Abriouel *et al.*, 2011). Reduced *Salmonella* colonization in liver and cecum has also been observed in *Bacillus subtilis* fed birds challenged with *Salmonella* Heidelberg (Hayashi *et al.,* 2018) and *Salmonella* Enteritidis (Knap *et al.*, 2011). The objective of the study was to evaluate the *in vitro* inhibitory effect of a new three-strain selected bacillus-based probiotic for poultry (GalliPro® Fit) on several *Salmonella* serovars using two different methods. The *Salmonella enterica* serovars tested were Albany, Braenderup, Enteritidis, Gallinarum, Heidelberg, Infantis, Kentucky, Mbandaka, Minnesota, Muenchen, Schwarzengrund, Stanley, Tennessee and Typhimurium.

The first method was an agar plate-based method. An agar plate was poured with pathogens on it. Wells were created into the plate. The bacillus-based probiotic was added to selected wells. After 24 hours of incubation, a zone of inhibition of up to 2 mm could be measured digitally.

The second method was a feed matrix based methodology. The feed was autoclaved, then the probiotic was added at 10^5 CFU/g of feed. The pathogens S. Heidelberg or S. Typhimurium were added at 10^3 CFU/g of feed. During the 24 hours time of incubation, samples were taken at different time points (4, 8 and 24 hours). Samples were added to plates, incubated and a counting of pathogens was conducted.

In these *in vitro* experiments the new bacillus-based probiotic was able to inhibit the growth of all *Salmonella* serovars belonging to different serogroups.The new bacillus-based probiotic inhibited type B, C, C1, C2, D *Salmonella* serogroups. When testing the probiotic vs. *Salmonella* spp. in an agar-based method, a radius of the inhibition zones of 1 to 2 mm was observed for all of them. When testing the probiotic vs. *Salmonella* spp. in a feed matrix, it inhibited the growth of *S.* Heidelberg (1.3 log inhibition after 4 hours, 4.6 log inhibition after 8 hours) and *S.* Typhimurium (1.6 log inhibition after, 4 hours, 2.2 log inhibition after 8 hours). Both *in-vitro* methods confirm the growth inhibition activity on numerous Salmonella serovars by this new three-strain probiotic.

Key Words: probiotic, bacillus, salmonella

M26, **Performance of broiler chickens fed a Adicox® as a stand alone anticoccidial program or in a "bioshuttle" program in conjunction with a coccidia vaccine** Greg Mathis*[1], Brett Lumpkins[2], Erik Wesoloski[3], Paulina Abramowicz-Pindor[3], Henryk Rozanski[3], Charles Hofacre[4] *[1] Southern Poultry Research, Inc., [2] Southern Poultry Feed & Research, Inc., [3] AdiFeed Sp., [4] Southern Poultry Research Group, Inc.*

Coccidiosis is the number one disease of the poultry industry. Control methods are through feed or water administration of anticoccidial products, coccidia vaccination, or a combination of both. Combination often referred to as a "biohsuttle" program, which is coccidia vaccination and a coccidiosis modulating/ control product given as immunity and disease progresses. "Bioshuttle" products are generally started with the grower feed. Several phytogenic products have shown anticoccidial activity and are candidates for use in a bioshuttle program. This study examined the performance of broiler chickens fed a phytogenic product AdiCox®, (Adifeed Sp) as a standalone anticoccidial program or in a "bioshuttle" program. For a comparison, Zoelene (Zoamix®, Zoetis) was also used as a standalone or in a bioshuttle" program. A 42-day broiler (Cobb 500 males) floor pen trial with built-up litter and was conducted using a randomized complete block (8 blocks) design. Each pen contained 50 chicks with 0.95 sq. ft per chick. All treatments consisted of a three-phase dietary program that used commercial feed formulation standards; starter (ST) d0-21, grower (GR) d21-35, and finisher (FIN) d35-42. Treatments were: T1 Zoalene 125 ppm d0-42, no coccidia vaccine (ZNV), T2, no feed additive, vaccine (NV), T3 Zoelene 125 ppm d21-42, coccidia vaccine (ZV), T4 Adicox® 200 ppm d21-42, vaccine (AV200), T5 Adicox® 300 ppm D21-42, vaccine (AV300), and T6 Adicox® 300 ppm d0-42, no

vaccine (AV300NV). 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. Coccidia oocysts per gram fecal material (OPG) were determined on d14, 21, 28, 35. D21 performances for ZNV and AV300NV were statically similar and better than vaccinated birds. D35 and 42 all treatments had significantly better performance than NV. D42 ZNV had the best performance with all "bioshuttle" trts and AV300NV better than NV. D14 and 21 opg were significantly lower for birds fed Zoalene or Adicox® compared to vaccinated. Coccidia immunity progressed with overall reduction of OPGs. D42 all treatments had less OPGS than NV. The feeding of Adicox® demonstrated significant improvements in performance as a standalone or in a "bioshuttle" program.

Key Words: Coccidiosis, Bioshuttle, AdiCox, Zoalene, anticoccidial

M27, **Development of a diagnostic PCR specific to the Cochlosoma anatis 28S ribosomal gene** Katherine Cupo*GS, Catherine Fudge, Kelly Keen, Robert Beckstead *North Carolina State University*

Cochlosoma anatis is a protozoan parasite that causes enteric disease in many species of birds including: turkeys, chickens, ducks, and quail. *C. anatis* can only be definitively diagnosed based on microscopic observation of the parasite in intestinal scrapings. This makes studying the parasite and disease outbreaks difficult. In order to more accurately diagnose *C. anatis* infections in poultry and study the transmission, pathogenicity and relationship of *C. anatis* with its hosts, a less invasive diagnostic tool is needed. Primers targeting the mitochondrial 16S gene of *C. anatis* have been published; however, these primers have demonstrated low sensitivity in our lab. Therefore, a more sensitive set of PCR primers is needed for diagnostics. To accomplish this, high molecular weight DNA was isolated from intestinal scrapings of turkeys infected with *C. anatis*. DNA sequencing was performed using PacBio, and the sequences were searched in nucleotide BLAST to identify those that did not align to turkey sequence in GenBank but shared similarity to *Trichomonas* species, the closest relatives of *C. anatis*. One 1622 bp sequence aligned to the *T. vaginalis* and *Histomonas meleagridis* 28S ribosomal gene. Two primer sets were designed along this sequence in A Plasmid Editor and were tested in a diagnostic PCR using DNA isolated from *C. anatis*. Both primers produced an amplicon of the predicted molecular weight. The species specificity and *C. anatis* strain specificity were tested in a diagnostic PCR using DNA isolated from: three field strains of C. anatis, *Tetratrichomonas gallinarum* isolated from turkeys, *H. meleagridis* isolated from turkeys, Ceva Immucox vaccine (containing two species of *Coccidia*), *Heterakis gallinarum* cecal nematodes, and a turkey. All three *C. anatis* isolates tested positive with both primer sets. Neither primer set was able to amplify DNA from any of the other species. The six amplicons from the three *C. anatis* isolates were Sanger sequenced. All amplicons aligned to the target sequence. Because both primer sets have demonstrated *C. anatis* specificity and the ability to amplify 28S target from distinct *C. anatis* isolates, the primers can be used for diagnostic PCR and to investigate *C. anatis* strain variation in poultry flocks through 28S sequencing.

Key Words: Cochlosoma anatis, PCR, diagnostic

M28, **Heat stress affects ileal digestibility and nutrient transporters in meat-type chickens infected with Eimeria maxima and subjected to heat stress** Ahmed Ghareeb*1GS, Jennifer Richter [1], Marie Milfort [1], James Foutz [1], Gustavo Schneiders [1], Alberta Fuller [1], Romdhane Rekaya [2], Samuel Aggrey [1] *[1] Department of Poultry Science, University of Georgia, [2] Department of Animal and Dairy Science*

Eimeria spp infection is a persistent disease in poultry. *Eimeria* (*E.*) *maxima* aggressively invades the middle jejunum and ileum, disrupting and damaging the intestinal mucosa. Heat stress a major stressor in poultry and contributes to oxidative stress. Our aim was to investigate the effect of *E. maxima* infection and heat stress (HS) on ileal digestibility and mRNA expression of nutrient transporters in broiler chickens. We designed a 2x2 factorial with two temperatures (20°C and 35°C) and two infection levels (2 x 10^5 *E. maxima* sporulated oocysts and water). There were 4 treatments, 6 replicates per treatment and 10 birds per replicate. The treatment groups were thermoneural control (TNc), thermoneutral infected (TNi), heat stress control (HSc) and heat stress infected (HSi). Birds (Ross708) were fed a non-medicated grower diet containing 0.2% titanium oxide. Ileal content was collected at 6-days post infection (dpi) and used to determine ileal crude protein, crude fat and amino acid digestibility. Ileal tissue collect at 6 dpi was used to determine the mRNA levels of sugars, lipids, and peptides *E. maxima* infection and HS would significantly reduce ileal digestibility and absorption, the HSc and HSi birds exhibited normal nutrients digestibility compared with TNc. The TNi birds had significantly lower apparent digestibility when compared with birds from the other treatment groups. The pepT1 and pepT2 genes were upregulated HSc compared to TNc. PepT1 and pepT2 were downregulated in the HSi group compared with the TNc group. Fatty acid binding protein (FABP) 1, 2 and 6 were significantly upregulated in the HSc group compared with the TNc group. Conversely, FABP1, 2 and 6 were downregulated in the TNi and HSi groups compared with the TNc group. The gene expression patterns of sugar transporters GLUT2, GLUT8, GLUT12 and SGLT1 were the same. The mRNA expressions of the sugar transporters for HSc, TNi and HSi were all downregulated when compared with TNc group. Our results show that exposing broiler chickens to heat can mitigate the disruptive digestion and absorption effects caused by *E. maxima*, however, mRNA expression of nutrient transporters is altered by both independent and combined effects of *E. maxima* infection and HS.

Key Words: Eimeria maxima, heat stress, ileal digestibility, nutrients transporters, gene expression

M29, **Molecular detection and differentiation of M. gallisepticum and M. synoviae in commercial poultry in Colombia** Magda Beltran [1], Arlen Gómez Ramírez [1,2], Belsy Gonzalez [1], Camila Daza [1], Gloria Ramirez-Nieto* [1,2] *[1] Universidad Nacional de Colombia, [2] Facultad de Medicina Veterinaria y de Zootecnia*

The main objective was to establish the presence of *Mycoplasma spp* in samples from commercial poultry submitted to the laboratory and to differentiate *M. gallisepticum* and *M. sinoviae* using molecular methodologies.

A total of 310 samples from commercial poultry were received in the laboratory on 2019. First, the samples were analyzed to determine the presence or absence of *Mycoplasma spp*. In order to o that, DNA amplification targeting genes *mgc2* y *vlhA2* for *M. gallisepticum* and *M. synovie*, respectively were used. Molecular analysis of selected positive samples included sequencing to determine phylogenetic relation to vaccine and/or field strains. In addition, as a diagnostic alternative, RFLP was attempted and compared with sequencing results. Cohen´s kappa coefficient was determined to compare agreement between test results.

The results showed that 31% (97/310) of the samples were positive to Mycoplasma spp. A co-infection was determined in 3.2% (10/310); *M. gallisepticum* was present in 24.1% (75/310) and *M. synoviae* in 10.3% (32/310) of the samples. Most of the positive results corresponded to a vaccine strain and they were detected in samples from birds at different ages.

As a conclusion, it was demonstrated the presence of *M. gallisepticum* and *M. sinoviae* in samples from commercial poultry in Colombia. There was a predominance of strains compatible to the vaccine strain used as a control measure. However, the identification of field strains, suggests that it is necessary to establish risk factors that could interfere with the protection conferred by the vaccine and/or a pharmaceutical treatment in particular. In terms of diagnosis, the molecular techniques were successful in detecting the presence of *Mycoplasma spp* and in the differentiation between *M. gallisepticum* and *M. synoviae*. On the other hand, the use of RFLP

represents an alternative for preliminary differentiation of Mycoplasma species in poultry.

Key Words: Diagnosis, field strain, Colombia

M30, **Assessment of bone marrow adipose tissue and bone mineralization on broiler chickens affected by wooden breast myopathy** Guillermo Tellez*[1], Barbara de Almeida Mallmann [1], Elizabeth Martin [1], Norma Calderon-Apodaca [2], Billy Hargis [1], Casey Owens [1] *[1] University or Arkansas, [2] Universidad Nacional Autónoma de México*

In humans, alterations in bone metabolism have been associated with myopathies. We postulate the hypothesis that perhaps similar pathologies can also be associated in modern chickens. Hence, this study aimed to assess the fat infiltration in bone marrow and its repercussion on broiler chicken affected by Wooden Breast (WB) myopathy. Ten Cobb 500 live birds with extreme rigidity of the *Pectoralis major* (PM) muscle were selected as WB affected chickens by physical examination of the muscle at 49 days of age, whereas ten chickens healthy with no physical signs of hardness in the breast muscle were considered to be unaffected. Macroscopic lesions in affected chickens included areas of firm and inflamed muscle with pale appearance, hemorrhaging, and viscous exudate on the surface. Bone marrow and sections of the PM muscle were collected and analyzed for light microscopy. Additionally, transmission electron microscopy was conducted in affected or unaffected muscle. Chickens affected with WB showed significant reductions ($P < 0.05$) in femur diameter, calcium, and phosphorous percentage but increased breast weight, compression force and fillet thickness when compared with non-affected chickens. Interestingly, bone marrow from WB chicken had subjectively, more abundant infiltration of adipose tissue, when compared with non-affected chickens. Histology of the P. major of birds with WB showed abundant infiltration of adipose tissue, muscle fibers degeneration with necrosis and infiltration of heterophils and mononuclear cells, connective tissue proliferation, and vasculitis. Ultrastructural changes of WB muscle revealed lack definition of bands in muscle tissue, or any normal ultrastructural anatomy such as myofibrils. The endomysium components were necrotic, and in some areas, the endomysium was notable only as a string of necrotic tissue between degraded myofibrils. The fascia appeared hypertrophied, with large areas of necrosis and myofiber without structural identity with degraded mitochondria adjacent to the disrupted muscle tissue. Further studies to evaluate the role of visceral fat, adipokines, and inflammasome, remains to be evaluated.

Key Words: bone marrow, adipose tissue, broiler chickens, wooden breast, electron microscopy

SCAD

M31, **Development and application of a multi-locus sequence typing (MLST) scheme for Eimeria maxima** Miranda Carrisosa*[1GS], Kenneth Macklin [1], Chengming Wang [2], Ruediger Hauck [1,2] *[1] Auburn University Department of Poultry Science, [2] Auburn University Department of Pathobiology*

Eimeria maxima is a eukaryotic parasite of chickens that can cause coccidiosis and predispose its host to secondary infection with *Clostridium perfringens*, the causative agent of Necrotic enteritis. *E. maxima* isolates have been typed by sequencing the internal transcribed spacer (ITS) region or sequencing the partial cytochrome oxidase I (COI) gene. More recently, a variant of MALDI-TOF mass spectrometry detecting single nucleotide polymorphisms (SNPs) was used, which requires equipment that is available only in few specialized laboratories.

Multi-locus sequence typing (MLST) is a nucleotide sequence typing system that reflects the population and evolutionary biology of bacterial pathogens. For MLST, five to six genes are sequenced, and the isolates are distinguished based on the detected allele variants. MLST schemes have also been developed for some eukaryotic pathogens like *Babesia* spp. and *Cryptosporidium* spp. The aim of the present investigation was to develop a MLST scheme for *E. maxima* and to compare strains from different farms.

Fecal and litter samples were collected from commercial broiler flocks in the Southeast. Oocysts were purified and tested by PCR for *E. maxima*. In a subset of samples, all *Eimeria* spp. present were identified by next generation amplicon sequencing with many being positive for *Eimeria acervulina* and *E. maxima*. If found positive for *E. maxima*, six gene loci that had been identified based on MLST schemes of several different parasites were then amplified and sequenced. Sequences were compared to the reference genome in GenBank.

With MLST, there was more than one allele in all six genes. In three of the genes, insertions and deletions were detected. Mutations were present in coding as well as in non-coding sequences. MLST was able to distinguish two commercially available vaccines and showed that most of the field isolates were not identical to these two vaccines.

The results show that MLST is a promising tool for epidemiologic investigation of *E. maxima*. The information gained from epidemiological investigations using the method will be useful in the development and evaluation of vaccine programs.

Key Words: Coccidiosis, Epidemiology

M32, **Effect of day of hatch inoculation with Gram-negative bacteria on gastrointestinal inflammation** Kaylin Chasser*[1GS], Kate McGovern [1], Audrey Duff [1], B. Danielle Graham [2], Whitney Briggs [1], Denise Russi-Rodrigues [1], Johel Bielke [1], Lisa Bielke [1] *[1] Ohio State University, [2] University of Arkansas*

Inappropriate microbial colonization, which can result in gastrointestinal (GI) inflammation, can act as a predisposing factor to a variety of opportunistic poultry diseases. Microbial introduction at the hatchery has been shown to manipulate GI microbial development. Two independent studies were completed to test wild-type Gram-negative poultry GI isolates for potential to induce GI inflammation and impact performance. In Exp 1, treatments included either no inoculation (NC), an oral dose of 10^3 CFU/chick of *Salmonella* Enteritidis E2 (E2), or *E. coli* LG (LG) on day of hatch. Yolk sacs (YS) and spleens were collected for bacterial enumeration (BE), and serum was collected for alpha-1-acid glycoprotein (A1GP) and fluorescein isothiocyanate dextran (FITC-d) analysis on d2, 6, and 13. In Exp 2, treatments consisted of NC and LG, with YS and serum samples collected on d2 and 14. In Exp 1, retained YS percentage was higher ($p<0.05$) in LG compared to NC on d6, though on d13, no differences were observed. Spleen BE showed elevated ($p<0.05$) levels in NC on d13, mirrored by high A1GP in NC, which suggested a systemic infection was being cleared. While A1GP was elevated for all treatments throughout, the only increase ($p<0.05$) was observed in E2 on d6. Serum FITC-d recovery was enhanced ($p<0.05$) on d13 for both E2 and LG compared to NC, which indicated increased intestinal permeability. In Exp 2, YS weight of NC was greater ($p<0.05$) than LG, but between d2 and d14, NC YS weight decreased by 70%, compared to a 30% reduction for LG, which suggested slower resorption of the YS in LG. Aerobic and Gram-negative YS BE were about 1 Log10 CFU/g higher in LG than NC on d14, yet not significantly different. Though A1GP marks systemic inflammation, added parameters of YS retention, weight, BE, and serum FITC-d further illustrate GI specific inflammation in chicks. Changes in YS parameters, BE, and FITC-d pointed toward increased GI inflammation for up to two

weeks following day of hatch bacterial administration. Mild GI inflammation may not cause stark changes in performance or inflammatory parameters, but these isolates may serve as predisposing factors for opportunistic disease, as well as candidates for studying the influence pioneer colonization on poultry intestinal health.

Key Words: E. coli, yolk sac, inflammation, Gram-negative

M33, **Age-related changes in gut permeability and optimal timing for experimentally induced leaky gut** Audrey Duff*GS, Kaylin Chasser, Whitney Briggs, Denise Rodrigues, Lisa Bielke *The Ohio State University*

Experimentally induced gut permeability, as a representation of inflammation, is critical for the study and development of intestinal health-promoting technologies in broilers. However, natural leakiness of the intestine in young birds may have substantial effects on ability to measure treatment differences. Fluorescein isothiocyanate dextran (FITC-d; 3-5kDa) has become a valuable tool for estimating the degree of leaky gut in broilers as it does not naturally cross the intestinal barrier, and when tight junctions are compromised, orally delivered FITC-d can paracellularly cross into the blood. Two experiments that highlight relationships between timing of leaky gut treatment and changes in gut permeability with age as measured by FITC-d were completed. Alongside untreated controls, permeability treatments included feed supplementation with dexamethasone (Dex), BMD50 (50g/lb), or a weekly 24h feed withdrawal (FR) prior to sampling. Experiment 1 included Dex (0.29ppm) d7-14 and BMD50+Dex, and Exp. 2 included Dex (0.57ppm) d21-28, BMD50, and FR. At weekly intervals, serum FITC-d (4.17mg/kg) levels were measured. All data were subject to ANOVA and means were separated with Student's t-test ($p<0.05$). In Exp. 1, Control and Dex d7 FITC-d was similar, and remained similar on d14 after treatment. However, on d21 and d28, levels of FITC-d in Control decreased, suggesting a decrease in natural permeability after week 2. In Exp. 2, FR had the highest ($p<0.05$) FITC-d on d7. As in Exp. 1 an increase in Control FITC-d, similar to consistently high levels in FR, was observed on d14. Importantly, when weekly FITC-d levels were compared for Controls, d14 was greater ($p<0.05$) than d7 or d21 which implied a natural increase in permeability of healthy intestines around d14. On d28 Dex FITC-d was significantly greater than FR and both were higher ($p<0.05$) than Control and BMD50. Additionally, Dex treatment had more noticeable effects when applied d21-28 than d7-14. These cumulative results suggest young birds undergo a natural increase in gut permeability during early life. Therefore, experimental intestinal permeability treatments applied and measured during this period may lead to inaccurate conclusions or predictions regarding later performance.

Key Words: Gut permeability, Broilers, FITC-d, Serum, Leaky gut

M34, **Evaluation of the effect of different 25-Hydroxyvitamin D3 materials in the response to coccidiosis and necrotic enteritis challenges in commercial broiler chickens.** Yun-Ting "Tina" Wang*, Anthony Pokoo-Aikins, April Levy *DSM Nutritional Products*

Chickens raised without antibiotics (RWA) face increased gastrointestinal challenges from coccidiosis, dysbacteriosis, and femoral head necrosis as opposed to broilers raised in a conventional program. Many published studies have shown Vitamin D/25-Hydroxyvitamin D3 is critical for skeletal development, meat yield, and immune support, as well as gastrointestinal functionality. A 21-day, 48-battery cage study, was conducted to evaluate the effect of two different sources of 25-Hydroxyvitamin D3 materials in performance and immune responses to a coccidiosis (Cocci) and *Clostridium Perfringens* (CP) challenge in commercial broiler chickens. The study comprised of 8 treatments: T1 (control diet with no cocci or CP challenge), T2 (control no cocci or CP), T3 (control with cocci but no CP), T4 (source 1: 25-Hydroxyvitamin D3 product (69 mg /MT), Cocci, No CP), T5 (source 2: VitD3/25-Hydroxyvitamin D3 mix product (34.8mg/MT) with cocci but no CP), T6 (Control with Cocci and CP), T7 (source 1: 25-Hydroxyvitamin D3 product (69 mg/MT) with cocci and CP) and T8 (source 2: VitD3/25-Hydroxyvitamin D3 mix product (34.8 mg/MT) with Cocci and CP) and 8 replicates per treatment. Before receiving the coccidiosis challenge at day 14, Trt 4 had the lowest mortality-adjusted feed conversion (adj. FCRm) at 1.746 followed by Trt 3 at 1.783 and Trt 5 at 1.820. At day 21 and 23, 7 and 9 days post *Eimeria* challenge, it showed similar trend for adj. FCRm. Trt 4 (1.958; 2.047) had lowest adj. FCRm followed by Trt 3 (2.011; 2.070) and Trt 5 (2.089; 2.154). Fecal samples were collected from Trt 1, 3, 4, and 5 to evaluate oocyst shedding. At day 21, 7 days post oral gavage *E. maxima* oocyts, Trt 4 had significant lower oocyst shedding (105,211/g) compared to Trt 3 (121,869/g) and Trt 5 (150,725/g). There was also a significant effect of the treatments (T1, T6, T7 and T8) on cytokine gene expression of IL-10 at day 21 post hatch (P=0.0001). Broilers fed source 1: 25-Hydroxyvitamin D3 product (69 mg/MT) had the lowest FCR before and after coccidiosis or CP challenge compared to control and source 2: VitD3/25-Hydroxyvitamin D3 mix product (34.8 mg/MT).

Key Words: 25-Hydroxyvitamin D3, coccidiosis, broiler, gastrointestinal, cytokine

M35, **Using novel phytogenic combinations to combat necrotic enteritis in broilers** Shelby Ramirez*[1], Johel Bielke[2], Antonia Tacconi[3], Raj Murugesan[1], Lisa Bielke[2] *[1] BIOMIN America, Inc, [2] The Ohio State University, [3] BIOMIN Holding GmbH*

Transition to antibiotic-free broiler production has driven the need for non-antibiotic solutions for disease challenges including necrotic enteritis. Therefore, the objective of this study was to evaluate novel formulations of herbs, essential oils and extracts on necrotic enteritis. *Eimeria maxima* (EM) viability was determined *in vitro* after 4-d incubation with either sterile water (CON) or 1 of 4 phytogenic feed additive (PFA) products diluted to 0.05%. By d 4, only 20% of EM were viable in the CON group; however, all PFA groups were able to further reduce viability to 10.43, 9.02, 4.64 and 4.23% for PFA1, PFA2, PFA3, and PFA4, respectively. Based on these *in vitro* results, 3 PFA products were tested *in vivo* using a necrotic enteric challenge model. A total of 800 Cobb-500 male broiler chicks were placed on day of hatch (BW=43.4±0.2 g) to 1 of 5 groups (8 pens/group, 20 birds/group): 1) non-challenge control (NCC), 2) challenge control (CC), 3) CC+PFA1 (PFA1), 4) CC+PFA3 (PFA3), and 5) CC+PFA4 (PFA4). All PFA products were included in the diet at 125 g/MT. On d 14, chicks in challenge groups were individually inoculated with 66,250 oocysts of EM Guelph strain. On d19, those same groups received 1×10^7 CFU/chick of *Clostridium perfringens*. Birds and feeders were weighed weekly to calculated performance parameters and all mortalities were necropsied to determine the presence of intestinal lesions. Data analyzed using GLIMMIX procedure of SAS. From d 0–21, BWG was increased ($P<0.05$) in NCC and PFA4 compared with PFA1 and PFA3 with CC being intermediate. A similar pattern was seen for bird feed intake during d 0–21; however, FCR was increased ($P<0.05$) in all challenge groups compared with NCC. From d 0–35, all challenge groups had reduced BWG ($P<0.05$) compared with NCC but did not differ from each other; however, there was no difference ($P>0.05$) in FCR. The mortalities confirmed with intestinal lesions were 7, 6, 8, and 11% in the CC, PFA1, PFA3, and PFA4, respectively. These results suggest that the combination of phytogenics may be a viable solution for necrotic enteritis and the extent of improvement vary depending on formulation.

Key Words: necrotic enteritis, phytogenic, broiler

M36, **Effects of glutamine supplementation on broiler performance and intestinal immune parameters during an experimental necrotic enteritis infection** Jarred Oxford*GS, Ramesh Selvaraj *University of Georgia*

The objective of the study was to evaluate the effects of glutamine (Gln) supplementation on performance and intestinal immune parameters of broilers during an experimental necrotic enteritis (NE) infection. 144-one-

day old Cobb 500 male broilers were randomly distributed to either an unchallenged control (UC, 0% of supplemented Gln), challenged control (CC, 0% of supplemented Gln), 0.5, or 1.0% Gln supplemented groups (n=6). Diets were formulated based on the breeder guidelines and balanced with powdered cellulose to ensure similar energy densities. Birds were raised to 21 d of age with each treatment having six replicates (6 battery cages) of 6 birds per replicate with water and feed provided *ad libitum*. On d 14 of the study, each bird in the challenge groups were given an oral gavage of 5,000 *E. maxima* oocyst. On d 19, 20, and 21 the challenged birds were given 10^8 CFU/mL of *C. perfringens* (CP, alpha toxin and Net B positive). BW, feed intake, and mortality were recorded throughout the study. On d 21, NE lesions scores, cytokine gene expression, cecal contents bacteria expression, intestinal permeability, and serum Gln were analyzed. Data were analyzed using the PROC GLM procedure in SAS, non-parametric data was analyzed via the Kruskal-Wallis Test. NE challenge increased mortality at all time points with the 1.0% Gln group having the most mortality (P<0.01). Gln supplemented at 0.5% increased IL-10 expression compared to the CC by 6.6-fold (P<0.05). The 1.0% Gln had higher cecal load of CP compared to the UC (P=0.02). A shift in NetB positive CP was observed in the challenge groups with the challenge groups having significantly more NetB positive CP compared to the UC group (P<0.01). Intestinal permeability was significantly increased in the CC compared to UC with an increase of 616% (P<0.05). The CC group had 267% higher intestinal permeability compared to the 0.5% Gln group (P<0.05). The 0.5% Gln group had an increase in serum Gln concentration of 135% compared to the CC and the 1.0% Gln group had an increase of serum Gln of 157% compared to the CC (P<0.05).The results show that during NE, serum Gln levels are significantly reduced and the supplementation of dietary Gln at 0.5% may help reduce the negative effects of NE.

Key Words: Glutamine, Necrotic enteritis, Broiler, Nutrition, Intestinal health

M37, **Next-generation random sequencing identification of infectious agents present in clinical samples from broilers affected by hypoglycemia and spiking mortality syndrome** Iryna Goraichuk*[1], James Davis[2], Arun Kulkarni[2], David Suarez[1], Claudio Afonso[1] [1] *USDA-ARS-USNPRC,* [2] *Georgia Poultry Laboratory*

Hypoglycemia-spiking Mortality Syndrome (H-SMS) is a malady of broiler chicks characterized by sudden increase in mortality. The affected birds are hypoglycemic, with blood glucose levels of less than 180 mg/dl (normal = 225-300 mg/dl) and often as low as 20mg/dl. Clinical signs may include tremors, ataxia, and blindness. The etiology of H-SMS is unknown; however, the successful experimental reproduction of H-SMS with the use of homogenized organs, indicates that an infectious agent is likely involved. Previous investigations have shown an association of H-SMS with reoviruses, adenoviruses or arenavirus-like agents.

Next-generation sequencing (NGS) technologies eliminates the need for prior knowledge of virus genomic sequences and provides advantages over traditional methods such as PCR or microarray hybridization, that are dependent on target-specific primers. To date, NGS has been instrumental in the discovery of novel viruses and identification of pathogens in samples with an unidentified source of disease.

In order to identify the infectious agent(s) of H-SMS, different field and experimental samples were analyzed by NGS. Total nucleic acids were extracted using the Qiagen DNeasy Kit, followed by DNase treatment to remove host DNA. The NGS libraries were constructed using SISPA in conjunction with the Nextera XT DNA library preparation kit. Pair-end sequencing was performed on an Illumina MiSeq instrument. Sequence data were assembled using a *de novo* approach as well as analyzed by the GOTTCHA Metagenomics classifier within a customized workflow on the Galaxy platform, which altogether allowed us to detect distinct infectious agents. Besides reference genome assembly, results were verified by target-based PCR and Sanger sequencing. Avian adeno-associated virus (*Dependoparvovirus*) and fowl adenovirus D (*Aviadenovirus*) were the pathogens more frequently observed to be present, however in some cases other viral agents were associated with these. It is generally believed that the adeno-associated virus is nonpathogenic, and that it is unable to replicate without the presence of a "helper virus". Also, it is not currently known to be a primary etiologic agent of disease in infected chickens. Future *in vivo* experiments are required to confirm our findings.

Key Words: NGS, hypoglycemia, spiking mortality syndrome, H-SMS, broilers

SCAD II

M38, **Effectiveness of a live Salmonella typhimurium vaccine for reduction in colonization of S. infantis in broilers** Charles Hofacre*[1], Roy Berghaus[2], Kalen Cookson[3], Manuel DaCosta[3] [1] *Southern Poultry Research Group, Inc.,* [2] *University of Georgia,* [3] *Zoetis Global Poultry*

A study determined the effectiveness of the live *S. typhimurium* vaccine (Poulvac ST) in broiler chickens administered at 1-day spray and 14 day in the water. The challenge was oral gavage of 15 birds per pen (direct) at 30 days of age with *S. infantis*. Each pen had 25 birds total with non-challenged (indirect) tagged and dyed. The samples collected were 37-day bootsocks (environment), ceca 42-day, whole bird carcass rinse 42-day (hot rehang), and liver/spleen (42-day). A typical 8-hour feed withdrawal was performed prior to catching and processing. Salmonella prevalence and enumeration were performed with tetrathionate, then XLT4. Enumeration was by micro most probable number (MPN) method of Berghaus, et al., 2013. Five horizontal birds/pen and five direct challenged were processed for carcass/rinses and ceca collected from each. Also, liver/spleen from each bird was cultured to determine presence of S.I. in internal organs. There was a consistent numerical reduction in S.I. $\log_{10}$ MPN/bootsock as well as in ceca (3.16^a $\log_{10}$ MPN/g) with the vaccine versus the challenge control (ceca, 3.26^a $\log_{10}$ MPN/g). There was also a consistent numerical reduction of S.I. MPN/g on both the direct and indirect challenged carcasses versus the challenge control. There was a significant reduction in S.I. in the liver/spleen for vaccinated broilers (83.3%[a]) versus the challenge control (96.3%[b]). There was also significantly lower S.I. numbers in the vaccinated broilers internal organs (1.87^a $\log_{10}$ MPN/g) versus control (2.91^b $\log_{10}$ MPN/g). In conclusion, the live *S. typhimurium* vaccine (Poulvac ST) demonstrated a numerical reduction in the ceca and carcass rinses to an *S. infantis*. Additionally, the significant effect of spleen/liver colonization may indicate the Salmonella vaccine (Poulvac ST) may play a role in reducing *Salmonella infantis* for ground products (Ciu, 2015).

Key Words: Salmonella infantis, broilers, carcass rinse, ceca, internal organs

M39, **Clinical manifestations and genomic analysis of a persistent *Salmonella Enteritidis* infection in a broiler flock** Grayson Walker*GS, Mitsu Suyemoto, Dawn Hull, Laura Chen, Sesny Gall, Rocio Crespo, Luke Borst *North Carolina State University College of Veterinary Medicine*

Virulent strains of *Salmonella* cause significant disease in poultry flocks, resulting in production losses and dissemination to human populations. Despite the eradication of *Salmonella* Pullorum and Gallinarum in many countries, other virulent strains of *Salmonella* have emerged. A flock of 5000 broilers reared for research and teaching experienced 7% overall mortality. Necropsies were performed daily and lesion samples were submitted for culture when a bacterial etiology was suspected. *Salmonella* was isolated continuously until 26 days of age and caused a variety of gross lesions, which predominantly consisted of polyserositis followed by yolk saculitis, arthritis, osteomyelitis, and spondylitis. Rep-PCR confirmed a clonal relationship among these isolates, which were determined to be *Salmonella* Enteritidis (SE) by serotyping. *E. coli* were also isolated from lesions. *E. coli* isolates were to PCR screened for virulence genes to differentiate commensal *E. coli* from avian pathogenic *E. coli* (APEC). Antimicrobial susceptibility phenotyping combined with whole genome sequencing methods were utilized to further characterize the SE and APEC isolates. The SE strain was resistant to nalidixic acid and sulphadimethoxine, while 3 of 5 sequenced APEC were resistant to multiple drugs including gentamicin and tetracycline. Assembled genomes were analyzed with ABRicate software, which utilizes 8 databases to detect antimicrobial resistance, virulence, and plasmid genes. This analysis detected an IncF plasmid, which was shared by SE and APEC isolates. These data demonstrated persistence of a virulent strain of SE that caused atypical lesions and elevated mortality in a broiler flock.

Key Words: Salmonella, E. coli, spondylitis, antimicrobial, resistance

M40, **Efficacy of Salmonella-chitosan nanovaccine to decrease colonization and shedding and provide cross-protection in broilers challenged with Salmonella enterica Enteritidis or Heidelberg** Keila Acevedo-Villanueva*1GS, Ramesh Selvaraj [1], Sankar Renu [2], Yi Han [2], Renukaradhya Gourapura [2] [1] *University of Georgia,* [2] *The Ohio State University Ohio Agricultural Research and Development Center*

This study analyzed the protective effects of an oral chitosan-*Salmonella* nanoparticle vaccine (CNP) engineered with *Salmonella* Enteritidis (SE) outer membrane proteins (OMPs) and flagellin proteins. A total of 264 broilers were orally gavaged twice with PBS, 1000µg CNP, or a live commercial vaccine (CV) at 1 and 7d. At 14d, birds were orally challenged with 10^5 CFU/bird of SE or *S.* Heidelberg (SH). At 8h-post-challenge, birds immunized with the CV (253%) or CNP (173%) had higher levels ($P<0.05$) of anti-SH OMPs-specific IgG, compared to control. Similarly, at 8h-post-SE challenge, birds in the CV (253%) and CNP (173%) treatments had higher anti-*Salmonella* OMPs-specific IgG levels, compared to that in the control group. However, birds in the CV (72.63%) and CNP (72.62%) groups had decreased ($P<0.05$) anti-SE OMPs-specific IgG levels at 4d-post-challenge, compared to that in the control. Birds in the CNP and CV groups had decreased cloacal-anti-SH specific-OMPs IgA by65.1% and 71.2% at 2d-post-challenge and 71.8% and 66.63% at 4d-post-challenge, respectively when compared to that in the control; Birds in the in the CV (64.36%) and CNP (51.2%) had decreased($P<0.05$) cloacal-anti-SH specific-flagellin IgA levels at 4d-post-challenge, compared to that in the control group. Cloacal-anti-SE specific-OMPs IgA levels decreased ($P<0.05$) for the CNP treatment (66.5%) at 2d-post-challenge, like that of the CV group (51.42%) and CV (65.13%); and CNP (59%) had decreased ($P<0.05$) cloacal-anti-SE specific-OMPs IgA levels at 4d-post-challenge, compared to control. At 8h-post-challenge, birds immunized with the CV (6.36%) or CNP (4.59%) had higher levels ($P<0.05$) of bile-anti-SH specific-flagellin IgA, compared to control. At 2d-post-challenge, *Salmonella* Enteritidis population in cecal content was decreased ($P<0.05$) by 97.5% and 98.8% in birds immunized with CNP and CV when compared to control. High levels of antibodies in the control group can be indicative of a persistent *Salmonella* colonization. Findings demonstrate that CNP can induce a specific immune response against *Salmonella*, with the potential to cross-protect against SH and to mitigate SE cecal colonization.

Key Words: Salmonella, Chitosan, Nanoparticles, Vaccination, Broiler

M41, **Current strategies and new approaches to boost Salmonella vaccination in broiler breeders** Gunnar Dunnam*1GS, Erin Riley [2], Randi Clark [2], Jaime Ruiz [3], Tim Cummings [4], Phil Stayer [2] [1] *College of Veterinary Medicine. Mississippi State University,* [2] *Sanderson Farms Inc.,* [3] *Elanco Animal Health,* [4] *Zoetis Inc.*

Salmonella species are important zoonotic pathogens that can cause gastrointestinal disease in humans and animals. Poultry products contaminated with these pathogens are one of the sources of human Salmonella infections. Vaccination, along with other intervention strategies, is an important tool that is currently used to control and reduce the levels of Salmonella in commercial poultry flocks, which may ultimately lead to lower rates of human Salmonella infections. This review examines some of the most common Salmonella vaccination programs in broiler breeders, including recent approaches to boost Salmonella protection using commercial Salmonella enteritidis vaccines. Descriptive statistics show that serological responses were not different between simultaneous and delayed injections of commercial with autogenous salmonella vaccines. Post-mortem examination associated with specific lesions scores show that all commercial products induced injection site plaques, especially when separated by three weeks from the autogenous products.

Key Words: Salmonella vaccination, broiler breeders

M42, **Physicochemical characteristics and immunogenicity of chitosan nanoparticles loaded with extracellular proteins of C. perfringens and Salmonella flagella proteins** Gabriel Akerele*1GS, Nour Ramadan [1], Sankar Renu [2], Gourapura . Renukaradhya [2], Ramesh Selvaraj [1] [1] *University of Georgia,* [2] *Ohio state univeristy*

There are currently no licensed vaccines against *Clostridium perfringens* which causes necrotic enteritis in poultry. Chitosan nanoparticle vaccines were formulated with native toxins (CN) or toxoids (CT) from extracellular proteins (ECP) of *C. perfringens.* Both the vaccines were surface-tagged with Salmonella flagellar proteins. CN vaccines incubated at 3.5 pH and 41°C for 3.5 hours released 8.2% of its cargo. The CN and CT vaccines incubated at 7.4 pH and 41°C for 10 days released a cumulative total of 10% and 20% of their antigens respectively.[RS1] The CN and CT vaccines incubated at 37°C for 3.5 hours released 17% and 8% hemoglobin respectively from chicken red blood cells. In an in vivo study, ninety broilers were randomly assigned to three treatments; sham-vaccinated (CTRL), CN-vaccinated (CN), and CT-vaccinated (CT) in six replicates. Each bird was orally gavaged with 50µg vaccine in 0.5ml PBS or 0.5ml PBS only on 0-, 3-, 7- and 14-days post hatch. On 10-, 17-, 21- and 28-days post hatch, sera and bile were analyzed for anti-ECP and anti-flagella IgY and IgA.[RS2] Bile Anti-ECP IgA was significantly higher in CN and CT groups than that in the CTRL group ($P<0.05$) at 21- and 17-days post hatch respectively. Bile anti-flagella IgA was significantly higher in the CN group than CTRL group at 10 days post hatch. Serum anti-ECP IgG and serum anti-flagella IgG was significantly higher in the CN groups than in the CTRL group ($P<0.05$) at 21- and 17- days post hatch respectively. [RS3] The recall response in splenocyte PBMCs was significantly higher by 60% and 56% in the CN group than in CTRL group when stimulated with 0.25mg/ml and 0.5mg/ml antigen respectively. Toxin neutralization by serum from CN and CT group was on average 40% higher than CT group at 17 days post hatch. The result from this study indicates the potential for chitosan loaded with *Clostridium perfringens* extracellular proteins to be applied to necrotic enteritis challenge studies.

Key Words: Chitosan, Nanoparticle, Poultry, Clostridium perfringens, Vaccination

M43, **Effect of Select Gram Negative Bacteria on Alpha-1-Acid-Glycoprotein and Ileal Histology** Kate McGovern*UG, Kaylin Chasser, Audrey Duff, Whitney Briggs, Denise Russi Rodrigues, Lisa Bielke *The Ohio State University*

Gastrointestinal (GIT) inflammation, caused by inappropriate microbial colonization, can predispose poultry to a variety of opportunistic infections and reduce growth performance, and parameters to quantify this inflammation are critical for research. Alpha-1-acid glycoprotein (A1GP) was tested for viability as an enteric GIT inflammation marker through histological analysis of the ileum using modified I See Inside methodology. Experiment 1 treatments included non-inoculated control (NC), and three challenge levels, 10^2 CFU/chick low (L), 10^3 CFU/chick medium (M), or 10^4 CFU/chick high (H) of *Citrobacter* and *Salmonella* Enteritidis, with 12 chicks per pen and three replicate pens per treatment. Experiment 2 chicks were inoculated with 10^3 CFU, 10^4 CFU, or 10^5 CFU/chick of *E. coli* on DOH. In both experiments, body weight was measured on d0, d7, and d14. On d14, blood and ileal samples were collected, and serum was used for A1GP ELISA analysis. To help determine correlation between A1GP levels and discernable GIT inflammation, 10 chicks with the highest and the 10 chicks with the lowest A1GP values were selected for histological analysis of ileum villi height, crypt depth, epithelial thickness, lamina propria thickness, lamina propria plasma cell clusters, goblet cells per mm^2 and epithelial thickness per mm^2. There was a significant increase ($p<0.001$) in epithelial plasma cells per mm^2 as well as a significant increase ($p<0.0402$) in goblet cells per mm^2 in birds with high A1GP levels as compared to low A1GP levels. No significant differences were found when comparing villi height, crypt height, epithelial thickness, lamina propria thickness, and lamina propria plasma cell clusters. Based on these results A1GP can be a good indicator for some types of gastrointestinal inflammation.

Key Words: gastrointestinal inflammation, alpha-1 acid glycoprotein, histology

M44, **Temporal and spatial evolution of Infectious Bronchitis Virus in North Carolina** Rocio Crespo*, David Roberts, David Rasmussen, Gustavo Machado *North Carolina State University*

The industry collects enormous amounts of surveillance data on poultry pathogens, but only a fraction is used for scientific purposes or analyzed to make informative decisions. Improved knowledge related to the circulation and spread of pathogens affecting poultry is critical to enable both public health and veterinary health sectors. The information and knowledge generated from disease spread models, especially those including spatial and evolutionary dynamics, has the potential to help the introduction of more effective prevention and control strategies and directly improve animal health, welfare of poultry, and to increase the profits for poultry farmers.

We compared and analyzed sequence data from IBV isolates between 2016 to 2019. From these flocks, we collected: farm location, flock size, total mortality, vaccination program, market age, market weight, etc, as metadata for subsequent analyses. We created a spatiotemporal map linking phylogeny, time, and location to track disease spatial spread and evolution. The data demonstrated that, in North Carolina, IBV spread North to South and East to West primarily. Poultry density, presence of wild birds, high humidity and proximity to crops were factors that promoted the spread of IBV. We will discuss how our model can be used to identifying newly and re-emerging strains.

Key Words: Infectious bronchitis virus, IBV, phylogeny, spatiotemporal

M45, **The use of monovalent or bivalent IBV vaccines significantly reduces clinical signs but not viral load after heterologous challenge** Brian Jordan*, Deborah Hilt, Mark Jackwood *The University of Georgia*

Infectious bronchitis virus (IBV) is an economically significant upper respiratory tract pathogen of poultry. Infection with IBV results in a mild respiratory infection but predisposes chickens to secondary bacterial infections leading to air sacculitis and condemnation at processing. Because of the economic significance of condemnation, nearly all commercial poultry are vaccinated against IBV. Vaccines are effective in a serotype specific manner, meaning they do not induce fully neutralizing antibodies against heterologous serotypes. The major challenge to control is that current vaccines are not effective against novel IBV variants, which seem to arise every few years. Recently, producers have begun to use multiple IBV serotype vaccines given together in an effort to induce cross-protection. This strategy has been used with some success, but variant IB viruses have still been detected in flocks indicating that the vaccine combinations have not prevented infection. In this study, we seek to determine how a single verses a combination of 2 different IBV vaccine types protect against DMV/1639, a currently circulating and economically important IBV variant. For this project, we vaccinated chickens by eye-drop on day of hatch with either a commercial Massachusetts (Mass), Georgia 08 (GA08), or Arkansas (Ark) type IBV vaccine, or with a combination of 2 Mass types, a Mass and GA08, or a Mass and Ark vaccines, and challenged with the variant DMV/1639 virus isolated from a clinical case in Georgia in 2019. Clinical signs were significantly reduced in all vaccinated chickens at 5 days post-challenge however, the DMV/1639 variant virus was detected by RT-PCR and by isolation in embryonated eggs in a majority of vaccinated chickens. Taken together, it seems the cross-protection strategy with these vaccines will aid in reducing or eliminating clinical presentations, but does not prevent infection or replication of the virus. Additionally, it was interesting to note that all vaccines, whether alone or in combination, performed equally in this experiment. In the future, it will be necessary to follow variant IBV viruses in flocks using this cross-protection strategy to evaluate the influence of these vaccines on viral evolution and development of new variants.

Key Words: Infectious bronchitis virus, Vaccine, Cross-Protection

M46, **The effect of Infectious Bronchitis virus live vaccination timings on the severity and growth characteristics of Mycoplasma synoviae Infection** Mohammadreza Ehsan*GS, Marianne Santos, Rachel Jude, Ruth Wooten, Naola Ferguson-Noel *Poultry Diagnostic and Research Center, University of Georgia*

Mycoplasma synoviae has been a major concern for the poultry industry due to economic losses as the result of subclinical effects (such as poor feed conversion) and other complications such as airsacculitis and tenosynovitis. The aim of the current study was to evaluate the effects of a common infectious bronchitis virus (IBV) vaccine, Arkansas strain, when administered in two different timings, i.e. prior or concurrently, to *M.synoviae* infection. In this trial, ninety-six SPF chickens were divided into 8 groups and vaccinated at 2, 4, 6 or 8 weeks of age via eyedrop with a commercially available IBV vaccine. *M.synoviae* was administered at 8 weeks of age and the groups were evaluated at 10 days post *M.synoviae* infection by gross lesion air sac lesion scoring, histopathological evaluation of tracheal lesions, serology, and real-time PCR. The results indicated IBV vaccination impacted not only the severity of lesions but also the replication rate of *M.synoviae* in the tracheas of infected birds.

Key Words: Mycoplasma synoviae, Infectious bronchitis, vaccination, Arkansas strain, timing

M47, **Effect of folic acid on the innate immune receptors in chicken B-lymphocytes infected with IBDV** Santiago Uribe-Diaz*[1GS], Alexander Yitbarek [2], Dario Vallejo [3], Daniela Losada [1], Marya Ahmed [1], Juan Rodriguez-Lecompte [1] [1] *University of Prince Edward Island,* [2] *University of Guelph,* [3] *University of Antioquia*

Folic acid (FA) has an immunomodulatory effect on chicken B cells, possibly affecting their ability to both recognize antigen through the Toll Like Receptor (TLR) and B Cell Antigen Receptor (BCR) pathways and their ability to present antigen via the MHCII presentation pathway. The objective of this study was to evaluate the effects of FA supplementation on chicken B cells innate immune response associated receptors during an infectious bursal disease virus (IBDV) infection. B lymphocytes (DT40) were cultured in triplicate at a concentration of 5x105 cells / well in 24 well plates. After 12h, the cells were supplemented with 3.96 mM FA and challenged with IBDV (ST-12 intermediate strain) at 1 multiplicity of infection (MOI). The experiment was repeated three times. Six genes associated with the presence of FA, virus infectious and innate responses were evaluated, which included reduced folate carrier (RFC), TLR-3, TLR-2b, TLR-4, BCR (Igβ chain) and MHC II (β) by real time PCR at different times (3, 6, 12, 24 and 36 hours post infection (hpi)). FA was found to significantly decrease ($p<0.05$) viral RNA load at 6 and 36 hpi and protecting the cells from infection with IBDV. However, the presence of FA did not modify RFC gene expression at all time points, except at 24 hpi, which coincided with the maximum virus load and the significant ($p<0.05$) upregulation of MHC II. The significant ($p<0.05$) increase in the transcriptional level of TLR3 gene expression in the presence of IBDV (dsRNA) may indicate an anti-IBDV role of TLR3. This also corresponded well with the highest virus load and RFC gene expression at those time points ($p<0.05$). In general in both groups (with or without FA) there were not significant changes on MHC II expression in the whole experiment, except at 24 hpi in cells with FA showing a significant up regulation of MHC II, which was positively associated with virus load, RFC, and TLR2b gene upregulation. In conclusion, this study evaluated the effects of FA supplementation on chicken B cells' innate response associated receptors during an IBDV infection and revealed that FA supplementation modulated the expression of important genes of the innate immune response and may have a effect in the control of IBDV replication in chicken B lymphocytes.

Key Words: IBDV, Folic acid, Innate immunity, TLR, BCR

M48, **A survey of wild type IBDV isolated from broiler flocks in the United States since 2014** Kalen Cookson*, Savannah Featherson, Andrew Barker, Jon Schaeffer *Zoetis*

Infectious bursal disease virus (BDV) is an extremely hardy double stranded RNA virus that can cause profound immune suppression—especially if infections occur prior to 3 weeks of age. This potential is high in the US because broilers are raised on built-up litter, resulting in a high infection pressure and keen competition between subtypes to override maternal antibodies and be the first to infect the next placement of birds. Not surprisingly, we see tremendous diversity of antigenic variant IBDVs in this country. This presentation will summarize the findings and trends in IBDV isolations from the last several years. In addition, results will be compared to a similar survey conducted between 2009-11 (Western Poultry Disease Conference, 2012) to see how the field challenge picture has changed over the past decade. Study Design: Five to six bursas were collected from over three hundred broiler flocks between 2-5 weeks of age. RT-PCR analysis was performed to amplify the VP2 gene and positive samples were then submitted for VP2 sequencing. The 139 IBDVs were divided into one of the following 5 variant categories: Del-E, AL2, T1, Group-6 and New-type. Results: The ranking of virus types by prevalence was AL2 family (52%), T1 (17%), New-type (15%), Del-E (8%) and Group-6 (8%). There were 4 unique AL2 subtypes with the original prototype still comprising the vast majority (82%). There were 3 subtypes of T1 virus, 5 subtypes of Group-6 and 4 subtypes of New-type—Del-E/GLS, Del-E/AL2, Del-E/Group-6 and Classic/Variant. Discussion: Compared to the 2009-11 survey, AL2 viruses remain the most prevalent type—making up over half of all field viruses. T1 viruses were again the second most common type recovered. New-types saw a big jump from 2% to 15%, perhaps at the expense of Del-E types, which fell from 21% to 9%.

Key Words: IBDV, PCR, survey, broilers, AL2

M49, **Pre- and post-challenge effects of essential oils (EO) on mRNA expression of antiviral and pro-inflammatory pathways genes on chicken macrophages infected with IBDV** Juan Rodriguez-Lecompte*[1], Santiago Uribe-Diaz [1], Blanca Martinez-Morales [2], Beatrice Despres [1], Julian Reyes [3] [1] *Atlantic Veterinary College -University of Prince Edward Island,* [2] *Promotora de innovacion en Biotecnologia (Promitec),* [3] *Research Group, Faculty of Agricultural Sciences, University of Antioquia*

A study was conducted to assess the pre and post-challenge effects of a single (Lippia origanoides)(Lo), Rosmarinus officinalis)(Ro) or blend of Lo and Ro of essential oils (EO) supplementation on innate immune response in chicken macrophages infected with Infectious Bursal Disease Virus (IBDV). Methods: Chicken macrophages (HD-11) cell line were cultured by triplicate at a concentration of 5x105 cells / well in plates of 24 wells. Macrophages were divided into five groups: 1. Negative control (No virus, no EOs); 2. Positive control (virus, no EOs); 3. No virus + EO (Lo); 4. No virus + EO (Ro); 5. No virus + EO (Lo + Ro); the macrophages received media supplemented with 50 ppm of Lo (group 3) or with 10-ppm Lo (group 5) and 100 Ro (groups 4 and 5). After 24 h of culture, cell were inoculated with a live vaccine of IBDV (ST-12) with a MOI=1 (Groups 2, 3, 4 and 5). Following of 1 h of virus absorption, the macrophages received again media supplemented with the same EO concentrations than before the challenge. This study evaluates periods of time 0, and 24 hours pre-infection and 0, 6 and 12 h post-infection (p.i). The research tested the effect of EOs single or blended supplementation on chicken macrophages gene expression of toll-like receptor 3, MDA5, cytokines IL-1β, IL-6, IL-12, IL-10, IRF-7, IFN-β, OAS and PKR as indicators of immunological cell activation during virus infection. IBDV virus was quantified and detected in chicken macrophages and supernatant using quantitative RT-PCR with SYBR green dye. An ANOVA model was calculated to determine the effect of Treatment group, gene and Time on the natural logarithm of CT values. Results: All groups with EO showed significant ($P < 0.05$) effect on virus load, decreasing it when compared with the positive control; however it was more significant with Ro EO ($P < 0.001$). Similar and correlated with virus infection EO affected significantly ($P <0.05$) the expression of IL-1β, IL-6, IL-12, IL-10, IRF-7, IFN-β, OAS and PKR genes in the pre-infection stage and the first hour post infection. Conclusion: The supplementation with a single or blend EO shown to reduce virus replication and enhance the innate immunity and correlates with both pro-inflammatory and antiviral pathways chicken macrophages

Key Words: Innate immunity, Essential oil, Macrophages, IBDV, Antivirus

Metabolism and Nutrition I, General Nutrition

M50, **Estimating energy utilization in laying hens using imaging technology** Alyssa Lyons*[1UG], Prafulla Regmi [2], Kenneth Anderson [2], Michael Persia [1] [1] *Virginia Tech,* [2] *North Carolina State University*

An experiment was conducted to evaluate the effects of dietary energy on the performance, and energy storage in first cycle laying hens housed in cages. Hy-Line W-36 laying hens were fed experimental diets from 36 to 52 weeks of age, including seven energy concentrations ranging from 2750 to 3050 kcal/kg with 50kcal/kg differences between each of the diets. Each dietary treatment was assigned 12 replicate cages and 3 birds per cage resulting in 36 hens per treatment or a total of 252 hens for the overall experiment. Diets were generated every two weeks. The hens were control fed 97 g of feed daily to mimic typical commercial feed intake. Eggs were collected and weighed on a daily basis. Egg production, energy intake, feed intake, egg weights, egg mass and feed conversion ratio were determined and calculated every two weeks. Hens were weighed every 4 weeks and all hens were euthanized and scanned using dual x-ray absorptiometry (DXA). Measurements taken over time (egg production, egg weight, egg mass, energy intake, feed intake, feed efficiency, and body weight) were analyzed using ANOVA with repeated measures and if significant differences were noted Tukey's test was used to separate means. Significance was accepted at ($P \leq 0.05$). Egg production (87.8 to 91.7% hen-housed egg production: SEM = 1.05), egg mass (52.9 to 55.1 g/d: SEM = 0.63), were not different ($P > 0.05$). These lack of differences in performance were expected due to the short-term nature of the experiment, as the laying hens continued to produce eggs at the expense of body energy reserves as indicated by the decreasing fatty tissue with reduced dietary energy. Unlike expectations with the controlled feeding protocol, hens on the two highest energy diets reduced feed intake in comparison to hens fed lower energy diets ($P \leq 0.05$). Fat storage, as indicated by carcass fatty tissue generally increased with increasing dietary energy with the exception of the 2850kcal/kg diet that resulted in increased carcass fatty tissue in comparison to both the 2900 and 2950 kcal/kg diets ($P \leq 0.05$). These results suggest that dietary energy did not have a significant effect on egg production but did generally increase carcass fatty tissue.

Key Words: dietary energy, laying hen, carcass composition, energy intake

M51, **Growth performance and carcass traits of broilers and Japanese quail fed selected grain sorghum varieties** Alissa Moritz*[1GS], Michael Blair [2], Robert Buresh [3], William Bridges [1], Richard Kaminski [1], Tiffany Wilmoth [1] [1] *Clemson University,* [2] *Devenish Nutrition,* [3] *Novus, International Inc.*

Modern varieties of U.S. grain sorghum (milo) are nearly 99% tannin-free; however, limited data is available to support that these varieties can be used as an alternative feedstuff to effectively replace corn in poultry and gamebird diets. Milo may be a viable alternative considering its resilience to adverse climate and soil types enabling it to be grown in locations coinciding with poultry and gamebird production in the southeastern U.S. This experiment determined growth performance and carcass traits of broilers and Japanese quail fed selected milo varieties: red/bronze, white/tan, U.S. No. 2 yellow and mixed. Trials evaluated the growth performance of Cobb 500 x Hubbard broilers (n = 550) 1 to 47 d of age, and Japanese quail (*Coturnix japonica*) (n = 480) 1 to 40 d of age randomly distributed at a density of 10 birds per pen. Birds were phase-fed 1 of 5 complete diets including a corn control and full-substitution milo treatments. Treatments for broiler trials included 10 replicates per treatment, and Japanese quail trials included 6 replicates for control, and 10 replicates for each of the milo treatments. Weekly measures of body weight and feed disappearance were used to calculate average daily gain, feed intake and feed conversion per treatment. At harvest, birds were evaluated for carcass and breast yields. An ANOVA was conducted to assess the effects of a full factorial of treatment, trial and treatment x trial. Broiler BW gain was significantly greater in white/tan and U.S. No. 2 yellow milo treatments than the control, red/bronze, and mixed treatments ($P \leq 0.0001$). Japanese quail diets showed no significant effects, but ADG and FC increased with increasing age ($P \leq 0.0001$). Results from growth performance trials indicate that modern milo varieties show potential for replacing corn in poultry and gamebird diets.

Key Words: broiler, growth, Japanese quail, grain sorghum, carcass quality

M52, **Effect of different inclusion levels of whole corn from 14 to 42 days on productive and processing performance of broilers.** Fozol Ovi*[GS], Wilmer Pacheco, Charles Starkey, Martha Rueda *Auburn University*

Previous researches have suggested that inclusion of whole grain has beneficial effects on broiler performance along with reduction of grinding costs. The objective of this study was to evaluate the effect of different inclusion levels of whole corn prior to pelleting on broiler growth performance, processing yield and organ development from 14-42 d. A total of 1,000 male Ross × Ross 708 broilers chicks were randomly distributed among 4 treatments with 10 replicate pens per treatment and 25 broilers per pen. The treatments consisted of four inclusion levels of whole corn (0%, 3%, 6% and 9%). A common starter diet was fed in crumbled form from 1 to 14 d of age. Treatment diets were provided from 14 to 42 d of age as 4-mm pellets. Body weight (BW) and feed intake were measured at 14, 28, and 42 d of age and feed conversion ratio (FCR) calculated by using the weight of the mortality. At 42 d, two birds from each pen were euthanized to determine relative weight of crop, proventriculus, gizzard, liver and ceca. 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 weight was 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 HSD procedure. The inclusion of whole corn did not influence BW, feed consumption, FCR, and organs weights (P>0.05) from 14 to 42 d of age. However, the weight of the proventriculus relative to BW decreased linearly as the inclusion of whole corn increased (P<0.05). Broilers fed diets with 9% whole corn had greater (P<0.05) carcass yield than broilers fed diets with 0% whole corn (77.86 vs 77.32%). Diets containing 9% whole corn had a significantly higher (P< 0.05) pellet durability index than feed containing 0% whole corn (61.12 vs 52.01). The results of this experiment indicated that up to 9% of whole corn could be used between 14 to 42 d with certain benefit on performance and carcass yield.

Key Words: Whole corn, Carcass, Yield, Proventriculus, Pellet

M53, **Evaluation of particle size, feed form and pellet diameter on broiler performance and processing yield from 1 to 39 days of age** Martha Rueda*[1GS]Susan Bonilla [1], Cleison de Souza [1], Fozol Ovi [1], Jessica Dunn-Starkey [1], Charles Starkey [1], Justina Caldas-Cuevas [2], Wilmer Pacheco [1] [1] *Department of Poultry Science, Auburn University,* [2] *Cobb-Vantress*

Particle size and feed form manipulation have emerged as alternatives to improve poultry performance. The objective of the study was to elucidate the effect of particle size and feed form on broiler performance and processing yield from 1 to 39 d of age. A total of 1800 d old male Cobb 500 birds were randomly assigned to 9 dietary treatments with 8 replicate pens per treatment and 25 birds per pen. The experiment consisted of a 3 × 3 factorial arrangement of 3 corn particle sizes (750, 1150 and 1550 μm) and 3 feed forms (mash, 3.0- and 4.0-mm pellets) provided from 1 to 39 d. Feed intake and BW were determined at 17, 27 and 39 d of age and FCR was calculated by using the weights of mortality. On d 40, 10 birds per pen were processed and on d 41, carcasses were deboned to determine meat yield. Data were statistically evaluated as a 3 × 3 (feed form × corn

particle size) factorial arrangement in a randomized block design. Broilers fed 3.0- and 4.0-mm pellets had higher BW, FI and lower FCR (P<0.05) compared to broilers fed mash at 39 d of age. Broilers fed diets with 750 µm corn particle size had higher BW (P<0.05) than broilers fed diets with 1550 µm at 39 d of age, while broilers fed diets with 1150 µm had an intermediate BW. Broilers fed diets with 750 µm corn particle size had higher FI (P<0.05) than broilers fed diets with 1150 and 1550 µm at 39 d of age. Particle size of corn did not influence FCR at 39 d of age. Broilers fed 3.0 mm pellets had the heaviest (P<0.05) carcass and breast weights, followed by broilers fed 4.0 mm pellets and the lowest weights by broilers fed mash. Tenders and wings weight were similar among broilers fed 3.0- and 4.0-mm pellets, but higher (P<0.05) than broilers fed mash. Broilers fed diets with 750 µm corn particle size had heavier carcass and breast weight (P<0.05) than broilers fed diets with 1550 µm, with 1150 µm having an intermediate carcass and breast weight. Furthermore, broilers fed diets with 750 µm corn particle size had higher tender weight and yield (P<0.05) than broilers fed diets with 1150 and 1550 µm. These results showed that feed form and particle size influence broiler performance and processing yield of Cobb 500 broilers.

Key Words: Cobb, feed efficiency, feed form, particle size

M54, **Effect of die retention time on pellet quality and phytase stability of a corn-soy diet** Caitlin Evans*[1GS], Marut Saensukjaroenphon [1], Joel McAtee [2], Cassandra Jones [1], Chad Paulk [1], Charles Stark [1] [1] *Kansas State University,* [2] *AB Vista*

The objective of this experiment was to determine the effect of pellet mill model (PM), die compression (L:D), and die retention time (DRT) on phytase stability (PS) and pellet quality. Treatments were arranged as a completely randomized design. Diets were pelleted using either a CPM 1012-2 HD Master Model or a 3016-4 Model equipped with a 4.8 × 50 mm (10.6 L:D), a 4.8 × 31 mm (6.6 L:D) or a 4.8 × 44 mm (9.3 L:D) with approximately 30 sec conditioning retention time at 85°C with a designated production rate. These processing conditions were used to create the following DRT treatments: 10.6 L:D with 4.3 sec DRT, 10.6 L:D with 2.9 sec DRT, 9.3 L:D with 1.7 sec DRT, 9.3 L:D with 1.1 sec DRT, 6.6 L:D with 2.6 sec DRT, and 6.6 L:D with 1.6 sec DRT. The PM were run 3 separate times to provide 3 replicates per treatment. There was an overall effect (P<0.001) of treatment on PS in cooled pellets. Phytase was more stable regardless of DRT when diets were manufactured with the 1012 PM using the 6.6 L:D die compared to the 10.6 L:D die (P<0.05). The hot pellet temperature (HPT) of 10.6 L:D die was 91–99°C, while 6.6 L:D die was 84–87°C; however, the PS was similar between the feed pelleted with 1012 PM equipped with 6.6 L:D die and the 3016 PM equipped with 9.3 L:D regardless of DRT (P>0.05). The HPT of feed pelleted with the 1012 PM equipped with 6.6 L:D die was 84–87°C, while the feed pelleted with the 3016 PM equipped with 9.3 L:D die was 82–84°C. As the die L:D increased (quadratic, P<0.001) PS decreased. Therefore, PM or DRT did not affect PS when the HPT was less than 87°C. Pellet quality increased (linear, P<0.001 for standard pellet durability index (PDI) or quadratic, P<0.001 for modified PDI) as die L:D increased. The die L:D had greater effects on both PDI methods than the DRT; however, increased DRT improved (P<0.05) pellet quality when the feed was pelleted with 6.6 L:D, but not when pelleted using the 9.3 or 10.6 L:D. In conclusion, the phytase that was produced by *Trichoderma reesei* strain could tolerate HPT up to 87°C, regardless of PM, L:D and DRT. However, PS was dramatically reduced when HPT ranged from 91–99°C. Therefore, HPT should be measured to monitor PS. Increasing L:D had the greatest effect on improving pellet quality.

Key Words: die retention, phytase stability, L:D ratio, pellet quality

M55, **The effect of screen hole diameter and hammer tip speed on the particle size of ground corn analyzed with and without sieving agent.** Nelsa Beckman*[UG], Caitlin Evans, Haley Wecker, Charles Stark, Chad Paulk *Kansas State University*

The objective of this study was to determine the effect of screen hole diameter, hammer tip speed, and the inclusion of sieving agent during analysis on the geometric mean diameter (dgw) and geometric standard deviation (Sgw) of ground corn. Treatments were arranged as a 4×6×2 factorial of screen hole diameter, hammer tip speed, and analysis method in a completely randomized design. Whole corn was ground using four common screen hole diameters (4.0, 4.8, 6.4, 9.5 mm) at 6 hammer tip speeds (6250, 5625, 5000, 4375, 3750, 3125 m/min). Ground corn samples were then analyzed for particle size with and without the inclusion of sieving agent according to ASAE standard S319.2. Each treatment replicate (n=3) was manufactured as a separate grinding queue with individual queue samples as the experimental unit. There was no evidence of interactions (P > 0.11) for screen hole diameter × hammer tip speed × analysis method or any of the two way interactions. Decreasing screen hole diameter from 9.5 to 4.0 mm decreased (linear, P < 0.01) dgw from 824, 706, 631, to 573 µm and Sgw from 3.18, 2.94, 2.76, and 2.79, and increased (linear, P < 0.01) percentage fines < 212 µm from 17.4, 18.4, 18.8, and 20.6%, respectively. Decreasing the hammer tip speed from 6250 to 3125 m/min increased (linear, P < 0.02) dgw from 473, 517, 596, 718, 853, to 944 µm and Sgw from 2.85, 2.89, 2.92, 2.90, 2.95, to 2.99, and decreased (linear, P < 0.01) percentage fines < 212 µm from 24.6, 23.1, 20.2, 16.8, 14.6, and 13.6%, respectively. Analyzing particle size using sieving agent decreased (linear, P < 0.01) dgw from 749 to 618 µm and increased (linear, P < 0.01) Sgw from 2.55 to 3.29 µm and percentage fines < 212 µm from 16.0 to 21.2%. In conclusion, reducing screen size and increasing the hammer tip speed reduced the mean particle size and standard deviation. Analyzing particles size using a sieving agent increases the movement of fine particles through the sieves, decreasing the average particle size and increasing the standard deviation.

Key Words: hammer mill, tip speed, particle size, sieving agent

M56, **Nutritional strategies for Cobb 500 broilers to maximize profitability and carcass yield** Maria Alfaro-Wisaquillo*[1GS], Edgar Oviedo-Rondon [1], Andres Ortiz [1,2], Yilmar Matta [1,2], Gherly Buitrago-Mejia [1,2], Jose Yanquen [1,2], Juan Martinez [1,2], Juan Hoyos-Torres [1,2], Hernan Cordova [1], Justina Caldas [3] [1] *North Carolina State University,* [2] *Universidad del Tolima,* [3] *Cobb Vantress Inc.*

Amino acid (AA) and energy levels during each feeding phase affect growth and carcass impacting profitability. This experiment evaluated the effects of strategies modifying either balanced protein or energy concentration in 4 feeding phases on broiler live performance, carcass yield, meat quality and profitability. Eight treatments resulted from a 4x2 factorial arrangement of 4 diets (T1: Control diet based on Cobb specifications; T2: T1 with +3%AA and -54 kcal/kg all feeding phases; T3: T1 with -3%AA and +40 kcal/kg only on finisher and withdrawal; T4: T2 for starter and grower and T3 for finisher and withdrawal) and sex with 6 replicate pens. After hatching and sexing, a total of 960-d-old Cobb 500SF chicks (480 each) were placed in 48 pens (20 chicks/pen) and fed Corn-SBM diets with DDGS. Group BW and feed intake (FI) were recorded and BW gain and FCR adjusted for mortality were calculated. Two chickens per pen were processed at 43 d, cut up parts were weighed, breast meat (BM) quality evaluated (pH, color, cooking and dripping loss) and BM myopathies scored. Interaction effects (P>0.05) were only observed on BW at 42 d of age and a* color value. Male broilers demonstrated to be heavier with BM more reddish when feeding T2. On live performance, diets affected (P<0.05) BW at 16 and 28 d while effects on FCR were detected between 17-28 d and 1-42 d. The best FCR was observed with T2 and the worst with T4, while T1 and T3 were intermediate. Despite no significant effects of diets were observed (P>0.05) on FI and flock uniformity, males ate more and were more (P<0.05) uniform (5.64%) than females (6.42%).

Dietary treatments did not caused effect (P>0.05) either on carcass yield or cut-up parts except for BM yield. The best BM yield (39.85%) was observed with T2 and the worst (38.39%) with T1. In BM quality, the pH 6-hour post-slaughter was higher for males than females, their dripping loss was higher and cooking lost lower than females. Male and female broilers had higher score of wooden breast (P<0.05) when fed T2 and lower when fed T1, while the other 2 were intermediate. In conclusion, modification of AA and energy levels on diets could be a strategy to improve Cobb 500 live performance, carcass yield, meat quality and profitability as it will be discussed.

Key Words: Broilers, nutrient levels, carcass, breast meat, profitability

M57, **Effects of rearing feeding programs in protein accretion, heat production, and body composition of broiler breeder pullets** Antonio Beitia[*1GS], Pramir Maharjan [1], Jordan Weil [1], Katie Hilton [1], Nawin Suesuttajit [1], Diego Martinez [1], Justina Caldas [2], Craig Coon [1] *[1] University of Arkansas, [2] Cobb-Vantress*

The objective of this study was to evaluate the effects of two different rearing programs on protein accretion (PA), heat production (HP), and body composition (BC) of broiler breeder pullets. 240-day old pullets were reared in 6 pens on 2 feeding schedules from 0-20 wks. Pullets were reared following the Cobb 500FF parent stock recommended growth curve. All treatments were fed the same Breeder 1 diet from 0-20 wks. The pullets were feed restricted with two rearing programs -skip a day (SD) and everyday (ED) with SD fed twice the amount of ED and weighed weekly with feed allotment adjusted to achieve desired body weight. At 16 wks, 6 birds from each feeding program were selected every 4 hours for sampling. This was done for a 24-hour period for the ED birds and for 48 hours for the SK birds. A CRD was employed with 2 treatments and 13 and 7 time periods for SK and ED respectively. 15N-phenylalanine (Phe) (150mM, 40% atom percent excess (APE)) was given to each selected bird by an intravenous flooding-dose of 10 ml/kg. After 10 min, birds were sacrificed by CO_2 inhalation and *Pectoralis major and* excreta were sampled for protein turnover (PT) determination. 15N-Phe enrichment and 3-methyl histidine quantitation were determined in skeletal tissue and 3-methyl histidine was quantified in excreta using GC-MS. Fractional protein synthesis (FSR) and fractional degradation rate (FDR) were determined as %/h. The HP was measured every 2 wks from 6 -18 wks using respiratory chambers. Volumes of oxygen (VO2) and carbon dioxide (VCO2) were measured, and HP calculated using HP (kcal) = 3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974). Body composition (BC) was measured every 2 wk from 6-20 wks by dual energy X-ray absorptiometry. ANOVA was performed using JMP software. Results showed that FDR was higher for SD (1.53%) in comparison to ED (0.48%) (P <0.05). PA was lower for SK than ED (P <0.05) at 12 and 24 hr post feeding (P <0.05). HP was higher in SK than ED pullets from 6-18 wk (P <0.01). SK pullets had a lower lean mass, protein mass, and higher fat mass g/kg BW at 6 wk (P <0.05) in comparison to ED pullets. Findings suggest that pullets mobilize body protein with SK feeding program in an attempt to sustain energy status in comparison to ED during the rearing period.

Key Words: Protein Accretion, Heat Production, Body Composition, Broiler Breeders

M58, **Apparent metabolizable energy content of 11 sources of distillers dried grains with solubles determined in broiler chickens at 3 weeks of age** Kenia Mitre[*1GS], Kevin Herrick [2], Samuel Rochell [1] *[1] University of Arkansas, [2] POET Nutrition*

Distillers dried grains with solubles (DDGS) is a co-product of the corn ethanol industry that is commonly used as a poultry feed ingredient in the United States. However, nutritional variability of DDGS, particularly in its metabolizable energy (ME) content, can be a limiting factor for its use. An experiment consisting of 2 identical trials was conducted to determine the ME and nitrogen-corrected ME (ME_n) of 11 DDGS samples obtained from different biorefinery locations operated by a single ethanol producer. The DDGS samples ranged in crude protein from 29.61 to 33.88%, ether extract from 5.82 to 9.66%, acid detergent fiber from 7.71 to 11.30%, and neutral detergent fiber from 24.08 to 31.35% (DM basis). In each trial, 576 off-sex male Cobb 500 breeder chicks were allocated to 72 battery cages. A total of 23 treatments included a basal diet with 0% DDGS and 22 test diets in which energy providing ingredients in the basal diet were replaced with 15 or 30% DDGS from each of the 11 sources. There were 12 replicates cages for the basal diet (6 per trial) and 6 replicates for the test diets (3 per trial) with 8 chicks per cage. Birds were provided a common starter diet from 0 to 14 d and experimental diets from 14 to 21 d, with a 48 h total excreta collection conducted from 19 to 21 d. Feed and excreta samples analyzed for DM, nitrogen, and gross energy content to determine the ME and ME_n of complete diets using the total collection method. For each source, dietary ME or ME_n was regressed on DDGS inclusion (%) using simple linear regression, and ME and ME_n estimates were based on extrapolation of dietary ME or ME_n at a dietary DDGS inclusion of 100%. Linear regression slopes for ME and ME_n were significantly different than zero for 9 of the 11 sources, with R^2 values ranging from 0.02 to 0.79 and 0.02 to 0.82, respectively. The ME values of the DDGS ranged from 2,477 to 3,263 and averaged 2,913 kcal/kg, while the ME_n values ranged from 2,284 to 3,088 and averaged 2,765 kcal/kg (DM basis). These results confirm that the energy content of DDGS for broilers can vary among biorefinery locations using similar ethanol production practices. Ongoing research to correlate and predict DDGS ME based on its nutrient composition will facilitate its use in feed formulation.

Key Words: Broilers, metabolizable energy, DDGS, energy prediction

M59, **Effect of Enogen® feed corn on broiler growth performance and carcass traits** Courtney Truelock[*GS], Christopher Delfelder, Adrian Baker, Chad Paulk, James Drouillard *Kansas State University*

This experiment evaluated the effect of corn type and conditioner retention time on broiler performance. Treatments were arranged in a 2 × 2 factorial of corn source (conventional [CON] and Enogen® feed corn [EFC, Syngenta Seeds, LLC]) and conditioner retention time (30 and 80 s). Twelve hundred male broiler chicks (Cobb-Vantress, Siloam Springs, AR) were used in a 45-d experiment with broilers randomly assigned to 1 of 80 floor pens with 15 broilers per pen. Treatments were randomly assigned to pens and blocked by location for a total of 20 replications per treatment. Diets were steam-conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and pelleted (1012-2 HD Master Model, California Pellet Mill), and starter diets were crumbled (Colorado Mill Equipment EcoRoll 7). Starter, grower, and finisher diets were fed from d 0 to 10, 11 to 24, and 25 to 45, respectively. Pen weights and feed consumption were measured on d 11, 25, 39, and 45 for calculation of body weight gain, feed intake, and feed efficiency. All chicks were harvested for determination of carcass traits. Data were analyzed using the GLIMMIX procedure in SAS 9.4. There was no evidence (P < 0.18) for a corn type × conditioner retention time interaction or a main effect of conditioner retention time. From d 0 to d 11, FCR was improved (P < 0.01) in chicks fed EFC, but there was no difference (P > 0.16) in BW, ADG, or ADFI between treatments. From d 11 to d 25, BW, ADG, and ADFI were greater (P < 0.01) for chicks fed EFC compared to those fed the CON. From d 0 to 39 birds fed EFC had increased (P < 0.04) BW, ADG, and ADFI compared to CON. From d 0 to 45, birds fed EFC had increased ADFI (P = 0.05) and poorer (P = 0.03) FCR compared to those fed CON. Chicks consuming EFC diets had greater (P < 0.03) carcass weights than those consuming CON diets at d 39 (2.57 vs 2.50 kg, respectively) and d 45 (3.11 vs 3.06 kg, respectively). Furthermore, carcass dressing percentage was greater (P< 0.02) for chicks on the EFC treatments than the CON treatments at d 39 (70.2 vs 69.5%, respectively) and d 45 (71.0 vs 70.3%, respectively). In conclusion, broiler

chicks receiving EFC had greater BW and ADFI resulting in heavier carcasses and greater dressing percentages.

Key Words: broilers, carcass weight, Enogen feed corn, growth performance

Metabolism and Nutrition II, General Nutrition / Vitamins & Minerals

M60, **Corn kernel hardness, drying temperature and storage affected the nutritional value of corn: a comparative analysis among NIRS prediction models** Hernan Cordova-Noboa[*1], Edgar Oviedo-Rondón [1], Andres Ortiz [1,2], Yilmar Matta [1,2], Juan Martinez [1,2], Juan Hoyos [1,2], Jose Yanquen [1,2], Daniela Buitrago [1,2], Ivan Ospina [1], Miguel Chico [1], Lina Peñuela [1,2] *[1] North Carolina State University, Prestage Department of Poultry Science, [2] Universidad del Tolima, Facultad de Medicina Veterinaria y Zootecnia*

NIRS is a cost efficient and fast indirect method to analyze the nutrient content of feedstuffs. This study evaluated the effects of kernel hardness, and drying temperature on nutritional content in corn stored for two or 24 wk. Six treatments resulted from a factorial arrangement of two kernel hardness (average and hard), and three drying temperatures (35, 80, and 120°C). Subsequently, five samples per treatment were ground and read in a FOSS NIRS DS2500. The non-standardized spectra of each replicate was sent to four companies to determine nutrient composition using their prediction models. Data were analyzed in a completely randomized design using ANOVA that contained 4 variables (kernel hardness, drying temperature, storage, and calibration model) with all possible interactions, and mean separation by Tukey's or t test. Effects were evaluated on CP, starch, and vitreousness. Results of corn stored for 2 wk from 3 models showed that average hardness kernels dried at 35°C had higher ($P \leq 0.001$) CP and less ($P < 0.001$) starch than similar corn but dried at 80 and 120°C. At each drying temperature level, hard kernel corn had greater ($P < 0.001$) vitreousness than treatments with average kernel hardness. Storing corn for 24 wk increased vitreousness ($P < 0.001$) when hard kernels where dried at 80 and 120°C, but an opposite effect was detected for corn with average hardness. Among treatments with hard kernel corn, drying at 120°C resulted in 2% less ($P < 0.01$) starch compared to corn dried at 35 and 80°C, and this was observed by 2 prediction models. For average kernel hardness, drying at 35°C resulted in more CP ($P < 0.001$), whereas for hard kernel corn the greatest CP was detected at 120°C. This effect was observed in 3 models. Results revealed that storing corn for 24 wk increased ($P < 0.001$) CP (R^2=0.89), and reduced the starch (R^2=0.97) content in all treatments except for average kernel corn dried at 120°C. Vitreousness (R^2=0.95) was increased in hard kernel dried at 120°C, but opposite response was observed in average hardness kernels. In conclusion, kernel hardness, drying temperature, and storage influenced corn nutritional value, suggesting possible chemical changes in complex compounds between protein and starch during storage related to vitreousness.

Key Words: NIRS, kernel hardness, drying temperature, stored corn, nutrient content

M61, **Growth performance, processing characteristics, and tibial breaking strength of broilers fed diets varying in calcium and Signis inclusion to 42 d of age** Stephanie Philpot[*1GS], Craig Wyatt [2], William Dozier, III [1] *[1] Auburn University, [2] AB Vista Inc.*

Excess Ca may buffer the upper gastrointestinal tract and reduce absorption of Ca, P, and amino acids. High phytase supplementation (1,500 phytase units) may allow for reduced dietary Ca and P inclusion. Signis is a novel feed additive consisting of xylanase and short chain xylooligosaccharides that may enhance amino acid digestibility through increased fiber fermentation in the cecae. An experiment was conducted to evaluate the interactive effects of Ca concentration and Signis. One thousand eight hundred male Yield Plus × Ross 708 chicks were randomly distributed to 60 floor pens (30 birds/pen; 0.08 m^2/bird). Six dietary treatments (10 replicates/treatment) consisted of a 3 × 2 factorial arrangement of 3 Ca feeding programs with or without 0.01% inclusion of Signis. All diets were formulated to contain 1,500 phytase units. The 3 Ca programs provided 0.95-0.85-0.75, 0.85-0.75-0.65, or 0.75-0.70-0.65% Ca in the starter, grower, and finisher periods, respectively. Birds and feed were weighed at 15, 30, and 42 d of age. Broilers were necropsied at 15 and 45 d of age for collection of tibias (8 or 4 birds per pen, respectively). Tibias were broken using a texture analyzer to calculate shear force required to fracture the bone. Data were analyzed for main and interactive effects using a two-way analysis of variance. Calcium inclusion had no effect ($P \geq 0.12$) on growth performance or processing characteristics. Signis inclusion increased ($P \leq 0.01$) feed conversion ratio compared to birds that did not receive Signis during all growth periods. Signis inclusion also decreased abdominal fat weight and percentage ($P \leq 0.0002$) and increased breast meat yield ($P < 0.0001$) compared with broilers that did not receive Signis. Interactive effects were observed for broilers fed diets with 0.75% Ca and Signis had elevated ($P = 0.0001$) feed conversion ratio compared with birds that received diets without Signis and either 0.75 or 0.85% Ca from 1 to 15 d of age. No differences were observed for tibial breaking strength at 15 or 45 d of age. These results indicate that Ca concentrations can be reduced when using high phytase supplementation (1,500 phytase units) without impairing growth performance, processing yields, or bone strength.

Key Words: Bone strength, Broiler, Calcium, Enzyme

M62, **The Effect of Dietary Calcium Inclusion Rate, Source, and Particle Size on Intestinal Health Parameters During a Naturally Occurring Necrotic Enteritis Outbreak** Christopher Eagleson[*1GS], Ariel Bergeron [1], Garrett Powell [1], Chance Williams [2], Craig Wyatt [3], Dan Klamfoth [4], Audrey McElroy [1] *[1] Texas A&M University, [2] Wayne Farms LLC, [3] AB Vista, [4] Lhoist North America*

Necrotic enteritis prevention and mitigation in commercial no antibiotic ever broiler flocks requires consideration of management as well as dietary strategies. Previously we reported research indicating differences in calcium inclusion rate, source, and particle size reduced broiler BW, increased FCR, and increased mortality during the grower phase. To better understand the interaction between live performance and intestinal health: pH, intestinal lesions from coccidia, bone mineralization, and digestibility were evaluated at d 14 and 25. A total of 3,600 d-old Ross male broilers were assigned to 8 dietary treatments (trt) fed in 3 phases (0-14 d, 14-28 d, and 28-35 d). Each trt was replicated by 9 pens. NE was allowed to occur naturally following mild intestinal response to day of age coccidia vaccination. Dietary treatments were a factorial of 2 Ca levels (1.05% total Ca and 0.75% total Ca), 2 Ca sources (source 1 and source 2), and 2 particle sizes (standard and fines). All data were subjected to ANOVA using the GLM procedure. Means were deemed significantly different at P≤0.05 and separated using Tukey's HSD. A 2x2x2 factorial analysis was used to determine the impact of dietary Ca inclusion rate, Ca particle size, and Ca source. There were no differences in the coccidia lesion scores or bone mineralization at d 14 or 25 with subclinical NE persisting. At d 14, there was a Ca level X source interaction ($p<0.05$) with birds consuming low Ca levels having increased Ca digestibility compared to the standard Ca level. At d 25, there was a difference in Ca levels with high levels resulting in reduced digestibility. At d 14 and 25, there was a Ca level X particle size interaction ($p<0.05$) with birds consuming low Ca levels having increased

P digestibility. The Ca levels resulted in a difference in ileal pH at d 14. At d 25, there was a Ca size X source interaction (p<0.05) with birds consuming standard particle size of source 1 having increased ileal pH compared to both fine particle sizes and source 2. Dietary Ca levels, source, and particle size not only had an effect on live performance and mortality, but indices of local intestinal health and function were also altered further suggesting a role of dietary Ca in NE pathogenesis.

Key Words: Broiler, Necrotic Enteritis, Calcium

M63, **Effect of maternal and post-hatch dietary 25-hydroxycholecalciferol on broiler chicken growth performance and carcass characteristics** Luis Avila*[1GS], Samuel Leiva [1], Gerardo Abascal-Ponciano [1], Joshua Flees [1], Kelly Sweeney [2], Jeanna Wilson [2], Anthony Pokoo-Aikins [3], Gilberto Litta [3], Charles Starkey [1], Jessica Starkey [1] *[1] Auburn University Department of Poultry Science, [2] University of Georgia Department of Poultry Science, [3] DSM Nutritional Products*

Dietary inclusion of the vitamin D3 (D3) metabolite, 25-hydroxycholecalciferol (25OHD3), has been previously demonstrated to positively impact broiler chicken growth performance and carcass yield. To evaluate the effect of combined maternal and post-hatch dietary 25OHD3 inclusion on broiler chicken growth performance and carcass characteristics, a randomized complete block design experiment with the main effects of maternal diet (MDIET) and post-hatch diet (PDIET) arranged in a 2 × 2 factorial treatment structure was conducted. All diets were formulated to provide an equivalent of 5,000 IU of vitamin D per kg of feed. From 25 to 38 wk of age, commercial parent stock broiler breeder hens reared without 25OHD3 were provided 1 of 2 MDIET: 5,000 IU D3 (MCTL; ROVIMIX D3 500) or 2,240 IU of D3 + 2,760 IU of 25OHD3 per kg of feed (M25OHD3; 69 µg ROVIMIX Hy·D). Their broiler chick offspring (n = 448) hatched from eggs collected from 37 to 38 wk of age were reared in floor pens (7 birds per pen; 16 replicate blocks) and fed 1 of 2 PDIET in 3 phases (starter d 0 to 14, grower d 15 to 28, and finisher d 29 to 40): 5,000 IU of D3 per kg of feed (PCTL) or 2,240 IU of D3 + 2,760 IU of 25OHD3 per kg of feed (P25OHD3) from 0 to 40 d of age. Data were analyzed using SAS (V9.4) PROC GLIMMIX and PDIFF for mean separation at $P < 0.05$. No MDIET × PDIET interactions were observed ($P > 0.05$). MDIET and PDIET did not alter d 0 to 40 broiler BW gain, FI, and FCR ($P \geq 0.1284$). Broilers from 25OHD3-fed hens were 2.7% heavier on d 40 than those from hens fed only D3 (2.911 vs. 2.834 kg; $P = 0.0397$). Tender wt (123 vs. 117 g) and yield (5.63 vs. 5.44%) were greater in M25OHD3 broilers than MCTL broilers ($P = 0.006$). Breast, wing, thigh, drum, and fat pad yields were similar among MDIET ($P \geq 0.2120$). Broilers fed 25OHD3 post-hatch (P25OHD3) tended to have heavier breasts (637 vs. 615 g; $P = 0.0503$), bone-in wings (215 vs. 210 g; $P = 0.0703$), and boneless thighs (279 vs. 270 g; $P = 0.0776$) compared with those fed only D3 (PCTL). Neither MDIET nor PDIET altered the incidence or severity of the meat quality defects, Wooden Breast and White Striping ($P \geq 0.1059$). Overall, dietary inclusion of 25OHD3 in maternal and post-hatch broiler chicken diets positively influenced carcass characteristics.

Key Words: 25-hydroxycholecalciferol, vitamin D, broiler breeder, maternal nutrition, broiler chicken performance

M64, **Additivity of apparent and standardized ileal digestibility of phosphorus in corn and soybean meal mixed diets fed to broiler chickens** Olufemi Babatunde*[GS], Saheed Osho, ChanSol Park, Olayiwola Adeola *Purdue University*

A total of 512 male Cobb 500 broiler chickens was used to investigate the additivity of apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of P in mixed diets containing corn and soybean meal (SBM) with or without phytase supplementation. To test the additivity of digestibility values in the mixed diets, the difference between the predicted values calculated from the measured values in the individual ingredients and the determined values in the mixed diets were examined. Birds were fed a starter diet from d 1 to 21 post hatching and then randomly allotted to 7 dietary treatments in a randomized complete block design. Four semi purified diets were prepared to contain corn or SBM as a sole source of P with or without the addition of phytase at 1,000 phytase units/kg diet. Two mixed diets were also prepared to contain corn and SBM with or without the addition of phytase at 1,000 phytase units/kg diet. A P-free diet (PFD) was formulated to estimate the basal ileal endogenous loss (BEL) of P. Chromic oxide was included in all diets as an indigestible marker. Birds were fed the experimental diets for 3 d. On d 24 post hatching, ileal digesta of birds was collected from the distal two-thirds of the ileum. The BEL of P as determined by the PFD was estimated to be 166 mg/kg dry matter intake. The addition of phytase improved ($P < 0.05$) the AID and SID of P in corn and SBM. The determined AID or SID of P in corn and SBM with or without phytase were used to predict the AID or SID of P in the mixed diets. The predicted AID and SID of P in the mixed diets with or without phytase supplementation were consistent ($P > 0.05$) with the determined values and were thus additive. The presence of phytase in the mixed diet did not affect the additivity of AID and SID of P. However, the extent of difference between the predicted and determined AID values for the mixed diet containing corn, SBM and phytase was higher than the difference in SID values, while the reverse was the case with the mixed diet containing only corn and SBM. In conclusion, phytase supplementation improved the digestibility of P in corn and SBM fed to broiler chickens. The AID and SID of P in corn and SBM were additive in the mixed diets with or without the addition of phytase.

Key Words: additivity, corn, digestibility, phosphorus, soybean meal

M65, **Effect of coarse corn particle size and organic mineral source in male turkeys' production.** Karlinton Flores*[GS], Adam Fahrenholz, Jesse Grimes *North Carolina State University*

Increasing the turkeys' feed efficiency and decreasing the cost of production are key challenges in the poultry industry. The increase of particle size of corn through feed phases, matching with the turkey's beak growth, could decrease the energy cost of corn milling. Chelated minerals with organic compounds could decrease the amount of minerals available to pathogens by increasing the absorbability of the minerals. The objective of this experiment was to determine the effect of increasing corn particle size through feed phases and the use of organic minerals on turkey performance, feed mill efficiency and processing at market age. Nicolas Select male poults (1200) were randomly assigned to 48 concrete-litter-floor pens. The experimental design was a completely randomized block design with a 2×2 factorial arrangement of 2 levels of corn particle size, increasing coarse (IC) vs fixed fine (FF) and 2 mineral sources, organic (ORG) vs inorganic (INO). Minerals chelated with methionine (organic) where Zn and Mn (Global Animal Products, Amarillo, TX). The particle size of the corn was increased in each phase by 500 microns form the starter (1000 µm) to the finisher 2 (3500 µm) by mixing pelleted feed with FF corn (270 µm) with coarse corn (2500 µm) and whole corn (10000 µm) after pelleting. Bird body weight (BW), weekly body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), corn particle size, pellet durability index (PDI), breast yield, pellet mill energy consumption were determined, and differences were considered to be statistically significant at $P \leq 0.05$ using a mixed model in SAS 9.4. The energy consumption of the pellet mill was lower in the IC when compared with the FF treatment (7.54 vs 11.71 ± 0.46 kW/hr/ton). Birds fed FF treatment had a higher BW when compared to IC fed birds at 18 wk (20.634 vs 20.375 ± 0.086 kg). The ORG treatment fed birds had a greater BW than birds fed the INO source (20.626 vs 20.383 ± 0.086 kg) at 18 wk and higher minor breast (5.98 vs 5.70 ± 0.08 %) and total breast yields (31.05 vs 31.73 ± 0.17 %) at 19 wk. In conclusion, ORG improved BW and % breast yield. Further economic analysis should be conducted to determine if the loss in BW by feeding IC could be outset by the energy savings at the feed mill.

Key Words: Turkey, Chelated, Corn particle size, Organic minerals

M66, **Effects of split feeding limestone particle size ratios on egg production, egg quality and eggshell strength of White Leghorn W-36 hens in the late production phase.** Ishab Poudel[*GS], Christopher McDaniel, M Schilling, Pratima Adhikari *Mississippi State University*

As we progress towards extending the production cycle of the modern laying hen beyond 65 to 70 wk of age, it is vital to develop effective nutritional strategies that could sustain egg production and maintain egg quality. A 12-wk study was performed to evaluate the effects of feeding various ratios of fine (F) and coarse (C) limestone in the diet continuously (conventional) or only in the afternoon (split) on egg production and egg quality. Six hundred and forty W-36 laying hens at 60 wk of age were randomly placed in conventional A-frame cages. Each cage contained 4 hens, and 2 sequential cages were considered as an experimental unit. The design was a randomized complete block with a split plot over hen age. A 2 X 4 factorial arrangement of two feed distribution strategies (Conventional and split) and four ratios of fine and coarse limestone (35 F: 65 C, 25 F: 75 C, 15 F: 85 C, and 0 F: 100 C) was utilized. Nine isoenergetic and isonitrogenous diets were formulated with 4 treatments that received the same diet in morning and afternoon and the other 4 treatments that received a special diet without limestone in the morning but with limestone in the afternoon. Morning diets were fed at 7:30 AM and afternoon diets were fed at 3:30 PM. The results showed that birds fed the 0 F: 100 C and 15 F: 85 C diets had higher eggshell breaking strength (EBS) as compared to the other limestone ratios (p=0.046). EBS was highest for the 0 F:100 C treatment group at 65 wk. Furthermore, an interaction between age and feeding strategy (p=0.041) was observed on EBS with the conventional feeding showing significantly higher EBS at 65 wk. A trend in the shell percentage (SP) was observed (p=0.057) with higher SP in 0 F: 100 C treatment group. Specific gravity (p<0.001) , percent albumen (p<0.001) , percent yolk (p<0.001), yolk color (p=0.014) and ST (p<0.001) significantly increased with age; whereas, egg weight (p=0.004), PS(p<0.001) and EBS (p=0.0013) decreased with age. In conclusion, conventional feeding at 65wk and birds fed coarse calcium diets (0 F: 100 C and 15 F: 85 C) laid eggs with higher EBS. Therefore, conventional feeding of a high coarse calcium diet can be an effective strategy to reduce egg breakage and maintain eggshell property at the late production stage.

Key Words: laying hen, split feeding, particle size, limestone

Metabolism and Nutrition III, Amino Acids/Enzymes

M67, **Effects of dietary amino acid and metabolizable energy reduction on growth performance and economic return of Ross 708 broilers** Bo Zhang[*GS], Sabin Poudel, Linan Jia, Wei Zhai *Mississippi State University*

Most broiler nutrient requirement suggestions are given to maximize growth rate and minimize feed conversion ratio (FCR). Economic return and profit analysis are often neglected. In a previous study, we found that 20% amino acid (AA) reduction increased FCR in all 5 commercial broilers tested, but the cost of feed required to correspondingly increase BW was decreased in 4 out of the 5 strains. In the current study, a randomized complete block design with a factorial arrangement of 12 treatments (3 ME × 4 AA) was used. The 3 ME levels were 100, 92, and 84% of the commercially recommended level. The 4 levels of digestible AA (lysine, TSAA, and threonine) were 100, 90, 80, and 70% of the recommended level. A total of 864 Ross × Ross 708 broilers were randomly allocated to 6 blocks. Each block contained 12 pens with 12 birds per pen. Data were analyzed using a two-way ANOVA. Significance level was set at $P < 0.05$. Mortality was not affected by any treatments ($P > 0.05$). The combination of lowest dietary AA level (70%) and highest ME level (100%) resulted in the lowest BW from d 10 ($P = 0.003$) and through the remainder of the study period, including d 24, 34, 41, 48, and 55 ($P = 0.007$, 0.009, 0.006, 0.0004, and 0.002, respectively). Birds fed 70% AA exhibited higher FCR from d 10 to 24, as compared to birds fed 80, 90, and 100% dietary AA ($P < 0.0001$); however, the 70% of AA decreased FCR from d 48 to 55, as compared to 90 and 100% AA ($P = 0.024$). The lowest ME level (84%) increased FCR from d 41 to 48 ($P = 0.015$). Due to the lower cost of feed with lower AA and ME, the economic return to produce the same amount of BW on d 41 was increased by feeding the birds 70 and 80% AA as compared to 100% AA ($P = 0.007$); similarly, the economic return on d 55 was increased by feeding the birds 70 and 80% AA as compared to 90 and 100% AA ($P < 0.0001$). The economic return was also increased by feeding the birds 84 and 92% ME as compared to 100% ME on both d 41 and 55 ($P < 0.0001$). In conclusion, adequate dietary AA is critical for early growth of young birds, and adequate ME is critical to feed utilization in older birds. Economic profit depends not only on growth rate and feed conversion efficiency of the birds, but also on the cost of the feed.

Key Words: amino acid, energy, broiler, economic return, growth performance

M68, **Determining the digestible isoleucine requirement of Ross x Ross 708 male broilers from 0 to 18 days of age.** Andrew Brown[*1GS], Jason Lee[2], Kelley Wamsley[1] [1] *Mississippi State University,* [2] *BIO Business for North America, CJ America Inc.*

Commercial poultry diets are typically formulated on a digestible amino acid basis using the ideal protein ratio concept. Continuous genetic improvement of high yielding broiler strains requires the reevaluation of essential amino acids. The objective of the study was to evaluate the dIle requirement of Ross x Ross 708 male broilers from d0-18. Experimental diets were created from a common deficient corn and soybean meal-based diet containing a dIle:dLys of 52%, after manufacture half of this treatment was retained for the creation of Treatment 7, the summit diet (82% dIle:dLys) by crystalline Ile addition. The remaining 5 experimental diets (Trts 2-6) ranged from 57 to 77% dIle:dLys, obtained by blending different proportions of deficient and summit dIle diets. A practical control diet (Trt 8) was formulated to 67% dIle:dLys and batched separately. A total of 2,400 Ross x Ross 708 male broilers were obtained from a commercial hatchery and equally allocated to 96 pens (12 replications/treatment). Birds were individually weighed and feed intake was recorded on d18 to determine average BW, BW gain, bird uniformity, feed intake/bird (FI), % mortality, and feed conversion ratio (FCR). All dIle requirements were estimated using quadratic regression (95% of the asymptote; QR), as well as linear and quadratic broken line models (LBL; QBL). The QR model for BW and BWG suggests the requirement for dIle to be 0.89% (73.2% dIle:dLys). When utilizing the LBL model, the requirement was estimated to be 0.76% (63% dIle:dLys), while the QBL estimated the requirement to be 0.80% (65.8% dIle:dLys). Additionally for d0-18 FCR, the QR model estimated the requirement to be 0.88% dIle (72.4% dIle:dLys) while the LBL model estimated the requirement to be 0.77% (63.3% dIle:dLys) and the QBL model estimated it to be 0.90% (74% dIle:dLys). These data are an important step in continued evaluation of the dIle:dLys requirement of Ross x Ross 708 male broilers which is continuously under genetic selection to improve performance and meat yield. Future research should determine the dIle requirement of Ross x Ross 708 male broilers utilizing similar methodology during the remaining grow-out phases to optimize performance and the use of nutrients in least cost commercial formulation.

Key Words: Amino Acids, Requirement, Digestible Isoleucine, Growth Performance, Live Performance

M69, **Determination of the optimal digestible isoleucine to lysine ratio for broilers from 28 to 42 days of age.** Tanner Wise*[1GS], Kevin Touchette[2], Paul Tillman[3], William Dozier III[1] [1] *Auburn University,* [2] *Ajinomoto Animal Nutrition North America Inc,* [3] *Poultry Technical Nutrition Services LLC*

Isoleucine is the fifth limiting amino acid (AA) for broilers fed diets containing feedstuffs of vegetable origin. An experiment was conducted to determine the optimal digestible (dig) Ile to Lys ratio for the growth performance and processing yields of broiler chickens from 28 to 42 d of age. Sixteen hundred Yield Plus × Ross 708 male chicks were placed in 64 floor pens (25 birds/pen) at 1 d of age. From 1 to 27 d of age, birds were fed a common diet formulated to meet or exceed nutritional requirements. Each pen received 1 of 8 dietary treatments (8 replicates) from 28 to 42 d of age. Diets were formulated with corn and soybean meal as primary ingredients, and diets 1 to 7 contained 2% spray-dried blood cells to create a negative control deficient in dig Ile. L-Isoleucine was added to create dose response diets of dig Ile. Diets were formulated to be adequate in all AA except for Ile and Lys. Diet 8 was a positive control (PC) formulated to contain 0.95% dig Lys with a dig Ile to Lys ratio of 0.70. Diets 1 to 7 were formulated to contain 0.95% dig Lys with dig Ile to Lys ratios ranging from 0.46 to 0.82 in increasing increments of 0.06. Feed and birds were weighed at 28 and 42 d of age to determine BW gain (BWG), feed intake, and feed conversion ratio (FCR). On day 42, 14 birds per pen were randomly selected and processed to determine carcass characteristics. Linear and quadratic regression analyses, as well as a preplanned orthogonal contrast between the PC and treatment 5, were performed. Broken-line regression analysis was also utilized to determine the optimal dig Ile to Lys ratio for BWG, FCR, total breast meat weight (BMW), and total breast meat yield (BMY). Male broilers fed gradient concentrations of dig Ile displayed quadratic responses for BWG, FCR, BMW, and BMY ($P < 0.001$). Linear broken-line regression indicated the optimal dig Ile to Lys ratio was 0.67 (±0.006 SE) for BWG ($P < 0.001$), FCR ($P < 0.001$), and BMW ($P < 0.001$). Broken-line regression also indicated that the optimal ratio for BMY was 0.68 (±0.01 SE; $P < 0.001$). Based on these data, the estimated optimal dig Ile to Lys ratio for BWG, FCR, BMW, and BMY ranges from 0.67 to 0.68 for Yield Plus × Ross 708 male broilers from 28 to 42 d of age based on linear broken-line analysis.

Key Words: isoleucine, broiler, amino acid

M70, **Determination of dietary digestible valine:lysine ratio for Cobb MV × 500 male and female broilers from 15 to 35 d of age** Craig Maynard*[1GS], Sonia Liu[2], Jason Lee[3], Justina Caldas[4], Edward Diehl[4], Samuel Rochell[1], Michael Kidd[1] [1] *Center of Excellence in Poultry Science, University of Arkansas,* [2] *The University of Sydney,* [3] *CJ America,* [4] *Cobb-Vantress*

Knowledge of dietary Val needs in commercial broilers is paramount for least cost diets and performance efficiencies as Val is typically the fourth limiting amino acid in diets based on cereals and oilseeds. Two concurrent studies were conducted to determine Val:Lys ratios needed for optimal performance and carcass traits for male (Exp 1) and female (Exp 2) broilers during 15 to 35 d of age. Two diets were formulated: a corn and soybean meal control and a valine deficient corn and soybean meal diet. Aliquots of L-Val were then added to the Val deficient diet, producing diets with Val:Lys ratios of 57, 64, 71, 78, 85, and 92. An additional diet was created using the titration diet with a Val:Lys ratio of 78 and an increased Ile:Lys ratio of 66 to 70. All diets were formulated to be equal in ME (3,100 kcal/kg diet) and Lys (1.06% digestible of diet). Diets were fed to 6 replicate pens of 21 birds in both Exp. Gain of BW, feed intake, and feed conversion ratio were determined for the 15 to 35 d period, and carcass traits and breast meat quality were evaluated on d 36. All data were analyzed by regression analysis and requirements were estimated at 95% of the quadratic response to prevent overestimation. In both Exp, the control diet performed better than the comparable titration diet with a 78 Val:Lys ratio. In Exp 1, no liner, quadratic, or cubic response was observed for any live performance measurement or carcass yield except a cubic response for feed conversion ratio. Increasing the Ile:Lys ratio in Exp 1 resulted in a decrease (P=0.033) in total breast yield of 0.88 percentage units. The male Val:Lys ratio requirement for feed conversion was estimated to be 78. In Exp 2, BW gain linearly increased as Val:Lys ratio increased. Similar to that observed in Exp 1, a cubic response to the Val:Lys ratio was observed for feed conversion, with no response for any processing yield. Digestible Val:Lys ratio did not impact the occurrence or severity of woody breast in either Exp. The female Val:Lys ratio needed for feed conversion was estimated to be 77. Results from this study indicate that formulating diets to contain a Val:Lys ratio of 78 is adequate to produce good performance for both male and female broilers.

Key Words: valine, requirement, male, female, feed conversion

M71, **Determining amino acid requirements for broiler breeders using the nitrogen balance method** Jordan Weil*[1GS], Antonio Beitia[1], Nawin Suesuttajit[1], Katie Hilton[1], Pramir Maharjan[1], Diego Martinez[1], Justina Caldas[2], Shivi Rao[2], Craig Coon[1] [1] *University of Arkansas,* [2] *Cobb-Vantress, Inc.*

In order to ensure that broiler breeders are reaching their maximal genetic potential in terms of hatching eggs, appropriate amino acid (AA) concentrations must be supplied in the diet. Amino acid requirements can differ across breeds, environments, gender, age, and body composition. Therefore, it is increasingly important to determine the AA requirements in broiler breeders to ensure optimal egg production, hatchability, and progeny performance. In this experiment, the nitrogen balance technique was used to determine the AA requirements in Cobb 700 FF broiler breeders. A total of 648 21 wk-old Cobb 700 FF broiler breeders were placed into 648 cages, with each cage containing one breeder. A total of eight test AA were studied in this experiment (10 reps/diet). Test AA included the total sulfur amino acids (Met + Cys), lysine (Lys), arginine (Arg), branched chain amino acids (Val, Ile), threonine (Thr), tryptophan (Trp), and glutamic acid (Glu). The concentration of AA that were fed to all treatments except for the test AA was equivalent to 120% Primary Breeder recommended AA intake (Cobb-Vantress, 2012) for peak production. Birds were fed for a period of 7 weeks (2 wks adaptation; 5 wks testing) in two phases. Phase I (P1) lasted from wks 30 through 37, while Phase 2 (P2) occurred from wks 44 through 51. The egg production, body weight and egg weight for all breeders was evaluated during the test and recovery period. Results were analyzed using a quadratic model (JMP Pro 13.1). Average digestible AA requirements for egg mass were as follows: Met+Cys, Lys, Arg, Ile, Val, Thr, and Trp are 808, 931, 900, 795, 916, 696, and 224 mg/day, respectively, for P1, and 847, 927, 920, 806, 955, 697, and 197 mg/d for P2. The digestible protein inclusion level was determined to be 20.9 and 19 (P1 and P2) g/ by feeding a series of glutamic acid levels plus digestible essential amino acids needed for optimum egg mass production. The digestible AA requirements were comparable, with the exception of a lower Arg requirement found for Cobb 700 hens, to previous digestible AA requirements determined for Cobb 500 FF hen at peak production. Knowledge gained from this study will provide the necessary information to determine the production requirements of Cobb 700 broiler breeders.

Key Words: broiler breeder, amino acids, requirements, nitrogen balance

M72, **Evaluation of a threonine fermentation product as a source of digestible threonine in broilers** Trevor Lee*[1GS], Jason Lee[2], Samuel Rochell[1] [1] *University of Arkansas,* [2] *CJ America, INC*

An experiment was conducted to evaluate a Thr fermentation product (FP) as a source of digestible Thr and other nutrients to support broiler performance and processing characteristics. A total of 1,260 Ross 708 male chicks (21 birds/pen) were allocated to 1 of 5 corn-soybean meal-based, isocaloric diets: 1) a positive control (PC) diet with L-Met, L-Lys•HCl, and L-Thr as the only added amino acids, 2) a reduced CP (RCP) diet with added L-Met, L-Lys•HCl, L-Thr, L-Val, L-Ile, and L-Arg, 3) a negative control (NC) diet identical to the RCP except that cellulose was added

at the expense of L-Thr, 4) the RCP diet with Thr FP used in place of L-Thr with only digestible Thr and energy contributions of the FP used in formulation (TFP), or 5) a RCP diet accounting for the full nutrient matrix of the FP (FM-TFP). Treatments were maintained across starter (0 to 14 d), grower (14 to 28 d), finisher 1 (28 to 39 d), and finisher 2 (39 to 48 d) phases with 12 replicate pens per treatment. Except for the NC diet, starter diets were formulated to contain 1.28% digestible Lys with minimum ratios of digestible amino acids relative to Lys set to 0.74, 0.67, 0.75, 0.67, and 1.05 for TSAA, Thr, Val, Ile, and Arg, respectively, and dietary specifications were adjusted according to primary breeder recommendations. Digestible Thr:Lys ratios for the NC diets were 0.56, 0.56, 0.53, and 0.53 for the starter, grower, finisher 1, and finisher 2 phases, respectively. Body weight gain (BWG), feed intake (FI), and FCR were evaluated, and at 49 d, 6 birds per pen were randomly selected for processing. From 0 to 14 d, FI ($P = 0.04$) and BWG ($P = 0.02$) were highest for broilers fed TFP, intermediate for those fed PC, RCP, and NC diets, and lowest for the FM-TFP group. During the same period, FCR ($P = 0.03$) was highest for birds fed NC, intermediate for RCP, TFP, FM-TFP fed birds, and lowest for PC-fed birds. From 0 to 48 d, FCR was highest ($P < 0.01$) for birds fed the NC diet, intermediate for those fed the FM-TFP diet, and lowest and similar among those fed PC, RCP, and TFP diets. The relative and absolute carcass and parts weights were similar ($P < 0.05$) among all treatments. In conclusion, these data indicate that the Thr FP was efficacious in replacing feed grade L-Thr in a reduced CP diet.

Key Words: threonine, fermentation product, amino acids, reduced protein, broiler

M73, **Effects of broiler breeder dietary amino acid levels during rearing on broiler progeny live performance, carcass traits, and meat quality** Gustavo Quintana*[1GS], Edgar Oviedo-Rondon [1], Andres Ortiz [1,2], Yilmar Matta [1,2], Gherly Buitrago-Mejia [1,2], Jose Yanquen [1,2], Juan Martinez [1,2], Juan Hoyos-Torres [1,2], Hernan Cordova [1], Justina Caldas [3] *[1] North Carolina State University, [2] Universidad del Tolima, [3] Cobb Vantress Inc.*

Broiler breeder (BB) nutrition during rearing affects progeny. This experiment evaluated the effects of four amino acid (AA) levels for BB grower and developer phases on progeny live performance, carcass yield and meat quality. Treatments resulted from a 4x2 factorial arrangement of four AA treatments during BB rearing (4 to 24 wk) and progeny sex (male and female) with 6 replicates for each treatment combination. Cobb 500SF hens were fed with diets containing balanced protein levels of 0.49, 0.54, 0.60, 0.66 and 0.51, 0.57, 0.63, 0.69% dig Lys for each phase, respectively, and common diets during laying phase. Eggs were collected at 43 wk of age and incubated with a single stage profile. After hatching and sexing, a total of 960-d-old chicks (480 each) were placed in 48 pens (20 chicks/pen). Progeny was fed according to Cobb 2018 recommendations. Group BW and feed intake (FI) were recorded at 7, 16, 28 and 42 d of age and BW gain and FCR adjusted for mortality was calculated. Two broilers per pen were processed at 43 d, cut up parts were weighed, breast meat quality evaluated (pH, color, cooking and dripping loss) and breast myopathies scored. No interaction effects ($P>0.05$) were observed indicating independency between treatments and sex. Levels of AA in BB caused a quadratic effect ($P<0.05$) on progeny BW and 17-28 d FI. Later, linear effects ($P<0.05$) of BB treatments were observed on FI between 29-42 d and 1-42 d of age. No significant effects ($P>0.05$) on flock uniformity were evident except for sex. Progeny FCR from 29-42 and 1-42 d of age worsen as AA in BB during rearing increased. A quadratic effect ($P<0.05$) of AA levels in BB were also observed on carcass yield. No effects ($P>0.05$) were detected on cut up parts yield. The AA levels of BB caused a quadratic effect ($P<0.05$) on breast meat pH 24h post-slaughtering. The L value of breast meat color increased linearly ($P<0.05$) in progeny as AA levels for BB increased. A quadratic effect ($P<0.05$) of BB treatments was observed on average white stripping scores and the biggest scores were observed on progeny from BB fed with the highest AA levels. In conclusion, AA levels during the BB rearing phase are a key factor that could influence Cobb 500 progeny live performance, carcass yield, and breast meat quality.

Key Words: Broiler breeders, amino acids, rearing, progeny, myopathies

M74, **The effects of L-Arginine supplementation on growth performance and intestinal health of broiler chickens challenged with Eimeria spp.** Fernanda Castro*[GS], Po-Yun Teng, Sudhir Yadav, Lorraine Fuller, Woo Kim *University of Georgia*

The aim was to study varying levels of L-Arginine (Arg) on the performance and intestinal health of broilers after a single challenge with *Eimeria* spp. A total of 720 d-old-male Cobb 500 chicks were randomly distributed in a factorial arrangement 5 by 2 (6 replicates/12 birds). The factors were Arg levels (1.04, 1.14, 1.24, 1.34, 1.44%) and challenge (CHA) or not (UCH) with *Eimeria* spp. At d 12, the CHA group received orally 12,500 *E. maxima* and *E. tenella*, and 62,500 *E. acervulina* sporulated oocysts. At d 17 (5 d post infection, dpi), 1 bird/replicate was gavaged with fluorescein isothiocyanate-dextran (FITC-d), and FITC-d in the serum was measured to attest intestinal permeability. At d 26 (14 dpi), BWG, FI, and FCR were measured, and intestinal samples were collected (1 bird/replicate) for morphology. Two-way ANOVA was performed, and means were compared by Tukey's test using SAS ($P<0.05$). No interactions were found for FITC-d and growth performance. At 5 dpi, CHA birds had the highest FITC-d concentration ($P<0.01$), and birds fed 1.34% had lower values than birds fed 1.04% of Arg ($P=0.01$). The BWG at 14 dpi was the highest in the UCH group ($P<0.01$), and higher for birds fed 1.44% than 1.04% of Arg ($P=0.03$). The FI was the lowest for CHA birds ($P<0.01$). The FCR was the lowest for UCH birds ($P<0.01$), and birds fed 1.14 and 1.34% had lower FCR than 1.04% of Arg ($P=0.02$). Significant interaction was only found for the villi:crypt ratio (V:C) in the duodenum ($P=0.04$). Within the UCH group, birds fed 1.24% had higher V:C than those fed 1.14 and 1.44% of Arg, whereas in the CHA group no differences were found between the levels. The V:C in the jejunum and ileum was lower for CHA *vs*. UCH birds ($P<0.03$), and the crypt depth was higher for the CHA *vs*. UCH birds in the duodenum and ileum ($P<0.01$). Moreover, birds fed 1.14, 1.34 and 1.44% had higher crypt depth than 1.04 and 1.24% of Arg in the duodenum ($P=0.04$). The challenge with *Eimeria* spp. reduced the performance and intestinal health during the challenge (5 dpi) and recovery (14 dpi) phases. Additionally, the use of 1.34% of Arg (0.1% above the recommended level by the line) appears to be beneficial, leading to lower intestinal permeability and better FCR than the non-supplemented group (1.04% of Arg).

Key Words: L-Arginine, Broilers, Eimeria spp., intestinal health

M75, **The effect of amino acid density on performance and processing yield in mixed-sex Cobb 700 x MV broilers** Dalton Dennehy*[1UG], Corey Johnson [1], Rocky Latham [2], Jason Lee [3], Gregory Archer [1], Christine Alvarado [1], Tri Duong [1] *[1] Department of Poultry Science, Texas A&M University, [2] Tyson Foods, Inc., [3] CJ America*

In this study, we investigated the phase effects of feeding increased digestible amino acid (dAA) density on the performance and processing yield of mixed-sex Cobb 700 x MV broilers. Experimental animals were allocated to 7 treatment groups with 10 replicate pens of 34 birds arranged as a randomized complete block design and were fed diets with varying dAA densities using a 4-phase feeding program (starter, 0-12 d; grower, 12-26 d; finisher, 26-36 d; and withdrawal, 36-49 d). Broilers were fed one of 3 dAA levels (Low, Medium, and High) during the starter phase and one of 4 dAA levels (Low, Medium, High, and High +) during the grower, finisher, and withdrawal phases. The treatment combinations consisted of LLLL, MLLL, MMMM, MHHH, HMMM, HHHH, and HH+H+H+. Diet formulations maintained equivalent dEAA:dLys ratios across all treatments within a given phase. The dLys content of the low diets was 1.22, 1.10, 0.95, and 0.90% for the starter, grower, finisher, and withdrawal phases, respectively. Body weight, ADFI, and mortality-adjusted FCR were evalu-

ated at 12, 26, 36, and 49 d. At 50 d post-hatch, 3 males and 3 females from each pen were processed to determine breast meat and fat pad yield. Data were analyzed using ANOVA, and means were separated using Duncan's Multiple Range test (P£0.05). During the starter phase, BW of broilers fed the medium and high dAA density diets was greater than that of those fed the low dAA diet (P<0.001). During the grower phase, BW of broilers fed the HH diet was greater when compared with those fed the LL, ML, MM, and HM diets (P<0.001). Cumulative FCR was reduced (P=0.001) by 8.7 points in broilers fed the HHHH diet compared to those fed the MLLL diet. Total breast meat yield (% of live weight) was increased in broilers fed the MLLL and HH+H+H+ diets by 0.96% and 1.85%, respectively, as compared with those fed the LLLL diet (P<0.001), whereas fat pad yield (% of live weight) decreased from 1.64% in broilers fed LLLL to 1.24% in those fed HH+H+H+ (p<0.001). The results of our study can be used by producers to inform decisions regarding the suitability of feeding diets with increased dAA density to Cobb 700 x MV broilers.

Key Words: Cobb 700, broiler, AA density, breast yield

M76, **Tryptophan requirement of 22 to 34-week-old caged-laying hens** Albaraa Sarsour[*1GS], Jason Lee[2], Keith Haydon[2], Michael Persia[1] *[1] Virginia Tech, [2] CJ America Inc.*

Tryptophan, the third or fourth limiting amino acid for laying hens, is important in protein synthesis and as a precursor in the serotonin and kynerunine pathways. An experiment was conducted to evaluate the digestible tryptophan requirement of first cycle laying hens from 22 to 34 weeks of age. A total of 252 Hy-Line W36 laying hens were selected at 16 weeks of age and allocated by weight (P = 0.90) to seven dietary treatments resulting in 12 replicate cages of three birds for each treatment. A Trp deficient basal diet was formulated using corn, corn gluten meal and soybean meal for each of the dietary phases and supplemented with synthetic L-Trp to generate diets that provided 105, 119, 133, 147, 162, 176, and 190 mg digestible Trp on a daily basis over the egg producing phase of production, respectively. Over the pullet phase, hens were fed complete diets that contained increasing amounts of corn gluten meal to gradually transition to experimental diets first offered at 16 weeks of age. Over all dietary phases, birds were provided a controlled amount of feed based on expected feed intake of birds in commercial management conditions. Hen-housed egg production (HHEP), feed intake, egg weights, egg mass (EM), feed efficiency (FE) and mortality data were collected on a daily basis and calculated over two-week periods to correspond with diet changes. Linear and quadratic broken-line, and quadratic polynomial models were used to estimate digestible Trp requirements based on HHEP, EM and FE. Overall, HHEP, EM and FE ranged from 88.3 to 94.2%, 46.7 to 50.0 g/d, and 510 to 540 g/kg, respectively, with increasing dietary Trp resulting in increased responses until a plateau was reached. Digestible Trp requirements were estimated to be 136.6, 183.3 and 192.2 mg/d for HHEP; 133.0, 180.0 and 183.1 for EM and 132.5, 177.4 and 173.0 for FE using linear broken-line, quadratic broken-line and quadratic polynomial analysis, respectively. Linear broken-line analysis of digestible Trp requirements resulted in lower estimates than other models, and HHEP, EM and FE resulted in similar estimated digestible Trp requirements in 22 to 34-week-old laying hens.

Key Words: Tryptophan, Laying hen, Requirement, First-cycle

M77, **In vitro and in vivo protective action of a thymol-based blend of botanicals against coccidiosis** Martina Felici[*1], Benedetta Tugnoli[2], Federico Ghiselli[1], Paola Massi[3], Giovanni Tosi[3], Laura Fiorentini[3], Andrea Piva[1,2], Ester Grilli[1,4] *[1] Universitiy of Bologna, DIMEVET, [2] Vetagro S.p.A., [3] Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Sezione Diagnostica di Forlì, [4] Vetagro, Inc.*

Chicken coccidiosis is a parasitic disease caused by *Eimeria* spp. Research has focused on finding new alternatives to fight these pathogens and botanicals have gained much interest in this field.

The aim of this study was to test the anticoccidial activity of a thymol-based blend of botanicals both in an *in vitro* model of coccidiosis and *in vivo* in broiler chickens.

For the *in vitro* assay, Madin-Darby bovine kidney cells were challenged with $5x10^4$ *E. tenella* sporozoites without (control) or with a thymol-based treatment (14 ppm). After 24 h at 37°C the efficiency of invasion was determined by counting the sporozoites left in the medium that were not able to invade the cells. Moreover, intracellular *E. tenella* DNA was detected by qPCR. Data were analyzed with T-test and differences considered significant at P<0.05. Sporozoites counts data showed that the treatment reduced the efficiency of invasion compared to control by 33% (P<0.0001). Accordingly, the presence of intracellular *E. tenella* DNA was reduced by 51.2% in treated cells compared to control cells (P<0.05).

For the *in vivo* trial, 1,800 day-old broiler chicks (50 chicks/pen) were assigned to 3 treatment groups (12 pens/group) as follows: (1) control, fed a basal diet; (2) salinomycin, fed a basal diet + salinomycin 60 ppm; (3) treatment, fed a basal diet supplemented with a microencapsulated thymol-based blend at 100 ppm (3). On d22 all chicks were infected with a multispecies *Eimeria* inoculum. *Eimeria* lesion score was measured on d28 and oocysts per gram (OPG) of feces determined on d29 and d36. Data were analyzed with ANOVA and differences considered significant at P<0.05. Compared to control, lesion score was reduced by salinomycin and, to a similar extent, by the treatment (P=0.01). OPG were not affected on d29 but both salinomycin and the thymol-based blend reduced oocysts shedding compared to control on d36 (P<0.05).

In conclusion, these studies showed both in vitro and in vivo anticoccidial properties for the thymol-based blend tested and it can be proposed as alternative to traditional anticoccidial products.

Key Words: coccidiosis, botanicals, Eimeria tenella, broilers

Metabolism and Nutrition IV, Enzymes/Feed Additives

M78, **The influence of a dacitic tuff breccia product on the performance of laying hens from 54 to 98 weeks of age** Cassandre Juzaitis-Boetler[*1GS], Matthew Jones[2], Adam Davis[1], Jon Farrel[2] *[1] University of Georgia, [2] AZOMITE Mineral Products, Inc.*

Aluminosilicates and other clay products have been added to poultry diets for a variety of reasons. Though historically incorporated to enhance pellet quality, they have also been reported to bind toxins, promote growth, and improve feed efficiency in research trials. One such substance, marketed as a hydrated sodium calcium aluminosilicate product (AZOMITE®), has been reported to promote growth in various production systems (poultry, aquaculture, and horticulture). In the current research, 6 replicate groups each consisting of 8 individually caged Hy-Line W36 hens were fed a control layer diet or this diet supplemented with 0.25% Azomite from 54 through 98 weeks of age. The hens were fed a standard molt diet or this diet supplemented with 0.25% Azomite from 71 through 72 weeks of age. During the experiment, body weights did not differ between the hens from the 2 treatments. At the end of the experiment, hen housed egg production was greater (P < 0.05) in the hens fed the diet supplemented with Azomite (242 versus 235 eggs per hen). After molting, hens fed the diets supplemented with Azomite returned to egg production quicker than the control-fed hens, and 6 of the 7 egg increase in hen housed egg production by the Azomite-fed hens occurred after molt. At 98 weeks of age tibia ash percent was greater in the Azomite fed hens than the control fed hens (P < 0.01). Plasma alpha 1 acid glycoprotein (AGP) levels did not differ be-

tween the treatments at 54, 70 and 72 weeks of age, but were greater ($P < 0.001$) at 72 weeks of age at the end of the molt period than prior to molt at 54 and 70 weeks of age. The results suggest that the addition of Azomite to laying hen diets especially during and after molt improves egg production.

Key Words: Azomite, Tibia ash, Molt

M79, **The effects of 2-nitro-1-propanol on growth performance, nutrient digestibility, gut health, and oocysts shedding in Eimeria-challenged broilers** Po-Yun Teng[*1GS], Sudhir Yadav [1], Tatiane Santos [2], Alberta Fuller [1], Woo Kim [1] [1] *University of Georgia,* [2] *Sao Paulo State University*

A 2 x 3 factorial arrangement study was conducted to evaluate 2-Nitro-1-propanol (NP) on growth performance and intestinal health of birds with or without *Eimeria* challenge. The main factors of the experiment were NP supplementation (0, 150, and 200 ppm) and *Eimeria* spp. challenge (challenge and non-challenge). A total of 432 13-day-old male broiler chickens (Cobb500) were randomly allocated to six treatments with eight replicate cages of nine birds per cage. All birds were fed with treatment diets from d 13 to d 21. Birds in the challenge group were gavaged with *E. maxima* (50,000 oocysts per bird), *E. tenella* (50,000 oocysts per bird), and *E. acervulina* (250,000 oocysts per bird) on d 15. BW, BWG, FI, and FCR was calculated from d 13 to d 21, and gut permeability was measured by fluorescein isothiocyanate dextran on d 20. The intestinal lesion, intestinal morphology and oocysts shedding were determined at the end of the trial. PROC GLM program of SAS software was used to analyze data in the study. The linear and quadratic orthogonal polynomial contrasts were used to evaluate the effects of increasing NP doses in responses to *Eimeria* challenge. The results showed that NP was not able to improved growth performance and gut leakage during *Eimeria* infection period. On the other hand, *Eimeria* infection increased gut permeability ($P < 0.0001$), and reduced ileal digestible energy (IDE) and apparent ileal digestibility (AID) of nitrogen. However, the increase of NP linearly enhanced IDE and AID of nitrogen ($P < 0.01$). Moreover, an interaction between challenge and linear dosages effects of NP was observed in IDE ($P = 0.0066$) and AID of nitrogen ($P = 0.0462$). The results indicated that NP improved nutrient digestibility in birds challenged with *Eimeria*. Also, broilers fed with higher NP numerically increased villi height in the intestine and reduced oocysts shedding of *E. acervulina* ($P = 0.059$). In summary, NP was not able to maintain growth performance of birds, but it presented positive outcomes on improvement of nutrient digestibility, intestinal morphology, and oocysts shedding during *Eimeria* infection.

Key Words: 2-Nitro-1-propanol, Eimeria, Coccidiosis, Digestibility

M80, **Eimeria challenge and feed additives affected broiler woody breast incidence** Linan Jia[*GS], Sabin Poudel, Wei Zhai *Mississippi State University*

Woody breast (WB) is a common breast muscle myopathy condition in the current commercial broilers. In this study, we investigated the effects of dietary supplementation of antibiotic and probiotic on WB incidence in male and female broilers with or without cocci challenge. A total of 672 male and 672 female Ross × Ross 708 day-old chicks were randomized assigned to 12 treatments with a 3 (diet) × 2 (cocci challenge) × 2 (sex) factorial arrangement in 8 blocks. The 3 dietary treatments were: control diet (corn-soybean meal basal diet), antibiotic diet (basal diet + 6.075 mg bacitracin /kg feed), and probiotic diet (basal diet + 2.2×10^8 CFU *Bacillus subtilis* PB6 /kg feed). One mL 20 × cocci vaccine was orally gavaged to chicks in cocci challenge treatments on d 14. Birds in non-challenged treatments received same volume of distilled water. On d 29, 35, and 43, breast conditions in live birds were determined by palpation and grouped into Normal, Slight, Moderate, and Severe conditions by the severity of WB. Data were analyzed using 3-way ANOVA. The significant level was set at $P \leq 0.05$. Cocci challenge increased incidences of Moderate WB on d 29 ($P = 0.043$), Slight WB in male birds on d 35 ($P = 0.041$), and total Moderate and Severe WB on d 43 ($P = 0.013$). On d 43, supplementation of antibiotic or probiotic to the basal diet increased overall WB incidence in birds not receiving cocci challenge; cocci challenge increased overall WB in birds fed control diet ($P = 0.040$). Birds fed antibiotic or probiotic diet exhibited higher Slight WB incidence than those fed control diet ($P = 0.036$). In addition, male birds exhibited higher incidence of Slight WB on d 29, Moderate WB on d 35, and Moderate and Severe WB on d 43, as compared to female birds ($P = 0.002$, < 0.0001, 0.0002, and < 0.0001), which may be due to higher growth rate in male birds. In summary, on d 43, antibiotic and probiotic supplementation increased WB incidence in non cocci-challenged group; and cocci challenge increased WB incidence in birds fed control diet. Manipulation of intestinal microbial by antibiotic or probiotic administration or cocci challenge may have disrupted the original microbiome balance in the chicken gastro intestinal tract and induced stress and subsequently increased WB incidence.

Key Words: Antibiotic, Bacillus subtilis, Broiler, Eimeria, Woody breast

M81, **Sodium bisulfate aids broilers in growth and intestinal health during a coccidiosis challenge** Elle Chadwick[*1GS], Jesse Grimes [1], Shaban Rahimi [2], John Pitts [3], Robert Beckstead [1] [1] *North Carolina State University,* [2] *Tarbiat Modares University,* [3] *Jones Hamilton Co*

Sodium bisulfate (SB) feed additive was evaluated on its ability to improve broiler growth and intestinal structure with(out) a coccidia challenge. One thousand two hundred Cobb500 males were randomly assigned within four experimental groups, which included combinations of diet (with(out)) SB) and disease (with(out) a 10X cocci vaccination). At D0, 14, 28 and 41 body and feed weights were collected. On D7, oocysts per gram of feces (OPG) was counted. At D21, twenty birds per treatment were subjectively scored for coccidia lesions and jejunal histological samples were collected for villi measurements. Twenty additional birds were given FITC-D to determine gut permeability. At D41, ten birds per treatment had histological samples collected. Statistical analysis was conducted in JMP Pro 14 using GLM procedure to compare disease state and diet. Means were separated using Dunnett's test ($p \leq 0.05$) with the non-challenged, no SB in the diet treatment considered the control. All parameters measured indicated an effect due to the coccidia challenge. Therefore, effects of diet on (non) challenged treatments were determined using a student t-test ($p \leq 0.05$). Limited differences due to diet were seen for the nonchallenged production data. SB had a thinner villi base width ($p=0.04$) on D21 and greater villus height ($p=0.03$), base width ($p=0.04$), muscularis ($p=0.03$) and lower crypt:height ratio ($p=0.01$) on D41. Challenged SB had similar gut permeability to the nonchallenged control ($p=0.94$) on D21. There was no difference in flock uniformity, feed intake, OPG or lesion scores between challenged treatments. Challenged SB had greater body weights on D14 ($p<0.0001$), 28 ($p<0.0001$) and 41 ($p=0.02$). FCR from D0-14 was also lower ($p=0.0002$). Challenged SB had smaller crypts ($p=0.02$) and therefore a smaller crypt:height ratio ($p=0.03$) on D21. Challenged standard had a larger apical width ($p=0.03$) and thicker muscularis ($p=0.04$) on D41. Overall, the addition of SB during coccidial enteropathy aided in body weights, FCR and villi health with no observed effects on parasite cycling.

Key Words: broiler, sodium bisulfate, cocci

M82, **Isolation and selection of Bacillus spp. as candidate direct-fed microbials based on qualitative in vitro enzymatic hydrolysis of indigestible non-starch polysaccharides and oligosaccharides found in soybean meal** Lucas Graham[*GS], Kyle Teague, Brittany Graham, Samuel Rochell, Billy Hargis *University of Arkansas*

In this experiment, 114 wild-type *Bacillus* isolates (BI) sourced from varying bird types and ages were isolated and screened. The objective was to identify isolates which would enzymatically hydrolyze the carbohydrates present in in soybean meal that are considered anti-nutritive in poultry diets. Isolates were subjected to a panel of in vitro tests to pre-determine their

efficacy as a candidate direct fed microbial (DFM). The first substrate used for screening was raffinose, an oligosaccharide found in soybean meal, and only 28 BI were able to utilize raffinose as a carbohydrate source in the absence of glucose. These 28 isolates were then evaluated for their ability to utilize cellulose, galactomannan, or arabinoxylan on soft agar overlay plates, which narrowed the library to 14 isolates that were susbsequently analyzed for 16S DNA sequencing. Speciation of the isolates were as follows: 7 - *B. amyloliquefaciens*, 6 - *B. subtilis*, and 1 - *B. licheniformis*. The 14 isolates were evaluated for biofilm production capacity and for bile tolerance for multiple time periods. Only 9 of 14 isolates produced a biofilm, while 12 were able to withstand 0.3% bile salts for at least two hours. The top performing isolate from each of the 3 *Bacillus* species was used in soft agar overlays to assess their ability to inhibit the growth of *Salmonella*. Only the *B. amyloliquefaciens* was effective at inhibiting the growth of *S.* Enteritidis, *S.*Typhimurium, and *S.* Kentucky. Additionally, the 9 isolates capable of biofilm formation were found to produce spores in solid state fermentation. Finally, recovered spores were tested for heat resistance, utilizing a hot water bath, as an indication of their ability to withstand temperatures regularly used during feed pelleting. The results of these in vitro assays identified potential DFM candidates that may be effective in vivo against anti-nutritional factors present in poultry feeds.

Key Words: Bacillus, direct fed microbial, soybean meal, non-starch polysaccharides, enzyme

M83, **In vivo evaluation of Bacillus isolates selected based on qualitative in vitro enzyme activity against soybean meal carbohydrates as direct-fed microbial candidates for broiler chickens** KYLE TEAGUE*GS, Lucas Graham, Brittany Graham, Billy Hargis, Samuel Rochell *University of Arkansas*

Bacillus isolates (BI) were selected as direct fed microbial (DFM) candidates in previous experiments based on their ability for *in vitro* degradation of carbohydrates found in soybean meal (SBM) that are poorly digested by poultry. In the current experiment, 6 in vivo trials were conducted to evaluate 5 different BI in broiler chicks reared to 21 d. In 3 floor pen trials, treatments were administered to 6 replicate pens of 12 birds ($0.09m^2$/bird) (trials 1 & 2) or 8 replicate pens of 20 birds ($0.11m^2$/bird; trial 3) housed on new pine shavings. In 3 battery (trials 4-6), treatments were administered to 12 replicate cages of 8 birds ($0.04m^2$/bird). In all trials, birds were fed high soybean meal (40%) diets formulated to contain 3,050 (CTL) or 2,925 [reduced energy (RED)] kcal/kg of apparent ME_n or the RED containing BI. Birds had ad libitum access to mash feed and water, and BW and feed consumption were recorded weekly to determine body weight gain (BWG) and feed conversion ratio (FCR). In all trials except trial 6, birds fed the CTL diet had higher BW and lower FCR ($P<0.05$) at 21 d than birds fed the RED. In trial 1, BI-46 tended ($P=0.08$) to increase BWG and lower ($P<0.07$) FCR when compared with birds fed CTL at 21 d. In trial 3, birds fed BI-40 tended ($P=0.09$) to increase BWG at 21 d compared to birds fed the RED. In trial 4, birds fed BI-46 had 10% higher ($P<0.008$) BWG and a 7 point lower ($P<0.03$) FCR than those fed RED, and similar performance with birds fed the CTL diet. In trial 5, birds fed BI-40 and BI-46 had higher BWG ($P<0.05$) and lower FCR ($P<0.05$) than birds fed the RED. In trial 6, BI-46 and the combination of BI-40 and BI-46 increased BWG ($P<0.05$) compared with birds fed the RED and was not different ($P>0.05$) from those fed the CTL. Data were pooled to further evaluate the responses across all trials in which BI-46 was used due to the consistent positive response. These pooled data showed that BWG and FCR values of birds fed BI-46 were 7% higher ($P<0.05$) and 11 points lower ($P<0.05$), respectively, than those of birds fed RED and similar ($P>0.05$) to those of birds fed the CTL diet. Thus, these data reveal a novel DFM candidate for broiler diets containing SBM and indicate that not all BI with promising *in vitro* enzyme activity yield benefits *in vivo*.

Key Words: Bacillus, DFM, Soybean Meal, Oligosaccharide, Nonstarch Polysaccharide

M84, **Evaluating the effects of Lactobacillus reuteri and antibiotic zinc bacitracin on the growth performance of French guinea fowl broilers** Alexis Pigg*GS, Samuel Nahashon *Tennessee State University*

Antibiotic growth promoters (AGPs) have been linked to antibiotic resistant pathogens, adding to the pressing issue in human and animal health. Therefore, regulation of antibiotic use in animal production has become more prevalent. Probiotics have the potential to be a viable method of promoting growth in livestock in comparison to AGPs, including poultry. This study investigates AGP zinc bacitracin and *Lactobacillus reuteri* and their effects on the growth performance of guinea fowl broilers.

320 one-day-old French guinea fowl broilers were purchased and divided into four dietary treatment groups with four replicates per treatment: control, antibiotic, probiotic (soy protein + *L. reuteri*) and soy protein (soy protein – *L. reuteri*). The dietary treatments included the basal control diet, and the basal diet plus 50mg/kg of antibiotic zinc bacitracin, basal diet with 100mg/kg of *L. reuteri* encapsulated in soy protein, and basal diet with 100mg/kg of soy protein only. Feed and water were provided ad libitum. Birds were individually identified and weighed once weekly for six weeks.

The antibiotic, probiotic, and soy protein treatments significantly reduced feed consumption in comparison to the control throughout weeks 1-6. The probiotic group consumed the least during weeks 1-4 and 6. The control group maintained the lowest body weights at weeks 4-6 and gained the least amount of weight each week. By week 6, the antibiotic, probiotic, and soy protein groups had significantly higher body weights when compared to the control treatment. Animals in the probiotic group consumed less than the antibiotic group during weeks 1-4 and 6, however, the body weights of these animals remained similar over the duration of the experiment.

In this study, zinc bacitracin and *L. reuteri* both significantly improved bird growth and performance. Zinc bacitracin was a common AGP. It had similar effects on feed consumption and weight gain in comparison to chickens. *L. reuteri* is a bacterium frequently used in probiotic formulations for humans and other livestock species. It has proven to reduce feed consumption without compromising weight gain in guinea fowl broilers. Thus, *L. reuteri* based probiotics may be a viable alternative to antibiotics used as growth promoters in poultry.

Key Words: Guinea fowl, performance, probiotic, agp

M85, **Response of Ross x Ross 708 male broilers fed varying phytase enzyme and activity during d 0-14** Courtney Ennis*1GS, Curran Gehring[2], Mike Bedford[3], Craig Wyatt[3], Kelley Wamsley[1] [1] *Mississippi State University,* [2] *Tucker Milling,* [3] *AB Vista*

Phytate (IP6) degradation is desirable for its effects on broiler performance and diet cost. Three phytases (A, B and C) with unique characteristics in their sequence or IP6 hydrolysis were evaluated for complimentary action upon IP6 via combinations of A, B, and/or C. The objective was to determine if any of these phytase inclusion strategies (PIS; A, B, C, A+B, A+C, B+C, or A+B+C) and total phytase activity (120 or 240 FTU/kg) affect d 0-14 performance and tibia ash using a 7 x 2 factorial arrangement within a randomized complete block design. Basal diets were antibiotic-free, all-vegetable with Ca and available P at 0.4 and 0.2%, respectively. On d 0, Ross x Ross 708 male chicks were randomly allocated into 140 raised wire cages (12 birds/cage; 10 replications/treatment). Variables calculated included average BW, BW gain (BWG), CV for BW, % mortality, feed intake, feed conversion ratio, and d 14 tibia ash. Due to space, only the 0-14 d BW, BWG, and tibia ash data will be explained. During d 0-14 a significant interaction was found between PIS and FTU/kg for BWG ($P=0.026$). Feeding A+B+C at 240 FTU/kg led to maximized BWG beyond birds fed C at 120 FTU/kg, A+B+C at 120 FTU/kg, C at 240 FTU/kg, and A at 240 FTU/kg. Birds fed PIS of A, B, C, A+B, B+C, or A+C had similar BWG at either FTU/kg. Feeding A alone at 240 FTU/kg led to the

lowest BWG; however, BWG was significantly increased by combining A with other phytases, such as A+B, A+C, and A+B+C at 240 FTU/kg. An interaction following the same trends was also observed between PIS and FTU/kg for d 0-14 BW (P=0.025). A significant interaction between PIS and FTU/kg was demonstrated for tibia ash % (P=0.036). Birds fed A+B+C had higher tibia ash % than B and C at 120 FTU/kg, as well as A+C and A at 240 FTU/kg. Lowest tibia ash % was found with feeding A+B+C at 120 FTU/kg and improved with higher FTU/kg. Birds fed PIS of A, B, C, A+B, B+C, or A+C had similar tibia ash % regardless of FTU/kg. A significant interaction was also found with tibia ash (mg/chick) that followed the same trends (P=0.036). These data suggest that phytase combinations improve IP6 breakdown, warranting further exploration using IP6 ester analysis and nutrient digestibility data from the current study.

Key Words: phytase, exogenous feed enzymes, broiler performance, tibia ash

M86, **Evaluating the effects of essential oil-based phytonutrient blends on the performance of meat type quail production** Chandler Keck*GS, Chris Ashwell, Zack Lowman *NC State University*

With the poultry industry progressively moving towards antibiotic-free production, the search for alternatives is at an all-time high. One avenue that has been considered is the implementation of feed additives such as Essential Oils (EO) due to their antioxidant and antimicrobial properties. Although not the predominant sector of the poultry industry, quail meat and egg production provide a possible alternative and an advantageous model for quickly simulating feed additives' effects on avian species. The objective of this study was to evaluate the effects of three different EO based phytonutrient blends (Ralco Nutrition) on all phases of quail production. 400 chicks were hatched, tagged and randomly assigned to one of four treatment diets: Control, Treatment 1 (Blend A), Treatment 2 (Blend B), or Treatment 3 (Blend C). All birds were brooded under the same conditions and were provided ad lib access to water and assigned feed throughout the trial. Birds were individually weighed at the day of hatch and weekly thereafter through d42. At d21, 48 birds were randomly selected from each treatment, sexed and moved to battery-style cages with eight replicate cages per treatment. All birds were challenged weekly from d21 to d42 with a nutrient gel containing bacteria cultured from their fecal material. At d42, the birds were transferred to layer cages for egg production and hatchability evaluations. Egg production was recorded daily for 8 weeks. Two groups of eggs were set to test fertility, mortality, contamination and hatchability. For each set, eggs were collected for 10 consecutive days, stored in egg cooler, incubated and hatched. All data were analyzed using a one-way ANOVA of JMP 13. The addition of each blend increased body weight (BW) throughout brooding and most of grow out (d7-d35) when compared to the controls (P<0.05). Birds given Blend A had significantly higher BW than all other treatments from d14 to d35. No significant differences in egg production were observed; however, in the second set of eggs, the addition of Blend C showed a significant increase in % hatch of fertile and a significant decrease in % mortality when compared to control. These results warrant further research in other species or inclusion rates to determine optimal levels.

Key Words: Feed Additive, Essential Oils, Phytonutrients, Quail

Metabolism and Nutrition V, Enzymes/Feed Additives

M87, **Comparing the efficacy of low and high specific activity corn-expressed phytase varieties, at equivalent FTU/kg in broilers** Eric Sobotik*1GS, Gabrielle House [1], Austin Stiewert [1], Philip Lessard [2], Gregory Archer [1] *[1] Texas A&M university, [2] Agrivida, Inc*

A study was conducted to compare the efficacy of corn-expressed phytase varieties, low specific activity (Grainzyme® Phytase PY203, PY203) and high specific activity (Grainzyme® Phytase PY1203, PY1203), at equivalent FTU/kg in broilers. Day-old Cobb 500 male broiler chicks were randomly allocated to one of 10 dietary treatments: T1, Positive Control (PC, standard Ca & aP); T2, Negative Control (NC, -0.15% Ca & aP); T3, NC+0.5kg/MT PY203; T4, NC+1.0kg/MT PY203; T5, NC+2.0kg/MT PY203; T6, NC+3.0kg/MT PY203; T7, NC+0.18kg/MT PY1203 (equal FTU/kg as T3); T8, NC+0.36kg/MT PY1203 (equal FTU/kg as T4); T9, NC+0.72kg/MT PY1203 (equal FTU/kg as T5); T10, NC+1.08kg/MT PY1203 (equal FTU/kg as T6). All birds were weighed on d14, d28, and d42 to obtain performance parameters: body weight (BW), feed consumption (FC), and feed conversion ratio (FCR). On d28, both tibias were sampled from 3 birds per pen, for a total of 30 birds per treatment to determine tibia bone ash. All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at P≤0.05. Treatment means that were determined to be significant were further separated using Fishers LSD Test. On d14 and d28, birds from treatments with PY203 (d14, 0.50±0.00 kg; d28, 1.80±0.00 kg) and PY1203 (d14, 0.50±0.01 kg; d28, 1.81±0.01 kg) were heavier (P≤0.05) than PC (d14, 0.41±0.00 kg; d28, 1.65±0.03 kg) and NC (d14, 0.41±0.00 kg; d28, 1.52±0.03 kg). On d42, birds from treatments with PY203 (3.41±0.04 kg) and PY1203 (3.44±0.03 kg) were heavier (P≤0.05) than NC (3.09±0.07 kg). On d0-28 and d0-42, birds from treatments with PY203 (d0-28, 1.41±0.00; d0-42, 1.61±0.01) and PY1203 (d0-28, 1.41±0.00; d0-42, 1.59±0.01) improved FCR (P≤0.05) compared to NC (d0-28, 1.52±0.01; d0-42, 1.67±0.01). On d28, PY203 and PY1203 (51.69±0.08 %) improved bone ash (P≤0.05) when compared to NC (47.95±0.95 %). In conclusion, both low specific activity and high specific activity corn-expressed phytase varieties improved BW, FCR, and bone ash compared to CON. Inclusion of either phytase improves broiler performance and bone mineralization in Ca and aP reduced diets, and that there were no discernible differences in the efficacy of these two enzymes at the tested loading rates.

Key Words: phytase, broiler, performance, bone ash

M88, **Protease and purified trypsin inhibitor in the diets of broiler chickens** Ayodeji Aderibigbe*1GS, Aaron Cowieson [2], Jose-Otávio Sobara [2], Guenter Pappenberger [2], Olayowola Adeola [1] *[1] Purdue University, [2] DSM Nutritional Products*

Trypsin inhibitors (TI) in soybeans influence protein utilization but whether this is true for purified TI and if exogenous protease administration will ameliorate such effects are not known. Therefore, this study was designed to evaluate the effect of dietary mono-component protease on growth performance and nutrient utilization of broiler chickens fed diets containing purified TI. Soybean meal (SBM) was pre-analyzed for basal TI concentration (2,996 TIU/g SBM) and formulated to contain 1,033 TIU/g diet. Purified TI was added at 9,000 TIU/g diet. The diets were corn-SBM based, formulated to be nutritionally adequate and supplemented with phytase (1,000 FYT/kg). Using a 2 × 2 factorial arrangement, with two levels of dietary TI (1,033 or 10,033 TIU/g) and exogenous protease (0 or 15,000 PROT/kg), 320 chicks were allotted to 4 experimental diets, each containing 8 replicate cages and 10 birds per replicate. On d 7, 14 and 21 post hatching, protease supplementation improved the BW gain ($P < 0.01$) and feed efficiency ($P < 0.05$) of birds fed diets with added purified TI. On d 14 and 21 post-hatching, the relative weight of the pancreas increased ($P < 0.05$) with increasing dietary TI but reduced ($P < 0.001$) with protease supplementation. Apparent ileal digestibility of all amino acids decreased ($P < 0.001$) with added TI, and protease supplementation improved ($P < 0.05$) the ileal digestibility of all AA except methionine. Protease supplementation upregulated the mRNA expression of MUC-2 ($P < 0.01$) and

SCL7A-2 ($P < 0.05$) in the jejunal mucosa, as TI concentration increased in the diets. The activities of trypsin and chymotrypsin in duodenal digesta were reduced ($P < 0.05$) with increased dietary TI but increased ($P < 0.01$) with protease supplementation. Birds fed diets supplemented with protease had longer villi ($P < 0.05$) and deeper crypts ($P < 0.01$) in the jejunal tissue. These results confirm that dietary TI, supplied in a purified form, negatively affects nutrient utilization by broiler chickens and show the efficacy of exogenous protease in ameliorating these effects by improvements in growth performance, ileal digestibilities of nitrogen and AA and digestive enzyme secretion.

Key Words: broiler, protease, soybean meal, trypsin inhibitor

M89, **Effects of dietary supplementation of two prebiotics as an alternative to antibiotic growth promoters on performance and intestinal morphometry of broilers** Jairo Florez Cardenas[*1,2UG], Lina Peñuela Sierra[1,2], Juan Javierre[3], Mayra Diaz[4], Yuri Tique Obando[1,2], Cristian Cardozo[1,2], Carlos Rodriguez[1,2], Paula Lozano[1,2], Juan Mejia[1,2] *[1] Research group in livestock agroforestry systems, Research Seed in Animal Nutrition, University of Tolima,, [2] University of Tolima, [3] Tekzol SAS, [4] University of Applied and Environmental Sciences (UDCA)*

Generally, in animal nutrition, the antibiotic growth promoters (AGP) are used for preventing different gut pathologies, thus it could slowly generate resistance in the pathogen's microorganism, however we found different alternatives how the prebiotics because this gives a balance to the microbiota, gut health, and possibly the best performance in the animal. This research had the objective to evaluate the effect of replacement of AGP's to prebiotics in diets for broilers in all one production cycle. As a result, were used 2520 males Ross 308, and 1134 were randomly marked to obtain individual cycle data, furthermore, those animals were vaccinated with an overdose of vaccine for coccidia. Therefore, we established 9 dietary treatments (7 replicate pen/treatment, and 40 birds/pen). They were: A) Positive control with AGP; B) Negative control (NC), free AGP; C) NC + 50 ppm of yeast culture (YC); D) NC + 100 ppm of YC; E) NC + 200 ppm of YC; F) NC + 50 ppm of Manan-oligosaccharides (MOS); G) NC + 100 ppm of MOS; H) NC + 200 ppm of MOS; and finally I) NC + 50 ppm of MOS and YC. A randomized experimental design was used. This experiment was carried out in the experimental unit of TEKZOL - University of Tolima (Armero - Tolima, height 285 m.s.n.m, average temperature 28°C and 70% relative humidity). The data was taken on days 1, 11, 22 and 42, for performance and intestinal morphometry the sample was taken for the development of the animals on days 21 and 42 of age and the statistical computations were performed using the SAS program (version 9.4; SAS Institute Inc., Cary, NC, USA) application analysis of variance and Tukey's test ($p<0.05$). We found significant differences between the treatments A, D, H, in the parameters of weight (A: 2679.57 g, D: 2697.85 g, H: 2661.85 g), feed conversion (A: 1.647, D: 1.646, H:1.632), and intestinal morphometry (Villi length; A: 901µm; D: 982µm; H: 941µm; Villi width; A: 102µm; D: 113µm; H: 104µm; Muscular width; A:220µm; D: 228µm; H: 209µm; Crypt depth; A: 73µm; D: 74µm; H: 78µm) because of this, data was statistically different. Finally, we conclude that the effect of both prebiotics in different concentrations is equal or better than the AGP, furthermore, the combination of YC and MOS must be evaluated in future researches.

Key Words: Prebiotics, Performance, AGP, Intestinal morphometry., Gut health.

M90, **Forsythia suspensa extract as an antibiotic substitute enhances growth performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function and intestinal morphology in broilers** Shenfei Long*[GS], Tengfei He, Di Wu, Ming Yang, Xiangshu Piao *China Agricultural University*

This experiment was conducted to determine the effects of *Forsythia suspensa* extract (FSE) on growth performance, nutrient digestibility, antioxidant status, anti-inflammatory function and intestinal morphology of broilers. A total of 168 male Arbor Acre broilers (one d old, weighing 45.6 ± 1.3 g) were randomly allocated to 3 treatments, 7 replicate pens per treatment, 8 broilers per pen. The treatments contain a control diet (corn-soybean meal basal diet; CON), an antibiotic diet (basal diet + 75 mg/kg chlortetracycline; CTC) and a FSE diet (basal diet + 100 mg/kg FSE; FSE). Compared with CON, broilers supplemented with FSE showed higher ($P < 0.05$) average daily gain in phase 2 (d 22 to 42) and overall period (d 1 to 42), and increased ($P < 0.05$) average daily feed intake in overall period. On d 21, the concentrations of serum catalase and total antioxidant capacity (T-AOC) were enhanced ($P < 0.05$) in broilers fed FSE, while the content of serum T-AOC was increased ($P < 0.05$) in broilers supplemented with CTC in comparison with CON. On d 42, broilers fed FSE had lower ($P < 0.05$) lightness in breast meat and a reduction ($P < 0.05$) of nitrogen and phosphorus excretion, whereas these broilers also showed higher ($P < 0.05$) contents of superoxide dismutase and glutathione peroxidase, as well as improved ($P < 0.05$) apparent total tract digestibility (ATTD) of dry matter, organic matter, gross energy, total carbohydrates and phosphorus compared with CON. Broilers fed CTC had higher ($P < 0.05$) bursa of fabricius percentage as well as increased ($P < 0.05$) ATTD of gross energy, total carbohydrates and phosphorus in comparison with CON. Broilers fed FSE also showed decreased ($P < 0.05$) content of tumor necrotic factor-alpha in liver on d 21 and d 42, while those broilers had reduced ($P < 0.05$) levels of IL-1β and IL-6 in liver on d 42 compared with CON. The villus height was higher ($P < 0.05$) in duodenum, jejunum and ileum of broilers fed FSE in comparison with CON. In conclusion, *Forsythia suspensa* extract can be used as an antibiotic substitute on enhancing the growth performance via the improvement of nutrient digestibility, antioxidant status, anti-inflammatory function and intestinal morphology in broilers.

Key Words: anti-inflammatory, antioxidant status, broiler, Forsythia suspensa extract, performance

M91, **Improvement in the performance and inflammatory responses of Ross 708 broilers when injected in ovo with various forms of vitamin D3** saman fatemi*[1GS], Katie Elliott[1], Abdulmohsen Alqhtani[1], Ayoub Amousstaaid[1], Abiodun Bello[2], April Levy[3], David Peebles[1] *[1] Mississippi State University, [2] University of Alberta, [3] DSM Nutritional products*

Broiler performance has been shown to increase and their inflammatory response to decrease by the use of supplemental dietary vitamin D3 (D3) or 25-hydroxyvitamin D3 (25OHD3) under commercial conditions. Therefore, the objectives of this study were to determine effects of the in ovo administration of vitamin D3 (D3) and its metabolite, 25OHD3, on the performance and inflammatory responses of broilers fed commercial diets. Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 5 in ovo injection treatments at 18 d of incubation (doi): 1) non-injected; 2) diluent; diluent containing either 3) 2.4 µg D3, 4) 2.4 µg 25OHD3, or 5) 2.4 µg D3 + 2.4 µg 25OHD3. A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. At hatch, 18 male chicks were randomly assigned to each of 30 pens and birds were fed commercial diets for the starter (0-14 d of age (doa)), grower (15-28 doa) and finisher (29-42 doa) dietary phases. The BW, BW gain (BWG), feed intake, and feed conversion ratio of the birds were determined in each dietary phase. At 14, 28, and 39 doa, the α-1-acid glycoprotein (AGP) levels in the blood samples of 30 birds (6 replicates per treatment) were determined. A randomized complete block experimental design was used and all data were analyzed using a one-way ANOVA. At 39 doa, the in ovo injection of 25OHD3 alone decreased (P=0.045) plasma AGP concentrations in comparison to non-injected, diluent-injected, and D3-injected treatment groups. In addition, birds that received 25OHD3 alone had a higher BW (P=0.011) and BWG (P=0.05) from 29 to 42 doa as compared to non-injected, D3, and diluent-injected treatment groups. These results indicate that the in ovo injection of 2.4 µg of 25OHD3 resulted in an improvement in the performance and inflam-

matory responses of broilers. A reduction in the inflammatory response subsequent to the in ovo injection of 2.4 µg of 25OHD3 may have led to an increase in broiler performance.

Key Words: In ovo injection, Broiler performance, inflammatory response, Commercial diets, 25-hydroxyvitamin D3

M92, **Evaluation of tributyrin supplementation in diets varying in lipid source and corn particle size on live performance and nutrient utilization in broilers reared to 21 d.** Brooke Bodle*[1GS], Stefan Vaessen[2], Jonathan Broomhead[2], Sami Dridi[1], Samuel Rochell[1] [1] *Center of Excellence for Poultry Science,* [2] *Perstorp*

Tributyrin (TB) is a glyceride ester of butyrate that has the potential to improve broiler performance and intestinal development, and previous research in our lab showed that TB efficacy may be influenced by dietary lipid source and concentration. In addition, feed ingredient particle size can influence gizzard function, reverse peristalsis, and enzyme secretion, and potentially, utilization of esterified butyrate. Therefore, an experiment was conducted to investigate the interactive effects of TB, dietary lipids, and corn particle size on broiler performance and apparent ileal nutrient (AID) and energy digestibility (IDE). Dietary treatments were a factorial arrangement of 2 lipid sources (poultry fat or soy oil) x 2 corn particle sizes (730 or 1,042 µm) x tributyrin supplementation (with or without 500 mg/kg tributyrin). Dietary treatments were fed in a single feeding phase throughout the 21 d experiment and were formulated to be isocaloric and isonitrogenous. Titanium dioxide was used as an indigestible marker in the feed for determination of AID and IDE following collection of ileal digesta at 21 d. Each treatment was replicated with 9 cages of 8 off-sex male chicks from a Cobb 500 breeder line. From 0 to 21 d, a lipid source x TB inclusion interaction was observed (P = 0.005) for FCR, where an increase in FCR was observed in broilers fed TB within soy oil diets but an inverse response on FCR in broilers fed poultry fat diets. A 3-way interaction among lipid source, corn particle size, and TB inclusion was observed (P = 0.028) for N digestibility which was driven by a tendency for TB to increase N digestibility in soy oil diets with large corn particle size that was not observed with the small corn particle size. A similar response led to a 3-way interaction for IDE (P = 0.014). In conclusion, these data indicate that dietary lipid source and corn particle size may influence the efficacy of TB on broiler growth performance and ileal nutrient digestibility, though inconsistencies among measurements and with previous experiments indicate that dietary lipid profile may not be a primary determinant of tributyrin efficacy.

Key Words: Tributyrin, Poultry Fat, Soy Oil, Particle size, Broiler

M93, **Effect of multi-enzymatic solution dietary addition on broiler chicken growth performance and carcass characteristics** Gerardo Abascal-Ponciano*[1GS], Rob Shirley[2], Mark Lemons[3], Jessica Starkey[1], Charles Starkey[1] [1] *Auburn University Department of Poultry Science,* [2] *Adisseo North & Central America,* [3] *Sanderson Farms*

Previous studies with corn-soybean meal diets demonstrate that a multi-enzymatic solution increases the degradation of non-starch polysaccharides, compensates for reductions in energy and amino acids, reduces feed cost, and maintains optimal broiler performance. A randomized complete block design was used to evaluate the effects of ROVABIO® ADVANCE T-Flex (ENZ) on the performance and carcass yield of male Ross 708 x Yield Plus broilers. The 4 experimental corn-soybean meal-meat and bone meal-based treatments were: 1) a positive control diet (PC) with no ENZ formulated to meet the birds' nutritional requirements, 2) a mid-control diet (MC) with a 277 kJ/kg reduction in energy, 3) a negative control diet (NC) with a 553 kJ/kg reduction in energy, and 4) NC + ENZ. The feed phases were starter (d 1 to 14; S), grower (d 15 to 28; G), finisher (d 29 to 42; F), and withdrawal (d 43 to 50; W). Within each phase, lowering the PC energy resulted in the MC and NC formulations hitting a minimum % fat which led to a fluctuation of other ingredients and nutrient concentrations among the 4 treatments. On d of hatch, chicks (n = 1,440) were assigned to 48 floor pens with 30 birds/pen and 12 pens/treatment. Means were separated with SAS V9.4 PROC GLIMMIX and PDIFF at $P \leq 0.05$. There were no differences in BW during any feed phase ($P \geq 0.4000$). FI, BW gain, and FCR for S, F, and W phase were similar among treatment ($P \geq 0.1583$). However, during the G phase, broilers fed PC had a lower FI compared with other treatments ($P = 0.0007$) and exhibited lower FCR when compared to NC and ENZ ($P = 0.0013$). Cumulatively (d 1 to 50), FCR for PC and MC were lower than NC and ENZ ($P = 0.0003$). Regardless of treatment, d 50 BW and FCR were superior to the genetic source performance objectives. PC-fed birds had heavier fat pads ($P = 0.0075$), indicating an oversupply in dietary energy relative to the other treatments. Breast, tender, wing, thigh, and drum weights were similar among treatments ($P \geq 0.4987$). Breast yield as a percentage of the chilled whole carcass weight was greater for ENZ and NC when compared to PC, while MC was intermediate ($P = 0.0410$). In this experiment, supplementing a calorically deficient set of diets with ENZ did not result in equal or improved broiler performance.

Key Words: broiler growth performance, caloric requirements, metabolizable energy, multi-enzymatic solution

M94, **Dose response determination of Dried Egg Product on broiler growth performance** Daniel Adams*[1GS], Nannette Olmeda-Geniec[2], Kimberly Livingston[1] [1] *North Carolina State University,* [2] *Elanco Animal Health*

Consumer concern regarding antibiotics and other commercial drugs in the poultry industry is ever growing, as is the push for finding alternatives to eliminate those drugs. Coccidiosis presence in the poultry industry presents large economic losses each year, especially subclinical forms which impact feed conversion and weight gain. Birds diagnosed with coccidiosis tend to exhibit higher levels of IL-10 throughout the gut. IL-10 is an anti-inflammatory cytokine which is decreased during a textbook immune response; however, Eimeria species have evolved the ability to increase IL-10 levels throughout the body allowing for decreased inflammation and an increase in colonization of the gastrointestinal tract. This study assesses the impact of a Dried egg product (DEP) containing IL-10 neutralizing antibody on performance of broilers challenged with 10x Coccivac B52® (Merck, Kenilworth, NJ) given at 7 days of age. DEP was analyzed through this study and compared to a control basal diet containing no product. Birds were housed in floor pens with new litter for 42 days. Each floor pen contained 20 birds with 24 replicates per treatment. DEP was administered during the starter phase (0-14D) and the grower phase (14-28D), no product during finisher phase (28-42D). Birds and feeders were weighed at Day 7, 14, 28, and 42 to analyze performance parameters including feed conversion ratio, average body weight, average daily gain, and feed consumption. Intestinal lesions were visually analyzed on a scale of 0 to 4 from 2 birds per pen at day 14 using methods pioneered by Johnson and Reid. Fecal samples were taken at 14 and 28 days of age to obtain oocysts per gram of fecal material using the McMaster egg counting method. Data were analyzed using one-way ANOVA. Dried egg product group had an improved overall feed conversion ratio (0-42D) when compared to the control (P<0.05). DEP group showed reduced number of *E. acervulina* at day 28 when compared to the control group (P<0.05). In conclusion, DEP resulted in overall improvement of broiler performance.

Key Words: IL-10, IgY, neutralizing antibody, dried egg product, Coccidiosis

Metabolism & Nutrition VI, Amino Acids/General

M96, **Protein-sparing effect of methionine and choline supplementation of low-protein diets on productivity in laying hens—WITHDRAWN** Islam Omara*, Marwa Suliman *Cairo University*

The purpose of the experiment: This study investigated the sparing effect of methionine (Meth) and choline (Cho) in low-crude protein (CP) diets offered to laying hens on productivity performance, egg quality, nutrients digestibility, and in reducing pollution of the environment due to lowering nitrogen in the excreta.

The experimental design: The control diet contained 18% CP, 0.40% Meth and 400 mg Cho/kg. The experimental diets that contained 15% CP were supplemented with 2 levels of Meth (0.30 and 0.40%), and 3 levels of Cho (400, 800, and 1600 mg/kg diet). Bovans White hens (n = 700) at 27-week of age, were assigned to 1 to 7 treatment groups (4 replicate/treatment; 25 birds/replicate). Performance data were subjected to ANOVA and difference test was used to separate means with significance reported at $P \leq 0.05$.

Results: Hen-day egg production and egg weight increased ($P<0.0001$ and $P=0.0004$, respectively) in hens fed diet contained 15% CP, 0.30% Meth and 1600 mg Cho/kg. Hens receiving the low-CP diets consumed ($P<0.0001$) more feed than those receiving the control diet. Moreover, the combination of 15% CP at different levels of Meth and Cho supplementation improved ($P<0.0001$) feed conversion ratio compared to the control group. Shell thickness improved ($P=0.0002$) with all the diets contained 15% CP at different levels of Meth and Cho compared to the control group. No significant effects on egg shell weight and Haugh unit were observed due to CP, Meth, and Cho combinations. Yolk and albumen weight increased ($P<0.0001$) due to interactions among low-CP diets contained 0.30 or 0.40 % Meth, and 800 or 1600 mg Cho/kg. Laying hens fed low protein diet supplemented with Meth excreted less nitrogen in their excreta, hence less pollution to the environment compared to the control diet.

Conclusion: The dietary combination of 15% CP, 0.30 Meth, and 1600 mg Cho/kg diet was adequate in meeting the birds dietary requirements, without adversely affecting hen's productivity performance, egg quality, nutrient digestibility, and with the added benefit of reducing environmental contamination with nitrogen. In addition, Cho can spare part of the Meth supplementation in layer's diets, and was as effective in improving performance and egg quality of the birds.

Key Words: Protein, methionine, Choline, performance, egg quality

M97, **Implementation of Box-Behken Design to evaluate potential dietary interactions among the branched-chain amino acids** Craig Maynard*[1], Sonia Liu [2], Jason Lee [3], Justina Caldas [4], Edward Diehl [4], Samuel Rochell [1], Michael Kidd [1] *[1] Center of Excellence in Poultry Science, University of Arkansas, [2] The University of Sydney, [3] CJ America, [4] Cobb-Vantress*

Previous branched-chain amino acid (BCAA) studies have indicated that dietary BCAA excess mitigates the negative effects of their interactions. As diet CP is lowered in diets based on corn and soybean meal, Val and Ile decrease and are least cost pressure points, but Leu increases. Therefore, a study was conducted using Box-Behnken design (BBD) to determine the impact of BCAA antagonism on broiler performance and carcass traits. Thirteen experimental diets were formulated to contain various levels of digestible (dig) BCAA supplementation according to BBD. Inclusion levels for the BCAA were based on ratios to dig Lys and included: 65, 75, and 85 for dig Val; 58, 66, and 74 for dig Ile; and 110, 130, and 150 for dig Leu. Fifteen-hundred and sixty male broilers were distributed across 78 floor pens and offered common starter from 0 to 14 d. On d 15, broilers were randomly assigned to experimental diets and BW gain, feed intake, and feed conversion ratio (FCR) were determined for a 15 to 34 d period. On d 35, 6 birds per pen processed for determination of carcass traits. Data were analyzed for linear, quadratic, and interactive effects. Statistical significance was considered at $P \leq 0.10$. Significant interactions ($P<0.10$) were observed between Ile and Val for BW gain, FCR, and total breast yield and between Leu and Val for total breast yield. Thirty-five d BW gain was maximized when Val was increased to its highest level and Ile was decreased to its lowest level. Observed FCR response displayed reciprocal antagonism between Ile and Val where it was minimized when either amino acid was at its maximal level while the other was at its lowest. Responses observed for total breast yield also displayed reciprocal antagonism between Ile and Val and Leu and Val, where at practical levels (midpoint) increasing Val reduced total breast yield while increasing Ile or Leu increased total breast yield. Results from this experiment highlight the importance of evaluating potential interactions between amino acids when conducting trials to determine amino acid requirements as well as modification of amino acid levels in broiler diet formulation and their impact on live performance and carcass traits.

Key Words: Box-Behken Design, branched-chain amino acids, broilers, performance, carcass traits

M98, **Supplemental DL-Methionine improved performance and nutrient retention in broiler chickens fed cassava chips based diets** Olugbenga Ogunwole*[1], Samuel Etop [2], Bamidele Adedeji [1], Folasade Jemiseye [1], Martha Olumide [3], Ireti oludoyi [1] *[1] University of Ibadan, [2] Grand Cereals Limited, [3] Babcock University*

This study was aimed at investigating the effects of DL-methionine in cassava based diets on performance and nutrients retention in broiler chickens. One-day old Arbor Acres broiler chicks (n-450) were allotted to cassava chips based diets with cassava chips incorporated at 0, 25, 50, 75 and 100% alongside DL-methionine at 0, 0.1 and 0.2% in a 3x5 factorial arrangement and a completely randomized design. Each treatment was in triplicate of 10 chicks per replicate. Data were subjected to descriptive statistics, regression and analysis of variance while means were separated at P=0.05 Feed intake (FI) of starter chickens on different dietary levels of cassava chips were similar (P>0.05). The final body weight (FBW) of chicks on 50% cassava chips (757.6g) was significantly lower (P<0.05) to those on 0% (855.3g), 25% (809.8g), but higher than 75% (688.2g) and 100% (701.4g) dietary cassava levels. The feed conversion ratio (FCR) of broiler chicks on 0 (1.68), 25 (1.78) and 50% (1.94) cassava chips dietary inclusions were similar (P>0.05) but differed significantly (P<0.05) from those on 75 (2.14) and 100% (2.09) cassava chips based diets. Supplemental methionine significantly improved (P<0.05) feed intake (FI), FBW and FCR. At the finisher phase, FI was only lowered by 75% dietary cassava while WG and FCR did not improve with increasing levels of dietary cassava chips. The relationships among FCR (R2=0.56), FW (R2 =0.57) and supplemental methionine in cassava based diets were cubic. Crude protein retention reduced (P<0.05) with cassava inclusion while CF and ash increased. Supplemental methionine increased CP and ash retention but lowered crude fibre and ether extracts. Effect of interaction of cassava and methionine on all monitored parameters was significant (P<0.05). Thus, performance and nutrient retention by broiler chickens fed cassava based diets improved with dietary supplement of methionine.

Key Words: Supplemental methionine, Cassava hydrogen cyanide, Cassava chips, Arbor acre broiler, Chicks performance

M99, **Influence of trypsin inhibitors in soybean meal on protein digestion in broiler chickens and peptide characterization of jejunal digesta.** Roselina Angel*[1], Nelson Ruiz [2], Jose Otavio Sorbara [3], Laerke Haahr [4], Tine Jensen [4] *[1] University of Maryland, [2] Nelson Ruiz Nutrition LLC, [3] DSM Nutritional Products, [4] Novozymes*

The objective of the trial was to determine the effect of different concentrations of trypsin inhibitor (TI) in soybean meal (SBM) on amino acid (AA) digestibility and peptide characterization of jejunal digesta in the

presence of graded concentrations of a commercial protease. SBM of different TI concentrations were produced from a batch of soy beans and 5 batches selected with KOH solubilities >80%, containing 0.64, 0.94, 1.67, 2.24, 3.46 mg/g of TI. The trial consisted of 21 treatments (Trt) (4x5 factorial arrangement of 5 TI and 4 protease concentrations (0, 15, 75, 150 PROT/g feed) plus one purified diet). One batch of a purified basal diet was mixed and batches of SBM replaced 40% of the basal in the SBM Trt diets. Each batch of SBM Trt diet was divided into 4 and protease added to achieve desired concentrations. Each Trt was fed to 8 pens of Cobb 500 broilers from 14 to 16d of age. Broilers were euthanized after 48 h and jejunal (for peptide characterization) and distal ileal content (for standardized ileal AA digestibility) collected. There were main effects of TI and protease on Ala, Arg, Cys, Glu, Gly, His, Iso, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val ($P<0.01$) but no interaction ($P>0.05$) on standardized ileal digestibility (SID). Effects of protease were driven by improvements in SID between 0 and 15 PROT/g protease with no further improvements at higher protease inclusions. SID for all AA were lower ($P<0.5$) when TI was 0.64 and 3.46 mg/g. The effect of TI appears to be confounded with effects of over processing at 0.64 mg/kg TI. Overall, addition of protease at 15 PROT/g improved digestibility of all AA. The level of free AA, di- and tripeptides in jejunum were not affected by an interaction between TI and protease. However, data showed a decrease in the jejunal concentration of free AA, di- and tripeptides with increasing TI going from 0.64 to 3.46 mg/g ($P<0.05$). And free AA increased 23% with protease concentration going from 0 to 150 PROT/g ($P<0.01$). Further work is needed to understand what in processed SBM is impacting digestibility differently and how addition of a protease is helping improve digestibility.

Key Words: trypsin inhibitors, soybean meal, digestibility, amino acids, broiler

M100, **Precision feeding enhances feed efficiency and carcass yield compared to broilers offered standard feeding programs** Amy Moss*[1], Peter Chrystal[2], David Cadogan[3] [1] *University of New England,* [2] *Baiada Poultry Pty Limited,* [3] *Feedworks Pty Ltd*

Feed represents 70% of total chicken-meat production cost; hence, nutrition is an important area to improve efficiency. Broiler chickens grow rapidly and nutrient requirements change daily (Aviagen, 2014). However, they are fed 3-5 diets, meaning nutrients are under and over-supplied throughout production. Thus, increasing diet number improves production efficiency as there is less time in the production cycle that nutrients are in under- or over-supply (Hauschild et al., 2014; Kleyn 2013; Warren and Emmert; 2000). Nevertheless, the process of administering 4 or more diets is often impractical. Modern feed delivery systems may be programmed to automatically blend a protein-dense concentrate that can be subsequently diluted with a low protein but energy-dense concentrate on a daily basis to achieve the desired nutrient profile. Thus, with the advancement of technology, such systems may now be possible to implement. To determine the effect and feasibility of precision feeding (PF), a study was conducted using 640 Ross 308 broiler chicks over 0-42 days post-hatch to compare a standard commercial feeding program (SF) with a PF program (Feedworks, unpublished data). Within the SF program Ross 308 broiler chicks received 500 grams starter, 1,200 grams grower, 1,500 grams of finisher, and a withdrawal diet to 42 days of age. The PF treatment used blends of the above diets to meet daily requirements of the broiler as predicted via EFG Broiler Model Software. Digestible lysine levels ranged from 1.3 to 0.82% and energy levels from 2950 to 3200 kcal/Kg for both feeding programs and data was analysed via a one-way ANOVA. PF of broilers improved mortality corrected FCR by 5.2% (1.83 versus 1.93; $P < 0.05$) at 42 days in comparison to the SF program. Dressed weight of carcasses was increased (2.502 versus 2.282; $P < 0.001$), reducing the cost per kilogram of chicken-meat from 71.4 cents to 66.3 cents, based on the retail value in November 2012. Therefore, despite the limited number of studies to date, PF of broilers has demonstrated promising benefits for growth performance and carcass yield, and may therefore improve long term sustainability and profitability of the chicken-meat industry.

Key Words: precision feeding, broiler, energy, protein

M101, **Enteric Deoxinivalenol exposure and epithelial integrity disruption** Wageha Awad[1], Daniel Ruhnau[1], Barbara Doupovec[2], Eduardo Vicuña*[3], Dian Schatzmayr[2] [1] *Department for Farm Animals and Veterinary Public Health, Clinic for Poultry and Fish Medicine, University of Veterinary Medicine, Veterinärplatz 1, 1210,* [2] *BIOMIN Research Center, Technopark 1, 3430,* [3] *BIOMIN Brazil*

Recent scientific research has shown that deoxynivalenol (DON) is able to break down the intestinal epithelia morphology. Since DON is a common mycotoxin found in numerous feed ingredients, this epithelial damage is broadly studied to determine its systemic repercussions. Important physiological processes such as barrier function, digestive capability, and nutrient absorption can be negatively affected after DON exposure with the enteric epithelia. Moreover, disruption of epithelial integrity due to DON exposure can result in bacterial translocation, leading to systemic infections, poor nutrients absorption, and low general performance. The objective of this study was to evaluate the potential damage caused by DON to the enteric epithelia through two methodologies of enteric permeability evaluation: mucosal to serosal leakage of 14C-mannitol in the small and large intestine and E. coli colonization and translocation to internal organs of birds exposed to DON. A total of 75 day-old broiler chicks (Ross 308) were randomly divided among three treatment groups, and housed in floor pens (n = 25 ; 5 replicates/group) . The treatments consisted of a non-challenged negative control (NEG), and two challenge groups, 5 mg DON/kg feed and 10 mg DON/kg feed. Birds were fed their respective diets for 5 weeks. All tests were performed using a multivariate general linear model, Duncan's multiple range test, and IBM SPSS statistics 24, SPSS software (Chicago, IL, USA). Significantly lower Body weight (BW) and BW gain were found in the birds fed with 10 mg/kg compared with the ones to 5 mg/kg DON and the control group. Additionally, significantly ($P < 0.05$) higher levels of mannitol leakage levels were found in jejunum and cecum in the groups provided feed containing DON when compared with control group. In agreement with these results, a higher count of E. coli bacteria were found in liver and spleen. Taken together, these results suggest that DON is able to negatively affect epithelial integrity and its barrier function leading to bacterial translocation, systemic infection of bacteria such as E. coli, and a negative impact on bird's general health and performance.

Key Words: Mannitol, Permeability, Translocation, Broilers, DON

M102, **Ulvans activates avian monocytes and neutrophils** Maria Rodriguez*[1], Maria Garcia[1], Frederick Bussy[1], Matthieu Le Goff[1], Pi Nyvall-Collen[1], Nathalie Guriec[2], Benoit Quero[3] [1] *Olmix Group,* [2] *Univesity of Western Britanny,* [3] *R&D Breizh*

Recent research has highlighted the potential of in-feed marine polysaccharides (ulvans) from the cell wall of green macroalgae as a reliable strategy to strengthen animal's immune response. The effect of an extract from *Ulva armoricana* ($MSP®_{IMMUNITY}$) on birds' monocytes and heterophils was evaluated *in vivo*. Three hundred male broilers (Ross 308) of 28 days of age were randomly distributed in 4 groups of 25 birds each and were given $MSP®_{IMMUNITY}$ *per os* at different concentrations (0, 10, 25, 50 mg/l) at day 0 of the trial (28 days of age). Blood samples (1 milliliter per animal) were daily collected from day 0 to day 3. Nitric oxide (NO) concentration and β-D-glucuronidase activity were measured individually in the plasma, the latter via the 4-methylum-belliferone formation. A dose- and time-dependent NO release was observed with maximal concentrations at day 1 with values of 9.99 ± 0.85 μM for the negative control, 29.72 ± 1.34 μM with 10 mg/l ulvan, 48.22 ± 1.51 μM with 25 mg/l ulvan, and 81.8 ± 6.50 μM with 50 mg/l ulvan. The groups without ulvan supplementation did not present any statistically significant variations over time, with NO concentrations between 10.41 ± 0.71 μM and 9.34 ± 0.35 μM. Increased

ulvan concentrations led to higher 4-methylum-belliferone concentrations, reflecting a greater subset of heterophils and/or monocytes being activated. All the series displayed a peak of β-D-glucuronidase activity at day 1, swiftly declining, with values no longer statistically different from that of the negative control at day 3. At day 1, β-D-glucuronidase in the plasma allowed 4-methylum-belliferone concentrations to rise to 6.29 ± 0.48 µM for the negative control, 48.7 ± 2.85 Mm with 10 mg/l ulvan, 74.67 ± 3.38 µM with 25 mg/l ulvan, 89.61+/4.23 µM with 50 mg/l ulvan. In the groups without any ulvan added, no variation was observed with 4-methylumbelliferone concentrations ranging from 6.12 ± 0.55 µM to 6.63 ± 0.39 µM. *In vivo*, when given per os, the ulvan extract modulates the activity, directly and/or indirectly of key players of the chicken innate immune system, i.e., heterophils and monocytes. This innovative bioactive compound can thus play an important role within the reinforcement of the immune response.

Key Words: ulvan, algae, immunomodulation, neutrophil, monocyte

M103, **In vivo mixed muscle and collagen turnover in P. major in two meat broiler strains and their characterization by heat production, microbiota and plasma metabolites for myopathy** Pramir Maharjan*[1], Jordan Weil [1], Antonio Guerra [1], Katie Hilton [1], Nawin Suesuttajit [1], Casey Hanning [1], Justina Caldas [2], Craig Coon [1] *[1] University of Arkansas, [2] Cobb-Vantress, Inc.*

Two fast-growing meat-type broiler strains were reared in 70 floor pens (25 birds/pen; 35 pens/strain) from 0-56d utilizing primary breeder nutrition and husbandry guidelines for *P. major* mixed muscle and collagen protein turnover (PT) study. Broilers (n=10, each strain) were selected at d 21, 28, 35, 42 and 56 and infused with ^{15}N- phenylalanine (Phe) and 1-^{13}C proline (Pro) utilized as amino acid tracers. Muscle and plasma samples were collected and enrichments of ^{15}N-Phe and 1-^{13}C Pro were determined using mass spectrometry. Fractional synthesis rate (FSR) and fractional degradation rate (FDR) were measured. At d 42, broilers were also studied for heat production (HP), microbiota and plasma metabolites after separating the sampled birds as myopathy (woody breast (WB) score > 2) and non-myopathy (WB < 1) (n= 5 each group). Data were analyzed using one-way ANOVA using t-test. Data from microbial 16s rRNA gene sequencing were analyzed using mothur package, whereas plasma metabolome was processed using skyline software. Results showed that mixed muscle and collagen FSR and FDR in *P. major* dropped (P <0.05) for both the strains as broiler aged. Mixed muscle FSR for Strain A was 33.75 %/D whereas it was 35.03 %/D for Strain B at d 21 and decreased to 8.01 %/D and 11.66 %/D by d 56 respectively. FDR for mixed muscle and FSR for collagen was higher for Strain B than Strain A (P <0.05). Total collagen was higher (P <0.05) for Strain B (4.50 and 12.12 µg/mg for Strain A and Strain B by d 56) with greater myodegeneration occurring as exhibited by histomicrograph. Fasting HP (P <0.05) was higher for non-myopathy bird which was indicative of higher body protein content. Microbiota results showed unclassified *Lactobacillus* was predominant genus in myopathy group. Differentially expressed significant metabolites (P <0.05) identified between myopathy and non-myopathy group in plasma were homocysteine, cyclic GMP, trimethylamine N-oxide (TMAO), tyramine, carnitine and acetylcarnitine, which were all associated to cardiovascular system. Findings suggest that higher myodegeneration occurring in Strain B (than Strain A) in could be due to higher mixed muscle degradation (likely from compromised vascularity) resulting in higher collagen content and greater myopathy incidence.

Key Words: muscle synthesis, muscle degradation, collagen, metabolite, myopathy

M104, **Ingredient quality influence on Ark NE compared to Classic NE** Diego Martinez, Antonio Beitia, Craig Coon, Jordan Weil, Katie Hilton*, Naiwin Suesuttajit, Pramir Maharjan *University of Arkansas*

The continuous development of accurate and precise methods for measuring energy is vital in the modern poultry industry. The classical method of net energy (NE) assesses the value of ME minus heat increment (HI). Previous research shows increasing amino acid levels increases body protein but also heat production (HP), while increasing ME increases body fat but lowers heat production. Classic NE can be misleading as more calorie efficiency is given to fat deposition than lean mass deposition. Therefore it's desirable to provide an improved process of calculating NE based on body composition plus and HP (Ark NE). Three test diets were developed for each feeding phase, with two different samples of soybean meal (SBM) or corn. Diets were evaluated for starter (0-10d, 3,008 kcals ME/kg, 21%CP), grower (10-22d, 3,100 kcals ME/kg, 19%CP) and finisher (22-42d, 3,200 kcals ME/kg, 18%CP). The highest concentration of lysine/CP/kcal for each ingredient was utilized as control ingredient. The control ingredient was formulated in a corn soybean diet to provide 80, 100 and 120% AA requirements for each phase with both AA levels and ME set according to the breeder recommendation. Each of the SBM or corn samples were fed equally on a percentage basis as determined for the control SBM and corn. Heat production (HP) kcal=3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d, Farrell, 1974) was measured for 1d. Fasting heat production (FHP) was measured for 1d. HI was determined HI=HP – FHP (Farrell, 1974). Body composition was measured throughout the experiments by dual energy X-ray absorptiometry (DEXA) to determine net energy gain (NEg). The objective of these studies is to determine the effect of ingredient quality (digestible nutrient content) on NEg and HP (NE maintenance) while comparing the Classic NE and Ark NE calculations. As quantity of AA increased in SBM, HP and protein:fat ratio increased indicating a positive correlation (R^2=0.94). However, as the quantity of AA decreased in corn, HP and protein:fat ratio decreased indicating a negative correlation (R^2=0.99). Based on the finding in the current study, HP is mainly impacted by SBM AA, however when additional corn is added HP becomes diluted, making the Classic NE system misleading.

Key Words: Net Energy, Amino Acids, Soybean meal, Corn

M105, **Realistic doses of mycotoxin deoxynivalenol, fumonisins, and their combination on broiler performance and immunological response following challenge with Salmonella enteritidis** Jundi Liu*[1], Barbara Doupovec [2], Raj Murugesan [3], Cristiano Bortoluzzi [1], Ana Villegas [1], Todd Applegate [1] *[1] Department of Poultry Science, University of Georgia, [2] BIOMIN Research Center, [3] BIOMIN America Inc*

The objective of this trial was to evaluate the effects of realistic doses of the mycotoxins deoxynivalenol (DON), fumonisin (FUM), and their combination on broiler performance after challenge with *Salmonella* enteritidis. A total of 320 Cobb-Cobb 500 male broilers were obtained on the day of hatch and distributed 8 birds per cage into 8 replicates battery cages. The experiment consisted of 5 treatments: (1) non-challenge + non-mycotoxin control (NCC); (2) challenge + non-mycotoxin control (CC); (3) challenge + DON 0.6 mg/kg (DON); (4) challenge + FUM 14.0 mg/kg (FUM); (5) challenge + DON 0.6 mg/kg + FUM 14.0 mg/kg (DON + FUM). All birds were fed *ad libitum* with a standard broiler starter diet (mash form). On d 4, birds from challenged treatments were orally inoculated with 1 x 10^8 CFU/mL *Salmonella* enteritidis. Total pen and feed weights were assessed on d 0, 4, 7, 11 and 18. Results showed no significant effect of mycotoxins on growth performance before and after the challenge. Treatment did not show effect on FITC-dextran (4 kDa) intestinal permeability on d 18. The FUM treatment significantly decreased the cecal tonsil pro-inflammatory cytokine TNF-α gene expression on d 18 (14 dpi) compared to the NCC (P < 0.05), while it significantly increased the cecal tonsil IL-10 gene expression versus the CC treatment (P < 0.05) on d 11 (7dpi). However, no significant differences were found for cecal tonsil CD8+ or CD4+CD25+ cell percentages on d 18. The cecal *Sal-*

*monella*relative prevalence was not different among different treatments. Mortality was unaffected by treatment through the trial. In conclusion, results from the current study showed that the realistic dose of mycotoxin DON (0.6 mg/kg), FUM (14.0 mg/kg) or their combination in the diet did not affect broiler growth performance through 18 d of age. Dietary FUM at 14 mg/kg potentially affected the cecal tonsil immune related gene expression after challenge with *Salmonella* enteritidis.

Key Words: mycotoxin, deoxynivalenol, fumonisin, broiler, Salmonella

M106, **Trends in mycotoxin contamination in the United States Corn** Chasity Pender*[1], Erika Hendel [1], Paige Gott [1], Shelby Ramirez [1], Ursula Hofstetter-Schähs [2], Raj Murugesan [1] [1] *Biomin America, Inc.,* [2] *Biomin Holding GmbH*

Mycotoxins are harmful secondary fungal metabolites that are of key concern to food and feed safety globally. These toxins are detrimental to animal health and can compromise performance even at low levels. The classic signs such as reduced feed intake and oral and intestinal lesions used as field indicators for exposure often underestimate other costs of mycotoxicosis, including increasing the frequency and severity of disease via immune suppression, inciting inflammation, and modulating the gastrointestinal environment. This survey examines initial samples of the 2019 crop and examines trends with previous years. New crop corn samples from various sources were submitted from September 2019 and consisted of corn (46%), corn silage (50%), and corn by-product (4%). Samples were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin-A (OTA). Mycotoxin quantity and co-occurrence data were analyzed by a Kruskal-Wallis test with a Dunn's correction (Prism 7) with significance reported at $P \leq 0.05$. Due to the delayed 2019 harvest, the current sample size is quite small compared to the fall of 2018 (50 samples in 2019 vs. 135 samples in 2018), thus risk profile of this crop year is likely to change as the sample pool expands. Co-occurrence (≥ 2 mycotoxins) is similar to 2017 and less than 2018. B-Trich prevalence has decreased compared with previous years, but average ppb is similar to 2018. ZEN prevalence and average ppb have numerically decreased from 2018 and is similar to 2017, while FUM is numerically less in prevalence compared to 2018, but has increased in average ppb. The current sample pool has not had Alfa detected, although corn going into storage with higher moisture content has increased risk for storage toxins. Mycotoxin risk of this harvest season is still coming into focus as harvest delays have affected sample submission. Due to continued risk of multi-mycotoxin contamination, mitigation strategies are needed beyond adsorption, including biotransformation and support of the immune system and liver function.

Key Words: mycotoxins, corn, feed ingredients, temporal, United States

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M107, **Does utilization of dietary protein differ between different broiler genetic lines?** Lan Zheng*[1], Shelby Ramirez [1], Chasity Pender [1], Eric Sobotik [2], Gregory Archer [2], Raj Murugesan [1] [1] *BIOMIN America Inc,* [2] *Texas A&M University*

The demand for dietary crude protein (CP) is high in fast-growing modern broilers as protein is utilized for fast growth and immune system. Two experiments were conducted to evaluate the effects of dietary CP on growth performance of Cobb broiler chickens. In Exp. 1, 360 1-d-old Cobb 500 broilers were assigned to two diets with different CP levels [(a corn-soybean meal-based diet (CON), and a diet contained 1.5% less CP than CON (LP)] on used litter. Each treatment had 10 replicate pens and each replicate had 18 birds. In Exp. 2, 288 1-d-old Cobb 700 broilers were assigned to 8 replicates of 18 birds per replicate on the same experimental diets on used litter as in Exp. 1. BW and feed disappearance were recorded weekly. Data were analyzed using the GLIMMIX procedure of SAS. In Exp. 1, birds fed LP diet had lower ($P < 0.05$) BW and feed intake (FI) during 0-21 d. They had lower ($P < 0.05$) BW and tended to ($P = 0.068$) have a lower FI during 0-28 d, while had lower ($P < 0.05$) BW during 0-35 d. In Exp. 2, birds fed LP diet had higher ($P < 0.05$) BW and FI during 0-7, 0-14, and 0-21 d. Although no significant differences in BW, FI, and FCR were observed between the two treatments in both experiments at the end of the trial (42 d), birds fed LP diet in Exp. 1 (Cobb 500) had 5.8% (183 g/bird) numerically lower BW than control birds which was not observed in Exp. 2 with Cobb 700. This is in agreement with our previously published data with Cobb 500 fed the same LP diet but raised in fresh litter, as they had lower BW (2.7%, 69 g/bird; $P < 0.05$) than the CON birds. Therefore, these differences among broiler genetic lines in utilizing dietary protein as well as the litter condition need to be considered when formulating diets.

Key Words: crude protein, broilers, growth performance

M109, **LED lighting intensity, feather coverage, and novelty behaviors in turkey hens** Brooke Bartz*[GS], Jesse Grimes *North Carolina State University*

Lighting involves three main categories: daylength, intensity, and chromaticity which may alter bird physiology. The objective of this study was to determine if spectral differences in combination with light intensity, measured in footcandles (fc), altered bird welfare, when turkey hens were grown under a fixed 14h daylength with LEDs. Treatments were: 5000K LED at 10fc (5KH), 5000K LED at 2fc (5KL), 2700K (27K) LED at 10fc (2KH), 2700K LED at 2fc (2KL), and natural ventilation (curtain-sided house) blocked at 14h of light with a 75W incandescent lighting with natural flux in fc (NAT). An LED spectroradiometer was used to determine wavelength output (380-780nm) in addition to fc readings. At 5 weeks of age (WOA) and 9 WOA, right eye tissue was taken for eye weight (EW), cornea diameter (CD), averaged medial-lateral diameter & dorsal-ventral diameter (MLDV), and anterior-posterior diameter (APD) using a digital caliper. Two novelty items were introduced into each pen at 7 WOA with novel interactions video recorded. Novelties included: 1) visual stimulation+no nutritional value (VS) and 2) no visual stimulation+nutritional value (NV). Breast feather coverage was completed at 9 WOA using infrared imaging and area of heat signature recorded on the breast tissue was measured using the area tool within ImageJ to obtain pixel percentage. Data were analyzed using JMP13 as a one-way ANOVA and statistical significance obtained using Tukeys HSD procedure at a $P<0.05$. Dimming of LED lights significantly altered the spectral output overlapping cone sensitive droplets for both 2700K LED (415-violet, 460-blue, and 510nm-green) and 5000K LED (560 – 580nm-yellow/orange). There were no differences in EW, CD, MLDV, or APD at 5 WOA. There was no difference in feather coverage pixel percentage between treatments at 9 WOA ($P=0.56$). There were no differences in EW, CD, and MLDV at 9 WOA. However, the APD was significantly longer in the 5KL TRT compared to the 5KH TRT with all other TRTs intermediate in response at 9 WOA ($P=0.05$). There were no differences in behavior novelty response time to peck VS, peck NV, or perch on NV by TRTS at

7 WOA (P=0.70, 0.99, 0.83, respectfully). It is still unclear at this time if spectral output has significant physiological changes in turkey hens.

Key Words: LED intensity, Turkey, Behavior, Novelty, Feather Coverage

M110, **Comparing various euthanasia devices and methods on 8 and 12 week old turkeys** Austin Stiewert*[GS], Eric Sobotik, Gabrielle House, Gregory Archer *Texas A&M university*

A study was conducted to compare captive bolt devices (CB) and cervical dislocation (CD) euthanasia methods on turkeys 8 and 12 weeks of age. The captive bolt stunners tested include the Zephyr EXL (ZEP), an experimental Crossbow device (CBD), a Jarvis pneumatic stunner (JAR), and the Turkey Euthanasia Device (TED). The manual cervical dislocation methods include, the Koechner Euthanizing Device (KED), a broomstick method (BRM) and manual cervical dislocation (MCD). Each method was used on 100 turkeys at each age (n=1400). The pupil dilation response (PDR), nictitating membrane response (NMR), cessation of movement (CM), and skin penetration (SP) were recorded. All data was analyzed via Kruskal-Wallis Test, where treatment means were determined to be significantly different at P<0.05. There were differences observed at 8 and 12 woa between CB and CD in PDR, NMR, CM, and SP with the CB have lower (P<0.05) values in all measures compared to the CD. At 8 and 12 woa, the KED had longer (P<0.05) PDR than all other treatments. KED was higher (P<0.05) at 8 and 12 woa in CM and NMR compared to all other devices. At 12 woa, ZEP had the longest (P<0.05) CM time compared to all other CB devices. The TED and ZEP had the most instances (P<0.05) of SP at 12 woa compared to all other methods. In conclusion, all CB devices have lower PDR, NMR and CM, but higher instances of SP compared to all CD devices. This suggests that CB devices are swifter and more effective means of euthanasia compared to cervical dislocation.

Key Words: euthanasia, turkey, captive bolt, cervical dislocation

M111, **Constant and cyclic chronic heat stress models differentially influence growth performance and carcass traits of broilers** Jean-Rémi Teyssier*[1GS], Aurélie Preynat [2], Mickaël Briens [2], Sami Dridi [1], Samuel Rochell [1] [1] *University of Arkansas,* [2] *Adisseo France S.A.S.*

Heat stress (HS) is one of the most important environmental stressors challenging poultry production worldwide, and several models have been used to investigate its effect on the metabolism of broilers. This experiment aims to compare several HS models on broiler performance and carcass characteristics. On day of hatch, 720 off-sex male chicks from a Cobb 500 line were allocated into 12 environmentally controlled chambers. Each chamber (244x366cm) was divided into two pens containing 30 birds, and separated feeders and water lines. All birds were kept at thermoneutral temperatures until 20 d post-hatch. At d 20, 4 chambers were set to 35°C until d 41 (constant HS), 4 chambers were set to 35°C for 12h and 24°C for the next 12h until d 41 (cyclic HS). The remaining 4 chambers were maintained at 24°C and half of the birds in each of these chambers was pair-fed to equalize FI with constant HS birds (PF), while the other half was fed ad-libitum (CTRL). Birds were provided common starter (0 to 13 d), grower (13 to 27 d) and finisher (27 to 41 d) diets. Feed intake and BW were measured weekly and carcass characteristics were determined at the end of the experiment. From 20 to 41 d, the FI and BWG of PF birds and birds under cyclic and constant HS conditions were decreased ($P \leq 0.001$) compared with CTRL birds, whereas FCR was only increased ($P = 0.002$ and $P \leq 0.001$) for PF and constant HS birds. The overall FI, BWG and FCR of PF birds was similar to that of constant HS birds. Cyclic and constant HS birds also had lower carcass weight ($P \leq 0.001$), breast yield ($P = 0.008$ and $P \leq 0.001$), total white meat (TWM) yield ($P = 0.014$ and $P \leq 0.001$) and a higher wing yield ($P \leq 0.001$) than CTRL birds. Each of these measurements was more impacted ($P \leq 0.001$) by constant HS than by cyclic HS. Although PF and constant HS birds had similar performance, PF birds had lower relative fat pad weights ($P \leq 0.001$) and a higher TWM yields ($P = 0.02$) than the constant HS birds. Thus, the detrimental effect of HS cannot be entirely explained by reduced FI observed in stressed birds and indicates that HS per se affects metabolic pathways associated with muscle and lipid accretion in broilers. Molecular analysis and digestibility are undergoing to characterize more specifically those HS models.

Key Words: Heat stress, Feed intake, Performance, Carcass characteristics, Broilers

M112, **An algae extract to improve vaccine response and performance in poultry** Le Goff Matthieu*, Frederick Bussy *Olmix Group*

Poultry can be faced to infectious challenges such as Newcastle disease (ND). ND protection can be achieved via vaccination programs and improvement of the animals "immune function". Three trials were set up in pullets, layers and broilers to evaluate the effect of a commercial product including a sulfated polysaccharide extract from seaweed called MSP®IMMUNITY on performance and reinforcement of adaptive immune response against ND. The product was administered via the drinking water at a dosage of 0.5 to 1 ml/L. Blood samples were collected on 20 animals per group to evaluate ND antibodies titers by ELISA. First trial was performed in pullets, two live ND vaccines were administered (Day 35 and Day 91) and one inactive ND vaccine was performed (Day 105). The product including MSP®IMMUNITY was supplied around ND vaccination, one day before and two days after. Blood samples were taken before each ND vaccine and after the transfer to the laying building. No significant effect on antibodies titers was observed. The coefficient of variation decreased in the group including MSP®IMMUNITY (Mann-Whitney test P<0.05 at D63). This better homogeneity suggests a better vaccine uptake against ND. Feed consumption and daily weight gain were equivalent, but a decrease in mortality was observed. The trial performed in layer showed a significant improvement of laying performance via an application of the product three times during three consecutive days, at the arrival of the layers, at 50% of laying rate and at the egg production peak (Mann-Whitney test P<0.05). Regarding the ND antibodies, a decrease was observed at the laying peak in both group but more intensive in control group. The last trial was conducted in a broiler farm and the product was administered 3 days during the first week of life and around both ND vaccines (Day 9 and Day 22). After the vaccination against ND, coefficient of variation was significantly lower in group including MSP®IMMUNITY (Mann-Whitney test P<0.05 at D21). This better homogeneity is also observed after boost shot. No significant difference was observed for antibodies titers even if they are higher at day 35. These trials showed the capacity of the product to improve homogeneity of ND antibody titers in combination with improved performance.

Key Words: Alagae extract, Immune response, Vaccination programs, Newcastle disease, Performance

M113, **Inclusion of Ocimum graticimum leaf meal in diets of chickens affects expression of genes regulating antioxidant capacity** Ufuoma Sorhue*[1], Emenim Onainor [1], Adimabua Moemeka [1], Ikenna Omeje [2] [1] *Department of Agricultural Education, Delta State College of Education Mosogar,* [2] *Department of Animal Science, Delta State University Asaba Campus*

The uses of antibiotics as growth promoters and disease control have resulted in the development of resistant strains of pathogenic bacteria and the retention of drug residues in poultry products. This has led to the advocacy for natural plant sources to replace antibiotics in poultry production. This study was therefore conducted to examine the effects of replacing in-feed antibiotics with Ocimum graticimum (OG) on expression of genes regulating the antioxidant capacity in chickens. A total of forty-five birds were randomly allotted into five dietary treatments at fifteen birds per treatment. Birds were fed diet containing varying levels of Ocimum graticimum leaf meal. Treatment one had 0% OG, while treatment two, treatment three, treatment four and treatment five had 0.5% OG, 1.00% OG, 1.5% OG and

2% OG respectively. Feed and water was provided adlibitum throughout the feeding trial. Three birds were slaughtered from each treatment at day 28 and 56, while liver samples were collected and stored using RNAlater in a -20°c freezer prior to RNA extraction. RNA cleanup was done using the RNA cleanup kit, while the quality and concentration was measured using a Nanodrop. cDNA synthesis was done in a thermocycler. Real-time qPCR was performed in 40cycles using the PowerUp SYBR Green reagent and analyzed using the ΔΔCt method. Gene expression data were subjected to analysis of variance in a completely randomized design. Genes studied includes; NRF2, SOD1, CAT, CASP6, GPx1, GST, GR. The results revealed that treatments diets had effects on the expression of genes controlling antioxidant capacity in chickens at 28days and 56days of age respectively. This study shows that dietary Ocimum graticimum leaf meal can act as regulating agent for oxidative stress in chickens.

Key Words: Scent leaf, Gene expression, Oxidative stress

Environment Management II Env. Factors/Bird Welfare

M114, **In silico reverse pharmacological investigation of the mechanism of action of a herbal gut function modulator in poultry** Bhaskar Ganguly*, Sunidhi Chauhan, Sameer Sapra, Ravikanth Kotagiri *Ayurvet Limited*

The use of herbal gut function modulators in poultry is often met with scientific skepticism with regards to the mechanism of their action. The polyherbal nature of such formulations is a significant impediment in the deciphering of their exact mechanism of action. In this communication, the results of an *in silico* reverse pharmacological investigation into the mechanism of action of a herbal gut function modulator, viz. Nbiotic™ (M/s Ayurvet Limited, India), are presented. A computational, reverse systems pharmacology approach combining chemical predictors based on molecular structure, chemo-genomics linking of compounds with pharmacological information, network-reconstruction for the targets, and target enrichment was employed. Briefly, the prominent phytochemical molecules in all the constituent herbs of Nbiotic™ were identified by extensive literature mining. Thereafter, the molecular targets of the phytochemicals showing favorable absorption and distribution characteristics (SwissADME; http://www.swissadme.ch/index.php) were identified with SwissTargetPrediction (http://www.swisstargetprediction.ch/). These molecular targets were further enriched with Panther v. 14.1 (https://www.pantherdb.org) and the interaction networks were identified with String v. 11.0 (https://string-db.org/). Lastly, the predicted pathogen targets were validated by molecular docking using DockThor (https://dockthor.lncc.br/v2/); all software were used at their default settings. Our results uncover the prominent phytoactive compounds, their molecular targets and the mechanisms that are responsible for the pleiotropic effects of Nbiotic™ such as the upregulation of immune mechanisms and the subjugation of inflammaging responses; molecular docking analysis showed the direct inhibitory activity of the phytochemicals against the pathogen targets. In conclusion, a computational reverse pharmacological approach was successfully employed to model the mechanism of action a polyherbal gut function modulator.

Key Words: gut function modulator, herbal, Nbiotic, mechanism, reverse pharmacology

M115, **The effects of water shower enrichment on Pekin duck growth, stress, and behavior** Gabrielle House*GS, Eric Sobotik, Austin Stiewert, Gregory Archer *Texas A&M university*

A study was conducted in order to determine the effects of water shower enrichment on Pekin duck behavior and welfare. Pekin ducks were reared on raised plastic slatted pens with access to one shower device per pen (Shower) or no access to water showers (Control) for 35 days. Showers ran beginning at 8:00 AM, and were turned on every 30 minutes until 6:30 PM every day beginning at 7 d of age. Fear response was analyzed using tonic immobility (TI) and inversion (INV) tests. Growth parameters included feed conversion ratio (FCR) and body weight (BW). Stress susceptibility was measured using heterophil to lymphocyte ratio (HL), and the physical asymmetry of three bilateral traits (metatarsal length, metatarsal width, and middle toe length; ASYM). All measurements were performed during Week 5 of the study. A one-way ANOVA was used for data analysis using the Minitab software, and significant differences were considered at $P < 0.05$. No differences were found between the two treatments for fear response parameters, growth parameters, or stress susceptibility measurements ($P > 0.05$). The results of this study indicate that Pekin ducks reared under normal environmental conditions did not have improved welfare or production parameters when provided water shower enrichment.

Key Words: Ducks, Enrichment, Production, Stress, Welfare

M116, **Resistance to fenbendazole in Ascaridia dissimilis and its economic impact on the production of turkeys** James Collins*1GS, Brian Jordan[2], Luke Baldwin[3], Claude Hebron[4], Kelsey Paras[1], Pablo Jimenez-Castro[1], Anand Vidyashankar[5], Justin Fowler[3], Ray Kaplan[1] *[1] University of Georgia, Dept. Of Infectious Diseases, [2] University of Georgia, Department of Population Health and Department of Poultry Science, [3] University of Georgia, Department of Poultry Science, [4] Prestage Farms, [5] George Mason University*

Ascaridia dissimilis is the most prevalent and one of the most economically important gastrointestinal nematodes of turkeys. Infections are most often subclinical, producing reduced feed conversion efficiency, with heavier infections causing symptoms such as lethargy, intestinal blockage, diarrhea, and enteritis. Currently, fenbendazole (FBZ) is the only FDA approved drug for use against this parasite in turkeys. We recently tested the efficacy of FBZ against 5 field isolates of *A. dissimilis* acquired from commercial farms. 3 of the 5 isolates (Wi, Ow, Po) demonstrated greater than 99% efficacy. However, two (Sn, Ad1018), yielded efficacies of 63.9% and 76.2%, respectively, indicating FBZ resistance in these isolates. Having proven FBZ resistance in *A. dissimilis*, we wanted to determine the economic impact of resistant worms on growth and productivity. 384 1-week old turkey poults were infected with either the Sn (resistant) or the Ow (susceptible) isolates. For each isolate, 8 replicates each of untreated and treated pens were established with 12 birds/pen. Turkeys were grown to 10 weeks of age, changing feed types at the recommended time points as per commercial growing practices. Birds were infected via a trickle dosage of 25 eggs/bird/week sprinkled onto their feed. At 4- and 8-weeks post infection, birds in the treated groups were administered FBZ via water (SafeGuard® Aquasol, 1.25mg/kg) for 5 consecutive days. Weight, weight gain and feed conversion were analyzed weekly for differences between groups. At weeks 7 and 8 post infection, feed conversion in the Ow-treated groups was significantly better than the other three experimental groups; this group used 700 grams less feed/bird than the other three groups. Since this was the only group with an effective treatment, these results strongly suggest that worms infecting the other 3 groups (due to either lack of efficacy or lack of treatment) caused a significant decrease in feed efficiency. Further work is needed to determine the full scope of *A. dissimilis* infections on feed conversion, but based on these data, there appears to be a large economic impact of *A. dissimilis* on turkey production. Consequently, resistance to FBZ also requires further study to investigate its prevalence and distribution.

Key Words: Ascaridia, Fenbendazole, Resistance, Feed Conversion, parasites

M117, **The prevalence and antimicrobial resistance properties of Campylobacter in Mississippi poultry** Tianmin Li[*1GS], Sadie White [2], Nikhil Nuthalapati [1], Anuraj Sukumaran [1], Aaron Kiess [1], Li Zhang [1] *[1] Department of Poultry Science, Mississippi State University, [2] Department of Food Science, Nutrition and Health Promotion, Mississippi State University*

Campylobacter is a leading cause of food-borne illness in the United States, and contaminated poultry meat is a major contributor to human Campylobacteriosis. Attempts to control *Campylobacter* using antimicrobials have become less effective with the widespread emergence of antimicrobial resistant strains. This study aimed to investigate the prevalence and antimicrobial resistance properties of *Campylobacter* in raw retail chicken and from commercial broiler farms in the state of Mississippi. Organic (n=70) and inorganic (n=90) chicken samples were collected from retail stores. Litter (n=103), feces (n=74), and cloacal swabs (n=72) were collected from commercial broiler farms. *Campylobacter* isolates were recovered from the samples using the USDA MLG41.04 protocol and confirmed by real-time PCR and 16S rRNA gene sequencing. The antimicrobial resistance properties were evaluated genotypically by examining the presence of 5 antimicrobial-resistant genes (*Tet(O), Aph(2")-lg, Aph(3")-llla, Oxa-184, Oxa-61*) and phenotypically by antimicrobial disk susceptibility testing (AST) against 9 antibiotics (azithromycin, ciprofloxacin, erythromycin, gentamicin, tetracycline, florfenicol, nalidixic acid, telithromycin, clindamycin). A completely randomized design was used to analyze the results; differences were evaluated using Chi-square test at P<0.05. Overall, out of 162 retail chicken samples, 66 contained *Campylobacter* (41% prevalence). The prevalence of *Campylobacter* in organic and inorganic samples were 31% and 44%, respectively (P>0.05). A total of 45 samples from the broiler farms were positive for *Campylobacter*, and the prevalence percentages were 4.8%, 7.4%, and 7.0% in litter, feces, and cloacal swabs, respectively (P<0.05). Most of the *Campylobacter* isolates carried 1 (22%) or 2 (11%) of the antimicrobial resistance genes. AST results showed that 41 isolates (37%) were resistant to at least one antibiotic and 22 isolates (20%) were multi-drug resistant. The representative *Campylobacter* isolates have been selected for whole-genome sequencing. Further research will focus on bioinformatics studies to identify potential vaccine candidates for the control of *Campylobacter* in poultry.

Key Words: Campylobacter, Antimicrobial resistance, Retail chicken, Commercial farm, Foodborn pathogen

M118, **Comparison of four bacterial DNA extraction methods in poultry litter and fecal samples** Sadie White[*1UG], Tianmin Li [2], Sabin Poudel [2], Aaron Kiess [2], Li Zhang [2] *[1] Mississippi State University, Department of Food Science, Nutrition and Health Promotion, [2] Mississippi State University, Department of Poultry Science*

Rapid and accurate detection of bacterial pathogens in poultry is crucial for the investigation and control of bacterial infection. PCR-based method proved to be a reliable means of detecting pathogen, but it requires high quality DNA for precise and reproducible results. Poultry feces and litter are known to contain large numbers of PCR inhibitors which may reduce or even completely impede the PCR amplification process. Thus, the appropriate, effective DNA extraction methods should be carefully assessed. The objective of this study was to assess different methods in their performance and effectiveness for the extraction of bacterial DNA in order to build a reliable technique for further detection of specific pathogens in poultry. Four different methods: phenol-chloroform, salt precipitation (Wizard), silica membrane (Qiagen) and magnetic beads-based (MagMax) extractions, were evaluated for the yield, purity and quality of extracted bacterial DNA from litter and fecal samples collected at a commercial broiler farm. Yield and purity were measured using Qubit fluorometer and NanoDrop spectrophotometer; quality was evaluated by agarose gel electrophoresis. The processing time, labor and cost for each method were compared. A completely randomized experimental design was used to analyze results; means were separated by Fisher's LSD at P<0.05. The results showed that the four extraction methods produced significantly different yields and purity of the extracted DNA products (P<0.05). Wizard method showed the highest DNA extraction yield (P<0.05) while Qiagen method showed the highest purity of extracted DNA (P<0.05). Phenol-chloroform method is the least expensive but is more time consuming and laborious than the other three methods. The efficacy of DNA extraction methods was highly dependent upon the type of sample, the results showed that the yield and purity of DNA extracted from fecal samples were significantly higher (P<0.05) than that isolated from litter samples. It is suggested that the Wizard and MagMax methods can be used to extract bacterial DNA from fecal and litter samples, respectively. Further research will focus on the detection of pathogenic bacteria by applying the above-mentioned DNA extraction technique in poultry fecal and litter samples.

Key Words: DNA extraction methods, poultry, feces, litter, pathogen detection

M119, **Does chick paper impact early chick performance on a commercial broiler farm?** Connie Mou[*GS], Michael Czarick, Brian Fairchild *University of Georgia*

A number of studies have found that reducing time between hatching and a chick's consumption of feed and water results in improved first week body weights, lower first week mortality, and better overall performance. To improve day-old chicks' access to feed, there is a growing trend for broiler producers to use chick paper sprinkled with feed in addition to the house feeder system and traditional supplemental feeders. Though anecdotal data indicates that chick paper has resulted in improved first week performance, to date there has been no objective study evaluating the effectiveness of this practice and whether the paper could have a negative impact on litter and footpad quality.

A study was conducted on a commercial broiler farm to examine the effect of chick paper on early chick performance, litter and footpad quality. The 21-day study was held in a 12 m X 152 m house divided into 8 sections by using a wired v-migration fence. All sections were equipped with Turbo Grow™ chick feeders placed between every other Big Dutchman Fluxx™ 360 dish feeder pan (35 cm, 16 arm). Chick paper (1 m X 36 m X 0.6 m) with 50 g of feed per chick (TRT) was placed in 4 sections and 2 large feed lids placed along the sidewall (CTL) in the other 4 sections. Each section was equipped with low flow water meters (20-5000 ml/min). In each section, 100 randomly selected chicks were crop scored at six- and 24-hours post-placement. One hundred randomly selected chicks were individually weighed in each section at Days 0, 1, 3, 7, 10, 14, and 21. Litter moisture and footpad quality were evaluated on Days 0, 7, 10, 14, and 21.

Six hours after placement, TRT used approximately 22% ± 4.8% more water and had 25% more chicks with full crops versus CTL (75% ± 10% vs 51% ± 16%). At 24 hours, there was no difference in total water usage (60.5 L ± 7.5 vs 60.5 L ± 7.5) or % of chicks with full crops between TRT and CTL (60% ± 5% vs 58% ± 3%). At Day 21, there were no significant differences between TRT and CTL in bird body weights (824 ± 21 g vs 818 ± 20 g), litter moisture (26% ± 2% vs 25% ± 2%) or occurrence of footpad lesions (3.2% ± 0.5% vs 3.3% ± 1%). During the 21-day study, TRT used on average 11% ± 4.4% more water than CTL.

Key Words: brooding practice, water usage, chick, management

M120, **Quantification and persistence of Clostridia on the surface and in the contents of chicken hatching eggs during incubation** Karely Cantu[*GS], Craig Coufal *Texas A&M University*

The purpose of this research was to quantify *Clostridia* on the surface and internal contents of chicken hatching eggs throughout the incubation period. Two preliminary trials were conducted to evaluate the presence of *Clostridia* on the surface and subsurface of visibly clean chicken hatching eggshells (10 eggs per trial). Eggs were collected from White Leghorn breeders at various ages. Surface *Clostridia* counts were enumerated by

rinsing the eggs with sterile PBS. Subsurface counts were evaluated using a modified crush and rub method. Average eggshell surface *Clostridia* counts obtained were 5.92 and 5.52 $\log_{10}$ cfu/egg for trial 1 and 2, respectively. Subsurface average enumeration results were 3.46 and 6.44 $\log_{10}$ cfu/egg for trial 1 and 2, respectively. An experiment was then conducted to assess *Clostridia* contamination on broiler hatching eggs. Eggs were obtained from a commercial hatchery from the same broiler breeder flock at 27 weeks of age. Eggs were evaluated at 0 (egg cooler), 6, 12 and 18 days of incubation. Five eggs were randomly sampled per incubation time. *Clostridia* was enumerated from the eggshell surface by rinse and plate methodology. Egg internal contents were evaluated by emptying the egg contents into a sterile Whirl-pak bag and diluting with sterile PBS. The surface and internal content samples were plated onto egg yolk agar and incubated in an anaerobic chamber for 48 hours. A one-way ANOVA was used for statistical analysis and significant differences were determined at $P < 0.05$. Surface rinse results demonstrated that 100% of the eggs sampled at day 0, 6, 12 and 18 were positive for *Clostridia* with an average of 5.25, 4.45, 5.37, 5.43 $\log_{10}$ cfu/egg, respectively. Data indicated that *Clostridia* increased from day 6 to 12 and 18 ($P < 0.04$ and $P < 0.03$, respectively). Furthermore, 75% of the eggs sampled for internal contents were positive for *Clostridia* with an average of 2.04, 0.78, 1.35, 1.92 $\log_{10}$ cfu/egg for 0, 6, 12 and 18 days of incubation, respectively. Results from this study suggest that *Clostridia* can persist on the surface and in the internal contents of eggs throughout the incubation period. Further research is warranted to evaluate persistence and impact of *Clostridia* on hatchability and chick colonization at hatch.

Key Words: Eggshells, Contamination, Clostridium, Incubation, Anaerobes

Environment Management III, Env. Factors/Bird Welfare

M121, **Comparison of dynamic and non-dynamic weighing methods for determination of individual broiler chicken body weight over time** Allan Calderon[*GS], Luis Avila, Joshua Flees, Charles Starkey, Jessica Starkey *Auburn University Department of Poultry Science*

Human error can be a cause of variation when conducting research projects, especially when experiments involve obtaining bird weight data as birds tend to move when performing this measurement. Additionally, weighing of individual animals can take a significant amount of time and, therefore, pen weights are often used instead. However, pen weights do not allow for the calculation of BW variation over time. Scale software has improved to mitigate some issues that often lead to erroneous readings, and thus, incorrect interpretation of experimental results. These improvements, which are often semi-automated, can also reduce the time spent weighing individual birds. Therefore, the objective of this experiment was to evaluate the accuracy and efficiency of using an Ohaus Defender® 7000 scale with a 2-s, semi-automated dynamic weighing mode (DM) compared with the conventionally used, non-dynamic weighing mode (NDM). DM measures the weight 10 times per s and provides the user with the most frequent weight observed during the weighing period. Broilers were reared in floor pens to 56 d and on d 21, 42, and 56, 20 birds were randomly selected to be individually weighed using both weighing methods to record a live weight (LIVEBW) using a static analytical scale on both methods. After measuring LIVEBW, birds were euthanized to obtain the true bird BW (TRUEBW). The absolute difference between LIVEBW and TRUEBW was calculated for both methods to assess accuracy. For both DM and NDM, the time required to obtain a still BW (TSBW) was measured from the moment that the bird was placed on the scale until the BW fluctuation was 0 g. Data were analyzed using the GLIMMIX procedure of SAS (v9.4) and means were separated using PDIFF when $P \leq 0.05$. On d 21, the use of DM reduced the absolute difference of LIVEBW from TRUEBW compared with NDM (1 vs. 4 ± 1 g; $P = 0.0210$) but was similar on d 42 or 56 ($P \geq 0.2453$). As importantly, on d 21, the TSBW using DM was 4-fold lower than NDM (3.0 vs. 15.7 ± 2.4 s; $P < 0.0001$), 10-fold lower on d 42 (3.0 vs. 34.7 ± 3.4 s; $P < 0.0001$), and 11-fold lower on d 56 (3.0 vs. 37.4 ± 3.1 s; $P = <0.0001$). Use of DM for weighing broilers resulted in a faster, more efficient, and in 3-wk-old birds, a more accurate method to determine individual broiler BW.

Key Words: body weight determination, broiler chicken, dynamic weighing, accuracy

M122, **Effect of light intensity and reduction of dietary energy and digestible lysine density on broiler chicken growth performance, breast meat yield, and meat quality defects** Joshua Flees[*GS], A. Jake Keel, Charles Starkey, Jessica Starkey *Auburn University Department of Poultry Science*

Previously, lighting programs and manipulation of dietary nutrient densities in different phases of broiler production have been aimed at optimizing growth and yield, while eliminating the meat quality defects, Wooden Breast (WB) and White Striping (WS). However, little research has been conducted to study their combined effects. The objective of this randomized complete block design experiment with a 2 × 2 factorial treatment arrangement to assess the effects of light intensity (LI) and starter phase dietary nutrient restriction (SDNR) on broiler growth, breast yield, and incidence and severity of WB and WS. On day of hatch, Yield Plus × Ross 708 broilers (n = 384;16 birds per pen; 3 replicate blocks) were randomly allotted to floor pens in the same facility that received either 2 (LOWLI) or 30 (HIGHLI) lux of light at litter level for equal durations from d 0 to 35. Birds were fed either: 1) a commercial starter diet (CON) or 2) the CON diet with a targeted 15% reduction in both ME and digestible lysine (dLys) density (RES) in crumble form from d 0 to 14. All birds consumed a common grower diet from d 15 to 35. Means were separated using SAS (V9.4) PROC GLIMMIX and PDIFF at $P < 0.05$. No interaction among LI or SDNR was observed ($P > 0.05$). Broilers reared with HIGHLI were heavier on d 35, had increased feed intake (FI) in all feed phases compared with those reared in LOWLI ($P \leq 0.0096$). Broilers reared with LOWLI had lower BW gain from d 15 to 35 and d 0 to 35 compared with those reared with HIGHLI ($P = 0.0073$). Broilers fed the RES starter diet had similar BW and BWG ($P \geq 0.1902$) from d 0 to 14, but increased FI and FCR than those fed CTL ($P \leq 0.0012$). Yet, d 0 to 35 growth performance was similar among RES and CON-fed birds ($P \geq 0.1902$). Neither LI nor starter diet altered breast weight, breast meat yield, and WB and WS incidence and severity ($P \geq 0.2050$). In conclusion, reductions in LI and dietary ME and dLys density during the starter period did not produce the expected reductions in breast yield and incidence and severity of WB or WS. Further reductions in starter diet ME and dLys density should be studied to better understand their impact on broiler performance, yield, and the incidence and severity of meat quality defects.

Key Words: light intensity, dietary nutrient restriction, digestible lysine reduction, wooden breast, broiler growth performance

M123, **Remedying Footpad Dermatitis Lesions in Broiler Chickens** Nathan Freeman*[1UG], Frank Tuyttens [2,3], An Garmyn [2], Leonie Jacobs [1] *[1] Virginia Tech, [2] Ghent University, [3] Research Institute for Agriculture, Fisheries and Food*

Footpad dermatitis (FPD) is an irritation of the skin, with necrotic lesions on the surface of the footpad. FPD can affect bird welfare due to pain and behavioral restriction. Furthermore, FPD can pose a health risk and reduce performance. Flock-level remedial approaches to developed lesions are unknown. Thus, our objective was to assess three remedial treatments to developed FPD lesions, and compare those to preventative approaches and a control treatment. Day-old male broilers (546 Hubbard*Ross) were housed in 42 pens (1.25 m2), with 13 birds/pen. We tested 7 treatments, with 3 flooring treatments provided either from day 1 (preventative approach; PREV) or day 29 (remedial approach; REM), plus a control treatment. The flooring treatments were: used litter from a previous flock (CONTROL), new pine shavings replaced every 4 days (CLEAN), disinfectant foam mat (0.42 m2) covered with polyester mesh filled with 1% povidone-iodine solution (MAT), and the mat with iodine placed on a plastic slatted floor (0.72 m2, SLAT). FPD and gait were assessed weekly (0-100 score, with 100 representing worst lesions/gait) from 7 birds per pen (n=294 birds). Data were analyzed in SAS 9.4. Results are presented as LSMeans. MAT and SLAT treatments showed worst FPD and gait scores when provided from day 1 or day 29, compared to other treatments, including CONTROL. Providing and replacing litter from day 1 (PREV-CLEAN) consistently resulted in healthier footpads (PREV-CLEAN FPD score 3.3, CONTROL 15.4, PREV-MAT 47.4, PREV-SLAT 52.4; SEM 3; P<0.001) and lower (better) gait scores (PREV-CLEAN gait score 7.8, CONTROL 11.2, PREV-MAT 13.8, PREV-SLAT 12.7; SEM 2; P<0.001) compared to the other treatments. Furthermore, providing clean litter from day 29 (REM-CLEAN) resulted in healing of FPD lesions (FPD score 12.4 in week 4 vs. 2.5 in week 7; SEM 3; P≤0.05), but did not result in improved gait scores (gait score 8.4 in week 4 vs. 23.6 in week 7; SEM 2; P≤0.05). While iodine is an effective antiseptic, it was ineffective as a treatment for FPD in our study. The continuous provision of clean litter worked best to avoid and counteract FPD lesion development and a similar approach may be feasible in a commercial setting.

Key Words: broiler chicken, animal welfare, footpad dermatitis, environment, litter

M124, **Comparison of male to traditional female diet on rooster semen quality and reproductive performance** Kelly Sweeney*[1GS], Santiago Garcia [2], Benton Hudson [3], Jeanna Wilson [1], Andrew Benson [1], Carla Aranibar [1], Laura Oxford [1] *[1] University Of Georgia, [2] University of Mexico Veterinary School, [3] Aviagen*

Adult broiler breeder males are often fed a hen diet that exceeds their nutrient requirements for protein, amino acids and calcium instead of a lower-density male ration. Two diets were mixed with 2750 kcal/kg and either 12.4% or 14% crude protein, respectively for male (STDm) or female diets (Aviagen, 2016). The hen (HN) ration had 3% total calcium from 25 to 41 wk and 3.2% calcium for the remainder of the trial. The STDm had 0.70% calcium. All treatment groups were fed equal feed amounts (112g/bird) so that energy intake was equivalent across all treatments. Starting at 25 wk, 63 Yield Plus roosters were assigned to each dietary treatment (9 replicates, 7 roosters per replicate), and weekly 25-30% of males in each treatment were weighed to maintain target body weights with the weigh groups rotating over time. The percentage of roosters in semen production and sample volume, concentration and mobility were measured every 4 wks from 33 to 65 wk. Individually caged Ross 708 hens were artificially inseminated with 0.05mL pooled and diluted semen from a replicate group of 7 males at 46, 52, 56, 60 and 64 wk. Semen was diluted with Avian Buffer to 7.5 x 10^7 sperm. Eggs were collected for 14 days' post insemination and incubated. The objective focused on whether a male diet would improve rooster semen production and quality. Data were analyzed by SAS 9.4 SLICE and means separated by LSD. Significance level was P<0.05. Diets had no significant influence on rooster body weight, semen volume or concentration to 65 wk of age. At wk 65 there was no significant difference in testes weight. Roosters consuming STDm diet had greater percentage of roosters producing semen at 31 wk (P=0.007) with the percentage becoming similar by 35 and 39 wk. Sperm mobility was not affected at 33 or 37 wk; however, at 41 wk the roosters consuming STDm had greater mobility than the roosters consuming the HN ration (P=0.02). There was no significant difference in fertility between STDm and HN treatments. Generally, roosters consuming the male diet came into semen production earlier than those on the hen diet; however, few differences in semen production or quality were noted as the caged males aged.

Key Words: Rooster/hen diets, Semen Production, Concentration, Mobility, Fertility

M125, **The effect of housing environment on commercial brown egg layer performance.** Benjamin Alig*[GS], Ramon Malheiros, Peter Ferket, Kenneth Anderson *Prestage Department of Poultry Science, NC State University*

Consumer demand for cage free eggs is driving the layer industry towards greater use of extensive housing systems, but there is limited research on how these systems affect egg production characteristics as was the focus of this study with commercial brown egg layers. Five housing systems were evaluated under typical industry conditions, including caged (C), enrichable (EN), enriched (E), cage free (CF) and free range. Eight different brown egg laying strains were housed in the different housing systems and managed according to standard husbandry practices and stocking densities. Data collection for the strains began at 17 weeks of age, with a base period of 28 days for feed weighbacks and egg quality measurements. Housing system had a highly significant (P<0.0001) effect on all egg production characteristics measured, including feed consumption (g/bird/day), egg production rates (% hen-day and % hen-housed), feed conversion (egg g/feed g), and mortality rate (%). Previous research revealed better egg production metrics for white egg layers in caged systems than extensive systems. In contrast, we observed brown egg layers had optimum production results for the FR housing systems. Hen-day egg production of these birds was 78.5±0.35% for C, 79.3±0.45% for CF, 79.3±0.36% for EN, 79.9±0.36% for E and 82.3±0.56% for FR. Feed consumption was the lowest for the Caged birds at 106.15g followed by CF at 106.8±0.57g, E at 112.0±0.46g, FR at 112.2±0.71g and EN at 113.7±0.46g. Feed conversion for these birds was the worst in the EN system at 0.434±0.0025 followed by E at 0.441±0.0025, C at 0.455±0.0024, CF at 0.459±0.0031 and finally FR at 0.470±0.0039. The free-range birds had the lowest mortality percentage with an average period mortality of 0.249±0.22% followed by C at 0.756±0.14%, E at 0.822±0.14%, CF at 1.027±0.18% and finally EN at 1.378±0.14%. In contrast to expectations, brown egg layers exhibited significantly better production characteristics in extensive housing systems than in cages. The brown egg layers performed better in the free range system in comparison to the other housing systems.

Key Words: Brown egg layers, Housing systems, Egg production, Feed conversion, Mortality rates

M126, **Mass mortality composting of spent laying hen carcasses during winter temperatures in the Upper Midwest** Kristina Bowen*[UG], Ethan Neiman, Brook Wilke, Dale Rozeboom, Zachary Williams *Michigan State University*

Mortality disposal has been an ever-growing issue in agriculture in recent years as farms increase in size. This issue is made worst by depopulation due to disease which raises concerns of pathogen spread through accepted carcass disposal methods. Of approved disposal methods composting can offer a safe, effective means of disposal and can inactivate pathogens and viruses through heat kill. Composting poses some challenges, as outside temperatures in the upper Midwest can stay below freezing for weeks at a time. Despite its success, composting has received little attention when air

temperatures are low. The aim of this study is to demonstrate the success of composting in cold climates. Two compost windrows were constructed on an open field using wood chips, pine shaving sawdust, and dried laying hen manure, with a C:N ratio of approximately 2:1. Approximately 50,000 Single Comb White Leghorn hen carcasses, averaging 1.58 kg, were used for each treatment. One pile was constructed by layering the substrate and carcasses, as described in the USDA Mass Mortality Composting Guidelines. The second was made by placing the carcasses in the middle of the substrate and covering in a roll design. Internal compost temperatures were taken in 10 evenly spaced locations on each pile every 7 days for 60 days. Temperatures were recorded at 0.91 m of depth. Each pile was turned twice to promote aeration, once when the USDA temperature constraint of 55º C was reached and a second time when internal temperatures began to fall. Statistical analysis was performed using repeated measures in SAS 9.4 at a $P<0.05$. Analysis of the means revealed that there was no significant difference between the two piles ($P>0.05$). Each pile rose to 65 and 66 ºC by day 35 before declining. Upon the second turn each pile rose to 67 and 68 ºC by day 53. Air temperatures fell to -28.11º C on day 14, while the highest temperature of 17.17º C was observed on day 60, with heavy snowfall and accumulation at times. [WZ1] Dismantling of the piles revealed no soft tissue. Results of this study reveal that necessary internal temperatures of windrows can be achieved despite unhospitable air temperatures and heavy precipitation.

Key Words: compost, mass mortality, laying hens, cold temperatures, disease

M127, **Evaluation of Commercially Manufactured Animal Feeds to Determine Presence of Salmonella, E. coli and Clostridium perfringens** Luis Munoz*GS, Wilmer Pacheco, James Krehling, Kaicie Chasteen, Aidan Tailorico, Lasheda Brooks, Jesse Evatt, Kenneth Macklin *Auburn University*

Animal feed can potentially expose animals to pathogens which may lead to infection or colonization of the gastrointestinal tract. Feeds can become contaminated with bacterial pathogens during harvesting and transportation of the ingredients, processing at the feed mill, transportation to the farm or during storage. These bacteria have the potential to colonize the animal leading to disease of the animal and during processing may lead to contamination of the carcasses with foodborne pathogens. The objective of the study was to establish if commercially manufactured animal feed serves as a source of contamination of *Salmonella*, *E. coli* and *Clostridium perfringens*. Four commercial feed mills and the research feed mill at Auburn University were sampled. A total of 140 samples (60 of ingredients and 80 of feeds) were collected from 5 different locations within each feed mill: ingredient receiving, post mixing, post pelleting (hot sample), post cooling the pellets and at loadout. All samples were assayed for *Salmonella*, *E. coli* and *Clostridium perfringens*using selective media. Resulting counts were log10 transformed and then analyzed using ANOVA, if significant ($P<0.05$), means were separated using Tukey HSD. The data shows that *Clostridium* spp. load in corn meal varied between the 5 feed mills (0.90 – 3.63). Feed mill A (0.90) and feed mills B, C and E (3.48, 3.48 and 3.63, respectively) were determined to be significantly different. For soybean meal, feed mill A showed the highest *E. coli* (3.00) counts, being significantly higher compared to the rest of the feed mills (B-E). From the processed samples, post mixing showed significance in *E. coli* values between feed mills C and B, with feed mill B having log10 4.84 CFU/g. In addition from processed feed *Clostridium perfringens* was isolated and confirmed via PCR from two samples from two different feed mills. No recontamination of *E. coli* was observed after pelleting the animal feeds and no *Salmonella* contamination was detected in ingredients or processed feed throughout the study. For future research, greater attention should be placed on *Clostridium perfringens* contaminated animal feed as a possible source of disease transmission to the birds.

Key Words: Animal feeds, Feed manufacturing, Clostridium perfringens, Salmonella, E. coli

M128, **Efficacy of probiotics in the broiler production environment for improved ammonia reduction and growth performance** Oscar Tejeda*[1GS], Casey Ritz [1], Justin Fowler [1], Caitlin Harris [2], Jeff Buhr [2] [1] *The University of Georgia, Department of Poultry Science,* [2] *USDA-ARS*

Ammonia is a volatile compound in broiler production that when elevated has both production performance and environmental implications. Probiotics are a group of microorganisms that when provided in adequate amounts, have the ability to positively modulate the animal's intestinal microflora and have been shown to have potential environmental benefits by the reduction of nitrogen-containing compounds such as ammonia. Our objective was to determine the efficacy of probiotics in the reduction of ammonium-nitrogen (NH4-N) and other environmental parameters associated with ammonia production in broiler chickens. The experiment consisted in four treatment groups with five replicate pens each in which the litter was treated (48 hours before chick placement) for the suppression of ammonia. The litter for the control group was treated with liquid alum as a positive industry control; the litter in the second group was once-treated with proprietary probiotics (LT). In addition to litter treatment with probiotics, birds for the third group received a daily supply of probiotics in the water (LT+P42). Finally, the fourth group, in addition to litter treatment with probiotics, received an every-other-week supply of probiotics in the water (LT+PBi). Measurements for litter pH, moisture, and NH4-N were taken twice a week for 6 weeks. Growth performance was measured on week 1, 2, 3, and 6 of age. Results of NH4-N collected from acid traps showed only differences ($P < 0.05$) on day 5 with the LT group significantly higher with 1747 ppm NH4-N compared to the rest with a range between 450-600 ppm NH4-N and with no difference in NH4-N between the treatments for the duration of the trial. No differences in the litter pH were observed overtime among treatments ($P > 0.05$). No significant differences in growth performance were observed when comparing the probiotic product with the liquid alum. All treatments had similar feed conversion ratio and body weight gain during the 6 weeks of the grow out period ($P > 0.05$). These data suggest that the use of probiotics in litter and water applications did not significantly improve performance nor serve as an effective litter amendment for the reduction of environmental ammonia production.

Key Words: probiotic, ammonia, broiler, performance, litter

M129, **Effect of protease supplementation in broiler chicken feed** Carlos Duque Ramirez*[1], Lina Peñuela sierra [1], Mayra Diaz Vargas [2] [1] *Universidad del tolima,* [2] *Universidad de Ciencias Aplicadas y Ambientales*

The use of enzimes in the feeding of broilers is currently one of the most used alternatives to reduce production costs as well as to reduce environmental pollution without affecting the performance of birds. Thus, this study evaluated the effects of proteases in broilers diet on performance, carcass yield and meat quality. 1290 one-day-old Ross AP male chicks were distributed in in a completely randomized design with five treatments Control diet (CP, diet fulfilling all the requirements Nutritional), Negative control diet (CN; is a diet with -1.0% PB and AA, -50 kcal / kg MS at each stage), CN diet + 50 ppm protease, CN diet + 100 ppm protease and diet CN + 200 ppm protease and six replicates of 43 birds each. The obtained results were analyzed using SAS statistical software 9.4. Following ANOVA, whenever a difference was found, the degrees of freedom were decomposed into polynomials and analyzed by regression for the different relationships – linear or quadratic ($P < 0.05$). As result, proteases supplementation increased feed intake from 6 to 11 d of age, also increased the weight gain ($P < 0.05$) from 28 to 43 d of age with 200 ppm of protease, observing a difference of up to 180 grams per bird when

compared with the CP. The feed conversion from experimental period, and carcass yield and the quality variables of meat (pH, color, water holding capacity and cooking weight loss) at 42 d of age were not influenced (P > 0.05) by treatments

Key Words: Protease, meat quality, performance, carcass yield

Metabolism & Nutrition VIII, Minerals/Probiotics/Feed Additives

T130, **Effect of Garlic Powder With and Without Protexin on Performance, Quality Traits of Carcasses and Morphology of Jejunum in Japanese Quail** farhad ahmadi *Department of Animal Science, Islamic Azad University, Sanandaj Branch, Sanandaj, Kurdistan, Iran*

This experiment was conducted to evaluate the effect of protexin, garlic powder and their composition on performance, quality traits of carcasses and the morphology of jejunum in *Japanese quail*. In this study, 240 day-old chicks were distributed in a completely randomized design (CRD) consisting of 4 treatments, 4 replicates and 15 birds in each pen from 1 to 42 days of age. The experimental treatments were: 1) control (base diet), 2) base diet + protein, 3) basal diet + garlic powder, 4) basal diet + combination of protexin and garlic powder with the same levels. The results showed that the addition of garlic powder and protein to base diet did not have a significant effect on feed intake during 1 to 21 days (P> 0.05). Feed consumption in chicks fed with supplements and their blend in the 22-42 and total period (1-42 days) was significantly lower than the control group (P<0.05). Also, the results showed that chicks fed with the combination of protexin and garlic powder increased LBW more than control treatment (P<0.05). The lowest feed conversion ratio was observed in the birds fed diet including combination of Protexin + Garlic powder (P<0.05). The results of this study showed that the blend of Protexin and garlic powder had significantly affected on carcass quality compared to the control and other treatments (P<0.05). The morphological traits of birds fed supplemented diet with protexin and garlic powder were significantly improved compared to control (P<0.05). From the results of this study, it is concluded that the addition of protexin with and without garlic powder improves performance, carcass quality traits, and the morphology traits of jejunum in *Japanese quail*. But the combination of supplements had more positive effects than any of them.

Key Words: Garlic, Performance, Protexin, Gut, Quail

T131, **Effect of Dietary Mixture of Fenugreek Garlic and Coriander on Performance of Broilers** Said Ali *University Of Agriculture Peshawar*

Phytogenic Feed Additives (PFAs) may be considered as alternative of antibiotics in broiler feed. Present study was evaluated to know effect on growth performance and economic efficiency of broiler chicks. There were randomly 4 groups (A, B, C and D) with four replicates, reared in open sided house. Group-A was kept control; fed basal diet without any additives. Group-B was fed basic feed including 0.33% of fenugreek seed powder (FSP), 0.33% of garlic bulb powder (GBP) and 0.33% of coriander seed powder (CSP). Group-C was fed with basic feed with FSP, GBP and CSP at 0.66% each. Group-D was fed with basic feed, supplemented with FSP, GBP and CSP at 1.0% each. Results revealed that feed intake (FI) was significantly lower for group B compared to other groups at 1 st week, while at day 28, FI was significantly lower for group C compared to other groups. Feed intake at finisher phase and overall FI was lower for group C compared to other groups. Overall weight gain was significantly higher for group B compared to other. Overall FCR was significantly lower in group D compared to other groups. Live body weight at day 21 was significantly higher in C. Carcass weight was significantly higher for group B compared to control group at days 21 and 42. There was no significant effect of dietary treatments on weight of giblets and dressing %age at starter and finisher phase. Various economics parameters at starter phase including total production cost/bird, profit/bird, total revenue/bird and benefit-cost ratio were similar at starter and finisher phase. At finisher phase, cost of feed intake per bird was significantly lower for group D followed by groups C, B and A. Significantly lower feed intake cost/Bird was recorded for birds in group C followed by D and B compared to A. Total revenue/Bird and profit/Bird was higher for birds in group B compared to other. It was concluded that inclusion of PFAs (mixture of Fenugreek, Garlic and Coriander at 0.33% each) at 1.0% in broiler feed resulted overall lower FI and FCR, and higher weight gain, live weight, carcass weight and dressing %age compared to other groups. Moreover, the use of PFAs at 1.0% was economically feasible. Therefore, dietary mixture of PFAs at 1.0% may be used for better growth and feed conversion in broilers.

Key Words: Broilers, Feed ingregients, Phytogenic Feed Additive, FCR, economic efficiency

T132, **Effect of a proprietary feed additive on both the performances and intestine microbial counts of organic raised broilers** Ashley WAGNER*[1], Bertrand MEDINA [1], James McNAUGHTON [2], Ivan GIRARD [1] [1] *Probiotech International Inc.,* [2] *AHPharma Inc.*

The objective of this trial was to examine efficacy of a proprietary feed additive (Alterna®OP, a blend of phytogenics, organic acids and functional oils[AW1] , Probiotech International Inc.) to support broiler enteric eubiosis and performance under National Organic Standard (NOS) guidelines. One-day old Cobb chicks (n=1248) were randomly assigned to 2 groups: CTL (standard NOS diet) *vs* AOP (Starter (0-10d): Alterna®OP at 1.5kg/MT; Grower-Finisher (11-39d): 1.0kg/MT). Each treatment was fed to 12 replicates of 52 mixed-sex broilers. Birds received a coccidiosis vaccine at the hatchery and were reared on built-up litter (moisture>25%) which contained controlled bacteria and coccidia counts. Individual live weights (LW) were recorded on day 10, 29 and 39. Feed intake (FI) was measured for each phase, and mortality was reported as percentage per time period. Intestinal lesion scores, *C. perfringens* and *E. coli* bacteria counts, *and Salmonella* incidence were conducted at 10 and 39d. *Pseudomonas spp*. counts were assessed every 2 days post-slaughter to determine breast meat shelf-life ($<10^6 \log_{10}$ per cm^2 skin slice). Treatment means were compared with a multi-factorial procedure ANOVA, and separated using a Duncan's LSD test with P<0.05 as significant threshold. Cumulative mortality (CM) of CTL averaged 5.56%, confirming challenged conditions, and was higher (P<0.05) than AOP (0.87%). Final LW was heavier (P<0.05) for AOP compared to CTL (2444 *vs* 2557g, P<0.05). Consequently, FCR was improved in AOP versus CTL (1.72 *vs* 1.63 P<0.05). *Salmonella* incidence was reduced (P<0.05) in AOP at d10(CTL:91.7, AOP:37.5) and d39 (CTL:83.3, AOP:44.2%). The same decrease (P<0.05) was seen for *E.coli* (d10- CTL:6.84, AOP:5.76; d39- CTL:6.75, AOP: $5.97 \log_{10}$ of CFU/g)and for *C. perfringens* enteric counts (d10- CTL:4.54, AOP:3.41; d39- CTL:4.47, AOP: $3.58 \log_{10}$ of CFU/g). Shelf-life was extended 6d with AOP (d8- CTL:5.604, AOP:4.461; d14- CTL:8.180, AOP: $5{,}930 \log_{10}$ of CFU/g). Lesion scores were reduced (P<0.05) with AOP at d10 (CTL:1.250, AOP:0.354) and d39 (CTL:1.308, AOP:0.367). The results indicate that Alterna®OP demonstrates a pathogenic microbial inhibition beneficial for broiler performance under NOS and challenged conditions.

Key Words: organic, broilers, phytogenics, feed additive, microbial counts

T133, **Strategies to manipulate early-life microbiota of poultry toward beneficial bacterial growth** Denise Rodrigues[*1], Jeremiah Cox [1], Alissa Hilsong [1], Whitney Briggs [1], Audrey Duff [1], Kaylin Chasser [1], Johel Bielke [1], Kim Wilson [1], Chad Risch [2], Debbie Jeffrey [2], Dan Shafer [2], Lisa Bielke [1] *[1] The Ohio State University, [2] Maple Leaf Farms*

Recent advances in multi-omics highlight the importance of early-life commensal microbiota for mediating poultry immunocompetence and health. Consequently, there has been a growing demand for early manipulation strategies of intestinal microbiota in order to reduce pathogen colonization, advance immune system development and optimize growth. We used two different experimental models to address how early commensal bacteria exposure could affect the modulation of intestinal microbiota at day of hatch (DOH). In Experiment (Exp) 1, embryos at 18 embryogenic day were introduced in ovo either 200 µL of saline (CON) or 102 CFU/mL of lactic acid bacteria (LAB) into the amnion. On DOH, the gastrointestinal tract (GIT) of chicks were collected for 16s RNA sequencing analyses. In Exp 2, treatments included a spray application of 9.0x106 CFU/mL of LAB or control (CON; without probiotic) through the top of the hatch cabinets containing eggs from poor-performing duck breeder flocks. The probiotic was applied 5 times every 4 hours on d27 of incubation. On DOH, GIT was collected for enumeration of bacteria using MRS, MacConkey and BD CHROMagar selective agars. Statistical differences were determined using Student's t-test. In Exp 1, there was a significant increase ($p<0.05$) of Lactobacillaceae and reduction of Enterococcaceae populations in LAB treated chicks. The relative abundance of Enterobacteriaceae was similar to CON. In Exp 2, recovery of LAB from ducklings GIT was significantly higher in LAB treatment as compared to CON. Similarly, the application of LAB-probiotic in the hatcher cabinets decreased ($p<0.05$) the recovery of Gram-negative from GIT of ducklings. In both experiments, the early exposure to LAB probiotics was able to modify the microbiota composition concerning desirable bacteria growth in DOH poultry. Application of LAB in the hatcher cabinets decreased colonization of Gram-negative in GIT of new hatched ducklings suggesting that these strategies have potential to minimize undesirable microbial colonization commonly acquired in the hatchery. Here, in this study, we provided practical interventions to manipulate the microbiota structure toward beneficial bacteria colonization as means of enhancing health and performance in poultry.

Key Words: Ducks, new hatched chicks, microbiome, probiotics, lactic acid bacteria

T134, **Feeding broilers a diet supplemented with a blend of organic acids and botanicals alters the microbial ecology in jejunal segments** Christina Swaggerty[*1], Kristina Feye [1], Michael Kogut [1], Steven Ricke [2], Andrea Piva [3,4], Ester Grilli [3,4] *[1] U.S. Department of Agriculture, Agricultural Research Service, [2] University of Arkansas, Center for Food Safety and Department of Food Science, [3] DIMEVET, University of Bologna, [4] Vetagro S.p.A.*

The use of natural products as feed additives in the poultry industry is increasing as antibiotic use decreases as a result of governmental and/or consumer demands. The purpose of this project was to determine if feeding a microencapsulated product comprised of a blend of organic acids and botanicals (AviPlus®P) impacts the microbiota composition of intestinal sections in broiler chickens. Day-of-hatch broiler chicks were assigned to either a control group with no additive or a group given 500g/MT provided in a starter diet (n=10/group; 2 replicate pens). Chicks were allowed ad libitum access to their diet and were euthanized at day 15 and jejunal and ileal contents were collected and the microbial composition determined using 16S rDNA sequencing of the V3 region. Following analysis using QIIME2.2019.I pipeline and considering the false discovery rate, there were significant ($Q<0.05$) differences in alpha (Shannon Diversity Index and Pilou's Evenness) and beta diversities (Bray-Curtis and Unweighted Unifrac Distance Matrix; ANISOM, $P < 0.05$; Pairwise $Q < 0.05$) between the treatment groups in the jejunum alone. Based on analysis of communities of the microbiome (ANCOM) to determine differential abundance of families of bacteria, the jejunal segments from chicks fed 500g/MT AviPlus®P had fewer Enterobacteriaceaecompared to tissue from control-fed chicks. Further, the relative abundance of Clostridiacea and Lactobacillaceaewere increased in the tissues from the supplement-fed chicks compared to the controls when the samples were evaluated with LEfSe. Typically, Enterobacteriaceae, Clostridiacea, and Lactobacillaceae population changes are indicators of a healthy jejunum. In conclusion, feeding a microencapsulated blend of organic acids (citric and sorbic acids) and botanicals (thymol and vanillin) changes the microbial ecology in the jejunum. A healthy gut would suggest this segment of the intestinal tract would be more likely to resist colonization by foodborne and poultry pathogens.

Key Words: Feed additive, Microbiome, Organic acids, Botanicals, Broiler

T135, **Effects of a synbiotic or synbiotic plus enhanced organic acid program on broiler performance and cecal and carcass Salmonella load.** Chasity Pender[*1], Shelby Ramirez [1], Raj Murugesan [1], Erik Sobotik [2], Greg Archer [2] *[1] Biomin America, Inc., [2] Texas A&M University*

Over recent years, the topic of food safety has quickly established itself at the forefront of media outlets, research, and government regulations. As producers shift away from usage of antibiotics, industry leaders and researchers are actively searching for tools to assist in managing food safety risks. Probiotics and organic acids have been considered potential options as they have been shown to limit the growth of several enteric bacterial pathogens. A trial was conducted to determine the effects of a synbiotic or a combined program of a synbiotic plus an enhanced organic acid on broiler performance and cecal and carcass Salmonella load. A total of 576, day-old Cobb 700 male chicks were allocated to 1 of 4 treatment groups: T1) standard control diet, T2) diet supplemented with a synbiotic (PoultryStar® me; 500g/MT; PS), T3) diet supplemented with PS in the starter phase and an enhanced organic acid (Biotronic® PX Top3; 500g/MT; BPX) in the grower and finisher phases, or T4) diet supplemented with PS in the starter and grower phases and BPX in the finisher phase. Each group consisted of 8 replicate pens with 18 birds/pen. The trial was conducted over a 42-day period and performance variables were calculated weekly as cumulative measures. Data were analyzed using the GLIMMIX procedure of SAS with significance reported at $P \leq 0.05$. For the 0-42 day period, body weight was increased in all supplemented groups. Feed intake was increased in birds in T3 and T4 groups for the 0-35 day period, while T2 was intermediate with T1. Feed conversion was improved for T2 and T4 for the 0-14 day period, while T3 was intermediate with the control. For the 0-28 day period, T2 demonstrated augmented feed conversion compared to all other groups. Though not statistically significant, the percentage of ceca that were positive for Salmonella in the supplemented groups was 38% compared to the control at 50%. Both T3 and T4 had reduced carcass Salmonella load by 1.6 and 1.4 log units, respectively, compared with T1 birds, while T2 was intermediate with a 0.8 log unit reduction. These data indicate that diet supplementation with the evaluated synbiotic and enhanced organic acid could be a potential tool to reduce carcass Salmonella contamination and increase performance of broilers.

Key Words: Salmonella, probiotics, organic acids, food safety, performance

T136, **Binding kinetics of IgY contained in a dried egg product to recombinant chicken IL-10** Jeffery Escobar[*], Desmond Adeniyi, Lori Armstrong, Wahala Wahala *Elanco Animal Health*

In coccidiosis susceptible birds, *Eimeria spp.* infection increases chicken IL-10 production in intestinal epithelial cells, which interferes with the ability of IFN-gamma to mount a robust Th1 response to this intracellular parasite. Thus, neutralization of intestinal secreted IL-10 may contribute to increased IFN-g production and elicit an appropriate Th1 response. We

have previously demonstrated significant improvements in growth performance of coccidiosis susceptible broilers consuming diets with a dried egg product (DEP) containing anti-IL-10 IgY neutralizing antibody. In this study we measured the binding kinetics of the IgY neutralizing antibody contained in DEP and of a purified rabbit anti-chicken IL-10 polyclonal IgG (Kingfisher Biotech, Saint Paul, MN) in a bio-layer interferometry device (Octet Red96E, Molecular Devices, San Jose, CA) using high precision streptavidin (SAX) biosensors. Recombinant chicken IL-10 (Kingfisher Biotech) was biotinylated using a Lightning-Link® Rapid Biotin–Type B kit (Expedeon, San Diego, CA) according to manufacturer's instructions. The IL-10 peptide used to hyperimmunize hens was biotinylated (GenScript, Piscataway, NJ) and used as a positive control in binding kinetics assays. Several assays were conducted to determine optimal assay conditions for accurate, reliable, and reproducible quantification of binding affinity constants (K_D) as well as association (K_{on}) and dissociation (K_{off}) rates. Biotinylated chicken IL-10 protein or peptide were bound to SAX biosensors and then exposed to anti-IL10 IgY or IgG antibodies. The IgY in DEP was able to bind the biotinylated peptide (K_D= 4.90×10^{-8} M, K_{on}= 2.30×10^{4} M^{-1}, and K_{off}= 1.20×10^{-8} s^{-1}). The binding kinetics for the IgY (K_D= 8.53×10^{-9} M, K_{on}= 1.09×10^{5} M^{-1}, and K_{off}= 9.28×10^{-4} s^{-1}) contained in DEP were comparable to those of purified rabbit polyclonal IgG (K_D= 9.64×10^{-10} M, K_{on}= 1.70×10^{5} M^{-1}, and K_{off}= 1.63×10^{-4} s^{-1}) indicating that both antibodies have a high affinity to recombinant chicken IL-10. In conclusion, these results demonstrate the ability of the IgY in DEP to bind recombinant chicken IL-10. Further, these results suggest a plausible mechanistic explanation for the reported improvements in growth performance of coccidia susceptible birds fed DEP.

Key Words: IL-10, IgY neutralizing antibody, Dried Egg product, Binding kinetics

T137, **Evaluation of a product derived from porcine plasma protein and a yeast product with similar biological activity in diets for growing broilers** Park Waldroup*[1], Mathew Vaugn [2], Michael Barnas [3], James McNaughton [3] *[1] University of Arkansas, [2] Puretein Bioscience, [3] AHPharma*

The efficacy of two bioactive protein products was determined. One originated from porcine plasma (betaGRO® (BG)) while the other was a yeast-derived product (celluTEIN® (CT)). Birds were grown under two different environments, a "Low Stress" using clean wood shavings litter and a "High Stress" with used litter from a commercial farm that had suffered high mortality. The litter was overseeded with coccidia and Clostridia. Each product was fed at 300 g/ton from 0-14 d and 50 g/ton to 42 d. Another group of birds in the low stress environment was fed 300 g/ton in the starter but none thereafter. Growing birds in the high stress environment had a negative impact upon performance; however, addition of either BG or CT successfully mitigated the detriments of the high stress environment and in many cases performance was equal to or better than performance of birds on the negative control diet in the low stress environment. Significant improvements in 42-day body weight and feed conversion were observed. Birds fed BG and CT were more uniform than birds grown on negative control diet. Mortality of birds in severe stress environment was significantly reduced by addition of BG or CT. Addition of BG and CT resulted in significant improvements in carcass and breast meat yield. The intestinal villi height was increased and crypt depth reduced by addition of BG and CT, suggesting an improvement in digestibility. Intestinal lesions scores were reduced as well as numbers of Clostridia organisms. The two products were equally effective in all responses. Failure to maintain BG in grower and finisher diets reduced performance compared to birds whose similar diets contained 50 g/ton. The data suggest that supplementation of BG and CT in a severe stress environment bolstered the broilers immune system to mitigate bacterial interference on the small intestine that result in performance detriments, while simultaneously promoting superior lean tissue growth.

Key Words: Broilers, Bioactive protein, Yeast product

T138, **Effect of feeding porcine or bovine Spray-Dried Plasma at graded levels for 5 or 10 days in the starter period on performance of broilers** David Cadogan [1], David Creswell [2], Robert Swick [3], Saksit Srinongkote [4], Ricardo Gonzalez-Esquerra* [5] *[1] Feedworks Pty Ltd, [2] Creswell Nutrition LTD, [3] University of New England, [4] Bangkok Animal Research Center Co. LTD, [5] APC Inc*

A trial evaluated the effect of feeding titrated levels of spray-dried Plasma (SDP) of bovine (SDBP) or porcine (SDPP) origins to broilers for 5 or 10 days during the starter phase on performance and mortality at slaughter age. Sixty-four floor pens of 16 male Ross 508 birds per pen were used. Pens were 1.5 x 1m and had fresh rice hulls as litter material. SDBP or SDPP were formulated into the starter diet at 0, 1, 2 or 3% aiming at having similar nutrient profiles across diets (ME 3000 Kcal/kg; SIDLys 1.2%). Crumbled diets were fed for 5 or 10 days. Treatments consisted of 1) No SDP; 2) 1% SDPP fed for 5d; 3) 2% SDPP fed for 5d; 4) 3% SDPP fed for 5d; 5) 2% SDPP fed for 10d; 6) 1% SDPP fed for 10d; 7) 1% SDBP fed for 10d; and 8) 2% SDBP fed for 10d. After SDP-added diets were fed, common crumbled starter and pelleted finisher diets were offered to all birds until 14d, and from 14 to 32d of age, respectively. Birds performance was excellent with the control group (T1) having BW, feed intake and FCR values of 2350 g, 3109 g and 1.344, respectively at 32d of age vs. 2058 g, 2895 g and 1.406 suggested by the Ross 308 performance 2019 objectives. At 10d of age, BW, BWG, intake and FCR improved across SDP-fed birds by 4.6, 5.2, 3.0, and 2.1% on average, respectively ($P < 0.001$; better FCR meaning lower FCR value). Birds fed SDP for 10d improved BW, BWG, FCR and intake than those fed SDP for 5d ($P < 0.05$), and the response was linear or quadratic by dose ($P < 0.05$). At 32d of age, BW, BWG, FCR, productivity factor and kg of live chicken per m^2 were all improved across SDP-fed birds by 2.0, 2.1, 0.9, 5.1 and 3.9% on average, respectively ($P < 0.05$) while overall mortality dropped significantly from 3.9 (T1) to 1.14% ($P = 0.02$). From all parameters measured, the only difference between SDBP vs SDPP birds was for FCR at 10d of age ($P < 0.05$), but this effect was lost with both SDP sources performing equally well at 32d. Feeding SDP in the starter period only, improved growth, FCR, and productivity, and reduced mortality at slaughter age in broilers. Final performance was influenced by SDP intake (g/bird) and by dose showing improved performance for most parameters in response to greater SDP intake levels; and was unaffected by Time of feeding or by source (SDBP vs SDPP).

Key Words: Broilers, Spray-Dried Plasma, Performance, Mortality, Feed efficiency

T139, **Antimicrobial probiotics to control Clostridia perfringens and necrotic enteritis in pre-harvest broilers.** Yiannis Kaznessis *General Probiotics Inc*

Statement of the purpose of the experiment

New technologies are needed to replace antibiotics in animal production and to treat gastrointestinal infections in animals and humans caused by *C. perf. C. perf*. is the major causative agent of necrotic enteritis (NE) in chicken. NE impacts the health of chickens, resulting in significant productivity losses.

Description of the experimental design

We developed three libraries:

Probiotic isolates from the GI tract of healthy broilers

Antimicrobial peptides (AMPs) with activity against C. perf.

DNA constructs for the expression and secretion of AMPs

We characterized isolates for virulence markers, and for antibiotic resistance markers, and chose non-virulent ones.

We characterized AMPs in terms of bactericidal activity.

We characterized expression and secretion systems in environments that mimic the GI tract.

Combining top performing components, we engineered over 300 distinct antimicrobial probiotics. We then screened systems through a series of experiments that measured the activity and metabolic profile of each system in environments that mimic the GI tract. We rank-ordered systems and selected the top five systems.

We tested these five systems in broiler chickens challenged with *C. perf*. The tests were run at a third party CRO, with an established battery cage model of NE, which involved an Eimeria challenge on day 14 and a *C. perf*. challenge on days 17 and 18. Mortality and lesion scores were measured on days 19-22.

Objective results and evidence of statistical analysis

The non-treated challenge control group showed mortality of 32.8% and average lesion score of 1.59. The best antimicrobial probiotic showed statistically significantly lower mortality (P<0.05) and statistically significantly lower lesion score (P<0.05). A 2-sided test of difference was conducted at the 0.1 significance level for each comparison. No multiplicity adjustment was applied. Statistical analysis was performed using SAS version 9.3 or later (SAS Institute, Cary, NC).

Informative conclusion

Administering the top performing antimicrobial probiotic to challenged birds resulted in a statistically significant reduction of NE and of lesion scores. To our knowledge, no other probiotic-based technology exhibits such high performance.

Key Words: necrotic enteritis, probiotics, feed additives, performance

T140, **A microencapsulated blend of nature identical compounds to control necrotic enteritis caused by Clostridium perfringens in broiler chickens** Benedetta Tugnoli*[1], Andrea Piva [1,2], Brett Lumpkins [3], Ester Grilli [2,4] *[1] Vetagro S.p.A., [2] University of Bologna DIMEVET, [3] Southern Poultry Feed and Research, Inc, [4] Vetagro, Inc*

Nature identical compounds (NIC) are pure botanical molecules well-known for their strong antimicrobial properties. The aim of this study was to evaluate the effects of a microencapsulated blend of NIC in broilers under a necrotic enteritis (NE) challenge model caused by *Clostridium perfringens* (CP). A total of 240 day-old chicks (Cobb500) were divided in battery cages (8 chicks/cage) and assigned to 3 groups (10 cages/group): negative control (NEG) group, challenged with cocci, fed a basal diet; positive control (POS) group, challenged with cocci and CP, fed a basal diet; treated (TRT) group, challenged with cocci and CP, fed a basal diet supplemented with a microencapsulated blend of NIC at 100 g/MT of feed. The challenge with cocci consisted of an oral inoculation with *Eimeria maxima* (5,000 oocysts) at day 14, while the challenge with CP was done by orally administering a strain isolated from clinical case of NE (alpha toxin and netB toxin positive, 10^8 CFU) for 3 days (d19, 20, and 21). The study lasted 28 days with small intestine lesions scored at d21 from 3 chicks/cage and growth performance recorded throughout the study. Data were analyzed with ANOVA and differences considered significant at P<0.05.

From d14 to d21, immediately after the challenge, there were no differences between the treated group and the positive control, that resulted in lower performance compared to negative control. Nevertheless, from d21 to d28 and for the overall period, feed conversion ratio and body weight gain of treated group were comparable to the negative control non-challenged group (P<0.001). Moreover, treated group showed reduced small intestine lesion score at d21 (P<0.0001) and lower overall mortality due to NE compared to positive control (3.8 % vs 15 % for POS, P=0.0002).

To conclude, the microencapsulated blend of NIC used in this study has the potential to contain loss of performance and lesions associated with NE challenge in broiler chickens.

Key Words: nature identical compounds, necrotic enteritis, C. perfringens, broilers

T141, **Efficacy of dried egg product administered to chickens during an enteritis infection caused by Eimeria spp. and Clostridium perfringens** Nannette Olmeda-Geniec*[1], Greg Mathis [2], Brett Lumpkins [2], Jordan Sand [3], Jeffery Escobar [1] *[1] Elanco Animal Health, [2] Southern Poultry Research, [3] Ab E Discovery*

Previously, a dried egg product (DEP) containing anti-IL-10 IgY neutralizing antibody improved growth performance of broilers during experimental necrotic enteritis (NE). Coccidiosis is believed to increase broilers' susceptibility to NE development, therefore control of coccidian infection may lessen NE incidence and severity. This study assessed DEP dose on growth performance of coccidiosis vaccinated broilers followed with NE challenged and fed antibiotic-free diets. Cobb 500 males were housed 50 birds/pen for 42 d in 50 floor pens with used litter. Diets were administered in 3 feeding phases consisting of a Starter (d 0-14), Grower (d 14-28), and Finisher (d 28-42). A randomized complete block design was used to evaluate a non-challenged control plus 4 doses (0, 130, 260, and 325 U/US ton) of a DEP under experimental NE, which consisted of Coccivac® B52 (Merck, Kenilworth, NJ) spray vaccination on d of hatch according to label, and 10^8 cfu/bird/d of *Clostridium perfringens* (Cp) in feed on d 15, 16 and 17. At d 17, 4 birds were randomly selected from each pen for NE lesion scoring. Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), unadjusted and adjusted for mortality (MA), and production efficiency index (PEI) were determined for each phase. Statistical analyses were conducted using JMP (v.14.1, SAS Institute, Cary, NC). Growth performance was not different (P=0.60 to 0.70) among treatments during Starter, *Cp* pre-challenge phase. *Cp* challenge during Grower negatively affected (P<0.0001) all growth performance measurements and survival, and increased (P<0.0001) NE lesion scores compared to the uninfected group, indicating a successful NE challenge. DEP improved (P<0.0001) growth performance and survival, and reduced (P<0.0003) NE lesions scores in a dose-dependent manner (dose linear, P<0.001) during Grower and the 42-d study. Growth performance and survival were not different (P>0.1) between DEP and the uninfected group. In conclusion, supplementation of DEP significantly improved overall bird performance (BW 7.6 to 9.3%; PEI 20.1 to 23.2%; MA_ADG 5.3 to 8.5% and MA_FCR 2.3 to 4.2 points) in the presence of an experimental NE infection model.

Key Words: IL-10 neutralizing antibody, dried egg product, Coccidiosis, Necrotic Enteritis

T142, **Use of AMN to prevent Clostridium perfringens induced necrotic enteritis in broilers** Sudipto Haldar [1], Shivaji Dey [1], Amrita Kumar Dhara [1], Anirvid Sarkar [1], Tusar Chowdhury [2], Carlos Domenech [3], Julia Pie* [4], David Diez [3] *[1] Agrivet Consultancy Ltd., [2] Doctor's Agro-vet Ltd., [3] Biovet S.A., [4] IFTA USA Inc.*

BACKGROUND

AMN is a natural biocide based on cimenol ring (botanical origin) effective against bacteria and fungi. It works as a feed preservative and intestinal microbicide to control the balance of the digestive flora.

OBJECTIVE

An *in vivo* experiment was conducted to determine the efficacy of AMN as a prevention for necrotic enteritis (NE) in 35-day-old broilers. Productive performance, mortality and lesions in the small gut mucosa were evaluated.

METHODS

160 Vencobb broilers were distributed following a random block design into four treatment groups, with four replicates per treatment: non-infected control (T1); infected control (T2); non-infected group receiving AMN as a prevention at 0.5 kg/Tm through feed, continuously during all the trial (T3); infected group receiving AMN as a prevention at the same dose and administration schedule (T4). Birds in T2 and T4 were challenged with toxigenic strains of *C. perfringens* inoculated orally (1 ml with 10^8 CFU/bird) on days 14, 15 and 16 of age. Water did not contain antimicrobial agents. All diets contained 23.5% crude protein and no antimicrobials, AGPs or coccidiostats.

RESULTS

Results showed that AMN as a preventive in infected birds (T4) was effective in controlling NE in infected birds (1.81% ADG increase ($P<0.10$), 4.72% feed conversion improvement, compared to T2).

AMN in non-infected birds (T3) had a positive effect compared to non-infected birds (T1) as it improved ADG, feed conversion and livability ($P<0.1$), probably thanks to a better balance of the digestive flora.

Regarding lesions in the small gut mucosa, infected birds in T2 showed the most severe unspecific lesions and the higher NE lesion score (1.7), compared to the other groups. Infected birds with AMN (T4) showed mild unspecific lesions and the lowest NE lesion score (1.3).

CONCLUSIONS

In conclusion, AMN in feed can be used as a preventive for NE in poultry farms. In addition, AMN in unchallenged birds also has a positive effect on performance, thanks to its beneficial activity towards the balance of the gut flora.

Key Words: necrotic enteritis, poultry, Clostridium, botanical, microbicide

SCAD III

T143, **Diagnostic Service for Avian Pathogenic Escherichia coli (APEC) Identification in Georgia: Comparison of Gene Profiles with Tissue of Isolation.** Nicolle Lima Barbieri*, darby newman, Lisa Nolan, Catherine Logue *University of Georgia*

Avian pathogenic *Escherichia coli* (APEC) are the etiological agent of localized and systemic extraintestinal infections in poultry. Poultry industries are an important branch of our economy, generating high-protein food of relatively low cost. Losses due to APEC associated disease leads to an increase of the cost of production and is important from a potential zoonoses perspective. Here, we assess the use of a multiplex PCR panel for classifying APEC from diagnostic cases and cecum and ileum samples.

In this study we used 66 isolates collected between August of 2018 and May of 2019 through PDRC diagnostic lab submissions and 838 *E. coli* from the cecum and ileum of healthy birds. All isolates were screened using a multiplex PCR panel that targets genes associated with APEC chromosomal and plasmid virulence genes.

Overall, isolates from diagnostic cases met the criteria for definition as well-developed pathogens with more than 90% of isolates being positive for the genes *iroN*, *ompTp*, and *hlyF*; 83% were positive for *aerJ* and 74% positive for *iss*; a significantly lower prevalence was observed for *cvaC*, *etsB*, *ireA* and *papC* (range 5-43%), also APEC gene prevalence varied significantly with tissue of origin. When overall gene prevalence in isolates was examined in regards to their sites of origin, APEC isolates from the liver, ovary, pericardium and bone marrow, had higher than average numbers of virulence genes compared to isolates recovered from the lungs and air sacs. In contrast, 34.5% of the *E. coli* isolates from the cecum and ileum of healthy birds harbored APEC genes and are considered APEC-like, suggesting that potential APEC strains are spread in the production flock.

The use of a multiplex panel to screen for APEC has shown good correlation with pathogenesis, and tissue source and correlates well with invasive strains. Epidemiological analysis provides valuable analysis of the defining traits of the environment and of the host that can potentially lead to *E. coli*-associated disease in production broilers.

The path panel diagnostics service is currently available through the PDRC diagnostic lab at University of Georgia and provides important information for APEC screening to poultry producers of Georgia and elsewhere.

Key Words: APEC, diagnostic, escherichia coli, colibacilossis

T144, **Characterization of a global collection of Avian Pathogenic Escherichia coli (APEC): genotype and phenotype analyses for virulence and resistance** Catherine Logue*, Nicolle Barbieri, Darby Newman, Aline de Oliveira, Lisa Nolan *University of Georgia*

Avian Pathogenic *Escherichia coli* (APEC) is a significant cause of mortality, carcass condemnations and economic loss to the poultry industry in the US and worldwide. APEC affects all facets of the poultry industry including breeders, layers, broilers and other avians such as turkeys, ducks, and geese. This study describes genotype and phenotype analyses of APEC and avian fecal *E. coli* (AFEC) isolated from production birds in the US and internationally with a focus on virulence and antimicrobial resistance. All isolates examined were subjected to antimicrobial susceptibility analysis using the National Antimicrobial Resistance Monitoring System (NARMS) panels to determine phenotype profiles against a range of antimicrobials including beta-lactams and colisitin. Genotype analysis was carried out to determine the presence of resistance genes using single and multiplex PCR for a range of virulence and resistance genes, efflux pumps, metalloproteases and other resistance mechanisms. Isolates were also assessed for the presence of plasmids using PCR to screen for plasmid replicon types and to determine the relationship between plasmid carriage, antimicrobial resistance patterns observed and virulence gene carriage. The data highlights the phenotypic resistances observed and the genotypic sources of these resistances in APEC from international and U.S. sources and provides insight into the influence of production practice on the selection of APEC and the challenges in controlling this pathogen.

Key Words: Escherichia coli (APEC), Antimicrobial resistance, Virulence, genotype, phenotype

T145, **Analysis of the role of the Type 6 Secretion System (T6SS) in avian pathogenic Escherichia coli** Aline de Oliveira*GS, Darby Newman, Nicolle Lima Barbieri, Catherine Logue *University of Georgia*

Avian pathogenic Escherichia coli (APEC) causes extra-intestinal infections in production birds, known as collibacilosis, that can manifest as localized or systemic infections. Colibacillosis is one of the most common and costly diseases, impacting all facets of the poultry industry and causing heavy economic losses. Despite the identification of several pathogenicity determinants in APEC, the role of secretion systems in their virulence remains poorly understood. The Type 6 Secretion System (T6SS) functions as a molecular syringe that secretes proteins into the external milieu or directly into the host cells. It is composed of a membrane complex, a baseplate and a tail-like structure and is involved in virulence mechanisms by several human and animal bacterial pathogens. Current knowledge on the role of T6SS in APEC pathogenesis is, however, limited. Here, we aim to

elucidate the role of the T6SS in APEC pathogenesis. To gain insight into the relationship between the T6SS and APEC pathogenicity, we screened a collection of 454 APEC, 106 avian fecal isolates (AFEC) and 102 litter E. coli isolates for the presence of 5 genes of T6SS cluster 1: hcp, which encodes a T6SS effector, vasK, impG, evpB and impK, which encode T6SS structural proteins so far uncharacterized in APEC. Except for evpB, the prevalence of these genes was significantly higher in APEC (6-35%) than in AFEC (5-14%) and litter isolates (1-11%), providing evidence that the T6SS contributes to virulence in APEC. To further characterize T6SS in APEC, we have generated mutants for these genes in APECO18 strain. Additionally, a recombinant version of Hcp expressing a FLAG tag in its N-terminus has been created in order to analyze the secretion of this protein by T6SS mutants. The functionality of the T6SS, and its influence on biofilm formation and Hcp secretion have also been assessed. Our data provides novel insight into the role of the T6SS in APEC virulence.

Key Words: APEC, T6SS, colibacillosis, virulence, pathogenesis

T146, **Characterization of Georgia poultry lesions infected with Avian Pathogenic Escherichia coli (APEC)** Darby Newman*GS, Aline de Oliveira, Lisa Nolan, Nicolle Barbieri, Catherine Logue *University of Georgia*

Colibacillosis is a disease of poultry caused by the organism avian pathogenic *E. coli* (APEC) that results in multi-million-dollar losses annually to the poultry industry. Here, we assessed the relationship between strains of *E. coli* recovered from different tissues of diseased chickens submitted to the poultry diagnostic research center (PDRC) and their ability to form biofilm as well as the relationship with virulence traits, antimicrobial resistance, and replicons they harbor. A total of 61 isolates were assessed for the presence of 86 total genes (virulence, antimicrobial resistance, and replicon types) with multiplex PCR. Each isolate was also screened for its antimicrobial resistance towards 14 antibiotics and ability to form biofilm, which was categorized as negligible, weak, moderate, or a strong biofilm former. Tissue site of isolation was also correlated to biofilm formation and the number of virulent genes detected. Overall, 58 strains were able to form biofilms and three strains formed a negligible biofilm. A total of 10 isolates were strong biofilm formers; 25 isolates were moderate biofilm formers, and 23 isolates were identified as weak biofilm formers. The data found that almost all strains showed the presence of genes associated with the ColV plasmid with over 90% of isolates harboring *iroN*, *ompTp,* and *hlyF.* In assessing antimicrobial resistance, we found that 20 isolates did not show any resistance to the 14 antibiotics screened. One isolate showed resistance to 9 out of the 14 antibiotics, but the remaining isolates showed resistance to between 1 and 5 antibiotics. This study suggests that current APEC causing disease in poultry harbor high numbers of virulence genes and some isolates harbor many antimicrobial resistance genes, which can lead to complications in treatment. The use of a multiplex panel to screen for APEC has shown good correlation with pathogenesis, and tissue source correlates well with invasive strains. This path panel provides significant value to APEC pathogenesis screening.

Key Words: APEC

T147, **Influence of season and age of flock on the virulence gene profiles of Escherichia coli isolates from 'no antibiotics ever' commercial broiler farms and their antibiotic susceptibility** Courtney Fancher*IGS, Hudson Thames [1], Alyssa Easterling [1], Alexis Dillender [1], Nikhil Nuthalapati [1], Li Zhang [1], Aaron Kiess [1], Thu Dinh [2], Anuraj Sukumaran [1] *[1] Mississippi State University - Poultry Science, [2] Mississippi State University - Animal and Dairy Science*

Avian pathogenic Escherichia coli (APEC) is the etiological agent of colibacillosis in poultry which causes severe economic loss to the industry. The objectives of this study were to determine the influence of season, age of flock, and the type of sample on the prevalence of APEC-associated virulence genes iroN, ompT, hlyF, iss, and iutA in E. coli from commercial 'no antibiotics ever' (NAE) broiler farms and to determine the antibiotic susceptibility of virulent isolates. Litter, feces, cloacal swabs, and tracheal swabs were collected from four NAE broiler farms on days 28 and 56 of one flock cycle each during spring and summer. E. coli were isolated on MacConkey agar and confirmed by PCR amplification of YbbW gene. Positive isolates were tested for the presence of 5 virulence genes, iroN, ompT, hlyF, iss, and iutA in a real-time PCR system. Across all seasons, ages of flock, and sample types, 100 isolates that contained all 5 virulence genes were screened for antimicrobial susceptibility to 11 antibiotic classes using the Kirby-Bauer disk diffusion assay (CLSI Guidelines). Proc Glimmix procedure of SAS 9.4 was used to analyze the data and statistical significance was determined at $P \leq 0.05$. Overall, the prevalence of E. coli was not affected by season, age of flock, or the type of sample. However, prevalence of all tested virulence genes decreased from spring to summer. The prevalence of E. coli with iroN, ompT, hlyF, iss, and iutA genes were 95.77%, 96.13%, 99.16%, 94.48% and 97.21%, respectively in spring as compared to 57.72%, 47.86%, 91.40%, 67.57%, and 83.25%, respectively in summer ($P \leq 0.002$). The prevalence of E. coli with ompT gene was greater in cloacal swabs (89.95%) and feces (86.32%) than in tracheal swabs (73.90%, $P \leq 0.040$). The prevalence of E. coli with the iutA gene was greater in cloacal swabs (97.91%) and feces (95.18%) than in litter (88.45%, $P \leq 0.040$). The prevalence of E. coli with hlyF and iutA genes decreased from d28 (99.21% and 96.69%) to d56 (96.02% and 92.30%; $P \leq 0.021$), whereas those with ompT increased from 76.69% to 90.06% ($P < 0.001$). Out of the 100 isolates tested, 55 were multi-drug resistant. In conclusion, virulence of E. coli in broilers varies with season and multi-drug resistant E. coli are prevalent despite the NAE policy.

Key Words: APEC, Escherichia coli, virulence genes, colibacillosis, poultry

T148, **Development of a wild-type Escherichia coli environmental bloom model to evaluate alternatives to formaldehyde fumigation** Danielle Graham*GS, Callie Selby, Lucas Graham, Kyle Teague, Christine Vuong, Guillermo Tellez-Isaias, Billy Hargis *University of Arkansas*

Horizontal transmission of opportunistic *Escherichia coli* (*E. coli*) during hatch can have detrimental effects on early performance, particularly as pioneer colonizers. Commercially, formaldehyde is commonly applied to combat the bacterial bloom. The purpose of these experiments was to develop a replicable *E. coli* horizontal challenge model to evaluate alternatives to formaldehyde sanitation. In Exp 1, two trials were conducted to determine the appropriate wild-type (WT) *E. coli in ovo* challenge dose, day of embryogenesis (DOE) for two isolates (I1, I2), and if placement of seeder (direct-challenged) embryos with contact (indirect-challenged) embryos during hatch affected contact hatchability. In trial 1, the most appropriate inoculation dose and time point was determined to be 10^2 CFU/embryo on DOE19. Trial 2 showed no differences in hatchability between groups. Subsequently, a 7-day experiment (Exp 2) was conducted to evaluate effects of I1 or I2 on horizontal transmission, Gram-negative bacterial (GNB) recovery from gastrointestinal tract (GIT), and impact on body weight gain (BWG). Compared to the negative control, seeder and contact chicks challenged with I1 or I2, had significantly ($P < 0.05$) more GNB recovered from GIT on day-of-hatch. There was a marked ($P < 0.05$) reduction in 7-day BWG between the I1 indirect-challenged group and negative control group. To further validate the model, two 7-day trials (Exp 3) were conducted to evaluate the effects of formaldehyde fumigation on coliform recovery from the hatching environment and on early performance using I1 for challenge. I1 positive control hatchers had increased levels of circulating coliforms compared to the negative control and formaldehyde treated hatchers, although there was no significant impact on performance induced by challenge or formaldehyde treatment in Exp 3. These data suggest that WT *E. coli* are feasible to administer at a low-dose at DOE19 to promote bacterial blooms under laboratory conditions.

Key Words: Escherichia coli, hatchery, Gram-negative, broiler, model

T149, **Pathologic and molecular characterization of variant avian reoviruses** Sofia Egana*[2GS] Ha-Jung Roh [1], Bart Weimer [2], , Darwin Bandoy [2], Rodrigo Gallardo [2], Beate Crossley [2] [1] *CEVA, Scientific support and investigation unit,* [2] *University of California, Davis*

We characterized two avian reovirus variants with tropism to heart and tendons and compared them with a conventional reovirus strain S1133. Samples were collected at different time points to study virus tropism to tissues, immunosuppression through bursal histomorphometry and gross pathology. Results of this characterization will be presented. In addition, insights on genomic analyses of several avian reoviruses will be discussed.

Key Words: Variants, Reovirus, Pathogenicity, Variability

T150, **Two clinical cases of pullets with severe lesions in lymphoid tissues and increased mortality associated with chicken anemia virus in Southern United States.** Eric Orozco*[1], Alejandro Banda [1], Rebecca Mackey [1], Candy Zhang [2], Martha Pulido-Landinez [1], Danny Magee [1] [1] *Poultry Research and Diagnostic Laboratory,* [2] *Mississippi Veterinary Research and Diagnostic Laboratory*

Two field cases of chicken infectious anemia (CIA) in Mississippi, involving egg and meat-type pullets of 15 and 14 weeks old, respectively, were diagnosed and identified. The birds showed depression, dehydration, and pale combs during the clinical evaluation. Atrophy of thymus, bursa and bone marrow were findings observed in the postmortem examination. Histologically, generalized thymic and bursal lymphoid depletion, heterophilic hyperplasia in bone marrow, lymphocytic infiltration in the pancreas and hepatocellular degeneration were observed in both the layers and the broiler breeder pullets. Packed cell volume (PCV) determinations demonstrated anemia with values below 27%. Detection of chicken anemia virus (CAV) DNA was performed with both conventional and real-time polymerase chain reaction (PCR), followed by sequencing and characterization of the viral protein (VP) 1 gene. Phylogenetic analysis demonstrated a close relationship between Alabama broiler-origin field isolates and one of the CAVs described in this report. *Staphylococcus aureus, Pasteurella multocida,* and *E. coli* were also isolated from breast muscle and hock joint from these birds.

Key Words: Chicken anemia virus, pullets, phylogenetic analysis

T151, **Comparison of poultry biochemical test results between a handheld blood analyzer and a conventional laboratory blood analyzer.** Fernando Ruiz-Jimenez*[GS], Maria Correa, Erika Gruber, Rocio Crespo *Department of Health Population and Pathobiology, College of Veterinary Medicine, North Carolina State University*

Clinical pathology is not commonly utilized to assess the health status or evaluate the effect of environmental manipulations or treatments of poultry in the field. A reason for the underutilization of clinical pathology in poultry is that rapid and proper sample handling is critical to obtain reliable measurements. Since many farms are far from a diagnostic laboratory, proper sample processing presents a significant challenge to poultry veterinarians. Therefore, the use of Handheld Blood Analyzers could be a good and cost-effective option for on-farm testing. Recently handheld blood analyzers (i.e. i-STAT) have been used to evaluate blood gases in chickens, but none of these studies compared the results to benchtop units. Furthermore, none of these studies consider the issue of proper and timely sample processing. The objectives of this study are to a) investigate time effect on the blood chemistry values; b) evaluate if the accuracy by a Handheld Blood Analyzer (i-STAT) is comparable to a traditional clinical pathology benchtop analyzer. We collected blood from 60 healthy chickens. Each sample was tested in triplicate with the cartridge CD8+ in the Handheld Analyzer (i-STAT) and once with the Conventional Laboratory Analyzer (GEM Premier 3000). All samples were analyzed within 60 minutes of collection. Pearson's Correlation Coefficient and Mountain Plots were used to determine whether there was a significant difference between the i-STAT results. A Bland-Altman assay was used to determine whether the results from the i-STAT and GEM 3000 were comparable. We obtained high (r= 0.7 – 0.89) to very high (r= 0.9 – 1.0) correlations in all blood gas analytes and electrolytes between different times after collection using the i-STAT analyzers. Bland-Altman statistics also demonstrated a good correlation for all blood gas analytes and electrolytes between the i-STAT analyzer and the GEM 3000 analyzer. In conclusion, the i-STAT was reliable and easy to use and had an overall acceptable accuracy in chickens. These features, together with portability and small required blood volumes, make the i-STAT suitable for point-of-care use in poultry.

Key Words: biochemical analysis, chickens, comparison, conventional analyzer, handheld analyzer

T152, **Evaluating synbiotic, enhanced organic acid, or combined supplementation for the reduction of Campylobacter from day-old chicks to ready-to-eat chicken carcasses** Mohamad Mortada*[1GS], Douglas Cosby [2], Revathi Shanmugasundaram [1], Ramesh Selvaraj [1] [1] *The University of Georgia,* [2] *USDA-ARS*

Campylobacter is a major foodborne pathogen associated with the consumption of poultry meat. *In vitro* and *in vivo* experiments were conducted to evaluate the effectiveness of synbiotic, enhanced organic acid (EOA), or combined supplementation for the reduction of *Campylobacter coli*. Supernatants from four probiotic strains were co-cultured with *C. coli* at different dilutions. *Enterococcus faecium*, *Bifidobacterium animalis*, and *Pediococcus acidilactici* probiotic supernatants decreased ($P < 0.05$) the *in vitro* proliferation of *C. coli* at 1:1 supernatant: pathogen dilution. The inhibitory dilution for *Lactobacillus reuteri* was at 5:1 ($P < 0.05$). Different concentrations of the enhanced organic acid were co-cultured with *C. coli in vitro*. EOA concentration of 0.08% significantly decreased the *in vitro* proliferation of *C. coli* ($P < 0.05$). For the *in vivo* experiment, 480 Cobb-500 broiler chicks were randomly assigned to four treatments: control (basal diet), synbiotic (0.5 kg/ton), enhanced organic acid (0.5 kg/ton), or synbiotic + EOA (synbiotic at 0.5 kg/ton from 0 to 28 d of age and EOA 0.5 kg/ton from 28 to 42 d of age). All birds were challenged with 1 X 10^8 CFU/bird of *C. coli* gentamicin-resistant strain on 14 d of age. At 42 d of age, the combined treatment (Synbiotic + EOA) had a 1.2-log reduction in *Campylobacter coli* load in ceca when compared to the positive control treatment ($P = 0.08$). Enhanced organic acid supplementation increased ($P < 0.05$) TLR4 mRNA levels in cecal tonsils when compared to the basal-fed and challenged control treatment at 28 dpi (42 d of age). There was a significant ($P < 0.05$) increase in TGF-β4 mRNA contents in the synbiotic-supplemented treatment when compared to EOA-supplemented treatment at 14 dpi. There were no significant treatment effects on FCR, BWG, or IL-10 and IFN-γ mRNA levels. The combined supplementation of synbiotic and enhanced organic acid reduced *Campylobacter* load in ceca ($P = 0.08$). However, none of the treatments altered the load on carcass at 42 d of age.

Key Words: Campylobacter, Probiotics, Organic Acids, Post-harvest, Food Safety

T153, **Assessment of lymphocyte and monocyte dynamics and tissular changes in the trachea of vaccinated challenged chickens against ILTV** Daniel Maekawa*[GS], Patrick Whang, David Hurley, Maricarmen García *University of Georgia*

Infectious laryngotracheitis (ILT) continues to negatively impact the poultry industry across the globe. The disease is primarily controlled by vaccination. Currently, there are two types of ILT vaccines utilized in the control of the disease: the live attenuated and the recombinant viral vector vaccines.The live attenuated vaccines include the chicken embryo origin (CEO) and the tissue culture origin (TCO) vaccines, whereas among the recombinant vaccines there are the herpesvirus of turkey – laryngotracheitis (HVT-LT) and the fowl pox – laryngotracheitis (FPV-LT). In particularly the CEO vaccines are capable to control severe outbreaks of the disease and the protection induced by CEO vaccine surpass that induced

by the TCO vaccine and the recombinant vector vaccines. Although, the protection efficacy among the vaccines is well characterized, the immunological cascade needed to establish protection among the ILT vaccines is unknown. Therefore, the objective of this study was to assess the relative amounts of lymphocytes and monocytes that reach the trachea of chickens vaccinated with CEO, TCO and HVT-LT after challenge with a virulent ILTV strain and to associate cell type infiltration with viral antigen. CEO vaccination induced robust protection showing a rapid virus clearance, maintenance of trachea structural morphology and controlled lymphocyte and monocyte infiltration. On the other hand, TCO and rHVT-LT vaccination conferred partial protection characterized by limited viral clearance and substantial infiltration of lymphocytes and monocytes which resulted in trachea morphology changes. Overall, better vaccine protection was not linked to increased immune cells migration to the trachea after challenge.

Key Words: Protection, Infiltration, Immunity

T154, **Evaluating nucleic acid preservatives as swab media for whole genome sequencing of avian influenza virus** Christina Leyson*, Sungsu Youk, Mary Pantin-Jackwood *Southeast Poultry Research Laboratory*

Advances in sequencing technology has made whole genome sequencing of influenza viruses more accessible and cost-effective. To be able to use whole genome sequencing for diagnostics it is important to refine methods that improve sensitivity and quality of the data generated. Sequencing of whole genomes from swab samples is particularly challenging since the starting material contains contaminants that either inhibit reactions during sample processing or degrade the material (e.g. bacteria). The aim of this study is to evaluate the use of nucleic acid preservatives as swab media, with focus on whole genome deep sequencing. To this end, a low pathogenic avian influenza virus was serially diluted in conventional swab media or in either one of two nucleic acid preservatives, RNAlater and Pursafe® DNA/RNA Preservative. Another set of samples was spiked with 10% (w/v) chicken feces to mimic cloacal swab conditions. To further validate our results, oropharyngeal and cloacal swabs were taken from mallards infected with an H7N9 virus using conventional or nucleic acid preservatives as swab media. We then evaluated the success rate of the initial RT-PCR amplification of influenza genome segments as well as its success rate upon deep sequencing with Illumina MiSeq. To test for virus viability in different swab media, samples were also inoculated into embryonated chicken eggs. Viability tests demonstrate the nucleic acid preservatives reduce viral titers about 10-fold. Results from this study will aid in advancing the application of deep sequencing as a diagnostic tool.

Key Words: Avian influenza, Next generation sequencing, Swab media

T155, **Evolution of H9N2 low pathogenic avian influenza viruses in Korea** Sungsu Youk* [1,2], Dong-Hun Lee [1,3], Jae-Hyun Jung [2], Mary Pantin-Jackwood [1], Chang-seon Song [2], David Swayne [1] [1] *Exotic and Emerging Avian Viral Diseases Research Unit, Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture,* [2] *Avian Diseases Laboratory, College of Veterinary Medicine, Konkuk University,* [3] *Department of Pathobiology & Veterinary Science, University of Connecticut*

While American H9N2 viruses are mostly found in wild birds, Eurasian H9N2 viruses have established in poultry causing great economic losses to the poultry industry. Among the five genetic lineages of Eurasian H9N2 viruses, the G1 and BJ94 lineages have been prevalent across Asian, Middle Eastern, and North African countries. Through the years, the Korean lineage H9N2 virus (also known as Y439 lineage) diverged and formed a distinct lineage circulating only in Korean poultry. To date, there has been no comprehensive analysis determining the epidemiology of H9N2 avian influenza viruses (AIVs) in Korea. In 10 years of surveillance (2006-2016) we have isolated and sequenced H9N2 viruses in Korean live bird markets (LBMs). To understand how H9N2 evolves and spreads in Korea, a statistical Bayesian phylogenetic model was used. Phylogenetic analysis indicated that three separate introductions of progenitor gene pools (Korean domestic duck-origin and two wild aquatic bird-origin AIVs) contributed to the generation of the five genotypes of H9N2 viruses in Korea. Phylogenetic reconstruction of ecological states infers that the LBMs are where chickens become infected with the virus, via domestic ducks playing a major role in the transmission and evolution of the H9N2 viruses. Following the most recent introduction of a wild aquatic bird-origin AIVs in 2008, six genes of the Korean lineage H9N2 virus were replaced, and the new genotype virus (K4) became widespread in Korean LBMs. Continuous surveillance and genetic analysis are strongly encouraged in order to investigate how the H9N2 viruses evolve in LBMs.

Key Words: H9N2, LPAI, Live bird market, Korea, Evolution

T156, **Evaluation of environmental sample collection devices and target sampling areas for Avian Influenza detection in layer cages** Jongseo Mo*, Erica Spackman *USDA Southeast Poultry Research Laboratory*

During recovery from an outbreak of avian influenza virus (AIV), ensuring that the infected premises is free of residual virus is critical for releasing a quarantine and restocking. Environmental testing may also be utilized as an adjunct to testing animals when attempting to identify infected premises. Currently there is not a uniform approach to testing wire cages. Thus, establishing an optimized and validated sampling protocol to ensure the removal of residual virus in such typical environments after an outbreak is essential. Optimization of collection procedures can impact the likelihood of detecting the virus in the environment, as more sensitive methods and tests will yield higher viral loads within the same level of residual virus. The primary objective of this study was to compare sampling devices and to determine optimal locations for AIV detection on environmental surfaces in wire cages to provide general guidance for environmental sample collection and testing for residual AIV after an outbreak. Another objective was to explore the possibility of reducing the number of samples collected and yet yield the same results to make the process less laborious. For that purpose, a laboratory-based, animal trial to simulate the environment of real poultry houses containing infected birds was conducted. Three sampling devices and multiple sampling locations, were evaluated.

Key Words: avian influenza, Environmental sampling

Metabolism & Nutrition IX, Gen. Nutrition/Enzymes

T157, **Performance of growing Japanese quails (Coturnix coturnix japonica) fed Pleurotus sajor-caju fermented cowpea husk** Rasheed Hamzat*, Mustapha Sabo, Jamilu Yahaya *Federal University Dutsin-Ma*

A five-week feeding trial was conducted to evaluate the effect of *Pleurotus sajor-caju* fermented cowpea husk inclusion at the expense of maize offal on the growth performance of growing Japanese quails. 300 two- week old Japanese quails were used in a completely randomized design with 5 treatments replicated four times with 24 quails per replicate. The treatments were 0% cowpea husk (RCH), 5% raw cowpea husk (RCH), 10% RCH, 5% *Pleurotus*-fermented cowpea husk (PFCH) and 10% PFCH.

Feed intake and weight gain were measured weekly. The results obtained showed no significant difference (P>0.05) among the treatment means in final weight, weight gain and feed intake. Feed conversion ratio (FCR) was highest (P<0.05) for quails fed the 10% RCH (6.14) compared to the 5% RCH (5.34). 0% RCH, 5% PFCH and 10% PFCH had similar (P>0.05) FCR to both the 5% RCH and 10%RCH. Carcass parameters assessed in this study were not significantly (P>0.05) affected by the inclusion of RCH or PFCH in the diets of growing Japanese quails. The results of this study indicated that RCH and PFCH can be included in the diets of growing Japanese quails without adverse effects on their performance.

Key Words: cowpea husk, maize offal, Japanese quails, weight gain, feed conversion ratio

T158, **Effect of proprietary vitamin-mineral premixes and two housing systems on some selected vitamins deposition in eggs at the late laying stage** Olugbenga OGUNWOLE*[1], Oluyemisi Oluremi [1], Ireti OLUDOYI [1], Martha OLUMIDE [2], Folasade JEMISEYE [1], Aderonke MOSURO [3]
[1] University of Ibadan, [2] Babcock University, [3] Lead City University

Chicken egg as food is a veritable source of dietary vitamin to humans. Vitamin composition in egg could be determined by dietary supplementation of different vitamins and minerals. Thus, the need to ascertain the effect of supplemental vitamin-mineral premixes (VMP) from different proprietors on deposition of vitamins in eggs produced from hens in different housing systems (HS). Thus, effects of five different proprietary VMP and two HS on depositions of thiamine, pyridoxine, cholecalciferol and folic acid in eggs of hens at the late laying stage were investigated. Black Bovan Nera pullet strain (n=240) aged 71 weeks were randomly allotted to five dietary treatments; each treatment was replicated six times with eight hens per replicate. The hens were raised in a conventional battery cage (BC) and an open-sided deep litter (DL) HS. Five isocaloric and isonitrogenous diets were formulated and each diet was supplemented with 0.25% of proprietary VMP 1, 2, 3, 4 and 5. The experiment was a 2x5 factorial arrangement in a completely randomized design. Eggs (n=60) from the housing systems were collected and used for the vitamin assay. Data were analysed using the analysis of variance and means separated at α0.05. Significantly higher (P<0.05) thiamine deposition (0.063 mg/100g) was in eggs of hens on VMP 5 with similar (P>0.05) content of pyridoxine, folic acid and vitamin D. The HS had significant impact on vitamin D (93.969IU) and foliate content (51.909mg/100g) of the eggs in the DL. Effect of interactions of HS x VMP on pyridoxine, folic acid and vitamin D deposition were similar (P>0.05) but differed significantly (P<0.05) for thiamine deposition in eggs. Hens from DL had better vitamins depositions than BC, while the vitamin in eggs were to a great extent influenced by dietary supplement of the different proprietary VMP and HS of hens.

Key Words: Deep litter, Battery cage, Vitamin-mineral premixes, Folic acid, Pyridoxine

T159, **The effect of feeding encapsulated butyric acid and zinc on disease signs of turkeys challenged with histomonas meleagridis and coccidia** Karen Vignale-Pollock*[1], Elle Chadwick [2], Robert Beckstead [2]
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The objective of this study was to determine if encapsulated butyric acid and zinc (EBZ) could reduce disease signs associated with the protozoan parasite *Histomonas meleagridis* with or without lesion exacerbation by a coccidia challenge. The experiment was a complete randomized block design using 384 turkeys in battery cages. Birds were assigned to one of six treatments (8 reps/trt; 8 birds/cage): 1- negative control on standard diet, 2- positive control infected with *Histomonas* while on the standard diet and treatments 3-6 were infected with *Histomonas* and a commercial turkey coccidia vaccine with the following diets: 3- standard, 4- EBZ (2.5 lbs./ton), 5- EBZ (7.5 lb./ton) and 6- EBZ (15 lb./ton). On day 3 post-placement, poults in treatments 3-6 were given a 10X dose of Immucox® T (CEVA Animal Health, Inc., Cambridge, Ontario, CA) diluted to 1 mL and administered by oral gavage. On day 11, four out of the eight turkeys in treatments 2, 3, 4, 5 and 6 were inoculated in the cloaca with 1 mL of *H. meleagridis* culture containing 100,000 *Histomonas* cells/mL. The other birds remained uninoculated, but free to interact with the infected birds to determine lateral transmission. Mortality was recorded daily, and necropsies occurred on all poults that succumbed to the disease prior to trial termination to determine cecal and liver lesions. The experiment was terminated when poults reached 32 days of age and the ceca and liver from each bird was scored for blackhead disease (0: no lesions to 4: severe lesions) to evaluate for infection rate and gut health status. Statistical analyses were performed using JMP® (SAS Institute, Cary, NC) software using the GLM procedure and means were separated using Tukey HSD with significance considered if $P \leq 0.05$. Treatment 4 led to a significant decrease in direct infection rates compared to treatments 2 and 3 (P<0.05). This treatment also had significantly lower cecal lesion scores when compared to treatment 3 (P<0.05) with similar liver lesion scores to treatment 1. No significant differences were seen in lateral transmission between treatments. EBZ at 7.5 lb./ton significantly reduced direct infection rates and cecal lesion scores for poults challenged with *Histomonas* and multiple coccidia species.

Key Words: butyric acid, zinc, Histomonas, coccidia, turkeys

T160, **A multi-component feed additive improves performance and lesions of birds challenged with Clostridium perfringens.** Nicholas Evans*[1], T Karnezos [2], Curtis Novak [1]
[1] Purina Animal Nutrition, [2] PMI Nutritional Additives

A variety of individual feed additives have been shown to improve performance and reduce disease severity in poultry. As such, feed additives containing multiple components may also be beneficial in poultry production. In this study, a multi-component feed additive (Beacon®) containing a blend of plant extracts, prebiotics, probiotics, and tributyrin was evaluated in a necrotic enteritis disease model. Birds (30 birds/pen @ 0.625 ft^2 and 15 replicates/treatment) were vaccinated with Coccivac®-B at day-of-hatch and challenged with *Clostridium perfringens* at day 19, 20, and 21. Experimental treatments included: 1) negative control (no challenge + 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); and 4) Beacon® (challenge + Beacon® 1.5 lb./US ton in starter, 1.25 lb./US ton in grower, 1.0 lb./US ton in finisher). Lesion scores were assessed at age 23 days and performance at age 40 days. As expected, the positive control had significantly ($p \leq 0.05$) higher lesion scores and significantly ($p \leq 0.05$) worse performance (body weights and mortality adjusted feed conversion) than the negative control. The BMD®/Stafac® treatment had the lowest lesion scores of all challenge groups, but the Beacon® treatment was also significantly ($p \leq 0.05$) lower than the positive control. Performance (body weights and mortality adjusted feed conversion) was similar between the BMD®/Stafac® and Beacon® treatments and both treatments were significantly ($p \leq 0.05$) better than the positive control. Mortality was not different between any treatments. Overall, Beacon® reduced necrotic enteritis lesion scores and improved performance of broilers challenged with *Clostridium perfringens.*

Key Words: Clostridium perfringens, Necrotic Enteritis, Broiler, Feed Conversion

T161, **Combination of feed additives with different modes of action in broilers under a coccidiosis challenge** Meghan Schwartz*, Marcos Rostagno *Phytobiotics North America, LLC*

A study was conducted to evaluate multiple commonly used feed additive combinations in broilers under a coccidiosis challenge. The objective consisted on an initial attempt to identify if one of the feed additive combinations would stand out in supporting the growth performance of broilers. A total of 540 day-old Cobb 500 males were assigned to six dietary treatments with 10 replicate battery cages per treatment (8 or 9 birds/cage) in a randomized complete block design. Bird placement density was

0.046 m^2/bird. The feeding program consisted of 3 phases using corn-soybean based diets. Dietary treatments were: 1) Control (No additive), 2) isoquinoline alkaloids (IQA) from *Macleaya cordata*, 3) IQA & triterpenoid saponins, 4) IQA & micro-encapsulated secondary plant compounds, 5) IQA & formulated phytogenic product, and 6) IQA & *Saccharomyces cerevisiae* fermentation product. At day of hatch, all chicks were orally gavaged with a commercial coccidiosis vaccine at 10X manufacturer's recommendation. Growth performance was evaluated on days 7, 14, 21, 28, and 35. No clear or consistent effect was observed, which is similar to field observations under commercial broiler production conditions. However, statistically significant differences ($P<0.05$) were observed for adjusted feed conversion ratio at 7 d and body weight at 22 d. Numerical trends were observed for body weight at 7 d and feed intake at 28 d and 35 d. A contrast analysis comparing Control versus IQA and Control versus all IQA treatment groups revealed a significant ($P<0.05$) higher body weight gain at 21 d for IQA and a trend ($P<0.10$) for improved FCR at 28d for IQA treatment groups. It is important to highlight the occurrence of a high variation on the growth performance variables across all treatment groups making it challenging to statistically detect differences of several FCR points. These results suggest that further investigation is warranted for commonly used feed additive combinations in broilers, especially for larger bird size beyond 35 days of age, and under floor pen conditions. Dealing with the complex intestinal challenges that impact the growth performance of broilers under commercial conditions very likely will require the adoption of multiple types of interventions.

Key Words: feed additive, broilers, coccidiosis

T162, **Diets contaminated with Fusarium mycotoxins affect immunity parameters of broiler chickens** Alexandro Marchioro*[1], Barbara Doupovec [2], Dian Schatzmayr [2], Ursula Hofstetter [1] *[1] BIOMIN Holding GmbH, [2] BIOMIN Research Center*

Climate change is a worldwide recognized issue leading to different problems in food and feed safety. Mycotoxin occurrence in different kind of commodities can affects animal health. One of the most important system affected by the mycotoxins is the immune system. Fumonisins (FUM) and deoxynivalenol (DON) are of major concern given their common co-occurrence in feed worldwide.

The aim of the presented feeding trial was to elucidate the negative effects of a mycotoxin-contaminated diet on immune parameters in broiler chickens and to investigate the efficacy of a counteracting strategy.

A total of 1280 broiler chickens was randomly assigned to 4 groups with 8 replicates (40 birds per replicates). Naturally mycotoxin-contaminated corn was used to prepare the diets: (A) negative control group (FUM < 200 µg/kg; DON < 100 µg/kg), (B) FUM group (1000 µg/kg FUM; DON < 100 µg/kg), (C) FUM/DON group (1000 µg/kg FUM; 200 µg/kg DON), and (D) FUM/DON group + 0.1% mycotoxin counteracting additive . Parameters evaluated were performance parameters, antibody titers for Infectious Bursal Disease, Infectious Bronchitis (IBD, IB) and liver parameters (AST, ALT, Albumin). The trial lasted for 35 days.

The contaminated diets had no significantly negative effect on performance parameters within the trial period. No acute intoxication was observed at the mycotoxin contamination levels applied via feed. IBD antibody titers were significantly decreased in group B (246 IU/L) compared to the other groups (A: 285 IU/L; C: 346 IU/L). This effect was counteracted by the additive (D: 374 IU/L). IB titers, however, were significantly lower in groups A and C (411 and 551 IU/L, respectively) compared to groups B and D (1027 and 852 IU/L, respectively). Liver parameters investigated showed negative trends in groups B and C compared to groups A and D.

To conclude, a combination of mycotoxins can impair immune response and show a negative impact on liver health in broiler chickens. The feed additive was able to counteract these negative effects. The effect of low levels of mycotoxins in broilers suggests that it is necessary to protect the animals, especially during the initial phases, against multi-mycotoxin contamination normally present in feed.

Key Words: Fusarium Mycotoxins, Immunity Parameters, Feed additive

T163, **Alpha-monolaurin improves the immune response in infectious bronchitis vaccinated broilers** Maarten De Gussem [1], Annelike Dedeurwaerder [2], Claudia Tersteeg [2], Isaura Christiaens [2], Ellen Damen [3], Olga Dansen*[3] *[1] Vetworks bvba, [2] Poulpharm bvba, [3] Framelco BV*

Alpha-monolaurin has been demonstrated to have antiviral and antibacterial properties and is therefore used as feed additive for broilers to prevent infectious diseases. Alpha-monolaurin is thought to exerts its antiviral effect by disintegrating the viral envelope. Therefore, the antiviral properties of α-monolaurin might counteract with the current vaccination programs used in poultry production. In this study, the effect of a commercial available formulation of α-monolaurin (FRA® C12 Dry) on the efficacy of infectious bronchitis (IB) vaccination, as well as its effect on the immune response after IB vaccination were analyzed in Ross 308 broilers. Two separate trials were performed with approval of the animal ethics committee. Broilers (Ross 308) were obtained from a commercial hatchery in Belgium. The test product, FRA® C12 Dry, containing α-monolaurin was provided by Framelco and a dose level of 3kg/ton of feed was used during the whole trial period in both trials. Trial 1 lasted for 49 days and trial 2 for 40 days. *Trial 1.* On day 0, 168 chickens were randomly allocated to 8 pens resulting in pens of each 21 animals. The study included 1 pen per treatment group. Half of the broilers received the product (groups E, F, G, H). The live attenuated IB vaccine (Nobilis IB 4/91®) was administered on day 2 and day 12 by individual oral (groups A, C, E, G) or spray (groups B, D, F, H) administration to all birds. On day 22, the number of animals per pen was reduced to n=16 and an intratracheal challenge with a 100x higher Nobilis IB 4/91® vaccine dose was performed on groups C, D, G and H. The main study parameters were body weight, feed consumption, feed conversion ratio and IB titre values. *Trial 2.* The study included 1 isolator per treatment group with 25 male broilers per isolator that were randomly allocated to the negative control group (no vaccination, no FRA® C12 Dry), positive control group (oral vaccination, no FRA® C12 Dry) or the treatment group (oral vaccination and FRA® C12 Dry. The live attenuated IB vaccine Nobilis IB Ma5 was orally administrated on day 1 and 15. The main study parameters were mortality, body weight, feed consumption, feed conversion ratio, vaccine uptake (presence of IB RNA was analysed by qPCR), vaccine response (antibody titres against IB were determined using ELISA) and immune response (IFN-γ was determined using ELISA). No negative effect of α-monolaurin was observed on the efficacy of IB vaccination using a live attenuated viral IB vaccine, as birds that received FRA® C12 Dry were still protected upon challenge with IB. Surprisingly, elevated IB titre values were observed in orally vaccinated unchallenged animals receiving FRA® C12 Dry (trial 1). Enhanced clearance of the IB vaccine after 30 days was observed in orally vaccinated unchallenged birds receiving FRA® C12 Dry, suggestively due to an increased immune response (trial 2). This was supported by increased IB titre values and increased IFN-γ levels in these birds, pointing to a more active immune system. The results demonstrate that α-monolaurin does not influence the IB vaccination efficacy and has the potency to improve the immune response. This further supports the use of α-monolaurin as a feed additive for broilers, not only by performance effects but also by an improved response on viral vaccination programs.

Key Words: Alpha-monolaurin, immune response, infectious bronchitis, vaccinated broilers

T164, **Conventional trypsin inhibitor levels of soybean meal and protease supplementation affect digestibility in broilers** Raquel Araujo*[1], Juan Ruiz [1], Ricardo Gonzalez-Esquerra [1], Sandra Rodrigues [1], Everton Krabbe [2] [1] *Novus do Brasil Com. e Imp. Ltda,* [2] *Embrapa Suínos e Aves*

Trypsin inhibitor (TI) analysis is considered the best *in vitro* method to predict anti-nutritional factors in soybean products. TI in solvent-extracted soybean meal (SBM) can vary according to genetics, under-processing, over-processing, etc, and high levels are associated to outbreaks of feed passage with consequent decrease in broiler performance. The objectives of the study were: 1) determine the impact of Brazilian commercial SBM with different levels of TI on digestibility of broilers; and 2) evaluate a protease enzyme [CIBENZA® DP100, Novus International, Inc. (PROT)] as a tool to mitigate the deleterious effect of TI on digestibility. A total of 17 samples of SBM were obtained from large companies of Brazilian poultry industry and analyzed for TI with five selected for the *in vivo* trial. The experiment was conducted with 624 Cobb male broilers reared in cages and fed a common corn-SBM diet from day 1 to 21. Test diets were fed from day 22 to 28 with birds submitted to five treatments (12 replicates each) with SBM (used as the sole protein source) varying in TI levels from 3.30 to 4.24 mg/g. The SBM with 4.24 mg/g TI was also fed along with PROT added at 500g/MT. SBM samples were standardized for particle size to avoid its influence on digestibility. Ileal digesta was collected on day 28 and digestibility data analyzed by ANOVA and Tukey test. The contrast between treatments with vs without PROT was also analyzed. Response curves were fitted using linear, quadratic and broken-line models. PROT use improved apparent digestibility coefficients of all AAs (except for Trp and His) on average 5.2% and gross energy from 74.3 to 79.9% (P<0.05). Digestibility decreased quadratically with increased levels of TI for Tyr, Ser, Trp and Leu while for Met, Cys, Thr, Ile, Val, Arg, Phe, Ala and gross energy the digestibility coefficients best fitted to broken-line model. There was a decrease in digestibility with increased levels of TI that reached the plateau between 3.47 to 3.99 mg/g TI depending on the AA. In summary, commercial TI levels affected broilers digestibility and the protease CIBENZA® DP100 was effective in mitigating risks related to variations in commercial SBM quality by increasing digestibility of high trypsin inhibitor-SBM.

Key Words: soybean meal quality, amino acids, energy, ileal digestibility

T166, **Effects of a specific blend of oleoresins of spices and essential oils on growth performance of broilers is influence by challenge acuity** Farouk Messad [1], Jean-Francois Gabarrou* [2], Bertrand Médina [3], Marie-Pierre Létourneau [1] [1] *Animal Science Department, Laval University,* [2] *PHODE,* [3] *Probiotec International Inc*

Some alternatives to antibiotics have been used in human medicine for centuries. Plant secondary compounds have recently been considered for use in animal production. Some studies have documented equivalent or even better responses than antibiotics but other reported no effect. The main goal of this study was to evaluate the effects of a blend of essential oils and spices, Oleo, with a specific focus on founding modulating factor of their response on growth performances of broilers from a meta-analysis approach. An exhaustive database including 25 trials with Oleo was built to quantify its impact on average daily gain (ADG) and feed conversion ratio (FCR) of broilers. Dietary treatments were either a positive control (C+) with antibiotic growth promoter, a negative control (C-) without antibiotic or a treatment with Oleo at 100 ppm. To test the hypothesis that Oleo response was influence by the challenge the bird experienced, when C+ and C- were both present in the same trial (n=9), the relative difference between them (C+/C-) was also calculated as Challenge Acuity Index and used as an X variable. Metabolizable energy and crude protein has been recalculated based on diet composition. Regarding the range of trial durations and slaughter ages of broilers, the Y variables were converted as relative difference in FCR and ADG expressed in percent between Oleo and C+ (ADG_Oleo/C+, FCR_ Oleo/C+) and Oleo and C- (ADG_Oleo/C-, FCR_ Oleo/C-). The mixed procedure of Minitab (Version 18.0) was used with the effect of the trial as random effect and the dose of Oleo and C+/C- as X variables. When comparing Oleo and C- treatments, results showed 2.8±0.70% (P<0.001; R2=65%) reduction of FCR and 3.6±1.2% (P=0.006; R2=56%) improvement of ADG with 100 ppm of Oleo. However, when comparing Oleo with C+ treatment there was no significant effect on FCR or ADG, indicating that Oleo and C+ are equal. The FCR_ Oleo/C- was negatively correlated (Linear, P=0.03; Quadratic, P=0.02; R2=85%) with the Challenge Acuity Index (C+/C-) indicating that in challenge condition (when C+ performed better than C-), the reduction of FCR with Oleo addition was higher. This work helps to better understand the variations observed between studies for the same alternative to antibiotics.

Key Words: Broilers, essential oils, oleoresins of spice, growth, challenge

T167, **Effect of dietary supplementation of essential oil and organic acid alone or in combination in broilers** Frances Yan*, Juxing Chen, Vivek Kuttappan, Deana Hancock, Mercedes Vazquez Anon *Novus International Inc.*

Both essential oil and organic acid have been demonstrated to improve growth performance and gut health of broilers. With their different mechanism of action, it is hypothesized that combining them could provide additional benefits. A floor pen study with 1728 day-old male broilers was conducted to evaluate an essential oil blend (EOB, NEXT ENHANCE® 150, 1:1 thymol carvacrol) and an organic acid blend (OAB, AVIMATRIX®, protected benzoic acid calcium formate and fumaric acid), either used alone or in combination, on growth performance and gut health of broilers subject to mild *Eimeria* challenge. The study consisted of 9 dietary treatments in a 3 x 3 factorial arrangement with 3 levels of EOB (0, 15, and 30 g/ton) and 3 levels of OAB (0, 250, and 500 g/ton). Each diet was fed to 8 replicate pens of 24 birds. All birds were orally gavaged with a coccidiosis vaccine at 5X recommended dose on day 14. Body weight, feed intake, FCR, and mortality were determined on day 21, 28, 35, and 41. On day 22, jejunal tissues were collected for cytokine mRNA expression. On day 42, footpad dermatitis lesions were scored. Data were subject to 2-way ANOVA to evaluate main effects and their interaction; means were separated by Fisher's protected LSD test. Body weight was not affected by dietary treatments (*P>0.05*). Feed conversion ratio (FCR) was more sensitive to dietary treatments and the best response was observed on day 28 and 35. There was a significant interaction between EOB and OAB (*P<0.05*) where except for EOB at 15 g/ton, all treatments significantly improved FCR on day 28 and 35, and the best FCR was observed with combining 15 g/ton EOB and 250 g/ton OAB. A significant interaction was also seen for footpad dermatitis where only combining EOB (15 or 30 g/ton) with OAB (500 g/ton) reduced footpad dermatitis lesion scores and increased percentage of healthy footpad (*P<0.05*). Essential oil blend at 30 g/ton also significantly reduced jejunal IL-10 mRNA expression (*P<0.05*) indicating its immune modulating function. In summary, OAB improved FCR, EOB improved FCR and modulated immune response, and combining OAB and EOB resulted in better FCR and lower incidence of footpad dermatitis, indicating additional benefits can be achieved with combination.

Key Words: Essential oil, Organic acid, Eimeria, Broiler

T168, **Efficacy of supplemented Bacillus subtilis DSM 17299 in broiler chickens fed standard and energy-, protein- and amino acid- reduced diets** A. Kehlet [1], M. Lang [1], J. Schleifer*[1], C. Bostvironnois [1], J. Zentek [2], F. Goodarzi [2], K. Männer [2] [1] *Chr. Hansen A/S,* [2] *Freie Universität Berlin*

Bacillus subtilis DSM 17299 (BS) can produce a wide range of digestive enzymes. potentially advantageous to digest feed in the gut of broiler chickens. The objective of this study was to evaluate the effect of addition of a BS in standard and energy - and protein/amino acid- reduced

diets fed to broilers from d 1 to d 42 of age. A total of 720-day-old Cobb male chicks were distributed in 48 floor pens (15 birds/pen), allocated to 6 dietary treatments (8 rep/treat): T1 (basal diet without probiotics), T2 (-20 MJ/kg -0.3 points CP), T3 (-40 MJ/kg -0,6 points CP), T4 (T1+BS), T5 (T2+BS), T6 (T3+BS). The concentration of BS in starter diet:1.6E+9 CFU/kg feed; grower diet: 8E+8 CFU/kg feed). All diets had the same CP:AA ratio. Body weight (BW), feed intake, and mortality adjusted FCR were assessed at each feed change. Apparent ileal digestibility (gross energy and crude protein) was measured at day 42. The results were analyzed by one-way ANOVA, $P \leq 0.05$ were recognized as statistically significant. The addition of BS improved BW by 13.5 g in the standard diet. In reduced T2 the addition of BS improved BW by 48.4 g and in T3 by 57.8 g. FCR was slightly (standard diet) or significantly (reduced diets) reduced by 1.8% or 3.1 and 3.0% respectively compared to the corresponding diets without addition of BS. Calculated ileal digestible for gross energy and protein showed improvements with the addition of BS in all diets. Digestibility of gross energy was improved by 1.7% comparing T1 and T4, 1.2% when comparing both T2 to T5 and T3 to T6. Crude protein was improved by 1.7% when comparing T1 to T4 and significantly by 3.2% and 3.0% comparing T2 to T5 and T3 to T6 respectively. The current study shows that BS can be used effectively to improve broiler performance and to overcome the performance loss associated with a reduction in nutrient density.

Key Words: probiotic, enzyme, Bacillus subtilis, digestibility, broiler

T169, **Metabolites produced by the probiotic Bacillus subtilis 29784 strengthen intestinal barrier and modulate inflammatory response** Pearl Choi [1], Eric Pinloche [2], Lamya Rhayat [2], Pascal Thiery* [3], Karoline Sidelmann Brinch [4], Estelle Devillard [2], Richard Ducatelle [1], Filip Van Immerseel [1] *[1] Ghent University, Faculty of Veterinary Medecine, [2] Adisseo France SAS, CERN, [3] Adisseo France SAS, [4] Novozymes A/S, Animal Health & Nutrition*

The probiotic strain *Bacillus subtilis* 29784 (Bs29784) produces a large number of metabolites. Niacin (NA), hypoxanthine (HPX) and pantothenate (PTH), are produced in high quantity in *in vitro* cultures, as well as in the digestive tract of poultry. The purpose of the present study was to determine the effects of those metabolites on the development and repair of the intestinal epithelium, on the enhancement of the intestinal barrier function, and on the intestinal inflammatory response, using Caco-2 cell models.

Caco-2 cells were subjected to different treatments through incubation with 1mM NA, HPX, or PTH and were compared to non-treated cells. Cell proliferation was measured after 9 days of treatment using Prestoblue staining. Differentiation of confluent Caco-2 cells was evaluated after a 72h treatment by measuring intestinal alkaline phosphatase. TEER (transepithelial resistance) and cytokine release were measured under an IL1-induced inflammatory stress after 6h treatment. For each condition, three biological replicates, with at least 4 repeats each, were carried out and data were subjected to the ANOVA procedure of XLSTAT.

All metabolites produced by Bs29784 increased ($p<0.0001$) cell proliferation, up to 73.5%, 59.8% and 50.4% for HPX, PTH and NA, respectively. On the contrary, cell differentiation was not impacted by Bs29784-produced metabolites. TEER was numerically improved by PTH. HPX treatment led to a decreased IL6 level ($p<0.0001$), representing 47.3% of IL6 production from the control treatment group.

The results of this in vitro study suggest that the main metabolites produced by Bs29784 have complementary effects on intestinal epithelial cells by improving cell proliferation (NA, PTH and HPX), intestinal integrity (PTH) and by decreasing IL6 production (HPX). This completes the previous data on the mode of action of Bs29784 showing that the probiotic promotes butyrate-producing bacteria in the gut. Taken together those results strengthen the comprehension of the effect of Bs29784 on the host and explain at least partly its positive effects on animal performance.

Key Words: Probiotic, Bacillus, Metabolites, Gut Health

T170, **Timely germination of Bacillus probiotics is key to product performance** Adam Nelson*[1], Jared Heffron [1], Pearl Choi [2], John Rice [3], Ross Wolfende [4], Damien Preveraud [5], Filip Van Immerseel [2], Richard Ducatelle [2], Lamya Rhayat [5], Estelle Devillard [5], Karoline Sidelmann Brinch [6] *[1] Novozymes Microbe Innovation, [2] Gent University, Faculty of Veterinary Medicine, [3] Novozymes Microbial Discovery, [4] Novozymes Poultry Research, [5] Adisseo, Centre of Expertise and Research in Nutrition, [6] Novozymes A/S, Animal Health & Nutrition*

Purpose: *Bacillus*-based DFMs are delivered in feed as spores. To exhibit their effects through production of metabolites, the spores need to germinate in the GI tract of the birds. Time of germination varies among strain of *Bacillus* and external factors (such as feed composition, additives) can influence the spores' ability to germinate. The purpose of this study was to show the germination profile of Bacillus subtilis (Bs) 29784 compared to other strains of *Bacillus* and to deliver *in vivo* proof of germination.

Experiments: Bs 29784 was grown *in vitro* together with a range of relevant feed components and additives to assess the impact on germination speed. *In vivo* studies were performed in chickens fed the Bs 29784 and samples taken from different gut compartments. Samples were analyzed for levels of *Bacillus* metabolites (nicotinic acid and hypoxanthine). Furthermore, *in vivo* samples were analyzed for levels of germinated cells.

Results: The ability to germinate was shown to differ between Bs 29784 and a range of other Bacilli with regards to germination time under gut-like conditions. The germination profile of Bs 29784 was similar in both wheat, corn and soy analyzed by OD-values over time. Also, inclusion of organic acids, coccidiostats and a range of additives showed no impact on germination. *In vivo* studies showed the ability of Bs 29784 to germinate in the gut and the increase of *Bacillus* abundance ($p<0.05$). In jejunum samples a 50-fold significant ($p<0.01$) increase in levels of nicotinic acid and hypoxanthine were observed.

Conclusion: Timely germination is key to product performance since Bacilli need to be metabolically active to exhibit their positive effects on gut health and performance. Bacillus strains differ in their ability to germinate inside the animal and a careful selection of strains is therefore needed. For most strains their ability to germinate inside the animal is unknown. Here we showed *in vivo* proof of germination and metabolic activity of a *Bacillus*-based DFM to support the mode of action of strain Bs 29784 and its beneficial effects in poultry.

Key Words: Probiotics, Bacillus, Gut health, DFM

Metabolism & Nutrition X, Enzymes/Feed Additives

T171, **Administration of yeast fraction reduces outcome of Salmonella Typhimurium challenge on broilers** Ruth Raspoet[1], Tadele Kiros[1], Breno Beirao[2,3], Celso Junior[2], Dany Mesa[2,3], Max Ingberman*[2], Luiz Caron[2,3] *[1] Phileo by Lesaffre, [2] Imunova Análises Biológicas, [3] Universidade Federal do Paraná*

In recent years, the market positioning for a reduction on antibiotic use in animal production gained strength with a focus to produce antibiotic free livestock. In this context mannan-oligosaccharides (MOS) have conquered the animal health industry. MOS could function as potential alternatives in a strategy for antibiotic reduction and substitution and help the animals to maintain their productive potential. Yeast fractions are rich in mannoproteins, mannans and β-glucans, enabling them to modulate the gut physiology. The occurrence of β-glucans allows them to modulate the immune system, while the presence of mannans can associate them with bacteria possessing type-1 fimbriae, such as *Salmonella*.

Therefore, the objective of this study was to evaluate the effect of the supplementation of a premium yeast fraction on *Salmonella* colonization in broilers.

At day 1 of the trial, newly hatched broiler chicks were divided over 3 isolators, each representing one treatment group; (1) the unchallenged control, (2) the challenged control and (3) yeast fraction supplemented and challenged group. At day 2, animals in the challenged control and the yeast fraction group were orally inoculated with 10^8 CFU of *Salmonella* Typhimurium ATCC 14028. Individual body weights were recorded at days 3, 7, 14 and 28. Immune parameters, histology scores and *Salmonella* Typhimurium loads in crop, liver and spleen were determined at days 14 and 28.

Challenge with *Salmonella* Typhimurium reduced body weights in the challenged control group in some dates. Additionally, the challenged control exhibited significantly increased *Salmonella* Typhimurium numbers in crop, liver and caecum compared to the negative control group, which remained negative for *Salmonella* throughout the experiment. The study also demonstrated the efficacy of the premium yeast fraction in decreasing the adverse effects of *Salmonella* infection on broilers. The yeast fraction was able to improve the performance of the challenged animals, while decreasing the lesion score in the cecum and the *Salmonella* colonization in liver and ceca. The results also demonstrated the effect of the yeast fraction on the immune system, since supplemented birds were able to produce higher antibody titers of IgA against salmonella LPS and flagellin.

Key Words: Salmonella, yeast fraction

T172, **Effect of different sources and levels of Cu on productive performance and ileal microbiota of broilers** Asal Forouzandeh[1], María Rodríguez[2], Alessandra Monteiro[3], Arturo Piñon*[3], José Francisco Pérez[1], David Solà-Oriol[1] *[1] Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, [2] PigCHAMP Pro Europa, [3] Animine*

In Europe, copper (Cu) supplementation is limited to 15 mg/kg in broiler diets. However, in many non-EU countries it is often used at high dosages (125 to 250 mg/kg) due to its positive effect on growth and intestinal health. The Cu sulfate ($CuSO_4$) is the most common Cu source used in the feed industry. Nonetheless, a newly authorized Cu source, dicopper oxide (Cu_2O, CoRouge®), has been claimed a superior growth promotion and antibacterial effect on pathogenic bacteria compared to $CuSO_4$. Therefore, the aim of the present study (Project E! 11780) was to determine the effect of two sources of Cu ($CuSO_4$ and Cu_2O) at three levels (15, 75 and 150 mg/kg) on growth performance and ileal microbiota. A total of 840 one-day-old male chickens (Ross 308) were randomly allocated to 7 dietary treatments: a basal diet (no Cu addition), a basal diet with the supplementation of 15, 75 or 150 mg Cu/kg as $CuSO_4$, or Cu_2O (12 pens/ treatment, 10 chicks/ pen). Broilers were challenged by reusing an old litter with high concentrations in *Clostridium* and *Coccidia*. The chicks were weighed individually, and feed intake was recorded at 21, 35 and 42 d of age. At d 43, one broiler per pen was euthanized to obtain ileum content. Birds fed Cu_2O at 150 mg/kg showed greater ($P < 0.05$) BW at d 35 (1.78 kg vs. 1.69 in average for other treatments) and tended ($P = 0.053$) to increase BW at d 42 (2.59 kg) compared to the other treatments (average of 2.47 kg). ADFI was significantly increased ($P < 0.05$) in birds fed 15 mg/kg $CuSO_4$, but FCR was not affected by the dietary treatments (average of 1.68). The influence of 0 and 150 mg/kg of Cu on microbial characterization of ileal content was determined by using 16S rRNA gene sequences. Compared to other treatments, Cu_2O at 150 mg/kg decreased ($P < 0.05$) the abundance of *Streptococcaceae* which is known to be involved in the development of metabolic disorders and increased ($P < 0.05$) the abundance of some of the main butyrate producers, *Clostridiaceae* and *Peptostreptococcaceae*. This study indicates that the supplementation of Cu_2O at 150 mg/kg established a healthy gut microbiota by increasing the abundance of beneficial microbial families in ileum, which may explain the positive impact on growth performance of broilers.

Key Words: Copper, broiler chicken, ileal microbiota, microbial characterization, growth

T173, **Microbiota analysis of commercial broiler flocks treated with antibiotic growth promoter or the direct-fed microbial Bacillus subtilis DSM 32315** Kristina Feye[1], Cristiano Bortoluzzi*[2], Anita Menconi[3], Steve Ricke[4], Michael Kogut[1] *[1] Southern Plain Agricultural Research Center, [2] Department of Poultry Science, Texas A&M Agrilife Research, [3] Evonik Corporation, [4] Center for Food Safety, Department of Food Science, University of Arkansas*

The objective of this study was to investigate the diversity and composition of the intestinal microbiota of broiler chickens raised under commercial conditions and treated with either an antibiotic growth promoter (AGP) or the direct-fed microbial *Bacillus subtilis* DSM 32315. For this purpose, the whole gastrointestinal tract of broilers with varying ages (from 14 to 46 d) was collected and placed in dry ice, and then the ileal and cecal contents were separated for further analysis. The cecal and ileal contents were used for bacterial DNA isolation, which was sequenced using the Illumina MiSeq V3 Platform, targeting the V3-V4 region of 16S rDNA. FASTQ sequences were imported into QIIME2.2019.7 using the q2cli interface, trimmed, filtered for chimeras, and aligned using SILVA database. Data indicated that there was only a location (all alpha indices) and time difference (Faith's PD) that was significant for alpha diversity by q2-longitudinal ANOVA ($P < 0.05$). Beta diversity showed greater effects, with a significant treatment effect as well as significant differences by compartment, treatment, and time (PERMANOVA/PERMDIST; Bray Curtis/Jaccard, $P < 0.05$, pairwise $Q < 0.05$). ANCOM (Analysis of Compositions of Microbiomes) was used to evaluate the main effects of treatment and time within compartment ($Q < 0.05$). In the ceca, direct-fed microbial treatment showed an increase in Verrucomicrobiota, Tenericutes, and Epsilonbacteria. In the ileum, direct-fed microbial showed an increase in Actionobacteria, and eventually demonstrated a contraction in Proteobacteria populations. The microbiota-by-age scores (MAZ) were numerically different, indicating that there was a trend for differences in microbiome stability between groups. This data is significant as it was a field trial, with few controls for variation; therefore, data suggests that the changes demonstrated in the microbiota are likely due to the differences between the AGP treated flocks and the flocks receiving the direct-fed microbial. Overall, the dietary inclusion of *Bacillus subtilis* DSM 32315 was capable of inducing changes to the microbiome that may be correlated to the benefits of maintaining an intestinal balance.

Key Words: broiler, microbiota, probiotic

T174, **Determining the effect of Lactic acid bacteria and Bacillus subtilis used alone and in combination on broiler chickens** John Slyvester[1], Adebayo Sokale[2], Normand St-Pierre[1], Randy Mitchel[1], Maria Mendoza[2], Mikayla Baxter*[1] [1] *Perdue,* [2] *Evonik Corporation*

Probiotics seed the intestinal environment with beneficial bacteria to maintain health and performance in chickens. The purpose of this experiment is to assess the effect of a Lactic acid bacteria probiotic blend (LAB-pro) and LAB prebiotic (LAB-pre), and Bacillus subtilis DSM 32315 (BS) used separately or in combination on performance, intestinal morphology, and bacteria profile of broiler chickens. Two thousand Ross YP 708 male chicks were randomly assigned to 4 treatments (10 replicates per treatment, 50 birds per replicate): T1 Basal diet with no probiotic; T2 LAB-pre-pro (1 x 10^4 CFU/g of feed) across all growth phase, T3: LAB-pre-pro (starter phase; 1 x 10^4 CFU/g of feed); LAB-pre + BS® (Grower and Finisher phase; 1 x 10^4 CFU/g of feed and 1 x 10^6 CFU/g of feed) and T4: BS (1 x 10^6 CFU/g of feed) across all growth phase. Chickens were vaccinated with 1× dose of COCCIVAC®-B52 at hatch, and raised for 42 days on a mix of fresh and used litter. Performance data was assessed within each feed phase (starter, grower and finisher) and overall (starter to finisher) and analyzed using a one-way ANOVA. Intestinal morphology was analyzed using a three-way ANOVA (comparing the effect of treatment, age, and intestinal segment). For the data analysis, significant *P*-value was set at ≤ 0.1. In the starter phase, chicks in treatments T2 and T3 had statically lower adjusted FCR than the control. At 10 days of age the duodenal crypt depth (CD) was significantly lower in T2 compared to T1, T3 and T4 and duodenal villus height (VH) to CD ratio was higher in T2 than all other treatments. At d 23 days of age, TI and T4 had higher duodenal VH than T2 and T3 and T3 had deeper crypts than all other treatment groups. In addition, T2 and T3 had lower duodenal VH:CD than T1 and T4. At 23 days of age T2 had thinner duodenal mucosa than T1 and T4 and T2 had thinner cecal mucosa than T4. At 23 days of age, probiotic supplementation altered the bacteria profile of the small intestine and ceca, reduced enteritis index, and incidence of swollen/red intestine and undigested feed. Overall, Lactic acid bacteria and Bacillus subtilis DSM 32315 probiotics used separately or in combination had significant effect on performance and intestinal morphology particularly in the starter phase.

Key Words: Probiotic, Broiler, Performance, Histology

T175, **Improvement of digestibility and performance of broiler chickens with an algae-clay based technology** Marie Gallissot[1], Raully Silva[2], Pedro Aleixo[2], Luiz Fernando Albino[2], Horacio Rostagno[2], Raquel Pereira[1], Mariel Tavares[1], Maria Angeles Rodriguez*[1] [1] *Olmix,* [2] *University of Viçosa*

Improving feed digestibility is a multi-faceted issue in animal nutrition. It contributes to improve livestock farming sustainability, by optimizing the use of available resources and by reducing environmental emissions, while increasing farming profitability. This study was set up to evaluate the effect of supplementing an algae-clay complex (ACC) on digestibility of the feed and performance of broiler chickens fed a corn-soy diet. Two experiments were set up: one digestibility trial, using ninety-six male chicks (Cobb 500) of 14 days of age with 8 replicates of 6 birds per treatment, and one performance trial using four-hundred one-day old male chicks (Cobb 500) with 10 replicates of 20 birds per treatment. Each trial had two treatments: a control group fed a standard corn-soybean based diet opposed to a test group receiving the same diet supplemented with the ACC (0.1%). All diets were formulated according to the recommendations of the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017). Results were submitted to ANOVA test (α=0.05) using R software (v. 3.6.1). Results of the digestibility trial showed an improvement in apparent metabolizable energy (AME) of 66 kcal/kg in the test group compared to control (P=0.048), and in nitrogen-corrected AME of 56 kcal/kg (P=0.032). Nitrogen retention was increased by 6% in the test group compared to control (P=0.052). In the performance trial, the supplementation in ACC improved the feed efficiency of broilers, with an overall 3 points gain on feed conversion ratio (P=0.046) and the weight gain at 42 days (+78 g, P=0.163). The highest effect was observed in the grower (day 21-33) and finisher phase (day 33-42). Overall, the two experiments implemented in this study demonstrate that the ACC improves digestibility and performance in broiler chickens fed a standard corn-soybean based diet. The improved digestibility came along with a decreased nitrogen excretion which is beneficial to the environment and contributes to more sustainable farming.

Key Words: Digestibility, Algae, Clay, Performance, Broilers

T176, **A comparative analysis of an enzymatically processed soy protein and non-SBM protein sources on broiler live performance and economic benefit under NAE conditions.** Josh Steed*[1], Alfred Blanch[1], Dan Moore[2], Simone Rasmussen[1], Christine Brøkner[1] [1] *Hamlet Protein A/S,* [2] *Colorado Quality Research*

Soybean meal (SBM) is the most widely used protein source as it represents an excellent source of essential amino acids. However, the anti-nutritional factors and immunogenic components in SBM have been reported to impair performance. A joint strategy is reducing SBM in early diets through implementing non SBM protein sources. Nevertheless, non SBM protein sources are often variable in nutritional content, can produce enteric disruptions, and many don't reduce inclusion of SBM at appreciable rates. As popularity of vegetarian diets increase, evaluating cost effective, consistent veg. protein sources that reduce inclusion of conventional SBM in early diets is necessary. An experiment was conducted to evaluate economic and live performance of enzymatically processed soy protein (EPSP) relative to common non SBM protein sources. Eight hundred and fifty day old Ross 708 males were placed in 50 pens (17 birds/pen; 0.27 m^2/bird). A completely randomized block design was utilized and pen replicates were assigned to 1 of 5 dietary treatments: 3%, 5% EPSP, 3% corn gluten meal (CGM), 3% dried distiller's grain with solubles (DDGS), or 3% meat bone meal (MBM). Broilers were fed a 3 phase program consisting of a starter (0-14d), grower (14-28d), and finisher (28-42d). Dietary treatments in each phase were iso-energetic and contained similar amino acid ratios relative to dig. LYS. At 35 days of age, body weight gain (kg/bird) in EPSP was significantly higher than CGM fed birds (P<0.05). BW adjusted feed to gain was significantly lower in EPSP compared to the CGM treatment (P<0.05). EPSP numerically reduced BW adjusted feed to gain in comparison to DDGS (-3.4). Treatment BW variation was reduced 2.50, 1.44, 1.35 fold when EPSP was equated to MBM, DDGS, and CGM, respectively. At 42 days of age, body weight gain (kg/bird) was significantly higher in EPSP versus CGM (P<0.05). BW adjusted feed to gain was numerically reduced in EPSP treatments in relation to DDGS and CGM (-2.5, -3.4). Treatment BW variation was reduced 2.89, 1.89, 1.58 fold when EPSP was fed versus MBM, CGM, and DDGS. EPSP, DDGS, and CGM were evaluated for their return on investment (cost and performance). In the present study, EPSP provided the greatest return on investment of the vegetarian protein sources.

Key Words: Enzymatically Processed Soy, Protein, Broilers, Soybean Meal, Corn Gluten Meal

T177, **Effects of in-feed phytogenic additive in broilers vaccinated for coccidiosis** sergio vieira*[1], Liris Kindlein[1], Andre Favero[1], Anja Pastor[2], Marcos Rostagno[3], Douglas Drebes[1] [1] *UFRGS,* [2] *Phytobiotics Futterzusatzstoffe GmbH,* [3] *Phytobiotics North America LLC*

The objective of the present study was to determine the effects of a coccidiosis vaccination or a chemical coccidiostat with or without a phytogenic in-feed additive on growth performance and intestinal health in broiler chickens. Permeability and inflammation markers were used (fluorescein isothiocyanate dextran - FITC-d; TNF-alpha and IL-10). A total of 1-day old 1,098 male Cobb 500 broilers were assigned to 7 treatments in a complete randomized block design, including 9 replicates per treatment, except for 2 treatments with 8 replicates each. Treatments were: Nega-

tive Control (NC; No feed additive or vaccine), Chemical (C; Nicarbazin 100 ppm), C + phytogenic 60 ppm (*Macleaya cordata* extract, CMCE60), C + phytogenic 90 ppm (CMCE90), Cocci Vaccine (V; Fortegra), V + phytogenic 60 ppm (VMCE60), and V + phytogenic 90 ppm (VMCE90). Birds were vaccinated for coccidiosis at placement. The bedding was of used pine shavings after 3 crops. Birds were *ad libitum* fed corn-soybean meal all-vegetable diets. No medication was included in feed or water throughout the study. Results showed that all cocci-vaccinated birds experienced an early reduction in weight gain and poor FCR (1-28 d) showing compensatory growth towards the end of the study. The other treatment groups performed better than the NC group. Moreover, the combination of both feed additives, chemical and phytogenic (at both dosages) showed positive synergism on the overall FCR (1-53 d). At 28 d, serum levels of FITC-d were evaluated 4 h after its oral gavage. FITC-d was significantly increased in the cocci-vaccinated birds compared to the remaining treatment groups, which indicates impaired gut integrity. The negative impact of vaccination was alleviated when combined with the phytogenic at either dosages. Similarly, TNF-alpha and IL-10 were significantly lower in birds fed both additives, either individually or combined. The results of the present study demonstrate that both feed additives evaluated were efficient in alleviating the loss of growth performance induced by a coccidiosis vaccination. This study also demonstrates the improvements provided by the phytogenic feed additive on the intestinal health of broilers, based on both a gut leakage model, as well as when evaluating pro inflammatory cytokines.

Key Words: broiler, coccidiosis, Macleaya cordata, phytogenic, feed additive

T178, **The efficacy of a phytogenic feed additive (PFA) in combination with an attenuated cocci vaccine in Eimeria-challenged broilers** Manu De Laet*[1], Stacie Appleton[2], Megan Koppen[2] [1] *Delacon GmbH,* [2] *Delacon USA Inc.*

The most common challenges affecting commercial broiler gut health are coccidiosis, necrotic enteritis, and bacterial enteritis. In the past, achieving production goals under challenge conditions relied largely on the use of antimicrobial growth promoters (AGPs). However, because of legislation against the use of AGPs and the increasing consumer demand for antibiotic-free animal production, the poultry industry is seeking for new alternatives. This pen trial study was conducted to determine the efficacy of a phytogenic feed additive (PFA), containing essential oils and saponins, in combination with an attenuated coccidiosis vaccine (Huveguard®, Huvepharma NV) in broilers under experimental infection using an *Eimeria* field isolate (sporulated oocysts of *E*. acervulina (118,000 oocyst/bird), *E*. maxima (9,000 oocyst/bird), *E*. tenella (17,000 oocyst/bird) and *E*. mitis (12,000 oocyst/bird)). This experiment was conducted at Poulpharm, Izegem, Belgium and lasted for 35 days. In total 800-day-old male broiler chickens (Ross 308) were allotted to 4 treatments, with 8 repetitions of 25 birds each. Treatments were 1) Non-infected non-treated (NINT); 2) Infected non-treated (INT); 3) Infected + vaccination at day 1 (IV); 4) Infected + vaccination at day 1 + 400 ppm PFA (IVP). Birds were infected with coccidiosis at day 14. At 35 days of age, INT group had significantly (P<0.001) lower daily weight gain (41.9 g/day) compared to the other three treatments (52.3 g/day for NINT, 45.8 g/day for IV, 50.6 g/day for IVP). FCR was significantly (P<0.001) different for INT (1.697) compared to NINT (1.537), IV (1.590) and IVP (1.524). Daily feed intake (DFI) at the end of the trial period was similar for NINT and IVP. Both groups had significantly (P=0.014) higher DFI than INT and IV treatments. Mortality was significantly increased by the challenge (INT vs NINT), and significantly (P=0.013) reduced by IV and IVP treatments. These results show that Biostrong® Protect in combination with an attenuated coccidiosis vaccine may improve bird's resilience against coccidial infections.

Key Words: coccidiosis vaccination, phytogenic feed additives, efficacy, broilers, coccidia

T179, **Alleviation of heat stress using a phytogenic feed additive** Manu De Laet*[1], Megan Koppen[2], Stacie Appleton[2] [1] *Delacon GmbH,* [2] *Delacon USA Inc.*

Heat stress in poultry can have a significant adverse impact upon performance, immunity and ultimately welfare. Some estimates suggest heat stress costs the US poultry industry up to $168M. Intervention strategies to manage heat stress have included management, nutritional solutions and feed additives. The objective of the current study was to evaluate the efficacy of a phytogenic feed additive (PFA) comprising flavonoids, essential oils and pungent substances (Biostrong® Comfort, Delacon BiotechnikGmbH) to alleviate the effect of heat stress on the performance of broilers. The trial was carried out at PNRC, Czech Republic. A total of 576 male day-old Ross 308 birds were randomly assigned to 3 treatments (6 replicates/treatment, 42 birds/replicate): 1) CON, basal diet; 2) POS, basal diet + Betaine (500g/T); 3) PFA, basal diet + PFA (400 g/T). Birds were reared for 42 days in floor pens on clean litter with access to feed and water *ad libitum*. From day 21-42, a cyclic heat stress was applied (32°C for 8 hours, 26°C for 12 hours) while humidity was 70-80%. Statistical significance was declared at $P<0.05$. From day 0-21 (no heat stress), PFA significantly reduced FCR (1.340) by up to 3 points versus CON (1.370) and POS (1.355) while bodyweight gain (BWG) was numerically higher in PFA than CON and POS (898g, 875g and 892g, respectively). From day 22-42 (heat stress) birds in the PFA and POS group had numerically higher BWG versus CON (1730, 1730 and 1623 g, respectively) while FCR also did not differ significantly between treatments (CON: 2.004, POS: 1.989, PFA: 1.956). Overall, there was a trend for PFA birds to improve BWG by up to 132g ($P=0.074$) versus CON and POS (2633g, 2501g and 2580g, respectively). Furthermore, PFA numerically improved ($P=0.084$) FCR (1.731) by 2.04% compared to CON (1.767) and 1.0% compared to POS (1.750). Mortality for the trial overall did not differ significantly between treatments, although POS had the highest mortality (8.3%) compared to CON and PFA (3.4% and 4.9%, respectively). Consequently, EPEF was 22 points higher in PFA (344) versus POS (322). EPEF in CON was 325. The current study demonstrates that a PFA (Biostrong® Comfort) can provide comparable performance to Betaine to alleviate the impact of heat stress in broilers.

Key Words: heat stress, phytogenic feed additives, efficacy, broilers, performance

T180, **Xylanase improves growth performance, enhances cecal short chain fatty acids production and increases relative abundance of fiber fermenting cecal microbiota in broilers** Amit Singh*[1], Rabindra Mandal[2], Michael Bedford[3], Rajesh Jha[1] [1] *University of Hawaii at Manoa,* [2] *Department of Microbiology and Immunology, University of Louisville,* [3] *AB Vista Feed Ingredients*

This study investigated the effect of supplementing xylanase in a corn soybean meal (CSBM)-based diet with graded levels of wheat bran (WB) on growth performance, cecal short chain fatty acids (SCFA) production and cecal microbiota diversity in broilers. A total of 180 straight run day-old chicks (Cobb 500) were randomly assigned to 30 pens (6 birds/pen). Six dietary treatments were tested using a randomized design in a 3×2 factorial arrangement (n=5). The treatments included 3 levels (0%, 5%, and 10%) of added wheat bran (WB) in a CSBM-based diet, supplemented without or with xylanase (0.01% Econase-XT). All diets contained 500 FTU/kg phytase and were fed in 2 phases (starter, d 0–21 and finisher, d 22–35) in mash form. Xylanase supplementation increased (P <0.05) the average daily gain (ADG) in the finisher phase and total period and increased (P <0.05) average total gain of broilers (2025 *vs* 1943 g). Xylanase improved (P <0.01) feed conversion ratio (FCR) in the starter phase while it produced a significant interaction (P <0.05) with WB on FCR in the finisher phase and total period. A greater improvement in FCR in response to xylanase was noted in the higher WB groups compared with 0% WB, which indicated that higher fiber diets were more responsive to xylanase. Independent of fiber level, xylanase increased (P <0.01) cecal SCFA (86.6

vs. 63.4 µmol/g) compared with no xylanase group. Amplicon sequencing of V3-V4 region of 16S rRNA gene from d 35 cecal samples revealed that xylanase had a tendency for higher count of operational taxonomic units (OTU; P =0.08) and larger beta diversity (P =0.054) compared with the no xylanase group. Xylanase increased the relative abundance of *Lactobacillus salivarius* compared with no xylanase groups (P <0.05). Xylanase also increased the relative abundance of *Ruminococcoceae* and *Lachnospiraceae* family belonging to order Clostridiales of phylum Firmicutes. Many members of these families are known butyrate producers and display antagonism with Eimeria in challenged birds. In conclusion, xylanase can enhance productive performance and health of broilers by improving feed efficiency and a portion of this response is related to increased cecal SCFA and enhanced abundance of beneficial microbiota.

Key Words: broilers, fiber, gut microbiota, volatile fatty acids, xylanase

T181, **Evaluation of xylanase alone or in combination with xylo-oligosaccharides on performance of broiler chickens fed corn-based diets** Gilson Gomes*, Tiago Tedeschi dos Santos, Xavière Rousseau *AB Vista*

Xylanases are frequently used in broiler feeds to improve animal performance. Despite this being a routine practice, there is still a lot of debate regarding the mode of action of xylanase-based products in broilers. Recently it was proposed that the main mechanism may be occurring by releasing xylo-oligosaccharides that may exert prebiotic effects on gut microbiota, playing an important role for the establishment of fibre-degrading bacteria, thereby positively affecting animal's performance. The aim of this study was to evaluate the effects of supplementing xylanase alone (XYL – Econase XT, AB Vista, Marlborough, UK) or a combination of xylanase plus a fermentable xylo-oligosaccharide (XYL+XOS - Signis, AB Vista, Marlborough, UK) on broiler performance fed low nutrient diets based on corn and soybean meal. Day-old male Ross 308 broiler chicks were randomly allocated to 6 pens per treatment, with 12 chicks per pen, and assigned to four treatments as follows: positive control (PC); negative control (NC - reduced by [g/kg] 0.14 dLys, 0.11 dM+C, 0.15 dThr, 0.16 dVal, 0.12 dIle, 0.20 dArg and 150kcal/kg); NC+XYL; NC+XYL+XOS. All diets contained 1,000 FTU/kg of phytase (Quantum Blue, AB Vista, Marlborough, UK) and fed as pellets in three dietary phases. Feed and water were available ad libitum throughout the trial. Body weight gain (BWG) and feed intake (FI) were measured from 0-40-d. Feed conversion ratio was calculated after correcting for mortality (FCR). Data was submitted to one-way ANOVA and means separated using Student's T-test (P<0.05). No differences in liveability, BWG or FI were seen throughout the experiment (P>0.05). Birds fed PC diets had the best FCR, while those fed NC diets presented the worst FCR (P<0.05). Birds fed NC+XYL but did not differ from either NC or NC+XYL+XOS (P>0.05). Birds fed NC+XYL+XOS showed similar FCR (1.62) when compared to either PC (1.59), NC (1.65) or NC+XYL (1.63) fed birds. Birds fed diets reduced in nutrients and supplemented with XYL+XOS showed similar performance when compared to PC fed birds.

Key Words: Xylanase, Xylo-oligosaccharide, Body weight gain, Feed conversion ratio

T182, **Butyrate affects broiler gut health depending on its presence in distinct digestive tract segments** Tim Goossens*[1], Pierre Moquet [2], Pascal Thiery [3], Haitham Yakout [4], René Kwakkel [2] *[1] Adisseo Belgium, [2] Wageningen University, [3] Adisseo France, [4] Adisseo North America*

Several reports have been published describing positive effects of butyrate on gut health, supporting the use of butyrate as animal feed supplements. However, the effects elicited by dietary butyrate supplementation are variable, especially when looking at the physiological responses that may underlie improvements in animal performance. We hypothesized that at least a part of this variation could be attributed to differences in gastrointestinal tract regions where butyrate was delivered by different butyrate-based feed additives.

A total of 320 male day-old Ross 308 broilers were assigned to a control group or four groups that were fed products with different butyrate release characteristics: 1 g butyrate per kg of complete feed was administered, either as unprotected pure sodium butyrate, tributyrin, a combination of the two, or a fat-protected butyrate product. For 22 days, birds were fed a diet high in rapeseed meal, to provide a dietary challenge, and evaluated on digestive, microbial and immunological parameters.

A higher butyrate presence in the proximal GIT was associated with a reduction of hindgut microbial diversity (P < 0.05) and a trend (P < 0.1) for a reduced apparent ileal digestibility of Ile, Leu, Phe, His and Lys. Moreover, an increased expression (P < 0.05) of immunity-associated genes (TLR-4, MyD88, IL-6, CAT and NOS2) in the ileum or colon suggests an inflammatory status. A similar inflammatory signature was observed when butyrate concentrations were elevated in the mid-gut, although no sign of bacterial hindgut dysbiosis was detected in these birds. With increased hindgut butyrate levels a longer intestinal retention time (P < 0.05) and a tendency (P < 0.1) for FCR improvement and Met digestibility could be observed, without negative effects on caecal microbiota composition or inflammatory status.

These results indicate that, at least under certain conditions, elevated butyrate concentrations in the fore- and midgut may induce negative effects on microbiota diversity and/or inflammation, as opposed to increased butyrate concentrations in the hindgut . Overall, the data emphasize the importance of product-dependent butyrate release kinetics in investigating the effects of exogenous butyrate supplementation in broilers.

Key Words: butyrate products, gut health, butyrate release profiles

T183, **Addition of a liquid extract of humic substances as growth promoter in broiler chickens with different body weight** Sergio Gomez-Rosales*[1], Maria Angeles [1], Yair Lopez-Garcia [1], Alejandra Domínguez-Negrete [2] *[1] National Center of disciplinary Research in Animal Physiology and Genetics, INIFAP, [2] Faculty of Natural Sciences, Autonomous University of Queretaro*

The objective of the study was to evaluate the productive performance, carcass components yield and the content of ashes, calcium (Ca) and phosphorus (P) in the tibia of broiler chickens supplemented with a liquid extract of humic substances (LEHS) in the drinking water. A group of 360 Ross male broilers from 8-42 d of age were allocated in holding cages and randomly assigned to 10 treatments in a factorial arrangement of 5 levels of the LEHS (0, 10, 20, 30 and 40%) mixed in the drinking water and 2 initial body weights (light = 125 and heavy = 135 g) at the start of the trial. The LEHS was extracted from a worm compost prepared with sheep manure using an alkaline solution of sodium hydroxide. The concentration of humic and fulvic acids in the LEHS were 0.33 and 0.27%, respectively. At 21 d of age, half of the birds were killed, and the other half were killed at 42 d of age, to get the carcass components and the left tibia. Data was analyzed using ANOVA and orthogonal contrasts. At 21 d, the body weight and WG were reduced linearly in light broilers, while the body weight and WG increased quadratically in heavy broilers, reaching the highest responses at 20% inclusion of the LEHS (Interaction of the LEHS and initial body weight, P < 0.05). The yield of the breast and the dry weight and weight of tibia ashes (P <0.05) were lower in light broilers compared to the heavy ones. The percentage and weight of Ca in the ashes showed cubic patterns (P < 0.01) due to the addition of the LEHS. At 42 d, the body weight and WG from 8-42 d and the yield of the carcass and breast were lower (P <0.01) in light broilers compared to heavy broilers. The tibia ashes and the weight of P in the ashes were higher in heavy broilers at 30% inclusion of the LEHS, while in the other treatments no differences were observed (Interaction of the LEHS and initial weight, P < 0.05). The content of Ca in the ashes showed quadratic patterns (P < 0.05) regarding the levels of the LEHS in the water. In conclusion, the addition

of the LEHS in broilers at 21 d of age increased the body weight and WG in heavy broilers and the Ca content in the ashes of all chicks. At 42 d of age, the ashes content, Ca and P in the ashes showed the higher values at 30% inclusion of the LEHS in the water.

Key Words: Broiler chickens, Humic substances, Productivity, Tibia calcium, Tibia phosphorus

T184, **The application of dual-energy X-absorptiometry to nutrition and yield dynamics research in poultry** Diego Martinez*, Katie Hilton, Pramir Maharjan, Antonio Beitia, Jordan Weil, Nawin Suesuttajit, Craig Coon *University of Arkansas*

The objective of the present study was to calibrate and validate the use of dual-energy X-absorptiometry (DEXA) for the determination of processing yield and to apply it to the study of yield dynamics in broilers. 242 male Cobb 500 birds coming from different nutrition experiments were sampled at different ages between 4 and 69 d (95 to 6300 g of body weight; BW), DEXA scanned and cut up to measure processing yield. Multiple linear regression models were developed on JMP 13.2 using the results from DEXA as predictors. The measured and predicted values were compared and correlation coefficients determined. In addition, a total of 220 male Cobb 500 birds fed a standard diet with or without the addition of an exogenous combined enzyme product were sampled and DEXA scanned (22 time points between 0 and 42 d; 5 birds sampled per treatment and age) and Gompertz curves were adjusted and compared (equivalence test for inflection point, growth rate and asymptote estimates) for BW, protein, fat and mineral contents in the body, and for carcass, breast, tenders, wings and leg quarters weights. Different non-linear models were adjusted to determine yield (%) dynamics of each part of the body. Finally, specific time points were compared using a t-student test. Multiple linear models were obtained for carcass, fat pad, breast, tenders, wings and leg quarters, being the adjR2 values 0.995, 0.801, 0.971, 0.974, 0.988 and 0.988 for the actual weight (g) of each piece, and 0.935, 0.752, 0.921, 0.878, 0.780 and 0.793 for the relative (%) values, respectively, with no statistical differences (P>0.10) found between measured and predicted values. No differences were found between treatments when the Gompertz curves of BW and body composition were compared (P>0.05); however, the birds fed the enzyme showed curves with different trends between 10 and 25 days of age and statistically different values (P<0.05) were detected in yield (%) (breast, wings, fat pad) at specific time points within that period, being the breast yield higher for the enzyme fed birds. The results of the present study support the capability of the specific DEXA machine evaluated to determine processing yield in broilers and its application to the study of yield dynamics in poultry nutrition research.

Key Words: dual-energy X-absorptiometry, breast, processing yield, broilers, body composition

Environment Management IV, House Factors/Welfare

T185, **A comparison of the growth performance of an exotic and a locally adapted meat chicken during the hot season in Nigeria – preliminary study** Akinniyi Dare *Center for Avian Resources Development and Services*

The experiment was conducted to compare the growth performance between the Nigerian Fulani Chickens (NFC) and a popular exotic meat chicken – Arbor acre (Aa) from 0 to 8 weeks of age during the hot season in Nigeria.

Eighty unsexed day old chicks per meat chicken group were used for the study. Chicks were reared at the Center for Avian Resources Development and Services, Ifewara, Osun State, Nigeria in a completely randomized block design of two meat chicken groups of 2 replicates per group. Chicks from each group were individually weighed at a day-old and randomly distributed into each group replicates. They were fed *ad libitum* with a straight commercial broiler mash for 8 weeks in 8 x 6 square feet per pen of 40 birds under an average temperature of 33.2 ± 0.11 ^{0}C. Free access to water was allowed throughout the experiment. Live weight (LW) and feed intakes were recorded weekly, average daily gain in live weight (ADG) and feed conversion ratio (FCR) were determined.

All data were analyzed using the general linear model (GLM) of SAS. Results revealed that all studied growth performance traits were significantly ($p < 0.05$) affected by age and breed. The Aa group was significantly higher in LW than the NFC from a day old (34.2 ± 0.83 %) to 8 week of age (87.4 ± 0.41%). The ADG was significantly higher in the Aa group throughout the experimental period (14.6 ± 0.53 to 59.8 ± 2.31 g/day) compared to the NFC (2.8 ± 0.07 to 7.5 ± 0.45 g/day). It was noted that the ADG increases with age in both group. However, a slump from 59.8 ± 2.31 to 50.6 ± 2.92 g/day was observed after the 6th week in the Aa group. There was a significant difference in FCR between the groups. The Aa group showed a lower FCR compared to the NFC at all ages except between a day old and 2 weeks of age. This parameter decreases with increasing age to the 6th week within the Aa group with ranges from 3.5 ± 0.38 to 4.6 ± 0.46. The FCR in group NFC increases with increasing age from 2.8 ± 0.07 in the first 2 weeks of life to 7.8 ± 0.19 at the 8th week. The study concluded that Arbor acre broilers have significantly better ($p < 0.05$) performance in LW, ADG and FCR at all age periods except the FCR within the first 2 weeks of life compared to the Nigerian Fulani chickens.

Key Words: adapted, chicks, exotic, feed, growth

T186, **Astragaloside IV improve hypoxic injury in tibial dyschondroplasia chickens through vascular endothelial cell based PI3K/Akt/HIF-1α signaling** Khalid Mehmood*[1,2], Hui Zhang[2], Jiakui Li[2] [1] *University College of Veterinary & Animal Sciences, The Islamia University of Bahawalpur,* [2] *College of Veterinary Medicine, Huazhong Agricultural University*

Thiram is a dithiocarbamate pesticide, which is commonly used to protect crop against pests and fungi along with protection of seed during storage and transportation. Many researchers reported that it's over use as pesticide and environmental contamination of crop with thiram can cause tibial dyschondroplasia (TD) in chickens. TD is a skeletal abnormality of broiler chicken in which growth plate cartilage fails to form bone and a plug of white avascular cartilage form in growth plate of tibia bone. Astragaloside IV (AST-IV) is a natural cycloartane-type triterpene glycoside extracted from *Astragalus membranaceus*. AST-IV promotes osteogenesis but its protective affects about TD did not study yet. So, we designed this study to use AST-IV for the treatment of TD through PI3K/Akt/HIF-1α signaling. For this purpose,180 Arbor Acres chickens was raised under the recommended conditions. The chickens were divided randomly into equal (n = 60) groups control, TD and Astragaloside IV (AST-IV). The control group was fed a standard normal diet ad libitum throughout the study period, while the TD group and AST-IV group were fed on normal feed with the addition of thiram at 50 mg/kg of feed from 3-7 days. After 7 days, the thiram was removed and Astragaloside IV was given orally @50 mg/ kg from 8-18 days. The chickens were slaughtered on 18 days, while tibia bone and liver were collected. The results were evaluated by using SPSS through student t-test and the data were presented as the means±SD, while P<0.05 considered significant. The results showed GP size was increased significantly, while feed intake along with weight intake was decreased in TD group. However, AST-IV administrations re-

stored the growth performance parameters and GP size of TD afflicted chickens. The gene and protein expressions of VEGF, VEGFR1 and HIF-1alpha were more, while Akt and PI3K were less in TD group as compared to control group. After the administration of AST-IV to chickens, the expression of VEGF, VEGFR1 and HIF-1alpha were decreased while Akt and PI3K were increased near to control group. Altogether, RT-qPCR, western blotting and immunohistochemistry analysis reveals that AST-IV treatment improves hypoxic injury in tibial dyschondroplasia chickens through PI3K/Akt/HIF-1α signaling.

Key Words: Thiram, Tibial dyschondroplasia, Chickens, AST-IV, HIF-1α

T187, **Solving floor egg laying in aviaries: can temporary litter restriction retrain hens without impacting welfare?** Ahmed Ali[*1], Janice Siegford[2] [1] *Clemson University,* [2] *Michigan State University*

A concern specific to cage-free systems, are the consequences of floor-laying such as more labor to collect eggs and more eggs damage. Temporary exclusion of birds from litter is a common strategy to reduce floor egg-laying. However, it may impact flock welfare and prevent hens from performing key behaviors. Our objective was to investigate the influence of excluding hens in midlay from litter areas during the morning on nest use, floor laying and hens' physical welfare. Pullets were raised in floor pens and placed into 4 multi-tier aviary units (100 hens/unit) at 17 WOA. Hens had continuous access to the litter until 54 WOA when ~60% of all eggs were being laid in the litter. Partial litter restriction (doors to litter automatically closed at 1:00 and re-opened at 11:30) was implemented for a 4-week period starting at 54 WOA. Egg production, damage and location, and hens' nest-use were recorded, and 15% hens/unit were randomly assessed for keel bone damage, foot health and plumage quality. Observations were conducted for 3 days at each time point: BEF = week before litter restriction; DUR = during the 4th week of litter restriction; 1WK and 4Wk = first and fourth weeks after restriction ended. Differences within measured parameters across time points were assessed using GLMM with Tukey's Post hoc test (α set at 0.05) in R 3.3.1. Hens occupied nests more frequently during morning observations in DUR compared to BEF (76.9, 39.6%, *P=0.01*), while nest occupancy during 1WK (66.98%) was like that of DUR but dropped dramatically at 4WK (44.9%, *P=0.02*). Hens laid more frequently outside nests during BEF, while, non-nest laid was remarkably reduced in DUR (34.1%, 8.31%, *P= 0.01*). However, there were slightly more non-nest laid eggs during 1WK (14.31%) than DUR, while during 4WK (30.11%) hens almost returned to their BEF lay pattern. Incidence of footpad and plumage damage markedly increased at DUR (56.9, 69.9 %) compared to BEF (33.7, 39.9%, all $P \leq 0.05$). However, incidence of footpad and plumage damage decreased during 1WK (41.2, 56.9%) and 4WK (45.6, 45.7%, all $P \leq 0.05$) compared to DUR. Temporary litter restriction failed to solve an already established floor-laying problem in a laying hen flock and reduced hens' foot health and plumage quality

Key Words: Litter, Welfare, Floor eggs, Restriction, Hens

T188, **Impact of natural beak smoothing feeders and outdoor vegetation density on pullet performance and welfare parameters.** Paul Patterson[*1], Nuket Acar [1], Xavier Florensa [2], Ivan Pineda [3], Inma Estevez [2] [1] *The Pennsylvania State University, USA,* [2] *Neiker Tecnalia, Spain,* [3] *Universidad Nacional Autonoma de Mexico, Mexico*

The goal of this study was to compare Natural Beak Smoothing (NBS) feeders made by Roxell to standard feeders (CON) for pullets raised in floor pens for their impact on pullet welfare and feather picking. All birds were provided outdoor access at the age of 8 weeks. A total of 496-day-old ISA Brown pullets were wing banded and randomly assigned to the following treatments at two weeks of age: two feeder treatments (NBS vs CON) and two different outdoor vegetation densities (LOW versus HIGH density) in a 2 x 2 factorial arrangement. All birds were weighed in even weeks and beak sharpness and beak length of 10 birds from each pen were assessed as well as welfare and feather parameters. The three scores for beak length were as follows: 0 = upper and lower mandible equal length, 1 = upper mandible slightly longer, and 2 = upper mandible significantly longer. The three scores for beak sharpness were, 0 = smooth beak tip, 1 = mildly sharp tip, and 2 = severely sharp tip.

There was no vegetation density effect or interaction with the feeder type; thus, these effects were combined. Body weights at 12 and 16 weeks were significantly heavier for the CON pullets compared to the NBS (1,182 g vs 1,138) and (1,434 g vs 1,400), respectively. Beak length was consistently longer among the CON birds at 8, 10, and 12 weeks of age and they had significantly sharper beaks at 8 and 14 weeks compared to those birds exposed to the NBS treatment. At the end of the 16 week study, there was no impact on feather picking or welfare as a result of either feeder type or vegetation treatments and no mortality was observed throughout the experiment. These finding suggest the Roxell NBS feeder can influence beak parameters in growing pullets, but in this study the level of feather picking/welfare issues was low and not impacted by the study treatments.

Key Words: Pullet, Welfare, Performance, Beak-smoothing, Vegetation

T189, **Deep Data- Statistical Review and Machine Leaning Resolves Risk of Avian Pathogenic E. coli Through Field Service and Epidemiology Modeling.** Jordon Gruber[*1], Elizabeth Kim [1], Terry Parrott [1], Mike Perry [1], Dave Seliner [1], Dan Bautista [2] [1] *DuPont Animal Nutrition,* [2] *University of Delaware*

Establishing risk and/or trajectory of bacterial disease provides application of field service data to improve production throughout complexes. However, field service can be under-powered or non-specific to elucidate enough data to build fundamental epidemiology models. Further complicating the impact of field service is that many bacterial pathogens in poultry fall within a 2-Hit structure of initiating disease. Avian Pathogenic E. coli (APEC) is one such pathogen that requires co-events for disease. The assessment of APEC is complex because resolving the infectious process requires meta-data from historic production and the production environment. A model-based system was created to incorporate meta-data of production, epidemiology of disease, and direct investigation of the isolated APEC from the system. To generate the priority list of meta-data and test factors related to APEC predictability, three years of field microbiology data (2015-2018) (1800 data points) was harvested from the DuPont Digital Poultry Database. Parametric (linear regression), Non-parametric (truth-test), and categorical data (pattern recognition) analytics identified both initial trends and organized data, while also relating to risk of isolates. Results from the study identified constructs of meta-data and microbiology of APEC were both actionable (strong relationship) and non-actionable (no strong relationship) through a machine learning algorithm. Factors such as global density of APEC or general virulence genes did not show any statistical relationship for disease, which is consistent with APEC and its 2-Hit model. However, incorporation of disease type, i.e. respiratory complex or Colibacillosis, and virulence genes, such as cvaC or iss, significantly improved the relationship. Meta-data including history of disease, management, and current vaccine and coccidiosis control strategies were also key in establishing risk for disease. To bring together the factors identified from the data investigation, a matrix was generated to organize data related to APEC and disease initiation. This matrix is currently deployed and is under further investigation as a program approach to provide innovative service to producers, and improve their ability to apply technology towards disease.

Key Words: APEC, Ecoli, Machine-learning, Modeling, Field-service

T190, **Effect of stocking density on crop fill and performance of broilers grown to 14 d** John Linhoss*[1], Joseph Purswell[2], Gary Chesser[1]
[1] Mississippi State University Agricultural & Biological Engineering, [2] USDA ARS Poultry Research Unit

Assessment of crop fill by poultry growers and service technicians is used to gauge appetite development and if chicks have found feed and water within the first 24 hours. Management guides suggest that at least 95% of birds should have a full crop 24 h after placement and that crop fill rates are indicative of proper chick management. Currently, there is no available data on the effects of stocking density on crop fill, nor any data which reports typical crop fill percentage distributions for broiler chickens. The objectives of this study were to 1) determine the effect of stocking density on chick crop fill and bird performance, and 2) to determine changes in crop fill percentage distributions during the first 48 h after placement. 720 straight-run chicks were randomly assigned to 16 pens (45 birds/pen). Two stocking density treatments (29.3 kg/m^2, and 43.9 kg/m^2) were used and each treatment was represented by eight replicate pens. Crop fill was measured on 15 birds/pen at 2, 4, 8, 12, and 48 h post-placement and on all birds at 24 h post-placement. Crops were scored using a 3-point scoring system, with 0 = empty crop, 1 = full but hard, and 2 = full, soft and rounded. Stocking density had no significant effect on BW gain, feed consumption, mortality, or feed conversion at 14 d. Crop fill percentages were not significantly influenced by stocking density; however, there was a significant interaction between crop fill score and time. Percentage of full crops increased from 41.7% at 2 h to 90% at 24 h for the 29.3 kg/m^2 stocking density treatment, and from 45.0% to 90% over the same period for the 43.9 kg/m^2 treatment. For both stocking densities, the percentage of full crops at all sampling times were lower than those recommended in management guides. The results indicate that stocking density has no significant effect on crop fill or performance in broilers grown to 14 d, but do suggest that broiler management guides may overestimate target crop fill percentages.

Key Words: broilers, crop fill, stocking density

T191, **Field comparisons of two natural product alternatives to a commercial antibiotic growth promotant (AGP) program in broiler diets** Leandro Redondo[1,2], Gaston Royero[3], Natalia Casanova[2], Ernest Pierson*[4], Juan Diaz Carrasco[1,2], Mariano fernandez-miyakawa[1,2]
[1] Consejo Nacional de Investigaciones Científicas y Técnicas, [2] Instituto de Patobiología, CICVyA, Instituto Nacional de Tecnología Agropecuaria, [3] Frigorifico de Aves Soychu, [4] The Pierson Consulting Group, LLC

Phytogenic compounds, probiotics and organic acids are used in broiler feeds as an option to AGPs due to their ability to emulate properties of antibiotics. This study's goal was to compare a field AGP (virginiamycin 100 g/MT) program (T1) with two antibiotic free programs: *Lactococcus* based probiotic+yeast MOS+a blend of Italian Chestnut-C/Argentine Quebracho-Q bioactive extracts (T2), or, a blend of C+Q alone (T3). A 42-day floor pen trial was conducted with a total of 1,575 of Arbor Acres® Max male broilers which were randomly distributed to 3 treatments (45 total pens); each treatment (525 birds) was fed to 15 pens of 35 birds/pen. Body weight, feed consumption and conversion were determined at 21 and 42 days of age. Livability and overall health were routinely measured and recorded. Intestinal health (lesion score) was evaluated at day 21 and 42. Caecal and litter samples were taken at these times for microbiota, bacterial, and moisture determination, and, measurement of the spread of environmental antimicrobial resistance. Body weight (T1; 1.052; T2: 1.072; T3: 1.077, kg, respectively) and feed conversion were significantly improved ($P < 0.05$) on day 21. No significant differences among treatments on day 42 were observed for body weight-kg (T1: 2.978; T2: 2.975; T3: 3.003) and feed conversion-Feed/Weight (T1: 1.728; T2: 1.714; T3: 1.715). Microbiota analysis showed a differential effect among treatments, *i.e.*, alpha diversity, number of species and specific groups of beneficial bacteria were improved by the non-AGP treatments. Food pad and intestinal health was generally good and similar among treatments. Both non-AGP treatments showed a tendency to reduce antibiotic resistance in representative bacteria from the intestine and litter.

Key Words: polyphenols, poultry, commercial farms

T192, **Genomic evaluation of chicken gut infected with Salmonella gallinarum & treated with Phytogenic growth promoter (AGP replacer) Herbiotic FS and Lincomycin** Vijay M[1], Shivi Maini*[2], M Reddy[3]
[1] Associate Professor,Department of Veterinary Pharmacology & Toxicology, Veterinary College,KVAFSU,Bidar (KS)-585401, [2] Deputy General Manager, Indian Herbs Specialites, [3] Principal Scientist, Division of Pathology,IVRI,Bareily (UP)

Demand for animal protein for human consumption is rising globally at an unprecedented rate. Modern poultry production practices are associated with regular use of AGPs, potentially increasing selection pressure on bacteria to become resistant. AGP-alternatives are being sought to improve poultry performance. While the industry is focused on developing alternate solutions; their mode of action still remains unclear. Present study is undertaken to establish probable mechanism of action of phytogenic growth promoter Herbiotic FS (M/S Indian herbs Specialities, India) in 120 Vencobb broiler by assessing global gene expression of chicken gut. There were 4 experimental groups (30 birds per group of 6 replicates); Healthy control, infected control (Salmonella gallinarum (1×108 CFU/ml) infection was induced on day 4th), infected & treated with Herbiotic@500g/ton and infected & treated with Linomycin@250g/ton of feed. High-throughput microarray technology provides comprehensive view of global gene expression changes in host. We report here chicken-specific microarray to profile host gene transcription of caeca between Salmonella infected & treated chicken to characterize their host response. Microarray was designed using mRNA sequences reported in NCBI database. 40944 target mRNA sequences were used to design probes. Cluster of differentially upregulated & downregulated genes, heatmap analysis and fold change(fc▲) were performed to analyze gene expression data. The normalization was done using GeneSpring GX Software. The functions & pathway analysis was done for the differentially regulated genes using DAVID database. Differential cut off Fold Value (> = 0.6) in the treated samples indicated Up Regulation & vice versa and was used to determine significant differential expression. Gene ontology performed for 45 upregulated genes with Herbiotic FS treatment compared to other treatments yielded 15 mapped gene ids, 28 unmapped gene ids and 2 multiple mapping ids. Supplementation of Herbiotic induced upregulation of genes of immune system process(XM_421205, fc▲5.84), transporter activity (XM_425129, fc▲2.39), metabolic process (CR353567, fc▲3.08), cellular processes & biological regulation(X56772; fc▲10.09), growth(CR87077, fc▲2.50) etc. Upregulation of genes suggest that Herbiotic FS possess multi-dimensional mechanism of action involving antimicrobial, anti-inflammatory, gut histomorphology & innate immunity promoting, growth promoting properties as compared to AGPs(Lincomycin). Gut microarray reveals that Herbiotic FS can successfully replace AGPs in poultry ration with additional advantages of no AMR & residues in meat.

Key Words: Antimicrobial resistence, Phytogenic, gut microarray, mode of action, AGP Alternates

T193, **Herbiotic FS modulated metagenomic profiling in Salmonella infected chicken gut** Vijay M.[1], Sushiel Agarwala[2], Shivi Maini*[3]
[1] Associate Professor,Department of Veterinary Pharmacology & Toxicology, Veterinary College,KVAFSU,Bidar (KS)-585401, [2] Chairman, Indian Herbs Specialities, [3] DGM, Technical, Indian Herbs Specialities

The relentless march of antimicrobial resistance (AMR) has emerged as a public health concern, especially in the light of the fact that newer antibiotic classes have been slow to develop and investments in novel antimicrobial drug classes have been receding. AMR problem transcends international borders with antibiotic stewardship. Increasing thrust for restriction on AGP's has instigated hunt for newer alternatives. Pres-

ent study is undertaken to establish mechanism of action of phytogenic growth promoter Herbiotic FS (M/S Indian herbs Specialities, India) in 120 Vencobb broiler based on metagenome profiling of chicken gut. There were 4 experimental groups (30 birds per group; 5 replicates of 6 birds); Healthy control, infected control (Salmonella gallinarum (1×108 CFU/ml) infection was induced on day 4th), infected & treated with Herbiotic@500g/ton and infected & treated with Linomycin@250g/ton of feed. Chicken gut metagenomics allows assessment of taxonomic & metabolic diversity of microbial communities. Metagenome of jejunum is carried to assess gut bacterial diversity. The present study illustrates analysis of the 16S rDNA based metagenome of the V3-V4 region, for DNA extracted from sixteen chicken jejunum. The Microbial composition within a sample was estimated using alpha diversity indexes, krona charts & piecharts. Rarefaction plot was generated to ensure that sampling depth was sufficient for the analysis. The microbiome diversity and species abundances was analysed using Metagenomic Rapid Annotation using sub-system technology (MG RAST) & QIIME analysis. Sample wise reports were generated that gives the abundance values from Phylum to Species referring Seed System Technology (Seed Database) & Ribosomal RNA Database (Greengenes, RDP II). Pie charts were generated using matplotlib for all unique taxa's at a cutoff of 0.5% & above, taxa's<0.5% represented as miscellaneous. Metagenomic bacterial diversity dominance of Lactobacillus (39.92%) in healthy control, dominance of pathogenic bacteria (40.58%) & clostridia (34.18%) in infected control, restoration of good bacteria lactobacillus (31.56%) & abnormally higher emergence of candidates bacteria (23.53%) in Lincomycin treatment and restoration & dominance of lactobacilli (61.11%, even higher than healthy control) in Herbiotic FS treatment. Herbiotic FS is efficacious for prevention of colonization of gut pathogens & augmenting peace keeping bacteria ensuring gut function modulation and attainment of eubiosis.

Key Words: antimicrobial resistance, metagenome profiling, Lactobacillus, bacterial diversity, AGP alternates

T194, **Does built-up litter competitively exclude Salmonella: lesson learned from Salmonella Heidelberg ?** Adelumola Oladeinde*[1], Kimberly Cook[1], Zaid Abdo[2], Samuel Aggrey[3], Torey Looft[4], Muhammad Rehman[5], Moussa Diarra[5], Casey RItz[3], Nelson Cox[1], Arthur Hinton[1] *[1] U.S. National Poultry Research Center, USDA-ARS, [2] Colorado State University, [3] Poultry Science Department, University of Georgia, [4] National Animal Disease Center, USDA-ARS, [5] Guelph Research and Development Center, Agriculture and Agri-Food Canada*

The practice of reusing "built-up" litter to raise multiple flock of chickens is known to promote the competitive exclusion of food-borne pathogens including Salmonella, however the mechanism and the microbiome associated with this process is poorly understood. Moreover, the general shift towards antibiotic-free live broiler production indicates the need for efficient litter management practices and the development of new antimicrobials alternatives for use in pre-harvest poultry. Using Salmonella enterica serovar Heidelberg (S. Heidelberg) as a pathogen-model and Big Data (Genomics, transcriptomics and metagenomics) generated from a series of in vivo and in vitro inoculation studies with "built-up" litter (bedding material that is intended for reuse on subsequent flock), we gained a better understanding of the interactions between the gut microbiome, litter microbiome and antibiotic resistance transfer in broiler chickens raised without antibiotics. Our results show that "built-up" litter reused after an adequate downtime (greater than 2 weeks between consecutive flocks, during which the broiler house is empty) harbors a stable and predictable microbiome dominated by the families Nocardiopsaceae and Bacillaceae. Members of these families were associated with a reduction in S. Heidelberg survival in reused litter (up to 6-log reduction). However, when day of hatch Cobb broilers were raised on "built-up" litter versus fresh pine shavings, Bifidobacteriaceae was the family associated with a lower rate of gut colonization (fresh = log 2.85±1.1 g/ceca ; reused=log 2.46 ± 0.8 g/ceca) and acquisition of resistance to tetracyclines and aminoglycosides (n = ~ 300 isolates; fresh = ~ 38.45 %; reused = <1%) by S. Heidelberg. Further, litter physicochemical parameters such as ammonia, pH, moisture and water activity were significantly correlated with the microbiome present in litter. Together, our study supports the notion that the practice of litter reuse promotes an "unfavorable" environment for Salmonella, therefore limiting their potential for survival or acquiring antibiotic resistance.

Key Words: Salmonella, Litter reuse, Competitive exclusion, Antimicrobial resistance, microbiome

T195, **Incidence of focal duodenal necrosis in layers fed diets with different calcium sources throughout rearing and production, and two levels of calcium during production.** Tennille Knezacek*[1], Henry Classen[1], Jenny Nicholds[2] *[1] University of Saskatchewan, [2] University of Georgia*

The role of nutrition in laying hen focal duodenal necrosis (FDN) is unknown. After exposure to two lengths of pre-lay period (7 or 28 d), a laying hen flock of 1,152 Lohmann LSL hens were fed different calcium sources (LM=limestone, OS=50% oyster shell, 50% LM) and calcium levels (R=recommended level, H=20% higher than the recommended level) to determine the effects on performance and gastrointestinal parameters during egg production, and the incidence of FDN. Each treatment had 12 cage replications (12 hens/rep) assigned in a completely randomized design with 8 cages for production data and 4 cages for tissue collection. Data were analysed using a three-way ANOVA with the MIXED procedure of SAS 9.4 and differences were considered significant when $P \leq 0.05$. FDN lesions were observed in hens at the end of production, with the overall incidence being 32 and 17% for tissue and production hens at 62 and 64 weeks of age, respectively. *Clostridium perfringens* was isolated from all three intestinal samples with gross lesions submitted for culture. Though there seemed to be no main treatment effects on FDN occurrence in the tissue birds, there was a higher incidence of FDN in production hens fed OS (22%) compared to LM (12%), and hens provided H (22%) versus R (12%) calcium level. There were no treatment effects on overall feed intake, feed efficiency measured by feed:egg mass, egg production or mortality to 63 weeks. Overall egg weights for LM treatments were heavier compared to OS (60.4 and 59.8 g, respectively); however, shell quality measured by specific gravity was superior for OS (1.088) than LM (1.086) treatments. With calcium level, H had better shell quality compared to R treatments (1.087 and 1.086, respectively). There were no main treatment effects on gizzard and duodenum measurements from tissue hens at 30 weeks of age, and limited, inconsistent effects on tissue measurements at 41, 52 and 62 weeks. There was significant pH variation between the proximal, mid and distal portions of the duodenum at all ages. In this study, feeding OS and H calcium diets resulted in a higher occurrence of FDN in 64-week old production hens.

Key Words: duodenum, pH, clostridium perfringens, oystershell, limestone

Teaching, Pedagogy, Extension

P196, **Eggs – Educating Rwandan Broiler Farmers to Eat This Protein Source** Niamh Schumacher[*1,2UG], Tom Gill[1,2], Brynn Voy[1,2] *[1] University of Tennessee, [2] Zamura Feeds*

Rwanda, a small country in East Africa, has an alarmingly high stunting rate, with the National Institute of Statistics in Rwanda reporting that 37.4% of Rwandan children under the age of 5 were stunted in 2015. In an effort to combat stunting and support the economic stability of the country, the University of Tennessee's Institute of Agriculture (UTIA) and Zamura Feeds (Ltd.) implements a project, Tworore Inkoko, Twunguke (TI), in Musanze, Rwanda to train farmers to raise broiler chickens to sell for profit. The purpose of this research was to determine the prevalence of eggs in the diet of TI project farmers and ways in which the TI project can encourage its farmers to eat more of this protein source.

Eight focus groups of 6-10 people were held with the TI project's farmers and their spouses between in May and June in Musanze. Each group was asked pre-determined questions in Kinyarwanda about their family's diet. After determining that many of the farmers were unfamiliar with eggs and rarely ate them, additional interviews were conducted with 19 TI project farmers at their homes about the nutritional value of eggs and glucose biscuits. A glucose biscuits is an energy-dense food frequently consumed by rural Rwandan families because of its convenience and low cost. The answers that the farmers gave were translated to English and recorded.

Almost all of the farmers were unaware of the nutritional value of eggs. Many had never eaten eggs or ate eggs only once every few months. Many of the farmers mistakenly believe that glucose biscuits are made entirely of sweet potatoes.

The results indicate a need to educate farming families in Rwanda about how to increase the amount of protein in their diet and how to make informed decisions about foods to purchase. Specifically, the TI Project should include information comparing the nutrition of biscuits to that of eggs, so that farmers can make informed decisions.

Key Words: Nutrition, Broilers, Africa, Eggs, Education

POSTER SESSIONS

Physiology, Endocrinology and Reproduction, Layer or Broiler Breeders

P197, **Transcriptome comparison of blastoderms exposed to prolonged egg storage and short periods of incubation during egg storage** Kristen Brady[*1], Julie Long[1], Dinah Nicholson[2], Nick French[2], Conover Talbot[3], Murray Bakst[1] *[1] Animal Bioscience and Biotechnology Laboratory, BARC, ARS, USDA, [2] Aviagen Group, [3] Institute for Basic Biomedical Sciences, Johns Hopkins University School of Medicine*

Cool temperature egg storage before incubation is common in the broiler industry; however, prolonged egg storage causes increased embryonic mortality, decreased hatchability, and decreased growth in surviving chicks. Exposing eggs to short periods of incubation during egg storage (SPIDES) reduces the adverse consequences seen with prolonged storage. SPIDES increases blastodermal cell viability by reducing apoptosis, though the counteracting molecular mechanisms are unclear. To further cognize the impact of prolonged storage and SPIDES, transcriptome analysis was used to compare blastoderm gene expression from eggs stored at 17°C for 4 days (CR) to expression from eggs either stored at 17°C for 21 days without SPIDES (NSR) or with SPIDES (SR) (n=3/group). Data analysis was performed using the CLC Genomics suite and Ingenuity Pathway Analysis. Differentially expressed genes (DEGs) were identified by pairwise comparisons of control (CR) and treatment (NSR or SR) groups. Sequencing results were confirmed by RT-qPCR and analyzed by the mixed models procedure in SAS. In total, 1468 DEGs were identified between CR and NSR blastoderms and 1125 DEGs were identified between CR and SR blastoderms ($q<0.05$, RPKM>20, |fold change|>1.5). NSR blastoderms showed up-regulation of endoplasmic reticulum stress, FoxO signaling mediated apoptosis, and ubiquitinated protein endocytosis pathways, as well as down-regulation of organelle biosynthesis, mitotic cell cycle progression, and transcriptional pathways compared to CR blastoderms. SR blastoderms showed up-regulation of cell-matrix adhesion related cell survival, carbohydrate/fatty acid oxidation, and translational pathways, as well as down-regulation of RNA degradation, p53 stress signaling, and DNA repair pathways compared to CR blastoderms. Prolonged egg storage without SPIDES resulted in enrichment of pathways associated with cell stress and death; while SPIDES resulted in enrichment of pathways associated with basic cell and anti-apoptotic functions. Although egg storage protocols have been examined through targeted gene expression approaches, this study provided a global view of the extensive molecular networks affected in blastodermal cells and helped to identify potential upstream regulators for future experiments.

Key Words: SPIDES, egg storage, blastoderm, transcriptome analysis, broiler

P198, **The effect of an advanced growth curve for early photostimulation at 15 wks using two different feeding regimes during rearing on performance and plasma corticosterone levels.** Renata Hoskova[*GS], Adam Davis, Jeanna Wilson, Andrew Benson *University of Georgia*

2X2 factorial designed experiment was completed to compare the effects of (a) 2 rearing growth curves/photostimulation ages (15 or 21 wks) with (b) 2 different feeding regimens, every-day spin feeding (EDS) vs skip-a-day (SAD), on performance and plasma corticosterone levels. 2,400 female Cobb-500 breeder chicks were divided into one of 4 treatments, with 3 replicates per treatment. From 2 wks of age until photostimulation, birds were fed on a SAD basis to reach target body weight (2.1 kg) for photostimulation at 15 wks (15-SAD) or 21 wks (21-SAD) with chain feeders; or EDS basis to reach target body weight at 15 wks (15-EDS) or 21 wks (21-EDS). Pullet body weights (BW) and coefficients of variation (CV) were calculated every three weeks. For corticosterone analysis, 30 birds from each treatment were randomly selected for blood sample collection at weeks 10, 15, 21, 24 and 30. Blood samples were collected at 7 h, 24 h and 48 h (SAD only) post-feeding. Plasma corticosterone was determined using the Corticosterone ELISA kit from Cayman Chemical. Samples (n=3) were analyzed in triplicate for each of the different ages and fasting states. All data were analyzed by GLM using SPSS with significance considered at $P < 0.05$. At both 10 wks and 15 wks of age, the 15-SAD at 48 h post-feeding time had significantly lower corticosterone levels than the 21-SAD at 48 h post-feeding. This lower level of corticosterone aligns with increased BW and lower CV in the 15-SAD at 10 wks (BW=1,420 g, CV=11.2%) and 15 wks (BW=2,091 g, CV=10.8%) when compared to 21-SAD at 10 wks (BW=1,082 g, CV=13.2%) and 15 wks (BW=1,450 g, CV=13.3%). Relatedly, the 15-EDS at 24 h post-feeding at 15 wks (BW=2,010 g, CV=10.2%) had significantly lower corticosterone levels than the 21-EDS at 24 h post-feeding (BW=1,410 g, CV=12.3%). Within the different growth curves, the SAD had lower levels than EDS at 7 h post-feeding at 15 wks in both growth curves, and at 10 wks, 21-SAD was lower than 21-EDS. In addition, at 15 wks, the 15-SAD at 48 h post-

feeding had lower corticosterone than the 15-EDS at 24 h post-feeding. Feeding on an advanced growth curve during rearing reduced plasma corticosterone levels, but EDS tended to increase corticosterone levels at the targeted collection times.

Key Words: Broiler Breeder, corticosterone, rearing, stress, skip-a-day

P199, **Post-hatch ontogeny of melanopsin gene expression in the brain of breeder ducks.** Brooke Van Wyk [1], Daniel Shafer [2], Gregory Fraley[*] [3]
[1] *Hope College,* [2] *Maple Leaf Farms, Inc,* [3] *Purdue University*

The hypothalamic-pituitary-gonadal axis (HPG) is known to be regulated by daylength through the deep brain photoreceptor (DBP) system. One of the DBPs, melanopsin, responds to blue light and is critical for normal HPG function in ducks. DBPs are known to respond to light and to activate the HPG via a pathway that involves the thyroid axis. The thyroid axis has numerous functions, and thyroid hormones are critical not only for reproduction, but also for post-hatch neuronal and somite development. It is currently unknown if thyroid axis activity during posthatch development is influenced by DBPs, nor is the posthatch ontogeny of any of the DBPs known. In order to understand the role of DBPs, specifically melanopsin, in posthatch development we first set out to determine the ontogeny of melanopsin gene expression. Ducks from the Maple Leaf Farms Elite breeder facility were euthanized by cervical dislocation and brains immediately removed, frozen on dry ice, and stored at -80C until processed for qRT-PCR. Brains were collected from 3 drakes and 3 hens on the day of hatch (Day 0) and again at 2, 4, 6, 10, 14, 19, 25 and 31 weeks of age. Whole brain RNA was collected using Trizol and cDNA produced using a commercially available kit (Invitrogen). Primers for melanopsin were designed based upon known chicken sequences, and confirmed using PCR and optimized for qRT-PCR. Beta-actin and GAPDh were used as reference genes. Data were analyzed between sexes at each age using an independent T-test. The qRT-PCR reaction was carried out using PowerUp Syber Green Master mix (Applied Biosystems) and relative gene expression determined using the 2DDCt method. Interestingly, results showed that on the day of hatch (Day 0) ducks showed adult-like levels of relative melanopsin gene expression. By week 2 through week 6, melanopsin gene expression was at its lowest levels. During week 10 melanopsin levels increased achieving highest relative expression levels at week 19 that maintained through week 31, typically peak fertility in ducks. These observations suggest that melanopsin may be active during the peri-hatch period, and again prior to the onset of puberty in ducks. Further understanding of the role of melanopsin and other DBPs may help us to better design lighting systems to maximize growth, performance and improve welfare of ducks.

Key Words: OPN4, OPN5, GnRH, GnIH

Physiology, Endocrinology and Reproduction, Laying Hens

P200, **Effects of tamoxifen on medullary remodeling and eggshell quality in aged hens.** Masayoshi Yamada[*1GS], Woo Kyun Kim [2], Toshie Sugiyama [1] [1] *Niigata University,* [2] *University of Georgia*

Aged laying hens often produce eggs with low eggshell quality, such as broken or soft-shell eggs, which causes tremendous economic loss for the hen industry. It has been shown that aged hens have inefficient Ca mobilization from the medullary bone (MB) to oviduct because MB supplies 40% of eggshell Ca. Thus, understanding of the relationship between MB and eggshell quality during physiological changes is critical. However, there is little information about the dynamics of MB and eggshell quality during the modification of ovary function. Therefore, the purpose of this study was to determine the MB remodeling and eggshell quality in aged laying hens with modified ovary function by tamoxifen.

A total of 11 Boris Browns at the age of 450-d old were used for the 35-day study. Tamoxifen was administrated to eight hens for 12 days to modify ovary function. Four hens were kept until the age of 485 when the egg laying cycle was returned to be stable. All hens were euthanized for serum and femur sample collection when eggs were in the shell glands. Serum was used for measuring calcium and phosphorus concentration. The femurs were used for Alcian Blue staining to detect MB area in the bone marrow. Eggs were also collected for percent eggshell, thickness and strength.

Student's T test were used to compared tamoxifen and post-treatment groups to pre-treatment group ($P<0.05$). Serum Ca and P were significant decreased during tamoxifen treatment. There was no significant difference between pre-treatment and post-treatment groups. However, P level tended to increase in post-treatment group. The percentage of MB in the femur was significantly decreased during tamoxifen treatment. There was no difference between pre and post-treatment groups. The percent eggshell was significantly increased in post treatment group. There was no significant difference in eggshell strength and thickness between pre and post-treatment groups. However, there was some tend for higher eggshell quality in post treatment group. This study showed that tamoxifen reduces Ca and P in blood and medullary bone formation, but post-treatment increases all parameters, such as serum Ca and P, MB, and eggshell quality, indicating that there are positive correlation among serum Ca/P, MB and eggshell quality.

Key Words: oviduct rejuvenation, antiestrogen, eggshell quality, bone histology

Physiology, Endocrinology and Reproduction, Turkeys

P201, **An algae extract to support digestive and metabolic functions as well as performance in turkey breeders** Matthieu Le Goff[*], Frederick Bussy *Olmix*

The digestive system of poultry breeders is highly solicited, especially late in the egg production period when the demand in lipids for yolk production increases. It is essential to support lipids metabolism and properly drain and stimulate digestive systems to maintain a good health status and optimal egg production. In this context, Olmix has developed a commercial product including a sulfated polysaccharide extract from seaweed called MSP®LIPIDS. A trial was carried out to assesse the impact of the commercial product on metabolism and laying performance. The trial was performed on breeding turkeys in two buildings, from 23 to 28 weeks of laying. During the trial one control group of 2,016 turkey breeders received a choline and sorbitol complex at 1L/1,000L of drinking water while one test group of 2,110 turkey breeders received the product containing the sulfated polysaccharide extract from seaweed at a dosage of 0.5L/1,000L of drinking water. The products were distributed during 2 periods, a first period of 10 days starting at 23 weeks of egg-laying and a second period of 5 days from 27 weeks of egg-laying. Histological evaluation of the liver was performed at the end of the trial. Statistical analysis was done with the R software (Mann-Whitney test and Anova with $\alpha=0.05$). The results showed a significant reduction of the biliary canal hyperplasia and blood capillaries leucocytosis in the portal area of the test group ($P<0.01$). Blood capillaries tended to have a lower congestion in the test group compared

to the control group (P <0.10). Before the start of the trial, both groups had equivalent laying performance (4.4 eggs / turkey / week). During trial period, the egg production in the test group was higher than in the control group (respectively 4.0 and 3.5 eggs / turkey / week), showing a slower decrease of egg production in the late laying period. Breeders are affected by loss of production in the last quarter thus it is crucial to support lipids metabolism to maintain a good health status and level of production. The use of the product containing a specific sulfated polysaccharide extract from seaweed, called MSP®LIPIDS may be beneficial to support metabolic functions and production level.

Key Words: Algae extract, Liver metabolism, Turkey, Egg production

Physiology, Endocrinology and Reproduction, Broilers

P202, **Evaluation of the impact of animal feed grade sodium bisulfate supplementation on performance, intestinal morphology and vitamin D3 status of broilers subjected to coccidiosis vaccine** Juan Suarez*[1], John Carey[1], Koyle Knape[1], Nasser Al Haj Ali[1], John Pitts[2] [1] *Texas A&M University,* [2] *Jones-Hamilton Co*

The poultry industry continues the rigorous search to find effective and practical alternatives to the use of antibiotics. This study aimed to analyze the effect of sodium bisulfate (SBS) supplementation on performance, intestinal morphology, and vitamin D3 status of broilers subjected to a 2x dose coccidiosis vaccine. Coccidiosis is a major concern within the poultry industry for its detrimental effects in poultry health and substantial economic losses. At day of placement, 600-day-old male Cobb500 broiler chicks were randomly assigned to a vaccinated or not vaccinated group for each of the two dietary treatments, control or SBS. Broiler chicks were placed in floor pens for a 21-day trial period. SBS from Jones-Hamilton Co (Walbridge, OH) was included in the experimental ration at .4% at the expense of salt. Replicates were arranged in a completely randomized block design with twenty-five broilers per replicate and six replicates per treatment, a 2x2 factorial arrangement. Body weights (BW), feed consumption (FC), and mortality-corrected feed conversion (FCR) were calculated at day 7, 14, and 21. Serum concentration of 25-hydroxycholecalciferol (25-OH-D), villi and crypt measurements from small intestine, and tibia breaking strength (TBS) were measured on day 10, and 21 along with lesion scores. There was a significant improvement ($p>0.05$) in 25-OH-D serum concentration, BW, FCR, and TBS from SBS compared to Control at day 21 in vaccinated birds. Duodenum villi length to crypt depth ratio was significantly ($p>0.05$) greater in not vaccinated treatments compared to vaccinated treatments at d10 and 21. Cumulative FCR was significantly lower ($p>0.05$) in the SBS treatment compared to Control at day 21 in both vaccinated and not vaccinated treatments. Lesion scores were significantly higher ($p>0.05$) for vaccinated groups compared to not vaccinated groups, suggestive of a good vaccine challenge. In conclusion, the data suggests that SBS can positively impact cumulative FCR, 25-OH-D concentration in serum, and TBS in broilers subjected to coccidiosis vaccine. Further research is necessary to determine the extent of SBS effect in gut health and morphology of vaccinated broilers as well as determine the mode of action.

Key Words: coccidiosis, sodium bisulfate, broiler, performance, Cobb500

P203, **Differential expression of insulin-like growth factor (IGF) binding proteins indicates potential differences in their endocrine or paracrine regulation of IGF signaling** Lauren Vaccaro*[GS], Laura Ellestad *University of Georgia*

The somatotropic axis is a key regulator of body growth and metabolism in mammals through insulin-like growth factor (IGF) induced signaling. Produced in the liver in response to pituitary growth hormone, the IGFs initiate pathways responsible for cellular growth upon binding to their receptor in target tissues. Signaling activity of IGFs is altered by IGF binding proteins (IGFBPs). However, the functional role of IGFBPs in birds is poorly understood. The objective of this study was to investigate putative endocrine and paracrine activities of IGFBPs during broiler juvenile development by measuring their expression patterns in liver and muscle between post-hatch day (d) 0 and d40. To accomplish this, liver and muscle were collected from male Ross 308 broilers on d0, 10, 20, 30, and 40 (n=8 replicate birds per age), and evaluated for expression of IGFs, IGF receptor (IGFR), and IGFBPs 1, 2, 3, 4, 5, and 7 by reverse transcription-quantitative PCR. Data were analyzed for significant interactive (tissue-by-age) or main (tissue and age) effects using a 2-way ANOVA followed by the test of least significant difference. All genes were detected in both tissues at all ages. Expression of IGF1 was similar between tissues at d0 but increased greatly in liver from d10 onwards, indicating that full maturation of endocrine somatotropic function occurs between hatch and d10 ($P<0.01$). Levels of IGFBP1 and IGFBP4 were consistently higher in liver ($P<0.01$). These IGFBPs may be regulating the endocrine signaling action of hepatic IGF. Raised liver expression was observed in IGFBP2 at both d0 and d20, suggesting that endocrine affects may appear at different ages for certain IGFBPs ($P<0.01$). Interestingly, IGFBP3 was the only binding protein with expression levels consistently greater in muscle ($P<0.01$). In mammals, IGFBP3 can act to prevent IGF-IGFR interaction, so the noticeable elevation of IGFBP3 expression in muscle may suggest paracrine regulation of growth in this tissue. Taken together, these results suggest that full maturation of the somatotropic axis occurs sometime between d0 and d10. Further, some IGFBPs appear to be important regulators of IGF endocrine function, while others may play a more substantial role in regulating IGF signaling in a paracrine manner.

Key Words: metabolism, endocrine, paracrine, signalling

P204, **Effect of a neonatal fast on the assimilation of yolk and IgY in broiler chicks.** Talaat Al-Alwani*[GS], Nassar Haj Ali, Jawad Al-Juboori, John Carey *Texas A&M University*

Protection of newly hatched chicks against disease depends greatly on innate immunity (maternal antibodies) acquired in a passive manner through the yolk sac. The impact of fasting on yolk utilization in fasted vs fed chicks has been well studied, however the impact of a neonatal fast on the assimilation of maternal antibodies is not well established.

Several studies were conducted to examine the impact of a 4-day fast on yolk and IgY assimilation in broiler chicks. The hypothesis being that fasted chicks will catabolize IgY to supply nutrients at the expense of maternal immunity.

All chicks hatching within a 2 hour period were immediately placed into one of three treatment groups (Fasted, Water only or Feed and water). Blood samples were collected from the jugular or brachial vein in the first hour post hatch and on d 4. Igy titers were measured by sandwich ELISA. Blood volume was estimated by treatment using the Evans Blue technique and whole body IgY levels were estimated. Body weights were recorded on d 0, BW and yolk weight was measured on d 4.

Day 0 BW was not significantly different among the treatments, d 4 BW of the fed group was significantly higher than that of water only treatment which in turn was significantly greater than that of fasted chicks. Absolute yolk weight d 4 was not significantly different among the groups, relative yolk weight (% of BW) was significantly lower in fed birds compared to fasted or water only treatments. IgY titers differed slightly on d 0. Day 4 IgY titers were significantly higher in fasted birds compared to those given water only which in turn was significantly greater than fasted birds. Blood

volume estimates indicated that the blood volume of the fasted chicks was greatly reduced compared to those receiving feed. When total IgY per bird was estimated from blood volume measurements, there were no significant differences among the treatments. These data indicate that compared to fed birds, fasted birds and those provided water only do not utilize yolk materials nor assimilate maternal antibodies at differing rates. Indicating that under these conditions, fasted or birds provided water only do not catabolize maternal antibodies at rates differing from fed birds.

Key Words: Fasting, Maternal Antibodies, Yolk sac

P205, **Delayed access to feed alters gene expression associated with cellular differentiation and protein metabolism in muscle of newly hatched broilers** Jason Payne*[1], Monika Proszkowiec-Weglarz [2], Laura Ellestad [1] [1] *University of Georgia,* [2] *Animal Biosciences and Biotechnology Laboratory, United States Department of Agriculture, Agriculture Research Service, Northeast Area*

Birds rely solely on the yolk sac as a means of nutritional support during embryogenesis and early post-hatch, before first feeding occurs. Newly hatched broilers are not given immediate access to feed, which can result in numerous alterations to developmental processes in several tissues. The objective of this study was to characterize gene expression profiles of newly hatched chicks' breast muscle with regards to regulation of growth, development, and differentiation, and determine impacts of delayed feeding on these processes. Birds placed in battery pens were given access to feed within 3h of hatch (Fed) or delayed access to feed for 48h (Delayed Fed). Breast muscle collected from male birds at hatch, or 4h, 1D, 2D, 4D, and 8D after hatch was used for analysis of mRNA expression. Under fed conditions, insulin-like growth factor receptor and leptin receptor mRNA expression decreased as birds aged; however, prolonged upregulation of these genes was observed in Delayed Fed birds so their mRNA levels were higher at 2D ($P \leq 0.05$). Levels of myogenin mRNA, a muscle differentiation factor, increased in Fed birds through 2D, after which they decreased. A similar pattern was deferred until 4D in Delayed Fed birds. Myostatin is a negative regulator of muscle growth, and its expression increased in Fed birds starting at 2D, while expression in Delayed Fed birds did not increase until 4D. These differences resulted in higher mRNA levels of myogenin and myostatin in Fed birds at 1D and 2D ($P \leq 0.05$). In Fed birds, levels of protein catabolism transcripts F-box protein 32 and forkhead box O3 began to decrease at 2D, while Delayed Fed mRNA levels did not decrease until 4D ($P \leq 0.05$). Mechanistic target of rapamycin mRNA levels decreased from 1D through 8D in both treatments, except for a transient increase in the Delayed Fed birds between 1D and 2D ($P \leq 0.05$). These data suggest that within breast muscle, delayed feeding alters sensitivity to hormones that could interrupt tissue differentiation, postpone onset of growth, and increase protein catabolism. Together, these processes may contribute to a reduction in proper growth and development of birds delayed feed at hatch, and ultimately hinder long-term muscle accretion in broilers.

Key Words: hormone receptor, protein metabolism, satellite cell, delayed feeding, development

P206, **Intestinal physiology associated with metabolic efficiency in commercial broilers** Laura Ellestad*[1], Michael Rothrock [2], Jean Guard [2], Samuel Latzsch [1] [1] *University of Georgia,* [2] *USDA-ARS US National Poultry Research Center*

Understanding feed nutrient use is crucial for developing strategies that maintain production efficiency in antibiotic-free systems. The objective of this study was to investigate differences in intestinal physiology and microbial ecology between high and low efficiency broilers. Between days 7 and 35, body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR; g FI/g BWG) were calculated on an individual basis for 108 male broilers. On D36, tissues from each intestinal segment were collected from the six highest (HE) and lowest (LE) efficiency birds based on FCR to evaluate gene expression associated with nutrient uptake, mucosal amino acid and organic acid levels, and genotypic and phenotypic microbial intestinal ecology. Data were analyzed with a t-test. While HE and LE birds did not differ in final BW, BWG, or FI, LE birds had a significantly higher FCR (1.69 vs 1.41; $P<0.0001$). Jejunal expression of glucose transporter 2 and sodium-coupled neutral amino acid transporter 2 were elevated in LE birds ($P \leq 0.05$), and cationic amino acid transporter 1 was higher in HE birds ($P=0.07$). Mucosal alanine, glutamate, serine, and threonine were higher in LE jejunum and ileum ($P \leq 0.05$). Lactate and succinate were consistently higher in all mucosal sections of LE birds ($P \leq 0.05$); citrate was higher in mucosa of HE jejunum and ileum ($P \leq 0.05$). Of note, 1-methylhistidine was detected only in mucosa of LE birds, suggesting it may serve as a biomarker for dysbiosis associated with poor metabolic efficiency. Genotypic microbiome analysis revealed significant differences in taxa between HE and LE broilers in all sections, including those within the *Firmicutes* and *Bacteriodetes* phyla ($P \leq 0.05$). Phenotypic analysis with single C-source arrays indicated that microbial respiration was higher in LE birds in duodenal, jejunal, and cecal segments ($p<0.0001$), with metabolism of glycogen, a-D-lactose, glucose-1-phosphate, and g-hydroxylbutyric acid differing between groups. These data suggest that LE birds may have decreased ability to absorb dietary nutrients or increased endogenous amino acid loss. Further, microbial signatures associated with differences in FE represent targets for novel strategies to maintain production efficiency in the absence of antibiotics.

Key Words: nutrient utilization, metabolism, intestinal microbial ecology, amino acid, organic acid

P207, **Evaluation of VANNIX™ C4 on performance, oocyst shedding and lesion scoring of coccidia vaccinated broilers** Vanessa Iseri*[1], Brett Lumpkins [2], Greg Mathis [2] [1] *Kemin Industries, Inc.,* [2] *Southern Poultry Research*

VANNIX™ C4 is a novel phytogenic product designed to help minimize the performance impact of coccidial infection in broilers. A broiler trial was conducted to evaluate the effect of VANNIX C4 on performance and oocyst shedding during a coccidia vaccination program. On day (d) of hatch, Cobb 500 male broiler chicks were vaccinated with COCCIVAC®-B52 (Intervet Inc.; except for no vaccine control group) and randomly assigned to 1 of 5 treatments (50 birds/pen; 12 pens/treatment): no vaccine control, vaccine control, VANNIX C4 (all feeds), VANNIX C4 (only grower and finisher; G/F) and Zoamix® (Zoetis) (G/F). Performance was monitored over the d35 trial. In addition, coccidial and necrotic lesion scores and *E. maxima* microscopic counts were measured on d16, d22 and d28; and oocyst shedding was monitored every 3 days starting from d10 up to d31. Statistical analyses were performed using JMP® (SAS Institute, Cary, NC) software and significance was determined at $P \leq 0.05$. There was no effect of VANNIX C4 on gain and adjusted (adj) FCR on d14 ($P>0.05$). On d28, both VANNIX C4 treatments improved adj. FCR by 3 pts compared to vaccine control, and Zoamix had further improvement compared to either VANNIX C4 treatments (4 pts; $P<0.05$). At the end of the trial (d35), there was a numerical increase in gain of VANNIX C4 and Zoamix compared to vaccine control. Both VANNIX C4 treatments significantly improved adj. FCR compared to vaccine control (6-7 pts), and Zoamix had a 3-4 pt. improvement compared to VANNIX C4 treatments ($P<0.05$). There was no effect of treatment on mortality. There was no effect of VANNIX C4 on oocyst shedding for *E. acervulina* and *E. maxima*, however there was a significant decrease with *E. tenella* compared to vaccine control ($P<0.05$). Zoamix significantly decreased oocyst shedding of all *Eimeria* species compared to vaccine control ($P<0.05$). Necrotic lesion, coccidial lesion and microscopic lesion scores were negligible across all treatments. Overall, VANNIX C4 improved performance and did not interfere with coccidial shedding in vaccinated broilers.

Key Words: broilers, cocci, phytogenic

P208, **Repeated intraperitoneal dexamethasone exposure alters gut barrier integrity biomarkers** Lizza Macalintal*, Kristen Brennan, Anthony Pescatore, Tuoying Ao, Michael Ford, Karl Dawson, Ronan Power *Alltech-University of Kentucky Nutrition Research Alliance*

Previous research suggested that repeated intraperitoneal (IP) dexamethasone (DEX) injection overtly retarded growth, lymphoid organ development, and metabolic acidosis persisted in young chicks. DEX in this study was used as an inducer of gut inflammation. To address the effects of IP DEX injection, on productive performance, biomarkers for gut barrier functions were assessed including; selected adhesion molecules (JAM2), mucin 2 (MUC2), inflammatory cytokines, heterophils (H)/ lymphocyte (L) count, fluorescein isothiocyanate dextran (FITC-D) and diamine oxidase (DAO). A total of 120 Cobb male broilers at 1-d of age were randomly divided into 2 groups each using 10 replicate cages with 6 chicks/cage. Birds were divided into 2 equal treatments: sham-PBS injected control and DEX- birds receiving either 1ml, PBS or 2mg/kg BW DEX (in PBS) via IP injection at d 7, 9 and 12. On d 13 and 20 (5 and 13 d post-initial [dpi] DEX injection), two bird/cage were randomly selected and orally gavaged with FITC-D (a marker for intestinal permeability) at 1.1 mg/ml/bird. Blood and segments of small intestines were collected 2.5 hrs post-FITC-D. On 5 dpi, differential cell counts showed a significant increase in the H/L ratio for the DEX birds, elevated H, and depressed L count is indicative of stress. The amount of FITC-D retained in the duodenal tissue was affected by the DEX ($P<0.05$). The lateral diffusion of FITC-D across the duodenal epithelial membrane was lower ($P<0.01$) for the control *vs* DEX birds, thus more FITC-D were detected in duodenal samples for the control birds. Ileal mRNA expressions of IL1b and IL10 in DEX birds were higher ($P<0.01$) compared to the control birds. By 13dpi, the H:L ratio, as well as the lateral flux of FITC-D across the duodenal epithelium, remained significantly elevated for DEX vs control birds ($P<0.05$). The plasma DAO level was lower for DEX vs control birds ($P<0.05$). Duodenal mRNA expression of JAM2 significantly fall while MUC2 increased for the DEX *vs* control birds ($P<0.05$). Taken together, repeated IP DEX exposure overtly affected growth performance by inducing leaky gut through alteration in the JAM2, MUC2, Il1B and IL10 expression with corresponding changes in H/L ratio, FITC-D, and DAO biomarkers.

Key Words: leaky gut, adhesion molecules, heterophils, diamine oxidase, interleukin

Processing and Products

P209, **Comparison of pulsed ultraviolet light systems for microbial reduction on chicken thighs** Joshua Cassar*GS, Edward Mills, Ali Demirci *The Pennsylvania State University*

Pulsed ultraviolet (PUV) light is an effective antimicrobial intervention that reduces the microbial contamination present on the surface of chicken. Pulsed UV light includes a broad spectrum, 100 – 1100 nm and is emitted in short bursts of very high intensity light and has been reported to produce measurable bacterial reductions in various food products. The current study compared reports showing antimicrobial effectiveness of PUV light using a laboratory benchtop unit and an industrial scale conveyor system equipped with PUV flashlamps. To evaluate the PUV light systems, *E. coli* K12 was inoculated on the surface of chicken thighs and exposed to a range PUV light treatments. Previous research has indicated *E. coli* K12 is an appropriate representative microorganism for *Salmonella*, *Campylobacter,* and the other pathogenic and spoilage microorganisms. Using both the PUV light benchtop unit and the conveyor system, boneless/skinless (B/S) chicken thighs were treated with approximately 10, 20, and 30 J/cm^2. The PUV light benchtop unit consistently produced greater microbial reduction values for equivalent energy exposure compared to the PUV light conveyor system ($p<0.05$). For example, microbial reductions for 10, 20, and 30 J/cm^2 treatments were reported as 1.49 versus 0. 51, 1.76 versus 0.74 and 2.04 versus 0.94 $\log_{10}$ CFU/cm^2 using the PUV light benchtop unit and conveyor system, respectively. The difference in microbial reduction between the two systems at equivalent PUV fluence values could be a result of the rate of temperature increase on the surface of the product. The benchtop unit produced greater temperatures at each respective treatment fluence compared to the conveyor system ($p<0.05$). At 10, 20 and 30 J/cm^2 treatments, surface temperature increased 6.2 versus 3.0, 10.2 versus 5.0 and 13.8 versus 9.0°C using the PUV light benchtop unit and conveyor system, respectively. Therefore, these results suggest that the greater microbial reduction observed in the PUV light benchtop unit could be a function of greater surface temperature. In conclusion, this study clearly demonstrates that further research is needed to better understand the effectiveness of PUV light for surface decontamination on an industrial scale.

Key Words: chicken, decontamination, pulsed ultraviolet light, E. coli

P210, **A method for characterizing instrumental quality measurements of turkey breast meat** Courtney Poholsky*GS, Edward Mills, John Boney *Pennsylvania State University*

The incidence of pale, soft, and exudative (PSE) poultry meat is associated with various meat quality parameters which supports the need to establish a method for examining breast tissue characteristics using instrumental quality measurements. The objective was to establish a method for monitoring breast tissue color (BTC), cook yield (CY%), and texture characteristics of three commercial tom turkey strains (treatments A, B, and C). Treatments (trt) were arranged in a randomized complete block design and applied to eight replicate pens from d1-133. On d119, one bird per pen was randomly selected, weighed, and harvested to examine BTC (n=16), CY%, and texture characteristics. After slaughter and evisceration, carcasses were placed in a chill tank overnight. Carcasses were then weighed to determine chilled carcass yield and each pectoralis major was placed in a vacuum sealed bag. Breast samples were stored in a -23.3°C freezer until further analysis. Upon analysis, samples were thawed at 4°C and then tempered to 20°C prior to determining color characteristics using the CIELAB color measuring system. Samples were cooked at 190.5°C until internal temperatures reached 80°C and CY% was recorded. Cooked samples were chilled overnight at 4°C and then tempered to 20°C. Texture characteristics were then determined using a Warner-Bratzler shear (WBS) force apparatus. The d119 live weight was highest for trt C, while trt A had the lowest live weight. The live weight for trt B was intermediate ($P=0.0220$).However, no significant differences in carcass yield or breast yield were observed between the trts ($P>0.05$). The L* value was similar for trts B and C, while trt A had a higher L* value ($P=0.0045$). The a* value was lowest for trts A and B, while trt C had the highest a* value ($P=0.0027$). The b* value for trt C was higher than both trts A and B ($P=0.0161$). No significant differences in CY% were observed between the trts ($P>0.05$). The WBS value was lowest for trt A and highest for trt B. The WBS value for trt C was intermediate ($P=0.0021$). In conclusion, this methodology for breast tissue characterization may be appropriate for establishing instrumental meat quality parameters that may be used to monitor the onset of PSE in future generations of commercial turkeys.

Key Words: PSE, texture, color, cook yield, Warner-Bratzler

P211, **Reliability of woody breast scoring through palpations in live versus processed broilers** Cirenio Hisasaga[*1GS], Sara Orlowski [2], Katy Tarrant [1] [1] *California State University, Fresno,* [2] *University of Arkansas*

The broiler industry has seen an increase in the prevalence of myopathies in recent years, which has negatively affected meat quality and has compromised consumer acceptance. Ultimately, a higher prevalence of myopathies can ultimately contribute to a decrease in yield and product value. Among these, woody breast (WB) has become an important disease of concern to both the scientific community and the poultry industry, prompting numerous studies on this condition. WB has been described as a myopathy that causes hardening and discoloration of the chicken breast muscle, subsequently decreasing the meat quality. Researchers choosing to induce WB for study may use live palpations of the breast tissue to estimate the presence of WB throughout the growout period. Through this experiment, we aim to understand if live palpation is a viable method for estimating woody breast incidence discovered after processing. Using 209 broilers at d47, WB incidence was measured using hand palpations externally from a single user on the left breast above the skin prior to euthanasia (EP) and internally through a postmortem palpation of the breast fillet (IP). Scoring of WB incidence was classified as present or absent. Other measurements that were obtained from these birds included sex and body weight. Both male and female broilers with and without WB did not vary significantly in weight (2.89 ± 0.04 vs. 2.91 ± 0.10 and 2.48 ± 0.04 vs 2.45 ± 0.08, respectively). Male broilers were correctly scored 67.6% of the time for a total of 73 correct calls out of 108 individuals. Female broilers were correctly scored 32.4% of the time for a total of 62 correct calls out of 103 individuals. Incorrect scores made up 36% of calls for both sexes, for which 24.1% EP scored as WB absent, but corrected to WB present when IP, and 11.8% initially scored as presence through EP, but corrected to absent when examined through IP. Bird weight did have an effect on the EP score ($P < 0.001$), but did not have an effect on IP scoring ($P = 0.28$). This data provides an insight into the reliability of using EP for woody breast scoring in live broilers. While this method can be used to gain general information as to the breast scores, internal evaluation of WB scoring is needed for accuracy.

Key Words: woody breast, myopathy

P212, **Effect of native and modified corn starch on the functional properties of chicken breast meat (pectoralis major) affected with woody breast myopathy** Aftab Siddique[*1GS], Eswin Ariza [2], Laura Garner [1], Jasmine Kataria [1], Adela Acosta [2], Amit Morey [1] [1] *Auburn University,* [2] *Zamarano University*

The increase of anomalous meat caused by the myodegeneration of *pectoralis major* has facilitated research to improve functional characteristics of woody breast meat. The objective of this study was to evaluate the quality effects associated with the addition of native versus modified corn starch in ground woody breast meat. Five ground woody breast meat treatments were evaluated over a 9 d storage period (n=375), a control without any additional ingredients, native corn starch (1 and 2%), and modified corn starch (1 and 2%). On each sampling day, 5 samples per treatment were procured from the refrigerator. Parameters analyzed on raw meat included, aerobic plate counts, proximate composition of the meat (fat, moisture, and protein), and color (CIE L*a*b*). Samples were cooked to an internal temperature of 165 °C and used to analyze cook-loss and texture profile analysis (TPA) on days 1, 3, 5, 7, and 9. The entire experiment was repeated in 3 separate trials. Data was analyzed using an analysis of variance and means were separated using Tukey HSD with a probability $p \leq 0.05$. No significant differences ($p > 0.05$) were observed between the treatments in the aerobic plate counts on individual days or proximate composition analysis during storage. A reduction in cook-loss was observed on 7 d of storage with the addition of 2% native and modified starch. Elevated values for L* (brightness) and a* (red) were observed by the addition of 2% native and modified starch. Hardness of the samples was not affected ($p > 0.05$) due to the addition of starch, however, springiness decreased with the addition of both modified and native starches. Modified starch (1 and 2%) reduced the chewiness of the cooked products compared to the control. Storage had a significant effect on the hardness, springiness and chewiness of the cooked samples. It was concluded that addition of native and modified corn starch had a significant influence on quality of ground woody breast meat and can have potential applications in further processed products.

Key Words: Woody Breast, Modified Food Starch, Cook-loss, Meat Quality

P213, **Systematic review of the different causes and effects of the wooden breast condition in poultry production** Dora Brown[*UG], Miyoung OH, Sang-Hyon OH *University of Maryland Eastern Shore*

The wooden breast (WB) condition is one of the most prevalent issues in the modern poultry industry. There are large amounts of research going on in the industry to try and improve meat quality without decreasing the performance of the bird or negatively impacting the poultry industry and human health. This study was to find the main causes and effects of the condition. About 90 studies were analyzed using PubMed. It was found that 34% of the research was done on how the condition affects the different qualities of the meat; 23% on how the feeding regimen/diet affects the incidence or severity of the meat; 15.5% on how different genetic lines affect the incidence of the condition and what genes were expressed due to the condition; about 18% on the histology and morphology of the affected meat; 5.5% on the pathology; 2% on the incidence of the condition is affected by the age of the bird; and just 1% on sex and how the time of hatch and incubation temperature can affect the morphology score. Most of the qualities affected by the condition had low or very low values; e.g. drip loss, cooking loss, fibrousness, springiness, cohesiveness, hardness, protein%, fat/lipid %, ash %, collagen %, magnesium, and calcium content. The qualities that were high were, a*, b*, shear force, chewiness, moisture %, potassium, and phosphorous content. The meat was more acidic in terms of pH, but the values were all close to neutral. The L* values and magnesium content were average; not low and not high. Most of the diet supplementations had little to no effect on the incidence of WB. There was no significant difference in the incidence of WB between high yield and standard yield genetics. Genes associated with WB were either upregulated or very dysregulated, which could be seen as a reason to why the WB condition occurs. The morphology characteristics were varied throughout the pectoralis major muscle. Wooden breast incidence occurs at a rapid rate as the bird gets heavier and older. Time of hatch and incubation temperature have no significant effect on WB. Lastly, males have a higher incidence of WB than females. In conclusion, it can be said that genetics and the feeding regimen could be two of the most important factors that can increase affect the incidence of the WB condition.

Key Words: woody, wooden, chicken, breast

P214, **Comparison of selective media for enumeration of Clostridium perfringens from broiler fecal, ileal and cecal samples in addition to pure culture samples** Whitney Briggs[*1GS], Daniel Boyes [2], Audrey Duff [1], Kaylin Chasser [1], Lisa Bielke [1] [1] *The Ohio State University,* [2] *Baldwin Wallace University*

Enumeration of *Clostridium perfringens* (CP) by direct plating has been widely practiced with many media choices available today, but selectivity for specific CP enumeration remains questionable and quantitative PCR detection has remained the favored choice for mixed species samples. The most commonly used media were developed prior to 1980, and have laborious costs associated with the making of and identification of CP. Since 2012, two new CP selective media have been developed, CHROMagar™ *C. perfringens* (CA) and CP *ChromoSelect* Agar (CS). The objective was to compare CA and CS for CP enumeration using broiler fecal, ileal and cecal samples, in addition to comparing these media against tryptic soy agar with sodium thioglycolate (TSAT) using a pure CP culture. During

a necrotic enteritis study, an un-inoculated control (UC) and *Salmonella* Enteritidis*, Eimeria maxima* and CP inoculated (NE) group had fecal samples collected before CP inoculation (d20). Pooled fecal samples were collected on d23, after NE challenge, in addition to ileal and cecal samples for CP detection. Each broiler sample was plated on CA and CS, with pure CP cultures plated on CA, CS and TSAT in three replicates. In all tests, CFU were subjected to ANOVA based on group and media type, with a significance of $p \leq 0.05$ determined using Tukey's HSD test. There were no differences between the media for CP enumeration with any of the samples tested, including the pure cultures. With the broiler samples, CA had higher counts than CS with the exception of the d23 NE fecal and UC ileal samples. With the pure culture samples, two out of three times, CS had the highest CP count, while in the third trial, CA had the highest. When counting the plates, there was clearer distinction of CP colonies on CA compared to CS (qualitative observation only). CP colonies on CA have orange color, and when plating the broiler samples, color differences enabled accurate counting of the CP colonies, with limited interference by other bacterial species. In addition, oxygen exposure did not influence the CP counts with either CA or CS, unlike with previous selective CP media. With these new CP selective media, CP enumeration by direct plating can become a reliable quantitative measurement.

Key Words: direct plating, selective media, Clostridium perfringens, necrotic enteritis, quantitative measurement

P215, **Germicidal ultra-violet light to lessen Campylobacter numbers on chicken liver** Burabari Bornu*UG, Mark Berrang, Richard Meinersmann, Eric Adams, Steven Knapp *USDA-ARS-U.S. National Poultry Research Center*

Campylobacter is a human pathogen associated with raw poultry including chicken livers. Multiple outbreaks of foodborne campylobacteriosis have been traced to undercooked chicken livers. The purpose of this study was to determine the effect of germicidal ultraviolet (UV) light as a means to reduce the number of naturally occurring *Campylobacter* on raw chicken liver lobes. Duplicate liver lobes were exposed to germicidal UV (λ=254 nm) at 800 $\mu w/cm^2$ for times of 0, 5, 15, 30, 60 or 120s. Lobes were sampled by rinse and blending. All samples were plated on Campy-Cefex Agar (CCA) plates incubated micro-aerobically for 48 h at 42°C. Typical *Campylobacter* colonies were counted; suspect colonies were confirmed by observation of cells under phase contrast microscopy and positive reaction to a latex agglutination test. Colony counts were $\log_{10}$ transformed and are reported as log colony forming units (CFU) per lobe. Mean Campylobacter numbers were compared by T test. No significant effect of treatments at 5, 15 or 30 s were noted, therefore, longer exposure times were tested. Exposure of liver lobes to 120 s of UV treatment resulted in log 3.6 or 3.4 log CFU *Campylobacter* when examined by rinse or blending respectively. This did not differ significantly from untreated control lobes which had log 4.0 or log 3.7 CFU *Campylobacter* also by rinse or blending. Treatment of raw chicken liver lobes with 800 $\mu w/cm^2$ UV light for 120 s is not adequate to significantly lessen *Campylobacter* numbers.

Key Words: Campylobacter, chicken liver, ultra violet light

P216, **Prebiotic Refined Functional Carbohydrates reduce adhesion of Salmonella and Campylobacter to chicken epithelial cells in vitro** Laney Froebel*UG, Lindy Froebel, Tri Duong *Texas A&M University*

The development of interventions to reduce *Salmonella* Typhimurium and *Campylobacter jejuni* in the gastrointestinal (GI) tract of chickens will be important to improving the microbial food safety of poultry. *Saccharomyces*-derived prebiotic Refined Functional Carbohydrates (RFC), composed primarily of β-glucans (β-Glc), mannanoligosaccharides (MOS), and D-mannose (D-man), have been demonstrated to reduce GI colonization of *Salmonella* and *C. jejuni* when administered to poultry. Although they are thought to inhibit adhesion of pathogens to epithelial surfaces in the GI tract, this potentially important functionality of RFC has not been well characterized. In this study, we investigated the effects of RFC and other prebiotics on the adhesion of *Salmonella* Typhimurium and *Campylobacter jejuni* to the LMH chicken epithelial cell line *in vitro*. Bacteria were co-incubated with prebiotic-treated LMH cells, and the reduction in adherent bacteria as compared with untreated cells was determined. Data were analyzed using logistic regression or ANOVA as appropriate (α=0.05). The dose response of pathogen adhesion to RFC was evaluated. The reduction of adherent bacteria was observed to be dose-dependent with *C. jejuni* (IC_{50} = 0.020%; slope=2.143; r^2 = 0.994) being more sensitive to RFC than *Salmonella* (IC_{50} = 0.048%; slope=0.935; r^2 = 0.989). The reduction of adherent bacteria by each primary constituent of RFC was also characterized. All 3 carbohydrates inhibited adhesion of both pathogens ($P < 0.001$). Additionally, reduction of adherent bacteria by β-Glc and MOS was greater than by D-man, suggesting D-man contributes less to adhesion inhibition by RFC than the others. Finally, the reduction of adherent pathogens by RFC was compared with that of fructooligosaccharides (FOS), galactooligasaccharides (GOS), and raffinose (RAF). All 4 prebiotics inhibited adhesion of *Salmonella* and *C. jejuni* to chicken epithelial cells ($P<0.001$). Reduction of adherent *Salmonella* was greatest with FOS (51%) and lowest with GOS (18%), whereas reduction of adherent *C. jejuni* was greater with RFC (95%) and RAF (94%) than with FOS (79%) and GOS (78%). The results of our study will inform future research elucidating the mechanisms of adhesion inhibition of pathogens by RFC and other prebiotics.

Key Words: Prebiotics, bacterial adhesion, Salmonella Typhimurium, Campylobacter jejuni, epithelial cells

P217, **New food safety challenges facing the poultry and meat industry with the advent of online meal delivery services** Charles Herron*UG, Kenneth Macklin, Amit Morey *Auburn University*

With over 100 companies and the market leader, Blue Apron, delivering 8 million packages a month, the market for online meal delivery kits has surged in popularity in recent years. Online meal delivery kits are pre-portioned ingredients and recipes that arrive in a box at a subscribing customer's door. The boxes with ice packs are not transported on refrigerated trucks, and are subjected to environmental conditions after they are delivered to the customer. The temperature abuse during the supply chain and after it is delivered to the customer can lead to significant food safety and spoilage issues. Research was conducted to determine the temperature abuse of meat and poultry products in commercially acquired meal delivery kits under various scenarios. The scenarios were as follows: boxes are held at 24 hours at 77-80ºF (Spring weather), 24 hours at 98ºF (Summer weather), and 16 hours at 40ºF followed by 24 hours at 98ºF (delay in shipping and delivery). Separate commercially obtained meal kit boxes were used for each scenario, and temperature was tracked using radio frequency identification tags (RFID). The tags were placed on all meats and produce in the boxes, and, in the first two scenarios, on the inside wall of the cardboard and the thermal layer. Temperature (40ºF) was set as a food safety cut-off. Time-temperature curves were analyzed using polynomial regression. Temperature increase was dependent on various foods and the scenarios tested. Raw chicken temperatures increased over 40ºF within 4-6 h in Scenario 3 compared to 10 h in Scenario 2. The temperature of chicken subjected to scenario 2 and 3 can be explained by 3rd order polynomial equations: $y = -2E\text{-}05x^3 + 0.0063x^2 - 0.3277x + 35.426$ (R^2=0.9965) and $y = -3E\text{-}07x^3 + 0.0004x^2 - 0.0705x + 38.36$ (R^2=0.9946). These findings expose the food safety lacuna in the supply chain making the consumers vulnerable to food safety risks.

Key Words: Food Safety, RFID, Meal Kit, Supply Chain

P218, **Employee exposure to formaldehyde at facilities using a formaldehyde-based feed disinfectant** Dillon Mellick*, Adam Cannon
Kemin Industries

Formaldehyde (FA) is labeled to maintain feed and feed ingredients *Salmonella*-negative for up to 21 days. Engineering and administrative controls are put in place at facilities to reduce employee exposure. Air monitoring of FA levels is compared to established regulatory safety levels. The objective of this study was to analyze air monitoring of FA at feed and ingredient facilities to determine employee exposure and locations requiring controls to minimize exposure. Data included personal employee exposure monitoring (124 samples, 35 facilities, taken 10/2012 to 6/2019), using passive samplers worn over a shift compared to the Action Level (0.5 ppm) and Permissible Exposure Limit (0.75 ppm). Samples ranged from 1-10 samples per facility with an average of 3.7 samples/facility. Additionally, data from area FA monitoring in facilities using handheld meters were analyzed (a total of 2,357 samples, 45 facilities, taken 1/2013 to 8/2019). Samples were taken in the same location within a facility, and general locations were assigned to represent similar areas with similar exposure potential. General locations include control room (high employee occupancy), hand-add (direct addition of ingredients into the mixer), loadout (bulk loadout of FA treated feed), mixer (application point), pellet mill (pelleting process), storage (from mixing to storage of treated feed), system (FA application equipment) and other (not related to FA application). Exposures were assigned to 3 groups, <0.5, 0.5-0.75 and >0.75 ppm. To determine the effect of sample location, employee exposures were analyzed using the GLIMMIX procedure of SAS (v 9.4, SAS Institute, Cary, NC) with facility serving as a random effect. The employee personal FA exposure, mean ± std dev, was 0.058 ± 0.064 ppm. The maximum and minimum employee personal FA exposures were 0.190 ± 0.009 ppm and 0.009 ± 0.000 ppm, respectively. For area samples, location was significant ($P < 0.001$) with 99% of control room, 94% of hand-add, 100% of loadout, 88% of mixer, 92% of pellet mill, 83% of storage, 80% of system and 98% of other samples <0.5 ppm. While employee exposure was below established regulatory safety levels, certain areas result in greater exposure potential, suggesting a need for implementation of appropriate controls.

Key Words: Formaldehyde, Salmonella, Employee Safety

P219, **Effect of dietary supplementation with industrial fruit waste on broiler meat quality** Elisa Ponsano*, Caroline Silva, Rodolfo Araujo, Mariana Rosetti *Unesp Univ Estadual Paulista - Faculty of Veterinary Medicine*

Demand for animal protein is increasing worldwide due to the rise in human population, while concerns about sustainability put pressure on minimizing the generation of industrial waste. In addition, consumers also want healthy food. All of these issues motivated us to investigate the effects of feeding broilers with grape, pineapple and guava meal (seeds and peels) on some meat quality parameters. Cobb 500 1-d-old broiler chickens were fed one of the following experimental diets for 21 d at 28 °C (5 treatments/6 replicates/15 birds per parcel): PC - basal diet for broilers (BD) with synthetic antioxidant (HBT); NC – BD with no HBT; GR - BD with 5% grape meal and no HBT; PA - BD with 5% pineapple meal and no HBT; and GV - BD with 5% guava meal and no HBT. Data on proximate composition and cholesterol in breast and thigh and on fatty acids in thighs were obtained by recognized methods and analyzed by ANOVA and Tukey tests at 5% probability. Feeding the fruit wastes to broilers reduced the cholesterol concentrations in breast ([mg/100 g] CN 61.48 x GR 32.65, PA 48.50 and GV 46.80; $p < 0.0001$) and thigh ([mg/100 g] CP 54.76 and CN 81.90 x GR 42.35, PA 52.56 and GV 48.65; $p < 0.0001$), which was assigned to their contents of phenolic compounds. Besides that, in breasts, grape meal decreased lipids ([%] NC 1.70 x GR 1.00; $p < 0.05$) and guava meal increased proteins ([%] PC 19.56 x GV 22.91; $p < 0.05$) and, in thighs, both grape and guava meals decreased lipids ([%] CN 6.18 x GR 4.28 and GV 4.70; $p < 0.0001$). The fruit wastes also influenced the fatty acids profiles by lowering saturated fatty acids and increasing polyunsaturated fatty acids (PUFA) in broiler thighs, besides providing better n-6/n-3 ratios (PC 16.24 and NC 16.10 x GR 12.55, PA 12.30 and GV 14.18; $p < 0.0001$). That was considered a positive result since the proper consumption ratio of PUFA n-6/n-3 ensures the balance of coagulation and inflammation processes in the body and modulates several genes involved in oxidative processes. Among the fruit waste investigated, grape meal was the best option to provide healthier chicken meat.

Key Words: grape, guava, pineapple, fatty acids, cholesterol

P220, **Effects of deboning time and thawing method interaction on sensory descriptive profiles of cooked chicken breast and thigh meat** Jian Zhang [1], Brian Bowker [2], Yi Yang [3], Bin Pang [4], Hong Zhuang* [2]
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The objective of this study was to evaluate the effects of freezing/thawing method and deboning time interaction on sensory descriptive profiles of cooked chicken breast and thigh meat. Broiler breast fillets (pectoralis major) and thigh meat were deboned at 45 min, 2 h, or 24 h postmortem. Meat samples were either cooked from a fresh state (no freezing/thawing) or freezing/thawing with three different methods. Sensory characteristics were profiled by a trained descriptive panel. Statistical results show that there were no significant deboning and thawing interaction effects for sensory characteristics regardless of sensory attribute and meat type. Deboning time had more impacts on cooked breast meat than thighs for attributes cohesiveness, hardness, bolus size, rate of break down, and chewiness. The thawing methods had more impacts on hardness and juiciness of cooked thighs than those of cooked breast meat. These results indicate that the effects of deboning time on sensory profiles are not altered by thawing method used for cooking frozen chicken meat products and by meat state (fresh versus frozen). The effects of the thawing methods on sensory characteristics are similar in cooked chicken meat no matter when meat is removed from bones.

Key Words: poultry, chicken fillet, sensory evaluation, flavor, texture

P221, **Growth of Campylobacter spp. in primary containers incubated aerobically** Arthur Hinton Jr*[1], N. Cox [1], Arturo Levican [2] *[1] U. S. National Poultry Research Center, Agricultural Research Service, [2] Pontificia Universidad Católica de Valparaíso*

The objective of this study was to examine the growth *Campylobacter* spp. in primary containers incubated in aerobic atmospheres. Ten ml of media composed of (g/L) beef extract, 50; tryptose, 10; soluble starch, 10; sodium bicarbonate, 5.0; sodium lactate, 3.0; and agar, 0.5 was inoculated with 10^4 cfu/ml of *Campylobacter coli* ATCC 33559*, Campylobacter fetus* ATCC 27374*, Campylobacter jejuni* ATCC 33560*,* or *Campylobacter lari* ATCC 35221. Inoculated media was transferred to 25 ml, 12.5 cm^2 culture flasks with a plug-sealed cap, a vented cap, or a vented cap covered with Parafilm. Containers were incubated aerobically at 37C for 48 h. *Campylobacter* were enumerated after incubation by plating serial dilutions of the media on Blood Agar Base #2 supplemented with 7.0% horse blood and Blaser-Wang antibiotic mixture. The inoculated plates were incubated microaerobically in BD GasPak jars with BD GasPak EZ Campy Container System sachets at 37C for 48 h. Cfu/ml recovered were converted to log10, and significant differences were determined using GraphPad InStat statistical software. Results indicated that after 48 h of incubation there was no significant ($p < 0.05$) difference in the number of *Campylobacter* spp. recovered from flasks with plug-sealed caps or with vented caps covered with Parafilm. The number of *Campylobacter* recovered from the closed flasks was significantly greater than the number of *Campylobacter* recovered from vented flasks not covered with Parafilm. During incuba-

tion, there was a 4-5 log increase in the number of *C. coli, C. fetus, C. jejuni,* and *C. lari* in media incubated in the plug-sealed or the Parafilm covered flasks; while there was a 2-4 log decrease in the number of *Campylobacter* recovered from flasks with vented caps not covered with Parafilm. Furthermore, there was no significant difference in the growth between the different species cultured in same type of container. Conclusions indicate that this medium can support the growth of *Campylobacter* species in closed containers that prevent exposure to aerobic atmospheres while the pathogen is unable to grow in containers that do not prevent exposure to normal breathing air. Findings will be used to refine methods for growing *Campylobacter* in primary containers incubated aerobically.

Key Words: Campylobacter, growth, aerobic, microaerobic, primary containers

P222, **Different sources of PAA may exhibit different antimicrobial effects** Kristina Feye*[1], Dana Dittoe [2], Casey Owens [2], Steven Ricke [2] *[1] USDA-ARS, [2] University of Arkansas*

Peracetic acid (PAA) in poultry processing is variable. Anecdotal evidence suggests that PeraClean may be more stable than other companies; however, the effects varying stability has on pathogen and microbial reduction are unknown. This is important as stability may enable the PAA to be more effective for longer periods of time. In order to evaluate the antimicrobial effects of PAA, one PAA (PeraClean; P) was qualitatively compared against two competitor products (Competitors 1 and 2; C1 and C2) at the University of Arkansas Pilot Processing Plant. A total of 150 Ross 708 broilers (42 d) were used in the current study (n=10 per treatment group). Prior to treatment, 10 birds were sampled post-evisceration to evaluate the incoming microbial load on the birds (C). The birds were dipped in either 400 ppm or 600 ppm PAA (A or B), chilled in either 25 ppm or 45 ppm PAA (1 or 2), and then manually agitated in 400 mL of nBPW for 1 min. There were 10 birds per treatment group in total. The resulting rinsates were transported to the Center for Food Safety and assessed for total microbiological load with total aerobic plate counts (Trypticase Soy Agar; APC), coliforms, (Eosin Methylene Blue Media; EMB), *Salmonella* (Xylose Lysine Deoxycholate agar, XLD), and *Campylobacter* (modified Charcoal Cefoperazone Deoxycholate Agar, mCCDA). The microbiological plates were incubated as per manufacturer's directions. Statistical analyses were calculated in JMP 14.0, with a significance level of $p \leq 0.05$. Data indicate that all three sources of PAA are effective sanitizers for poultry processing. But, each product qualitatively resulted in differences in prevalence and load. PA1 was the most effective antimicrobial inclusion rate for PeraClean, effectively reducing *Campylobacter* prevalence 100%, with load differences in Coliforms (1 log 10), *Salmonella* (0.8 log10), and *Campylobacter* (2.58 log10). Yet, other products were mostly effective on *Campylobacter* at different antimicrobial inclusion rates. However, additional studies will be required to determine if those differences are quantitatively significant and whether the product effects are due to stability.

Key Words: PAA, Poultry Processing, Salmonella, Campylobacter, E. coli

Pathology

P223, **Presence of Eimeria species in chicken farms from four major poultry provinces of Colombia** Carolina Mesa Pineda*[1GS], Luis Gómez Osorio [2], David Villar Agaiz [2], Sara López Osorio [2], Edimer David Jaramillo [1], Jorge Duque Noreña [1], Oscar Múnera Bedoya [1], Jenny Chaparro Gutiérrez [2] *[1] Grupo de Investigación Nutri-Solla, SOLLA S.A, [2] Grupo de Investigación CIBAV, Facultad de Ciencias Agrarias, Universidad de Antioquia*

Background: As human population continues to grow; food security is threatened. Poultry is becoming an important source of protein for humans. Global chicken production, with an estimated annual growth of 2%, will reach 97.8 million tons (MT) in 2019. In Colombia, production for 2019 is estimated at 1.7 MT, and 62.8% is located in four provinces. Coccidia (*Eimeria* spp.*)* infections are one of the main challenges confronting the industry because they are ubiquitous in poultry operations, and although they rarely cause clinical disease, they decrease growth rate and increase production costs, resulting in large economic losses. No epidemiological studies have been conducted on *Eimeria* spp. in broiler farms from Colombia. Objective: To establish the presence of *Eimeria* spp. in broiler farms in several Colombian provinces Methods: A total of 192 broiler farms were sampled in four provinces (Antioquia, Santander, Valle del Cauca and Cundinamarca). Pen litter samples were taken from birds aged ≥21 days and tested for oocysts presence per gram of bedding material (OPG) using the McMaster technique. Identification of *Eimeria* spp. was conducted according to morphological characteristics. Results: Positive farms in Antioquia were 77.42% (n= 31), Santander 59.72% (n= 72), Valle del Cauca 38.6% (n= 57), and Cundinamarca 34.4% (n= 32). The average OPG (±SD) in positive farms were Antioquia (4,671±9,551), Santander (1,386 ±3,185), Valle del Cauca (1,002 ±3,683); and Cundinamarca (1,657 ±4,969). The presence of the different *Eimeria* species in each province (Antioquia, Santander, Valle del Cauca and Cundinamarca) was: *E. acervulina*: 20.19%; 38.85%; 37.32%; 39.62: *E. maxima*: 35.52%; 21.38%; 8.54; 12.58. *E. tenella*: 36.01%; 22.61%, 36.10%; 36.90% Other species: 8.27%; 17.16; 18.05 and 10.90, respectively. A PCR confirmation will be performed. Further studies are underway to evaluate resistance to anticoccidial drugs with pooled samples from each province. Conclusion: Coccidia were detected in 52.08% (n=192) of the Colombian farms. The type of *Eimeria* species varied from farm to farm and among provinces. These results warrant further studies to assess the impact of coccidia on performance in Colombian broiler farms, and whether methods of control should be implemented.

Key Words: Broiler, E. acervulina, E. maxima, E. tenella

P224, **Survival and sporulation of coccidia oocysts on different litter conditions** Maria Tereza Bethonico Terra*[1GS], Ruediger Hauck [1,2], Isai Franco [1], Shanhao Jin [1] *[1] Department of Poultry Science, Auburn University, [2] Department of Pathobiology, Auburn University*

The environmental influence in sporulation and deterioration of coccidia was investigated in order to better understand the dynamics of this parasite on litter. Two studies were done in used litter comparing different temperatures and moistures content. For the first trial, 1200 g of dry litter and 200 g feces containing E. maxima oocysts were mixed and divided into four treatments: cool-dry, cool-wet, warm-dry, and warm-wet. For the wet treatments, 20 ml of tap water were added, and the warm treatments were stored 20 cm under a heat lamp. Seven samples were collected on a four-day interval. Oocysts were counted in a McMaster chamber and the proportion of sporulated was calculated. Of the two cool aliquots kept at a cooler temperature, the dry one started with the highest counts and the reduction in numbers overtime was 88%. Its percentage of sporulated oocysts had a linear increase while the wet litter had a greater variation on this percentage of sporulated oocysts over time. Between the two warm groups, in terms of oocyst counts, there were no large differences and, by the fifth day of sampling, their counts were close to zero. There was no clear difference in sporulated oocysts which was around 5%. Comparing cool and warm treatments, counts and percentage of sporulated oocysts were higher for both cool-dry and cool-wet treatments. A second trial was conducted with the same litter. This time, there were two groups with four replicates per group: wet-cool and dry-cool. Eight samples were collected

every three days for counting in a McMaster chamber and for taking photos of 50 different areas of the chamber. Those photos were used to measure the length and width of the oocysts and evaluate oocyst deterioration. The average number of oocysts for the wet treatment was higher (149,422) than the dry (143,698) and the percentages of decrease over time were 45 and 5%, respectively. There was no difference in size between groups and the percentage of damage oocysts was slightly higher in the wet litter. The results from both trials indicate that coccidia oocysts deteriorate quicker in warm and wet conditions. This information is essential for us in order to set up future tests exploring how different litter treatments influence the infection pressure.

Key Words: Coccidiose, Litter

P225, **Heat stress reduces sexual development of Eimeria maxima in chickens** Gustavo Schneiders*, James Foutz, Marie Milfort, Ahmed Ghareb, Alberta Fuller, Romdhane Rekaya, Samuel Aggrey *University of Georgia*

Coccidiosis, caused by *Eimeria* spp. presents a self-limiting intestinal infection of poultry. Intestinal replication of the parasite causes severe morphological alterations to the host gastrointestinal tract. The objective of this study was to investigate the pathogenesis and gene expression of *E. maxima* infection in broiler chickens under heat stress (HS). Three hundred 14-days old Ross708 broiler chickens were infected via gavage with $2x10^5$ *Eimeria maxima* sporulated oocysts suspended in water and housed at two different temperatures: 20˚C (TNi) and 35˚C (HSi). 300 control chickens were mock infected with water and housed at 20˚C (TNc) and 35˚C (HSc). All chickens were raised in batteries with wired floor, with *ad libitum* access to water. Individual body weights (BW) and feed consumption were recorded. From 4 to 7 dpi, 10 chickens from each treatment were euthanized by cervical dislocation, following with collection of Ileum samples for histology and gene expression. Intestinal lesions and the presence of *E. maxima* developmental stages (micro-scores) were recorded and fecal oocyst shedding was estimated. Quantification of *E. maxima* developmental stages in the ileum was assessed by qPCR. Statistical analysis of BW, feed intake, FCR, relative growth, lesion scores, micro-scores and oocysts shedding was conducted in Prism 7.0 software. Gene expression was conducted in SAS software by GLM procedure with multiple comparisons corrected with Tukey's method. All tests were performed at the 5% level of significance. There is a significant detrimental effect of HS on the pathogenesis of *E. maxima* infection in broilers. There was a restricted replication of the parasite in heat stressed chickens evidenced by significantly reduced oocyst shedding and disruption of the intestinal blood barrier. Gene expression of parasite genes demonstrated curtailed sexual reproduction of *E. maxima* in HSi chickens marked by downregulation of *Eimeria* spp. genes related to gamete fusion (HAP-2), oocyst formation (GAM56 and GAM82), mitosis and spermiogenesis (protamine) as compared to TNi.

Key Words: coccidiosis, gene expression, cytokine, heat stress, parasite development

P226, **Prevalence and characterization of quinolone resistance in Campylobacter spp. isolates in chicken livers from various supermarkets in Georgia** Hung-Yueh Yeh*, Nelson Cox, Tory Thompson, Arthur Hinton, Jr. *USDA ARS USNPRC*

Purpose: Bacteria that harbor various antibiotic resistance genes present a serious threat in public health worldwide. One such resistance gene is the *qnr* family genes. *Campylobacter* is the leading foodborne pathogen worldwide, and has been shown to develop resistance to fluoroquinolones, which is commonly used in human and veterinary medicine for combating multi-drug resistant bacteria. The aim of this communication was to determine the prevalence and characterization of *Campylobacter* isolates from retail chicken livers.

Experimental design: *Campylobacter* spp., originally isolated from poultry livers in Georgia, USA (n = 250), were revived from the glycerol stocks on Campy Cefex agar plates. After 48 hours incubation under microaerobic conditions, a single colony from each isolation was cultured on fresh Campy Cefex agar plates. Speciation of *Campylobacter* spp. was performed using the end-point PCR assay as described previously. Antimicrobial susceptibility tests were carried out using the Sensititre *Campylobacter* CAMPY plates according to the manufacturer's instructions.

Results: The results show 181 isolates and 69 isolates were identified as *Campylobacter jejuni* and *Campylobacter coli*, respectively, from the endpoint PCR assay. A total of 93 (37.2%) of 250 *Campylobacter* spp. isolates were detected to have resistance to at least one antibiotic. Specifically, for 90 *Campylobacter jejuni* isolates, 33 isolates were resistant to ciprofloxacin and nalidixic acid; 25 isolates to tetracycline; 18 isolates to nalidixic acid and tetracycline; nine isolates to ciprofloxacin, nalidixic acid and tetracycline; two isolates each to ciprofloxacin and tetracycline, and ciprofloxacin, nalidixic acid, azithromycin and erythromycin, respectively; and one isolate to ciprofloxacin, nalidixic acid, azithromycin, tetracycline and erythromycin. For *Campylobacter coli*, two isolates were resistant to tetracycline, and one isolate to both nalidixic acid and tetracycline.

Conclusion: Based on our results, *Campylobacter jejuni* were prevalent in chicken livers and were resistant to the quinolone class and other antibiotics. Further evaluation of the *qnr* genes to determine whether these genes play a role in the quinolone resistance is needed.

Key Words: Campylobacter jejuni, Campylobacter coli, PCR, Quinolone, Resistance

P227, **Establishment of a blackhead challenge model using nitarsone as a reference treatment** Makenly Coles*[1GS], Lesleigh Beer [1], Brittany Graham [1], Brian Wooming [2], Billy Hargis [1], Christine Vuong [1] *[1] University of Arkansas, [2] Cargill Inc.*

Blackhead, also known as histomoniasis, is a problem the turkey industry and broiler breeders are currently facing. This disease, caused by Histomonas meleagridis, has been recently known to cause up to 80% mortality within turkey flocks. There are no approved treatments currently on the market, with the last (the arsenic-based antiprotozoal drug nitarsone) voluntarily removed from the market in 2015. Because nitarsone was apparently effective against blackhead, some have considered nitarsone as a potential reference treatment for evaluation of new potential compounds for prevention of blackhead. This pilot study aimed to elucidate a challenge model based on efficacy against nitarsone to be used in blackhead disease models for future evaluation of prophylactic compounds. Groups included negative control, positive challenge control, and dietary inclusion of 0.01% nitarsone treatments. Twenty-three DOH turkey hens were allocated per pen, with four replicate pens per treatment. At 14 days-of-age, turkeys were challenged with 105 virulent H. meleagridis cells intracloacally. During the challenge phase, all mortalities were weighed, necropsied, and lesion scored. Surviving turkeys were terminated 14 days-post-challenge for final lesion scores and body weights. Mortality data were analyzed using the chi-square test of independence testing all possible combinations, while all other data were analyzed using ANOVA with post-hoc Tukey's HSD. Significant differences were reported at $p \leq 0.05$. Nitarsone treatment had significantly lower mortalities, liver lesion, and cecal lesion scores compared to the positive-challenge control turkeys (reaching levels comparable to the non-challenged negative controls). Based on the results of this trial, the challenge dose of 105 H. meleagridis cells per turkey was appropriate for direct challenge, with the nitarsone reference treatment able to effectively control disease incidence.

Key Words: Blackhead, Histomoniasis, Histomonas, Nitarsone, Turkey

P228, **In-house hatching and early feeding to improve chick performance during a necrotic enteritis challenge** Siobhan Boothe*GS, Nima Emami, Mallory White, Rami Dalloul *Virginia Tech*

As the welfare of newly hatched chicks attracts more attention, the issue of hatchery stress has garnered more interest. A 2×2 factorial study explored the effects of place of hatch [in-house (IH) vs. hatchery (HH)] and necrotic enteritis (NE) challenge [non-challenged (NC) vs. challenged (CH)] on broilers. Cobb 500 eggs (~E19) were acquired from a commercial hatchery; 840 eggs were placed in pens on clean shavings in pre-warmed floor pens and allowed to hatch out, while 927 eggs were placed in a hatcher set at 36.5 °C. On day of hatch, all chicks were weighed, sorted evenly and assigned to 4 treatments (8 replicate pens each and 30 birds/pen). The IH birds were placed immediately after sorting while HH birds were moved back to the hatcher for 24 h to simulate commercial hatchery procedures. After placing HH birds, feed and litter in the CH group pens were sprayed with a live oocyst coccidia vaccine as a predisposing factor to naturally occurring NE. The small intestines of three males per pen were scored for NE lesions on day 8 (onset of disease) and jejunal samples collected from one bird to measure mRNA abundance of peptide transporter (PepT)-1 and sodium glucose co-transporter (SGLT)-1. Data were analyzed using JMP Pro 14 and significance between treatments identified by LSD ($P \leq 0.05$). Hatchability for IH chicks was lower (87%) than H chicks (96%) ($P < 0.05$). Mortality was significantly higher for CH (2.38%) compared to NC birds (0.71%), but there was no effect of place of hatch on mortality. Lesion scores were significantly ($P < 0.01$) higher in the duodenum, jejunum, and ileum of CH birds compared to NC birds. Interestingly, IH birds had significantly higher average daily feed intake and average daily gain compared to HH birds for days 0-28 regardless of the challenge. There was a 2-way interaction effect on mRNA abundance of PepT-1 and SGLT-1 where higher levels were observed in IH/NC birds and HH/CH birds. In conclusion, in-house hatching may improve early post-hatch performance and nutrient transporter gene expression by allowing birds to have early access to feed and water. This may hold potential for further exploration by the industry as an alternative practice to traditional hatching to improve the welfare and productivity of broilers, post-hatch.

Key Words: In-house hatching, necrotic enteritis, broiler, nutrient transport, welfare

P229, **Broiler response to a necrotic enteritis challenge after varying in ovo doses of commercial probiotics** Mallory White*GS, Siobhan Boothe, Rami Dalloul *Virginia Tech*

Necrotic enteritis (NE) is a major poultry disease caused by the opportunistic bacteria Clostridium perfringens. Using probiotics to establish balanced and beneficial microbiota helps reduce the harmful effects of NE, a method that may be more effective with earlier application during embryonic development. The objective of this 21-day study was to determine optimum dosage levels of two different commercial probiotics (P1 or P2) given in ovo, by measuring performance and intestinal lesion scores (LS) during a NE challenge. In total, 336 embryonic day 18 (E18) Cobb500 eggs were obtained from a commercial hatchery and each egg received 100 µL of one of seven in ovo treatments: sterile water (CTRL), low (P1L or P2L: 1x105 CFU), medium (P1M or P2M: 1x106 CFU), or high (P1H or P2H: 1x107 CFU) probiotic doses dissolved in sterile water. After hatch, birds were placed in floor pens (12 birds/pen, 4 reps/treatment) and fed a mash corn/soybean meal diet. One day post-placement, coccidiosis vaccine was sprayed on feed and litter to promote a naturally occurring NE. Bird performance measurements were taken weekly and all data were analyzed using JMP Pro 14 with significance reported as $P \leq 0.05$. Interestingly, from D4-D8, P2L had a lower BWG compared to the CTRL and all P2 treatments. From D0-D8, FCR and mortality were also higher in P2L. During the last week, P1L, P1H, and P2H all had similar BWG and FCR compared to the CTRL, while P2L had lower BWG and higher FCR. Cumulatively, BWG tended ($P = 0.06$) to be lower in P2L and P2M compared to CTRL. For D8 LS, there were no significant differences in the duodenum or jejunum, but in the ileum, P1M had higher LS compared to other treatments. Within both P1 and P2, the high doses tended to perform better than the lower doses. In conclusion, P2L birds tended to perform lower than expected, with the higher doses of each probiotic performing better overall than the low doses. This could be due to the probiotic concentration, with smaller doses having different effects on performance compared to higher doses. Future research will include testing the two high doses of probiotics in a larger study, with additional parameters in order to better understand how these probiotics affect bird health and performance during a NE challenge.

Key Words: Broiler, probiotics, in ovo, performance, necrotic enteritis

P230, **Effects of a naturally occurring necrotic enteritis challenge on performance, lesion Scores and expression of tight junction proteins in broiler chickens** Nima Emami*GS, Siobhan Boothe, Mallory White, Rami Dalloul *Avian Immunobiology Laboratory, Department of Animal and Poultry Sciences, Virginia Tech*

Necrotic enteritis (NE) continues to present major challenges to the poultry industry with subclinical forms being responsible for the greatest economic impact on production. The objective of this study was to identify the effects of a naturally occurring NE challenge on performance, intestinal lesion scores and mRNA abundance of interferon (IFN)-γ, mucin (MUC)-2, and tight junction (TJ) proteins. On day of hatch, 480 Cobb 500 mixed sex broiler chicks were randomly allocated to two experimental groups, non-challenged (NC) and challenged (CH), each with 8 replicate pens and 30 birds/pen. Birds in both groups had ad libitum access to a corn/soybean meal commercial diet. One day post placement, feed and litter in the CH group pens were sprayed with a live oocyst coccidia vaccine as a predisposing factor to naturally occurring NE. At the onset of NE on day 8, the small intestines of 3 male birds/pen (24/group) were examined for NE lesions, and jejunum samples from one of those birds were collected to measure mRNA abundance. Statistical analysis was performed using the JMP 14 software and significance between treatments ($P \leq 0.05$) determined by LSD. Birds in the NC group had significantly higher average daily feed intake and average daily gain compared to CH birds for days 0-28. Feed conversion ratio tended to be higher ($P = 0.09$) in the NC birds compared to the CH group for the same period. Challenged birds had significantly higher ($P \leq 0.001$) lesion scores in the duodenum, jejunum and ileum compared to the NC birds on day 8. mRNA levels of IFN-γ ($P = 0.003$) and zonula occludens (ZO)-1 ($P = 0.02$) were higher in the CH birds compared to NC. Despite similar levels for MUC-2, occludin and claudin (CLD)-3, expression of CLD-1 tended ($P = 0.08$) to be higher in the CH group than in the NC. *Clostridium perfringens* toxins attach to the extracellular domain of CLD proteins, disintegrate TJ and increase the paracellular permeability of the gut. However, ZO are intercellular components of TJ proteins with no extracellular domain. In this study, NE challenge caused differential changes in TJ mRNA abundance in conjunction with higher lesion scores and lower performance. This might be a defense mechanism employed by enterocytes to alleviate the negative effects of NE.

Key Words: Broiler chickens, necrotic enteritis, performance, , lesion scores, tight junction

P231, **Assessing intestinal permeability: FITC-d versus mRNA expression of tight junction genes** Gustavo Schneiders [1], James Foutz*[1], Alexandra Rivera [1], Marie Milfort [1], Alberta Fuller [1], Romdhane Rekaya [2], Samuel Aggrey [1] [1] *Department of Poultry Science, University of Georgia,* [2] *Department of Animal and Dairy Science, University of Georgia*

Intestinal tract functions as a barrier against commensal pathogens and antigens and protects the body from secondary infection and sepsis. The gut barrier is breached by stress, inflammation, dysbiosis and infection among many others. Intestinal barrier integrity can affect gut health, however, there is no universal method for assessing intestinal barrier permeability.

The objective of this investigation was to assess gut barrier permeability using fluorescein isothiocyanate dextran (FITC-d) and mRNA expression of tight junction (TJ) genes in broiler chickens infected with Eimeria spp and subjected to heat stress. Three hundred 14-days old Ross708 broiler chickens were infected via gavage with 2x105 Eimeria maxima sporulated oocysts suspended in water and housed at two different temperatures: 20˚C (TNi) and 35˚C (HSi). Three hundred control chickens were mock infected with water and housed at 20˚C (TNc) and 35˚C (HSc). Prior to sample collection, chickens were gavaged with FITC-d and blood was collected 2 hours later for the quantification of FITC-d in the serum. Intestinal permeability data were compared to the mRNA expression of TJ genes in the ileum. Genes analyzed where occludin, claudin-1, zona occludens 1 (ZO-1) and junction adhesion molecule 2 (JAM-2). Gene expression differences among the treatment was done with the GLM procedure with Tukey's method (SAS software). Significant difference was declared at 5%. Changes in intestinal permeability occurred independently of changes in TJ gene expression. At 5 dpi, intestinal permeability was increased in TNi chickens, however this did not correlate with changes in the expression of TJ genes. Moreover, at 6 dpi, the peak of intestinal permeability in TNi and HSi, the expression of occludin, JAM-2 and ZO-1 were generally statistically similar between infected and control groups. Our results also indicate that HS of the host did affect intestinal permeability. This study demonstrates that changes in intestinal TJ expression do not necessarily correlate with changes in intestinal permeability and the quantification of FITC-d in the serum should be used as gold standard method to assess intestinal permeability in broilers.

Key Words: gut permeability, tight junction, FITC-d, gene expression

P232, **onstruction and Efficacy of a Recombinant HVT-ND Vaccine Against NDV and MDV Challenge in SPF and NDV Challenge in Broiler Birds** Yugang Luo, Kelly Turner-Alston, Megan Bosserd, Lauren Taylor, Tyler Brown, John Dickson, Jennifer Embrey, Sing Rong *Zoetis - VMRD*

The etiologic agent of Marek's Disease (MD), Marek's disease virus (MDV) serotype 1, is a member of the family Herpesviridae. Herpesvirus of turkeys (HVT), is an avirulent turkey virus that is capable of replication in chickens. NDV (Newcastle disease virus) causes a highly contagious and fatal disease, and the NDV fusion protein (F) is one of the major protective antigens. We constructed a HVT-ND recombinant vaccine and demonstrated efficacy in SPF (specific pathogen-free) birds against a velogenic NDV challenge (93% protection for *in ovo* and 100% protection for subcutaneous route), and against a virulent MDV challenge was also observed (83% protection for *in ovo* and 80% protection for subcutaneous route). Additionally, NDV efficacy was observed in commercial broiler birds, when the vaccine was administered *in ovo*. Recombinant vaccine stability was demonstrated with *in vitro* passaged viral culture by PCR, IFA and DNA sequencing of the insertion site.

Key Words: vaccine, HVT-ND, HVT vector vaccine, NDV, MDV

P233, **Duration of Immunity of a Recombinant HVT-ND Against a Velogenic NDV Challenge in SPF Birds** Lauren Taylor, Megan Bosserd, Kelly Turner-Alston, John Dickson, Jennifer Embrey, Sing Rong *Zoetis - VMRD*

A recombinant HVT-ND was developed as a bi-valent vaccine for protection against Newcastle disease (ND), a highly contagious and fatal disease affecting all species of birds; and Marek's disease (MD), a common cause of condemnations and immune suppression in broilers. In this study with SPF leghorns, recombinant HVT-ND was injected either *in ovo* at E18 or subcutaneously at hatch. On Day 63, 30 vaccinated birds of each treatment group were challenged with a velogenic NDV. Protection of 100% (30/30) was observed for both *in ovo* and subcutaneous route of administration. The details of experimental design and study results will be further discussed.

Key Words: vaccine, HVT-ND, HVT vector vaccine, NDV, MDV

SCAD

P234, **High zinc glycinate concentration on host immune response in necrotic enteritis in broiler chickens** Theros Ng[*1GS], Alexandra Blanchard[2], Ramesh Selvaraj[1] *[1]Department of Poultry Science, University of Georgia, [2]Pancosma SA*

Necrotic enteritis (NE) costs over US$6 billion a year. The causative agents of NE in chickens is Clostridium perfringens predisposed with coccidiosis. Since the discontinued use of antibiotics in poultry production, alternative methods to alleviate NE infections are being explored. Zinc is involved in pathways in metabolism and immunity such as the zinc-finger motif in DNA polymerase, the NFκB inflammation transcription factor, and the antioxidant enzyme, zinc/copper superoxide dismutase. The current recommendation of zinc in chicken diets is 100 mg/kg by poultry producer guidelines. Therefore, we hypothesized that a higher concentration of zinc is required to alleviate NE in chickens. In this study, we conducted two experiments to evaluate the effects of zinc glycinate on necrotic enteritis infection in broiler chickens. In experiment one, the birds were supplemented with 40, 80, and 120 mg/kg of zinc glycinate. The birds were infected using our necrotic enteritis infection model. Briefly, *Eimeria maxima* at 5,000 oocysts/ml were orally gavaged on day 14 and *Clostridium perfringens* at 10^8 CFU/ml was orally gavaged on day 19, 20, and 21. Samples were collected on day 21 for analyses. In the follow-up experiment, the birds were supplemented with 100, 120, 140, and 160 mg/kg of zinc glycinate.In the first experiment, growth performance was not affected by zinc. However, intestinal damages as evaluated by lesion scoring was alleviated by zinc glycinate at 120 mg/kg ($P < 0.01$). Cytokine gene expressions were downregulated by higher concentration of zinc glycinate in infected birds, but not statistically different. In the follow-up experiment, feed conversion ratio was improved by zinc glycinate at 160 mg/kg in infected birds ($P < 0.01$) and weight gain was improved by zinc glycinate at 120 mg/kg ($P < 0.01$). Lesions was alleviated ($P < 0.01$) and mortality was reduced ($P < 0.01$) by zinc glycinate at 120 mg/kg. In conclusion, our findings suggested that zinc glycinate concentration at 120 mg/kg would be beneficial to alleviate NE infection. Given that zinc glycinate has a higher bioavailability than zinc sulfate, a higher concentration than 120 mg/kg zinc sulfate may be optimal.

Key Words: Nutrition, Poultry, Zinc, Necrotic Enteritis, Immunology

P235, **Evaluation of a Novel Microbial Muramidase Ingredient as a New Tool for Supporting Gastrointestinal Functionality and Animal Performance in a Necrotic Enteritis Challenge Model** Matthew Jones[*1], Charles Hofacre[1], Greg Mathis[2], Brett Lumpkins[2] *[1]Southern Poultry Research Group, [2]Southern Poultry Feed & Research, Inc.*

Chicken intestines contain large numbers of normal bacterial flora (10^9 to 10^{11} CFU/g). A peptidoglycan (PGN), also known as murein, is a polymer that is an essential component of the bacterial cell wall. Ninety percent of Gram positive bacterial cell walls are made up of PGNs. PGNs only make up 10% of Gram negative bacterial cell walls. As bacteria in the intestines

turn over, a large amount of free PGNs are released, which can increase mucus production and interfere with digestion. Increased mucus has also been shown to result in the proliferation of *Clostridium perfringens* (CP) (Collier, 2008). This novel microbial muramidase has been shown to hydrolyze dead cell debris that contains PGNs. In this 60 battery-cage study with ten replicates (10 birds per replicate), broilers were challenged with *Eimeria maxima* at 13 days and CP on days 19 and 20. The treatments were 1) non-challenged controls, 2) challenged controls, 3 & 4) muramidase (25,000 LSU/ton) without and with coccidiosis vaccine, respectively, 5) muramidase plus a bioshuttle with salinomycin (60g/ton) from 15 to 28 days, and 6) muramidase plus zoalene (113.5g/ton). Percentage of mortality due to clinical necrotic enteritis (NE) was highest in the CP challenged control (29%^{a}) and the muramidase only (31%^{a}) group, and significantly lower in the muramidase plus zoalene (20%^{b}) group, muramidase plus cocci vaccine (13%^{b}) group and the salinomycin bioshuttle with muramidase group and the negative control (0%^{c}). During the peak of the CP challenge, the muramidase with salinomycin group had the lowest non-adjusted feed conversion (FCR; 1.87^{bc}) and weight gain (0.79 kg^{a}) versus CP challenged control FCR (2.74^{a})/weight (0.67^{c}). At 28 days, all of the muramidase treatments had significantly heavier weights and lower FCR compared to the CP challenged control. In addition, muramidase with the coccidiosis vaccine group was able to perform as well as muramidase with the bioshuttle (salinomycin) treatment and much better than muramidase plus the zoalene treatment. In conclusion, even though muramidase did not affect mortality and NE lesion scores, this study suggests that muramidase supports gastrointestinal functionality by helping to reduce the negative impact on body weight and FCR in CP challenged birds.

Key Words: peptidoglycan, Clostridium perfringens, gastrointestinal, coccidiosis

P236, **Codon pair deoptimization of the HN and F of NDV LaSota strain does not sufficiently attenuate the virus for in ovo vaccination** Fatma Eldemery*, Changbo Ou, Taejoong Kim, Stephen Spatz, John Dunn, Robert Silva, Qingzhong Yu *US National Poultry Research Center, Agricultural Research Service, United States Department of Agriculture.*

In ovo vaccination is an attractive immunization approach for poultry industry. Currently available Newcastle disease virus (NDV) vaccines cannot be administered *in ovo* because of the reduced hatchability and embryo mortality. Codon pair deoptimization (CPD) approach has been used for efficient and rapid attenuation of a variety of RNA viruses by targeting the virulence genes through synonymous substitutions to reduce protein production of the target genes. In this study, we aimed to attenuate the NDV LaSota (LS) strain for *in ovo* vaccination by CPD of the fusion (F) or/and hemagglutinin neuraminidase (HN) genes. Three NDV LS recombinants expressing codon deoptimized LS F (rLS/F-d), HN (rLS/HN-d) or both genes (rLS/F+HN-d) were generated by using reverse genetics technology. Biological assays showed that the codon deoptimized viruses maintained similar growth ability and hemagglutination activity in embryonated eggs as the parental rLaSota virus. The pathogenicity of the rLS/HN-d and rLS/F+HN-d viruses were slightly attenuated with a lower intracerebral pathogenicity index than the rLaSota and rLS/F-d viruses. However, all three codon deoptimized viruses were still lethal to 10-day-old specific-pathogen-free chicken embryos with a mean death time less than 128 hours. These data suggested that the CPD of the surface glycoprotein genes of the LaSota strain does not sufficiently attenuated the virus for *in ovo* vaccination. Apparently, other virus attenuation approaches are needed to develop a safe *in ovo* NDV vaccine.

Key Words: Newcastle disease virus, Codon deoptimization, In ovo vaccination

P237, **Impact of diluent pH and holding conditions on Newcastle Disease vaccine virus infectivity titer** Nassar Haj Ali*GS, John Carey *Texas A&M University*

Newcastle disease is a viral disease causing severe economic losses worldwide. Despite routine vaccination, severe outbreaks continue to occur. Vaccine failures can result from mutations in the virus; however, virus inactivation may also occur in lyophilized Newcastle disease virus (NDV) live vaccines due to diluent properties at the time of administration.

The present study evaluated the virus infectivity titer of NDV live vaccine reconstituted in diluent at different pH levels and holding temperatures. Two 3x2 factorial trials were conducted, using 3 diluent pH levels (pH 5, 7, and 9). At each pH level, reconstituted virus was held one hour at room temperature or on ice.

The first trial utilized 124 fertilized SPF eggs (20 eggs per group, 4 eggs for negative control). The second trial utilized 217 fertilized eggs (35 eggs per group, 7 eggs for negative control). The second trial's eggs came from a commercial laying hen flock with known low titers of anti-NDV antibodies. Comparison of the two trials verified that the commercial laying hen flock low level of anti-NDV antibodies did not affect the virus infectivity titer measurement.

All eggs were incubated under standard conditions. Eggs were inoculated with 100 µl NDV vaccine on day 9 of incubation. Dilutions from 10^{-3}x to 10^{-7}x were used to measure the virus infectivity titer. Four days after inoculation, all eggs were chilled for at least 2 hours. Allantoic fluid was harvested from the 13-day old embryonated eggs and virus infectivity titer was measured by hemagglutination test.

Minimum reduction in virus infectivity titer was recorded in pH 7 diluent held on ice (1st trial: $10^{8.00}EID_{50}$/dose, 2nd trial: $10^{7.69}EID_{50}$/dose). The virus was less stable at pH 5 dileunt held on ice (1st trial: $10^{6.67}EID_{50}$/dose, 2nd trial: $10^{6.84}EID_{50}$/dose). Maximum reduction of infectivity titer was observed in pH 9 diluent held at room temperature (1st trial: $10^{6.25}EID_{50}$/dose, 2nd trial: $10^{6.13}EID_{50}$/dose).

The results of these studies indicate that optimum diluent pH for effective delivery of the NDV live vaccines for administration is 7. However, holding virus on ice may have beneficial effects on preserving the infectivity titer of the virus, even at pH 5 or 9 conditions.

Key Words: Newcastle disease, vaccine, diluent pH, Infectivity titer

P238, **IBD challenge studies comparing inactivated vaccine protection against different AL2 types** Kalen Cookson*, Manuel Da Costa, Andrew Barker, John Dickson, Jon Schaeffer *Zoetis*

Infectious bursal disease virus (IBDV) is an extremely hardy double stranded RNA virus that can cause immune suppression—especially if infections occur prior to 3 weeks of age. For well over a decade, the AL2 type viruses have dominated the broiler landscape of IBDVs. Its antigenic uniqueness has been documented in progeny challenge studies starting as early as 1999. This was further supported in 2004 when a partial dose study compared several commercial inactivated vaccines on protection against a panel of IBD viruses—and only the AL2 challenge resulted in suboptimal protection levels in some of the products. This presentation will cover three studies which compare protection levels of inactivated vaccines against prototype AL2 as well as other AL2 subtypes, including 9109. Study Design: SPF leghorns were vaccinated with a partial dose of killed IBD vaccine at 4 weeks of age and those designated for challenge received 3.5 EID50 of IBDV by eye/nose drop 4 weeks after vaccination. 10 days later bursa to body weight ratios (B:BW) were determined to calculate protection. In Study 1, Vaccine A 2-way protection was measured against AL2, 9109 and an AL2-like isolate. In Study 2, Vaccine A 2-way and 4-way vaccines were compared on AL2 protection. In Study 3, Vaccine A 4-way was compared to 4-way vaccines B, C and D against AL2 and two AL2-like isolates. Results: Trial 1. Vaccine A 2-way gave excellent protection against all three AL2 viruses including 100% protection

against 9109. Trial 2. Vaccine A 2-way and 4-way gave 95-100% AL2 protection and practically identical IBD seroconversion. Trial 3. Vaccine A gave over 90% protection against all three AL2 viruses. Vaccine B gave over 90% protection against 2 of the 3 AL2 viruses. Vaccines C and D gave between 6-63% protection against the three AL2 viruses, with both vaccines averaging less than 50% protection. Discussion: The AL2 family of viruses continue to show an ability to override immunity produced by certain inactivated IBD vaccines—perhaps underscoring the importance of IBDV antigen selection (Vaccine A contains a Group-6 variant and Vaccine B contains GLS). In addition, these studies suggest that vaccines that protect against the prototype AL2 are more likely to protect against other AL2 subtypes.

Key Words: IBDV, AL2, protection, inactivated, vaccines

P239, **New Avian Pathogenic Escherichia coli O Serogroup Epidemiology for Poultry Health in Georgia.** Caleb Austin*[1UG], Darby Newman[1], Robert Pasko[1], Aline Oliveira[1], Lisa Nolan[2], Catherine Logue[1], Nicolle Barbieri[1] [1] *Department of Population Health, College of Veterinary Medicine, University of Georgia,* [2] *Department of Infectious Disease, College of Veterinary Medicine, University of Georgia*

Colibacillosis caused by Avian Pathogenic E. coli (APEC) is a costly disease for the poultry industry worldwide, significantly affecting one of the world's cheapest sources of high-quality protein, resulting in mortality at the farm and carcass condemnation at slaughter. For years, geographic variation in APEC distribution has been known, with the most common E. coli serogroups representing >90% of characterized APEC which includes serogroups O1, O2, and O78. Currently, commercial vaccines are available specifically targeting these serogroups. Here, we propose to fill the gap in knowledge of APEC and perform an epidemiological study to investigate emerging O serogroups that may be contributing to poultry disease in Georgia.

Our research group has developed a PCR-based-assay that identifies the 13 most frequently found O serogroups (including O1, O2, O8, O18, O24, O25, O44, O78, O86, O88, O103, O117 O119 and O186). We found that 72.2 % of isolates from diagnostic APEC, and 25.3 % of the fecal isolates were positive for one of these 13 serogroups tested. The most prevalent serogroups identified among strains of APEC implicated in disease in Georgia include O78, O24, O2 and O25 with a prevalence of 22.8, 14.3, 10, and 8.6% respectively. While serogroups O8, O44, O18, and O186 (6.4, 6.3, 4.9 and 3.2% prevalence) were most common among fecal isolates collected.

Epidemiological analysis of our data has allowed us to identify new emerging clones, such as O25 and O24 that were not previously associated with APEC before, and defining traits of the environment of the host that can potentially lead to E. coli-associated disease in production broilers in Georgia.

Interestingly we found O8 and O18 serogroups in fecal chicken isolates that are common serogroups found in E. coli associated with human urinary tract disease.

With better knowledge of the populations of microbiota present, we can better predict vulnerable flocks and identify strategies for collibacilosis control at the farm level. Having a better knowledge of the current APEC causing disease we can identify new potential targets for vaccine development which could potentially reduce losses for producers in Georgia and elsewhere.

Key Words: APEC, AFEC, O serogroup, Colibacillosis, isolates

P240, **A tale of two Kentuckys: the case for increased surveillance of Salmonella serotype Kentucky** Nikki Shariat*, Bryan Larsen, Jenny Nicholds *University of Georgia, PDRC*

Salmonella serotype Kentucky is the most common serotype isolated from broilers, but is rarely associated with salmonellosis in the United States, unlike other serotypes found in poultry, such as Enteritidis and Heidelberg. Outside of the United States, including Europe, Kentucky is also associated with poultry but with a higher association with human illness. There are two Kentucky lineages, Group I (Kentucky-I) and Group II (Kentucky-II), that share antigenic O and H profiles, but which are otherwise genetically distinct. Antibiotic susceptibility testing revealed that the two groups exhibit distinct antibiotic resistance profiles, with Kentucky-I resistant to penicillins and cephems, and Kentucky-II resistant to quinolones. This suggests the two groups have different environmental, zoonotic, or geographical reservoirs.

The experimental goals were to examine the prevalence of these two lineages as they relate to poultry and to human illness. CRISPR Typing distinguishes between the two groups and this analysis of a collection of 40 Kentucky human clinical isolates shows equal numbers of Kentucky- I and -II isolates. We show that Kentucky-I, but not Kentucky-II, is frequently associated with domestic food animals, including poultry. A total of 100 Kentucky whole genome sequences isolated from broiler carcasses at processing that were submitted by FSIS to NCBI were downloaded and assembled. These were screened bioinformatically for their CRISPRs; the majority, 96%, were Kentucky-I, and 4% were Kentucky-II. We developed a CRISPR-based qPCR assay that was able to rapidly distinguish between the two Kentucky groups; this was used to screen 100 diagnostic samples of Kentucky, identifying a single Kentucky-II isolate. Finally, CRISPR-SeroSeq is a next-generation sequencing tool that reveals whole *Salmonella* serotype populations in individual samples. Using this population analyses, we identified two instances of Kentucky-II in two different processing facilities, and in both cases, the frequency of Kentucky-I was greater than Kentucky-II.

Given the association with human illness, Kentucky-II frequency should be closely monitored. We present three approaches to surveying Kentucky populations, and data showing that the Kentucky-II lineage is found in domestic poultry.

Key Words: Salmonella Kentucky, CRISPR, Broilers

P241, **Antibacterial effects of green tea extract on multidrug-resistant Salmonella spp strains isolated from commercial poultry** Sierra Muzquiz*[1UG], Temitayo Obanla[1], Devendra Shah[2], Yewande Fasina[1] [1] *North Carolina A&T State University,* [2] *Washington State University*

Acquired resistance to in-feed antibiotic growth promoters continues to be an important problem in the poultry industry, thereby necessitating continuous search for alternatives. Green Tea (*Camellia sinensis*) contain antioxidant polyphenols (catechins) with antibacterial activity. An experiment was conducted to evaluate the potency of Green Tea extract (GTE) against multidrug-resistant *Salmonella* (SAL) strains isolated from poultry flocks. The impact of graded GTE concentrations (0 to 100 mg/mL) on the growth kinetics of four strains of *SAL* (*S.* Kentucky*, S.* Enteritidis*, S* Typhimurium *and S.* Heidelberg) was determined. Antimicrobial activity of GTE was assessed on SAL strains using broth dilution method. Bacterial culture was adjusted to McFarland standard 1, then exposed different concentrations of GTE. Growth kinetics was determined by measuring turbidity at 2 h intervals over an 8-hour period. The number of viable *Salmonella* colonies was then estimated by plating culture mixture on Xylose lysine tergitol 4 agar, incubating at 37°C for 24 h, and counting suspect black colonies. The minimum inhibitory concentration (MIC) of GTE was also determined using a serial microdilution method with tetrazolium violet as indicator of growth. Results indicated that GTE reduced the growth of all SAL strains at each concentration tested. The MIC value of GTE for all SAL strains was 12.5mg/mL, and there was about 2-log reduction ($P <$

0.001) in SAL population at this concentration. It was concluded that GTE possess antibacterial activity against *Salmonella* spp.

Key Words: Green Tea Extract, Salmonella spp., Poultry, Bacterial growth kinetics, Minimum Inhibitory Concentration

P242, **Correlation of Salmonella infantis Enumeration of Bootsocks, Ceca, Liver/Spleen, to Carcass Rinses from Broiler Chickens** Virginia Baxter*[1], Angela Crawford[1], Charles Hofacre[1], Roy Berghaus[2], Manuel Da Costa[3], Kalen Cookson[3] *[1] Southern Poultry Research Group, [2] University of Georgia, [3] Zoetis Global Poultry*

Salmonella is the most commonly reported bacteriological agent of human foodborne diseases. The CDC estimates *Salmonella* to cause 1 million illnesses, 380 deaths per year, and the cost associated to be 2.7 billion dollars annually. In the U.S. broiler industry, the regulatory approach to foodborne pathogen control is focused primarily on processing plants. It has been determined that contamination of poultry carcasses by fecal material and enteric pathogens are most likely to occur during processing. Understanding the load and prevalence of *Salmonella* pre-harvest may be a key role in reducing carcass contamination. In 2013, Berghaus et al. found in a farm study that *Salmonella* prevalence and load of bootsocks was correlated to the *Salmonella* load in whole bird rinses with feathers on. In this 48 pen study, birds were sampled to evaluate the correlation of *Salmonella* numbers in ceca, liver/spleen, carcass rinse, and bootsocks. On day 31, fifteen of the twenty-five birds per pen were challenged with $8x10^8$ CFU/bird *Salmonella infantis.* The remaining birds were tagged and were the horizontal (indirect) challenged. On day 37, bootsocks from each pen were collected. On day 40 and 41, after an eight hour feed withdrawal, five tagged and five non-tagged horizontally challenged birds per pen were cooped and processed. After scalding, the carcass rinse (hot rehang), ceca, and liver/spleen were collected from the ten birds. The data from this study was used to evaluate the correlation of ceca, liver/spleen, and bootsock samples to carcass rinse samples. There were statistically significant correlations between individual bird ceca and liver/spleen MPN ($P<0.001$), individual carcass rinse and liver/spleen MPN ($P=0.042$), pen level ceca and liver/spleen mean MPN ($P=0.002$), and pen level carcass rinse and liver/spleen mean MPN ($P=0.005$). Enumeration of *Salmonella* level in internal organs can be an indication of an intervention's effectiveness to lower the load of *Salmonella* entering the processing plant. This data shows that liver/spleen enumeration could be used to predict the incoming load of *Salmonella infantis* in the ceca and carcass rinses.

Key Words: Salmonella

P243, **Evaluating the potency of ginger root extract against multidrug-resistant Salmonella spp strains isolated from commercial poultry** Caitlin Moore*[1UG], Temitayo Obanla[1], Devendra Shah[2], Yewande Fasina[1] *[1] North Carolina A&T State University, [2] Washington State University*

It is undeniable that emergence of antimicrobial resistance is a major threat to the development of poultry industry globally, therefore prompting the search for alternative natural feed additives. Ginger (Zingiber officinale) contains gingerol, a naturally occurring phenol which disrupts the cell wall of bacteria, thereby causing cytoplasmic leakage and death. This study was conducted to ascertain the effectiveness of ginger root extract (GRE) against multidrug-resistant Salmonella (SAL) strains isolated from poultry flocks. The antimicrobial effect of different GRE concentrations (0 to 100 mg/mL) on the growth kinetics of four strains of SAL (S. Kentucky, S. Enteritidis, S Typhimurium and S. Heidelberg) was determined using broth dilution method. Specifically, bacterial culture was adjusted to McFarland standard 1 and exposed to different concentrations of GRE over an 8-hour period. Impact on growth was determined by measuring turbidity at 2h intervals. The population of viable cells was determined for after 8h of exposure by plating culture mixture on Xylose lysine tergitol 4 agar, incubating at 37oC for 24 h, and counting suspect black colonies. Minimum inhibitory concentration (MIC) was determined using a serial microdilution method with tetrazolium violet as indicator of growth, and results were analyzed using one-way ANOVA and Tukey tests. Results showed that GRE inhibited growth of all SAL strains in a dose- dependent manner over an 8-hour exposure period. Approximately 3-log reduction in SAL population was observed at the MIC concentration of 6.25mg/mL determined for GRE ($P < 0.001$). It was concluded that GRE possess antibacterial activity against Salmonella spp.

Key Words: Ginger Root Extract, Salmonella spp., Poultry, Bacterial growth kinetics, Minimum Inhibitory Concentration

P244, **Mucosal immunity of broilers vaccinated with an oral polyanhydride-nanoparticle based Clostridium perfringens vaccine expressing Salmonella enterica flagella** Nour Ramadan*[2GS], Sankar Renu[1], Ramesh Selvaraj[2] *[1] Ohio State University, [2] University of Georgia*

Two-separate studies were performed to analyze the protective effects of an oral polyanhydride-based Clostridium perfringens nanoparticle vaccine (PNP) loaded with inactivated Clostridium perfringens crude supernatant protein and surrounded by Salmonella enterica flagella. Study A constituted a complete randomized design of 3 treatments with 6 replicates. Day-hatched male Cobb-500 broilers (n=30) were orally gavaged with PBS (mock-vaccination) or 50ug/0.5ml of polyanhydride-based C. perfringens inactivated supernatant toxin vaccine (PN) or polyanhydride-based C.perfringens inactivated supernatant toxoid vaccine (PT). Boosters were administered at 3, 7- and 14-days post-hatch. Study B comprised a complete randomized design of 2 treatments with 6 replicates (n=6): Unimmunized or PN-vaccinated. Results of study A and B demonstrated no significant differences in BWG and FCR between treatments. Similarly, no significant differences were observed in ELISA IgA and IgY-specific anti-CP supernatant protein. Results of study A; ex-vivo toxin neutralization revealed significantly higher percentage ($P=0.0024$) of neutralizing antibodies in PT treated broiler sera compared to PBS and PN. No significant differences were observed in ELISA IgA and IgY-specific anti-Salmonella flagellin protein between treatments at different ages. The proliferation of T-cells stimulated with 0.05, 0.1, 0.25 and 0.5mg/ml of antigen was significantly increased ($P<0.05$) in PN vaccinated birds via splenocyte recall response. In study B, higher percentage ($P=0.0487$) of CD4+CD8+ population was found in caecal tonsils of PN vaccinated birds compared to control. C.perfringens DNA load in caecal contents demonstrated a significant decrease ($P= 0.0220$) of CP bacterial population in broilers treated with PN vaccine when compared to control. PNP-C. perfringens candidate vaccines were safe with no systemic reactions or adverse effects on bird performance and immunogenic via the oral gavage route of administration.

Key Words: Clostridium perfringens, Broilers, Vaccine, Immunity

P245, **Optimization of a Clostridium septicum bacterin-toxoid as cost-effective prophylactic for Turkey Cellulitis/Dermatitis** Aaron Forga*[UG], B. Danielle Graham, Lucas Graham, Kyle Teague, Christine Vuong, Guillermo Tellez-Isaias, Billy Hargis *University of Arkansas*

There are limited prophylactic and/or therapeutic treatment options outside of vaccination and antibiotics to control Turkey Cellulitis. An efficacious, cost-effective vaccine against *Clostridium septicum* (CS) is of significant importance to the industry. Our laboratory has shown that parenteral immunization with a formalin-inactivated CS bacterin-toxoid (CSBT) with Seppic Montanide 71 R VG water-in-oil adjuvant (SM) improves antibody response against CS α-toxin and reduces CS-associated mortalities in commercial turkeys. We conducted *in vitro* experiments to optimize antigenic α-toxin production (Exp 1) and a comparative study to determine if a conventional oil-emulsion adjuvant (CV) induced a similar immune response as SM (Exp 2). In Exp 1, α-toxin production was evaluated by fermenting small (20mL volumes) or large batch cultures (46L volumes). Selected field isolates were cultured and two isolates (CS2, CS3) were selected based on hemolytic activity of supernatants *in*

vitro. Experiments were then conducted to determine selected incubation conditions (temperature, shaking vs. static) to enhance α-toxin production. Temperature had a considerable effect on hemolytic activity (HU) in 46L cultures, with temperatures below 38.2C producing the highest titer (128HU), and the lowest titer (16HU) at 43.3C when incubated in an agitated state for 24h. In small batch cultures, shaking significantly ($P < 0.02$) reduced HU at 18h when compared to static incubation for only CS3 (shake 179.20±31.35 vs. static 256.00±0.00; n=5/group). However, similar results were not observed in large cultures indicating further issues with up-scaling antigen production. In Exp 2, turkeys (n=25 turkeys/group) were subcutaneously vaccinated (0.5mL) at 5-weeks-of-age with CSBT + CV or SM. At 7-weeks-of-age, turkeys vaccinated with CSBT + SM had significantly ($P < 0.0006$) higher sample to negative values (S/N ratio) than turkeys vaccinated with CSBT + CV (0.84±0.05 vs. 1.68±0.23 S/N ratio) indicating that vaccination with CSBT + commercial adjuvant induced a more robust immune response. Further studies are to be conducted to consistently elevate large-scale α-toxin production and identify the effects on immune response of CSBT in conjunction with commercially available oil-emulsion adjuvants.

Key Words: Cellulitis, turkey, oil-emulsion, vaccine, Clostridium

P246, **Campylobacter jejuni intervention screening model for broilers** Nathaniel Ollis*[1], Charles Hofacre [1], Virginia Baxter [1], Roy Berghaus [2]
[1] Southern Poultry Research Group, [2] University of Georgia

While *Campylobacter* doesn't always have a significantly negative effect on broilers, it is a major cause of foodborne illness in humans. There are increasing efforts to find interventions to reduce *Campylobacter* in broilers. To screen these products quickly before going into a forty-two day floor pen study, a battery cage challenge model was developed. In this challenge model, birds were divided into two treatments of high and lower challenge doses and placed in battery cages at a day of age. At placement, ceca and liver/spleen samples were taken from five birds to screen for *Campylobacter jejuni and C. coli*. The birds were gavaged with the J.B. strain of *C. jejuni* at seven days of age. To determine the correct dose for a seven day old broiler, two challenge levels were used. Treatment one received a dose of $1x10^3$ CFU/chick, while treatment two received a dose of $1x10^5$ CFU/chick. Cloacal swabs were collected from 20 birds per treatment on day of age 14, and from 10 birds per treatment on day of age 23. On days of age 23 and 29, ceca samples were aseptically collected from 20 birds per treatment. The birds sampled at placement were all negative for any detectable *Campylobacter*. The *Campylobacter* prevalence in cloacal swabs did not differ significantly by challenge dose (P=0.302) or by day of sample collection (P=0.654). The *Campylobacter* prevalence in ceca also did not differ significantly by challenge dose ($P = 0.668$), while the effect of day could not be statistically evaluated because none of the day 29 samples had a negative culture result. The counts of *C. jejuni* in ceca did not differ significantly between treatments (P=0.318), however there was a significant effect of day of collection ($P<0.001$) with day 29 being higher. This model was effective in determining an in vivo method to successfully colonize *C. jejuni* and for testing products. The next steps in evaluating the effectiveness of a *Campylobacter* intervention in broilers, would then be a larger scale floor pen study to go to a processing age.

Key Words: Campylobacter, model development, screening

Environment, Management and Animal Well-Being, Stress Responses, Behavior

P247, **Determination of some qualitative descriptors of the Nigerian Fulani chickens from four agro-ecological zones of origin** Akinniyi Dare*[1], Emmanuel Sonaiya [2]
[1] Center for Avian Resources Development and Services, [2] Obafemi Awolowo University

This study was carried out with a goal of determining some qualitative descriptors of the Nigerian Fulani chickens (NFC) from four different Agro-ecological zones of origin.

Two hundred and forty adult NFC comprising 30 males and 30 females from each of four different agro-ecological zones of origin (AEZO); Sudan Savannah (SS), Guinea Savannah (GS), Derived Savannah (DS) and Humid Forest (HF) were used for the study. Data on 11 qualitative traits were collected by visual observations. These includes Eye color (EC), Skin color (SkC), Feather structure (FS), Feather distribution (FD), Ear lobe color (ElC), Beak color (BC), Nail color (NC), Shank color (SC), Plumage color (PC), Skeletal variation (SV), Comb type (CT).

Data were analyzed with Chi-square statistics of SAS. The Kruskal-Wallis test, a non-parametric test was used in assessing for significant differences in the qualitative traits across AEZO. At all level of analysis, a significant level of $p < 0.05$ was used.

The results from this study shows that the AEZO has no significant effects on all traits in males NFC. However, in females, the AEZO has a significant effect on FD, ElC, SC and PC. The NFC was predominantly orange eyed (100 %), white skinned (100 %) with a predominant normal FS (99.17%) irrespective of sex. Black BC was predominant with frequencies of 60.83 % in males and 60.00 in females. The predominant NC was white (82.50 % in males and 80.00 % in females). The CT was predominantly single (87.50 % and 85.00 % in males and females, respectively), while the SV was also predominantly normal with frequency values of 86.67 % in males and 87.50 % in females.

The study concluded that the Nigerian Fulani Chickens can be qualitatively described as having orange eyes, white skin, normal feather structure and distribution, black beak color, white nail color, single comb type, normal skeletal variation, plumage color was predominantly red, ear-lobe color was predominantly white, while shank color was yellow irrespective of sex. There are more phenotypic diversity in NFC females than in males due to the effect of their ecological environment. This is likely to be adapted strategies in coping with the physically induced stress of their diverse natural environment.

Key Words: qualitative, descriptors, chickens, agro-ecological, variations

P248, **Effect of feeding spray-dried plasma in broilers raised under heat stress conditions** Jared Ruff*[1UG], Thaina Barros [1], Joy Campbell [2], Ricardo González-Esquerra [2], Christine Vuong [1], Sami Dridi [1], Elizabeth Greene [1], Billy Hagtis [1], Guillermo Tellez [1]
[1] University or Arkansas, [2] APC, Inc.

Feeding spray-dried plasma (SDP) reduces gut permeability in mammals subjected to stress or disease challenges. We aimed at studying the effect of feeding SDP to broilers using heat stress (HS) as a model to impair gut permeability and bone health. Twelve environmental chambers with two pens of 20 chickens each were used. A group of birds was kept at thermoneutral conditions (TN-Control); a second group was subjected to HS and fed a Control dietary program (HS-Control); and a third group were kept under HS and fed SDP fed at 2, 2 and 1% for the starter (1-10 d), grower (11-28 d) and finisher (29-42 d) periods. Diets were corn-SBM-DDGS based and had similar nutrient profiles across treatments. A smooth temperature reduction (from 32°C to 24°C) was imposed in all chambers from 1 to 21 d of age. From then on, constant temperatures of 24 or 35°C were imposed for the TN and HS chambers, respectively until 42 d. Core body temperature was monitored with a Thermochron temperature logger.

Two birds per pen randomly selected were orally gavaged with Fluorescein isothiocynate-dextran (FITC) at 21, 28, 35 and 42 d of age. Serum FITC levels were assayed one hour later. Tibias were removed on days 21 and 42 (before and after HS) to assess bone breaking strength. Feeding SDP improved BW at 11 d of age (P<0.05). Core body temperature increased in birds under HS vs TN as soon as 2 hr after initiating HS; and decreased BW, feed efficiency and feed intake at 28, 35 and 42 d of age vs TN (P<0.05). Birds under HS showed greater BW at 28 d (1335 vs 1260 g) and 42 d (1850 vs 1715 g) when fed SDP vs a control dietary program (P<0.05). HS increased intestinal permeability, as indicated by higher serum FITC levels (P<0.10), and reduced bone breaking strength (P<0.06) while feeding SDP reduced intestinal permeability and increased bone breaking strength in birds housed under HS. Subjecting chickens to chronic HS proved to be a robust model to induce leaky gut. Bone health was also negatively affected by HS. Feeding SDP to heat-stressed chickens can help in reducing its negative effects on gut permeability, bone health, and performance.

Key Words: chickens, inflammation, heat stress, intestinal permeability, leaky gut

P249, **Evaluating the effect of a concentrated yeast fermentation product on performance and stress response of broiler chickens** Zach Heinsohn*[1UG], Eric Sobotik [1], Gabrielle House [1], Austin Stiewert [1], Hilary Pavlidis [2], Gregory Archer [1] *[1] Texas A&M University, [2] Diamond V*

A study was conducted to evaluate the effect of a concentrated yeast fermentation product on performance and stress response of broiler chickens. Day-old Cobb 500 male broiler chicks were randomly allocated to one of two dietary treatments: T1, Control (CON); T2, Concentrated yeast fermentation product (CYFP, 0.625 kg/MT). On d18, feed was removed from all pens, and litter temperature was increased (32°C35°C) for a period of 12 hours. On d19 and d42 blood was collected from 5 birds/pen. Blood was analyzed for plasma corticosterone (CORT) and heterophil to lymphocyte ratio (HL). All birds were weighed on d18, d19, d28, d35, and d42 to obtain performance parameters: body weight (BW), feed consumption (FC), and feed conversion ratio (FCR). All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at $P \leq 0.05$. On d19 CYFP birds had lower ($P \leq 0.05$) CORT (5320.3 ± 804.1 ng/ml) and HL (0.14 ± 0.01) than the CON birds (9049.6 ± 1464.7 ng/ml and 0.21 ± 0.01, respectively). On d42 CYFP birds had lower ($P \leq 0.05$) CORT (1623.8 ± 177.8 ng/ml) and HL (0.74 ± 0.04) than the CON birds (2920.2 ± 721.1 ng/ml and 1.05 ± 0.06, respectively). There were no differenced observed in mortality or in BW between treatments ($P > 0.05$). However, CON birds had a higher ($P \leq 0.05$) FCR than CYFP through all phases of the trial. Differences in FC were also observed as CON birds consumed more feed than CYFP birds through all phases of the trial. In conclusion, CYFP reduced stress and improved feed conversion when compared to CON making it a viable feed additive to improve welfare and production.

Key Words: Broiler, Stress, yeast fermentation product, production

P250, **Euthanizing chicks with carbon dioxide or nitrogen gas in conjunction with various wave lengths of light** Alfred Dimas*[UG], Austin Stiewert, Gregory Archer *Texas A&M university*

A study was conducted to compare euthanizing chicks with carbon dioxide or nitrogen gas in conjunction with various wave lengths of light. Two experiments were conducted. Experiment 1 was conducted using day old layer chicks and Experiment 2 used day old broiler chicks. Chicks were euthanized using carbon dioxide (CO2) or Nitrogen (N) gas at displacement rate of 20%. Chicks were euthanized under one of the following lighting displacements green (G), blue (B), red (R), white (W), or dark (D). All data was analyzed via One-Way ANOVA using the GLM model (Minitab Software) with treatment means deemed significantly different at P<0.05. Treatment means were determined to be significant were further separated using Fishers LSD Test. In Experiment 1, layer chicks euthanized using N (55.48±0.43) took longer (P<0.05) to lose posture than those euthanized with CO2 (23.68±0.16).Cessation of movement was greater (P<0.05) in layer chicks euthanized with N (145.81±1.08) compared to CO2 (78.12±0.52). Greater vocalizations (P<0.05) were observed in layer chicks euthanized with N (99.12±21.48) compared to CO2 (7.25±0.24). Cessation of movement was greater (P<0.05) in layer chicks euthanized using D (117.43±3.49) compared to G, R, B, and W (110.74±1.29). In Experiment 2, loss of posture was greater (P<0.05) in broiler chicks euthanized using N (57.92±0.70) when compared to CO2 (24.21±0.17). Cessation of movement was greater (P<0.05) in broiler chicks euthanized with N (155.85±1.20) compared to CO2 (76.8±0.40). Greater vocalizations (P<0.05) were observed in broiler chicks euthanized with N (129.78±22.34) compared to CO2 (5.17±0.20). Cessation of movement was greater (P< 0.05) in broiler chicks euthanized using G and D (120.35±2.27) compared to R, W, and B (113.65±1.77). In conclusion, both layer and broiler chicks euthanized using N experienced longer latency to loss of posture, cessation of movement, and vocalizations than those using CO2. Chicks euthanized under G and D generally had longer time to death than other lighting conditions. Therefore, not only gas type but also the lighting conditions during euthanasia are important to the welfare of the chicks and the efficacy of the euthanasia.

Key Words: euthanasia, chicks, light, welfare

P251, **Sex differences in serum levels of corticosterone and cortisol in duck serum: An increase in serum corticosterone levels in hen but not drake breeder ducks in response to transportation stress.** Brooke Van Wyk [1], Daniel Shafer [2], Gregory Fraley*[3] *[1] Hope College, [2] Maple Leaf Farms, Inc, [3] Purdue University*

Some concerns have been raised recently about the assay of corticosterone vs. cortisol in poultry species. We tested the hypothesis that ducks secrete both glucocorticoids. Separation of cortisol and corticosterone was accomplished using HPLC under reversed-phased, isocratic conditions with UV-Vis detection. The retention times for cortisol and corticosterone are 7.92 and 4.72 minutes, respectively. Further we validated two commercially ELISA kits for the two glucocorticoids by first charcoal stripping duck serum in order to remove all steroid hormones. We ran serial dilutions of spiked, charcoal-stripped serum on kits of opposite glucocorticoid as well as a serial dilution using the respective ELISA buffer of the opposite assay kit. We found that the glucocorticoid standard curve in duck serum matched the respective curve in that kit's own buffer. However, when the opposite hormone was run in each kit in both duck serum or ELISA buffer, a near zero slope was obtained. These results suggested to us that each kit was specific for corticosterone or cortisol. Second, we tested the hypothesis that each glucocorticoid would have different serum levels in response to shipping stress. To test this hypothesis, we collect serum from 15 drakes and 15 hens from 2 flocks: 24 hours prior to shipping, at shipping, 24 hours after shipping, and 1 week after. Data were analyzed by 2-way repeated measures ANOVA. Results showed that serum corticosterone levels were higher ($p < 0.01$) at all time points then cortisol levels. Surprisingly, we also observed a sex difference in both glucocorticoid levels in that hens showed higher ($p < 0.01$) serum levels than do drakes at all time points. Finally, we observed that hens show an increase in glucocorticoid levels compared to drakes at the time of transport whereas no differences were observed in serum cortisol levels in either sex. Our data suggest that corticosterone may be the predominant glucocorticoid in ducks and that hens may be more sensitive to shipping stress than are drakes. The fact that both glucocorticoids are released in measurable amounts lends to the possibility that they may be differentially regulated.

Key Words: breeder ducks, glucocorticoids, stress response, duck welfare

P252, **Selecting broiler platform enrichments for commercial houses** Rodrigo Lopez*GS, Katy Tarrant *California State University, Fresno*

As broiler producers increase their usage of environmental enrichments, more information is needed to determine how these enrichments should be implemented in a conventional environment. Our group aims to identify platform designs that can act as broiler enrichments, but that also meet fast growth broiler needs, with consideration of implementation by farm employees. Platforms evaluated were constructed from plywood to mimic designs currently used in industry and approved by a third party welfare certifier. Three replicates of wooden platform prototypes were examined with the following designs: 1) slatted ramps with five vertical angles (angled), 2) 0.91 m x 0.41 m rectangles raised to seven heights (raised), and 3) 1.52 m x 1.52 m pyramids with one, two, and three levels of steps (pyramid). Platforms were weighed before being placed in a commercial broiler barn, and were weighed after removal on the day of ship-out. While data have previously indicated a broiler preference for the raised design, the purpose of this study was to determine pitfalls in designs which focuses on the ergonomics of the birds, and the workers manipulating the platforms. The pyramid platforms had the highest average weight at 36.03 ± 6.44 kg. The pyramid designs also experienced a significantly larger amount of gain from fecal and moisture content at 47.2 ± 1.9 kg versus 0.37 ± 0.75 in raised and 0.33 ± 0.89 in angled ($P < 0.001$). To control for the size of the platforms, % gain was calculated as (platform weight gain) / (platform final weight). Pyramid platforms had a significantly larger % gain at 0.56 ± 0.05, versus 0.07 ± 0.02 in raised and 0.05 ± 0.02 in angled ($P < 0.001$). The weight the pyramid platforms reach by the end of grow-out required moving with at least two people. This also caused a safety issue when moving through the broilers. While the number of flip overs were not counted in the area around these designs, workers did note an increased number in the general area of the angled ramps compared to all other designs. On one occasion, a broiler was found stuck between slate in an angled platform at d10. This information has helped us to identify best practices in utilizing platforms in a commercial broiler house, that goes beyond evaluation of broiler usage alone.

Key Words: Welfare, Behavior, Enviornment, Enrichment, Conventional

P253, **Histological changes after ventilation shut down in turkeys and broilers** Sesny Gall *North Carolina State University*

Ventilation Shut Down (VSD) is a method of mass euthanasia used in poultry production when rapid depopulation is needed, such as during highly pathogenic avian influenza outbreaks. This method consists of intentional cessation of adequate ventilation so that animals are euthanized by suffocation and hyperthermia. As VSD alone is often not sufficient to euthanize all of the birds in a typical house, it can be supplemented with additional carbon dioxide (CO_2) or heat in order to achieve 100% mortality as rapidly as possible.

The purpose of this experiment was to characterize the histopathologic changes in tissues of birds following VSD euthanasia.

Clinically normal broilers and turkeys were euthanized using VSD with or without supplemental CO_2 (VSD+CO_2)or heat (VSD+heat). Bird densities were similar to those in commercial flocks. At the end of the experiment, samples of liver, lung, spleen, brain, and breast muscle were placed into 10% buffered neutral formalin. The same organs were taken from control birds prior to VSD. Histologic sections were prepared using conventional paraffin embedding, sectioning at approximately 5µm, and staining with hematoxylin and eosin. Tissues were scored for control and experimental birds.

Greatest changes were in the lungs. Cuboidal atrial epithelial cells and vascular thrombosis occurred more frequently in VSD birds than control, VSD+heat or VSD+CO_2 birds ($p = <0.05$). Congestion was a common finding in the brain. Muscle damage including myofiber degeneration and cell swelling approached being significantly higher in VSD+heat and VSD+CO_2 birds. No changes were identified in livers and spleens.

In conclusion, changes in the lungs are consistent with prolonged hypoxia. Use of VSD alone without additional heat or CO_2 could be a potential animal welfare concern due to the microscopic changes in the lungs indicating the birds experienced prolonged hypoxia.

Key Words: Euthanasia, Histology, Ventilation shut down

Environment, Management and Animal Well-Being, House Environmental Factors and Bird Welfare/Performance

P254, **Age-related effects of feeder space availability on biochemical, enzymatical, and electrolytes parameters of broilers grown to heavy weights (> 3.0k)** Hammed Olanrewaju*, Stephanie Collier, Joseph Purswell, Scott Branton *USDA-ARS, Poultry Research Unit*

Demand and consumption of poultry meat has increased dramatically, and is expected to continue in the future due to the relative price-competitiveness compared to other meat products. Consumer concern nowadays is about rapid growth and increased production efficiency is perceived to negatively impact the well-being of the animal. Changes in the levels of various plasma biochemistry and enzyme activity biomarkers are indicators of internal organs' (heart. liver, kidney) health and overall systemic homeostasis. This study investigated the effects of feeder space availability on biochemical, enzymatical, and electrolytes parameters of broilers grown to heavy weights (> 3.0 k). A total of 1440 1-d-old chicks (straight-run) were obtained from a commercial hatchery. Chicks were equally and randomly allocated to 32 groups inside 32 pens based on feeder space treatment assigned. Treatments were four different feeder space allocations: 2.30 (Single feeder), 2.30, 4.60, and 6.90 cm/bird. In order to maintain uniform bird:feeder floor space except for the single feeder, three feeders were installed in each pen. Blood samples (3 ml) were collected from the brachial wing vein of 3 birds per pen on d 27 and 55. The collected blood samples were centrifuged to separate the plasma and used to determine the electrolytes levels, concentration of biochemical (ALB, TBILI, BUN, CREAT, TP, UA, CHOL, LDL-C, HDL, TRIG, GLUC) parameters and enzyme (ALT, ALP, AMYL, AST, CK, GGT, LDH, LIP) activities using an ACE-AXCEL automatic analyzer. Results show that only UA was affected by feeder space. However, age had significant effects on most examined variables. Plasma corticosterone was not affected by feeder space and age, indicating absence of physiological stress. The results are in broad agreement with those reported in the literature and contribute to our knowledge of blood metabolites and homeostatic variation in developing broilers. In conclusion, expanding feeder allowance does not enhance the welfare of broilers grown to heavy weights.

Key Words: Feeder-space, enzyme, biochemistry, broiler, welfare

P255, **Combining variable-rate sprinkling and evaporative pads to cool broiler birds** Yi Liang *University of Arkansas*

The objective of the study was to demonstrate the efficacy of incorporating intermittent sprinkler cooling in the evaporative-cooled and tunnel ventilated commercial houses. Although intermittent sprinkler cooling systems have been adopted gradually by integrators and contract growers, there is a learning curve in understanding and operating the two different cooling systems, namely the conventional evaporative cooling system and the new variable-rate sprinkler cooling system. Two of the four broiler houses (13.4 × 152 m) on a commercial broiler farm were evaluated in the

summer, one managed as the majority sprinkler-cooled house, the other as the majority cool cell-cooled house. Temperature and relative humidity were monitored continuously for two summer flocks, while surface temperature of birds were measured using an infrared camera at age of 28 day and 43 day. Maximum daily cooling water of the sprinkler-cooled house were 1,100 gallons/day, representing significantly lower cooling water consumption. The latent heat loss at house level dominated the sprinkler-cooled house during the summer daytime. Surface temperature of sprinkler-cooled birds averaged 3 degrees lower than those in cool cell-cooled houses. Results of the study demonstrate that sprinkler cooling can be employed effectively in modern broiler houses to support competitive bird performance.

Key Words: Broiler, sprinkler cooling, latent heat loss

P256, **Evaluation of tris phosphate carbonate Salmonella pre-enrichment media for various ingredients and feed types** Shannon Clay*[1], Kurt Richardson [1], Douglas Cosby [2], Mark Berrang [2], Nelson Cox [2] *[1] Anitox Corp, [2] USDA, ARS, US National Poultry Research Center*

Feed is a major source of *Salmonella* on poultry farms. Detecting *Salmonella* in feed is challenging due to its non-uniform distribution and it exists in a stressed state requiring resuscitation with pre-enrichment media. Studies have shown that pre-enrichment media lack the buffering capacity to prevent an acidic pH. Exposure of *Salmonella* to acidic conditions can result in cell injury, death or altered biochemical pathways preventing detection. A tris-phosphate-carbonate (TPC) pre-enrichment media has been developed to maintain a near neutral pH during incubation of feed. It has not been evaluated against a wide variety of feed and ingredients. Eight ingredient types and ten feed types were weighed out into 5g samples and individually placed into 45mL of different pre-enrichment broths: lactose broth (LB), buffered peptone water (BPW), 2xBPW, universal pre-enrichment broth (UPB), and TPC. Media were incubated for 0, 18, 24, and 48 hours at 37°C and the pH determined (five replicates/treatment). Data were analyzed by ANOVA and least significant difference T-test. For cereal grains and by-products, the initial pH of LB ranged from pH of 5.06 for DDGS to pH of 6.8 for sorghum. After 18 hrs of incubation, the pH had decreased to 4.4 to 4.6. Significant ($p<0.05$) decreases in pH were observed with longer incubation periods. BPW exhibited similar buffering capacity to that of LB. Although, 2xBPW and UPB were slightly better, the pH at 18-24 h was slightly acidic at 5.2-5.5. TPC maintained a near neutral pH during incubation. The pH of media containing vegetable protein meals decreased during incubation to pH 4.7 -5.0 in LB and BPW, 5.1 - 6.0 in 2xBPW and UPB and 5.7-6.3 in TPC. The pH of media containing meat and bone meal was near neutral for all media. The pH of media containing poultry feeds had an initial pH of 6.5 to 8.4. After 24h incubation, the pH had decreased to 4.6-5.5 for LB and BPW. More variability was observed among feed types incubated in 2xBPW and UPB where the pH ranged from 4.8-6.4 at 24h. The pH of TPC varied less among feed types ranging from 6.5-7.4 at 24h. Results suggest that TPC is able to maintain a near neutral pH during pre-enrichment for a wide variety of ingredients and feed which can improve the ability to detect *Salmonella*.

Key Words: Salmonella, pre-enrichment, feed, ingredients

P257, **Comparison of 3 phytogenic supplementations on broiler chickens raised under overheated conditions** Thibaut Chabrillat*[1], Mamadou LY [2], Camille Boroch [2], Sylvain Kerros [1] *[1] Phytosynthese, [2] UniLasalle*

Use of plant bioactives in poultry feed becomes more popular in order to improve health and welfare. This trial had the objective to compare 3 different phytogenic programs on heat challenged broilers. 1440 mixed 1day ROSS 308 broilers were used and raised in an experimental farm until D35. 4 treatments were allocated in 6 pens of 4.5m² with 60 birds. Individual weight and pens' feed consumption were recorded at 9D, D23 and D35. Heat challenged consisted to apply an overheating fixed at 24°C (75°F) from D18 to D35. A negative control group (C) was fed with a starter feed D1-9, a grower D9-23 and a finisher D23-35. It was compared to 3 experimental groups: a low level of essential oil (EO) with 60ppm in starter, 90ppm in grower and finisher (EXP1), a high level of EO (EXP2) with +30% of EO compared to EXP1 in all feeds, a low level of EO with addition of gut integrity bioactive (EXP1+curcuminoids+flavonoids) (EXP3). Anova was used for statistical analysis with HDS for live weight (LW) and Newmann-Keuls for FCR analysis. Results indicate a lower live weight (LW) at D9 for EXP2 ($p<0.01$); at D35 birds achieve 2365.14 for C, 2350.00 for EXP1, 2284.49 for EXP2, 2401.14 for EXP3. Mixed sex and heat stress have generated high LW heterogeneity; LW data were separated inside 2 groups: the 50% with the lowest LW (50LLW) and the 50% with the highest LW (50HLW). EXP3 allows a better LW ($p=0.05$) for the 50LLW instead of the EXP2 reduces the LW of 50HLW ($p<0.05$). During the D23-35, ADG were ascending classed with EXP2 (83.35 g/d), C (85.05), EXP1 (87.06) and EXP3 (89.01). For all treated groups, a strong and significant effect was observed on FCR ($p<0.05$), C achieved 1.44, 1.36 for EXP1, 1.34 for EXP2, 1.37 for EXP3. Mortality difference is not significant, we achieved on average 5.3% and 2.8% between D23-35, the overheating period. To conclude this study confirm the benefit of plant extracts on broilers performances, however uses have to be adapted: EXP1 shows a better trend before heat challenge and EXP3 gained performances during the heat challenge. In opposite, too high level of essential oils seems to be negative for the growth performances of high potential birds.

Key Words: Natural bioactives strategy, Heat, FCR

P258, **Efficacy of Phytogenic Respiratory tonic 'Animunin' in therapeutic management of CRD in poultry broiler** Vijay M*[1], Shivi Maini [2] *[1] Associate Professor,Department of Veterinary Pharmacology & Toxicology, Veterinary College,KVAFSU,Bidar (KS)-585401, [2] Deputy General manager, Indian Herbs Specialites*

Chronic Respiratory Disease (CRD) is of high economic importance as this causes heavy production loss & mortality. Mycoplasma gallisepticum(MG) & Mycoplasma Synoviae(MS) is involved in polymicrobial "chronic respiratory disease" of chicken. Various antibiotics are used for management but pose the threat of MDR, & human food safety risks. Hence, the need of the hour is to find suitable alternatives for alleviation of sub-clinical infections and prevention of local pulmonary colonization of respiratory pathogens. A study was conducted to assess efficacy of polyherbal respiratory tonic Animunin®(M/S Indian Herbs Specialites, India) in Krisbro chicks positive for MG-MS. Day old broiler chicks from infected parent flock were obtained from ICAR Directorate of Poultry Research, Hyderabad, India. Chicks were screened for MG-MS infection at day 0. Positive 150 chicks for MG-MS infection were allocated to 3 groups(n=50). T0 being control group wasnt given any treatment; T1: Animunin @ 40 ml in drinking water/ 100 birds for 10 days (from day 16th-25th) ; T2: Tiamutin @ 25mg/kg BW for 5 days from 16th -20th day. Choanal cleft swabs were collected for identification of Mycoplasma by PCR method.MG/MS tires were recorded on day 0, 21st & 42nd . MG-A mean tires were 3136 in control, 1270 (59.5% reduction) in Animunin supplemented group T1, 1591(49.26% reduction) in Tiamulin supplemented group T2. MS-A mean tires were 9846 in control, 462 (95.5% reduction) in Animunin supplemented group T1, 683 (93% reduction) in Tiamulin supplemented group T2. Pulmonary histological changes were mild in T1 & T2 and severe in T0 revealing beneficial effectS of treatments. Airsacculitis score was significantly higher in group T0 (23) and lowest in T1 (8). Mortality was significantly lower in treatmentS (16% & 8% reduction in T1 & T2 than control). Mean Body weight at 6th week was significantly higher in Animunin supplemented group (2187.04g) followed by 2081.52g in Tiamulin supplemented group than infected control (1786.81g). trial results demonstrates that Animunin is as efficacious as Tiamulin for therapeutic management of CRD in birds. Animunin is

unique combination of essential oils and herbal bioactives that do not exert any disadvantages of antimicrobial resistance and residues in meat.

Key Words: Mycolasma, respiratory, antibiotic resistence, titre, phytogenic

P259, **Disinfecting persistent Salmonella and associated dry-surface biofilms following vaccine challenge studies in poultry isolation chambers** Taylor Kindlespire, Sonja VanHolland, Kris Skorseth, Jaydin Rowbotham, Katelyn Groetsch, Melissa Malakowsky, Brianna Beckler, Sara VanderWal*, Kipp Erickson *RTI*

Vaccine development often requires challenge studies to be performed in bio-secure isolation chambers, where challenge pathogens can be administered. *Salmonella* strains can be quite robust, often capable of forming dry-surface biofilms, long-lived and very resistant to disinfection. This study evaluated methods of scrubbing vs. no scrubbing, various disinfection agents, and biofilm indicator gels on ability to decontaminate surfaces. Subsequent to a 7-week *Salmonella* challenge study conducted in six poultry HEPA isolators (~40 birds/chamber) we explored two different disassembly/cleaning approaches and three different disinfection agents. Pairs of isolators were either: 1/ disassembled and hand-scrubbed or 2/ remain assembled and hand-washed, all followed by three spray disinfectant schemes using 1/ Dish Soap, PI Quat, 2/ Biosolve Plus, PI Quat, 3/ Sterilex, all followed by Bleach and Virkon S disinfectants. After drying, all isolators were closed, tested for biofilm presence (Indicon Gel), powered up to 90°F and humidified for one week, after which swabs were collected for *Salmonella* enrichment and recovery. Several surface areas were swabbed within each isolator and all tested positive for *Salmonella* prior to cleaning. After cleaning and drying (room temp), every isolator tested negative for *Salmonella*, however after 90°F incubation and humidification for seven days, the three isolators that were not disassembled and hand-scrubbed (sprayed/wiped/power-washed only) tested positive for *Salmonella*. The three isolators that were disassembled and hand-scrubbed, regardless of disinfection agent, tested negative. Minimal hand-scrubbing to a visible clean only is not adequate to disinfect against *Salmonella*. Disassembling isolators and hot water, hand-scrubbing with abrasive brushes/sponges, disinfection agents and use of biofilm detection is required to rid putative and non-visible dry-surface biofilms, capable of growth following heated incubation.

Key Words: Salmonella, Biofilms, biosecurity, disinfection

P260, **Plasmid and antibiotic resistance genes present in the ceca of broiler chickens infected with Eimeria spp** Jodie Lawrence*[1], Adelumola Oladeinde [1], Alberta Fuller [2], Romdhane Rekaya [3], Samuel Aggrey [2] *[1] U.S. National Poultry Research Center, USDA-ARS, [2] Poultry Science Department, University of Georgia, [3] Department of Animal and Dairy Science, University of Georgia*

Eimeria is a genus of parasites that can cause coccidiosis in poultry. The onset of this disease has been characterized with an imbalance in the microbiome of chickens infected, however little is known of the resistome (antimicrobial resistance genes and plasmids) associated with coccidiosis. The goal of this study was to investigate changes in the broiler gut resistome that are correlated with *Eimeria* infection. To address this, male chicks of equal numbers of Cobb500 and Athens Canadian Random Bred (ACRB) genotypes were raised in coccidian free rooms under standard husbandry management. Cobb500 is a commercial meat-type strain developed by Cobb Vantress Inc., which has been selected for growth and feed efficiency. The ACRB is an unselected control population that has been compared with commercial broilers in numerous studies. At 14 days of age, the chicks were randomly assigned to 6 treatments groups in a 2 x 3 factorial design, with 7 replicates per treatment, and 10 birds per replicate. The treatment effects consisted of bird genotypes (ACRB or COBB) and three infection levels (oral gavage of sporulated *E. acervulina* oocysts, mixed culture of sporulated *E. acervulina, E. maxima* and *E. tenella* or distilled water). Birds were fed on a non-medicated grower diet and given food and water ad libitum. At 6 days post infection (dpi), 5 birds from each treatment group were randomly sampled and their ceca were removed and frozen for further analysis. DNA was extracted from 250 mg of cecal contents and used in TaqMan®-based real-time qPCR assays targeting a total of 39 genes including 27 antibiotic resistance genes/plasmids, total bacteria (n =1), *Campylobacter* spp. (n = 3), *Salmonella* (n =1), *E. coli* (n =2), *Enterococcus* spp. (n =2) , *Staphylococcus* (n =1) and *Clostridium* (n =2). A principal component analysis revealed that the concentration of total bacteria, *E. coli* and antimicrobial resistance genes/plasmids (*bla-TEM*, *ermB* and IncI1) could explain ~ 64.2 % of the total variability in our data. This observed variability was significantly influenced by bird genotype rather than the *Eimeria* strain used for infection. This result suggests that bird genotype and *Eimeria* infection may perturb the chicken gut resistome in different ways.

Key Words: Eimeria, Antibiotic resistance, Gut microbiome, Coccidiosis, Bird genotype

P261, **Effects of housing environment and laying hen strain on tibia and femur bone properties.** Milan Sharma*[1GS], Dima White [2], Woo Kim [2], Pratima Adhikari [1] *[1] Mississippi State University, [2] University of Georgia*

An experiment was conducted to determine the effect of housing environment (HE) and laying hen strain (HS) on tibia and femur bone properties: tibia breaking strength (TBS), tibia ash percentage (AP), femur bone mineral density (BMD), femur bone volume percentage (BVP), femur trabecular thickness (TbTh) and porosity percentage (PP). The experimental design was completely randomized design (CRD) in a 3 × 2 factorial arrangement of three HE (conventional cages (CC), enriched colony cages (EC) or cage-free free-range (CF)) and two HS (Hy-Line Brown (HB) and Hy-Line W36 (HW)). Six left tibias from each treatment were analyzed every seven wks (38, 45, 52, 59, 65, 72, 79 and 85 wks of age) at eight different time points whereas the same number of femur was analyzed only at the end of the trial. Instron machine was used to determine the TBS, and fat-free soxhlet procedure was used to measure the AP from previously broken tibias. A 3-D image was generated using x-ray microtomograph to analyze the bone properties of the femurs. Data were analyzed as a CRD with a split-plot in time. The main effect of HE ($P = 0.0005$), HS ($P < 0.0001$), and age of the birds ($P = 0.0026$) was observed for TBS. The highest TBS was observed in CF and lowest in CC, with HW having higher strength than HB. The TBS increased with the age of the birds with the highest observed at 72 wks of age. An interaction among HE and age ($P = 0.0048$) and HS and age ($P = 0.0005$) was observed for AP. The AP was higher in both EC and CF than CC at wk 79, but on wk 85, EC and CC had highest AP than the CF. Similarly, HW had higher AP at wk 45 than HB, but on wk 79 and 85, HB had the highest AP. The effect of HS was observed for total BMD ($P = 0.0350$), which was higher in HW than HB. Cortical BMD ($P < 0.0001$) and BVP ($P = 0.0030$) were higher in the HW than HB, whereas bone PP ($P = 0.0030$) was higher in HB than HW. There were no differences between HS and HE for trabecular BMD or TbTh ($P > 0.05$). These findings indicate that the CF and EC had positive effects on measured tibia bone properties. Laying hen strain but not the HE, had effect on femur bone properties. However, to fully understand the effect of HE on femur bone properties, longitudinal study should be done at different time points using different HS.

Key Words: bone properties, housing environment, laying hen strain, x-ray microtomograph

P262, **Housing environment and laying hen strain impacts on cloacal microbiology and incidence of avian pathogenic Escherichia coli (APEC) in the late phase of production** Milan Sharma*, Li Zhang, Aaron Kiess, Pratima Adhikari *Mississippi State University*

Avian pathogenic Escherichia coli (APEC) is well-known for causing avian colibacillosis and is associated with the economic losses in the poultry

industry. The current study was aimed to evaluate the effects of the housing environment (HE) and laying hen strain (HS) on cloacal microbiology as well as the incidence of APEC genes. The experiment was conducted in three HE (conventional cages (CC), enriched colony cages (EC), or cage-free free-range (CF)) with two HS (Hy-Line Brown (HB) and Hy-Line W36 (HW)) from 72 to 85 wks of age. Cloacal swabs were collected at 72, 79 and 85 wks of age and cultured in TSA for aerobic and anaerobic bacteria, and in EMB for coliforms. Potential E. coli colonies from EMB were isolated on MacConkey agar and confirmed for the presence of E. coli by PCR amplification of ybbW gene. These positive E. coli isolates were screened further for the presence of five major virulence genes (iroN, ompT, hlyF, iss, and iutA). Data were analyzed by using a completely randomized design with split-plot in time. An interaction among HE and age of the birds was significant for cloacal aerobes (P = 0.0001), anaerobes (P = 0.019), and coliforms (P = 0.0068). The total aerobic bacterial count for CC increased from 72 to 85 wks and became similar to the CF and EC at 85 wks of age. Total anaerobic bacterial count for EC decreased, while it increased for CC at 85 wks of age and became similar to CF. The coliforms count was higher in the CF than both CC and EC at 72 wks of age, but on 85 wks, the count was similar between all three HE. The incidence of the E. coli was found higher in the CF than CC and EC (P = 0.033), but there was no difference for APEC (P > 0.05). The incidence of virulence genes, ompT (P = 0.019) and hlyF (P = 0.0164) were higher in CF than CC and EC. The incidence of APEC (P = 0.0216) as well as the incidence of virulence genes ompT (P = 0.0537), hlyF (P = 0.0433), iss (P = 0.042) and iutA (P = 0.0448) were higher in the HB than HW. It can be concluded that the HE and HS affect the cloacal microbiology and the incidence of the virulence genes for APEC. These results challenge us to further investigate the incidence of APEC with respect to bird health and its significance towards food safety in alternative HE as well as different HS.

Key Words: cloacal microbiology, Escherichia coli, housing environment, hen strain

P263, **Feed intake by broiler breeder pullets is altered by dietary fatty acid profile and mild stress.** Nikolas Faust*GS, Kristen Moncada, Tara Price, Rosemary Walzem, Marvin Vides, Kimberly Gardner *Texas A&M University- Department of Poultry Science*

Broiler breeder pullets are feed restricted at ~ 3 weeks post-hatch to improve livability and chick yield. Management strategies that reduce voluntary feed intake are of interest. Dietary fatty acid profile influences feed intake; but effects may be lost with stress. We tested the effect of altering dietary fatty acid towards a Mediterranean (MED) profile in breeder pullets in the presence and absence of mild stress during a 2wk interval post-hatch.

Twenty groups of similar starting body weights (BW) were formed using 100 day-old, ROSS 708 breeder pullets. Groups were randomly assigned to one of 4 treatments (n =5 groups/treatment). Group was housed in separate battery compartments (pen) within two 6-tier battery cages for the first 7 days post hatch. Birds were then further divided into pairs day 8 - 14. The 4 treatments were 1) MED diet, 2) Usual diet, 3) MED diet + stress (MED+), 4) Usual diet + stress (Usual+). Stress was introduced by daily handling to administer 1 ml of a .125% gelatin solution by gavage. The diets were formulated in accord with recommendations from Aviagen for ROSS 708 pullets. Daily feed intake was measured for each pen. Individual BWs were measured weekly. We report 14-day BWs (n = 24/treatment) and per pen feed intake day 8-12 (n=12/treatment). Two-way ANOVA (JMP) tested the main effects of diet and handling with Tukey-Kramer post-hoc means testing. Values are means ± SEM. The day 14 BWs were 174.9A, 156.8AB, 148B and 143B grams for Usual, Usual+, Med and Med+, respectively. The main effects of both diet (p< 0.0004) and handling (p< 0.038) was significant (adj. R2 = 0.148). Average feed consumption was 308.6A, 306.1AB, 257.2BC and 217.1C grams for Usual, Usual+, Med and Med+, respectively. The main effect of diet (p< 0.0001) was significant, but handling (p = 0.12) was not (adj. R2 = 0.38). The results show that voluntary feed intake was reduced dietary fatty acid profile modification; i.e. increased monounsaturated fatty acids in the MED diet. Handling stress did not affect feed intake but did limit BW. BW decreased with stress by 11% in the Usual diet and by 3.4% in pullets fed the MED diet. Studies are needed to determine the effects on egg production and hatchability. Agrilife project no. 9778 & NIFA-AFRI 2019-67015-29708 (RLW).

Key Words: Appetite, Dietary fatty acids, Welfare, Breeder, Performance

P264, **The Effect of Low pH Formic Acid Salts on Litter Performance** Kaicie Chasteen*GS, Kenneth Macklin, James Krehling, Luis Munoz, Adian Talorico *Auburn University*

In the poultry industry ammonia reduction between flocks is often accomplished through use of acidifying chemicals like sodium bisulfate. A potential alternative is sodium formate which acts as an antimicrobial. In this study the pH was adjusted to 5.5 and 4.5 in order to make it more effective in controlling ammonia($NH_{3)}$ as well. Efficacy of SF as a litter amendment was tested by placing approx. 68 kg of broiler litter in a room at 35C. 3 days before collection initial moisture % was determined and adjusted to 25-30%, activating the litter. On D0 3 kg of litter added to 20 boxes, providing 5 replicates for each of the 4 treatments. The treatments were: Control (C), Sodium Bisulfate (SB), sodium formate at pH 4.5 (SFL), sodium formate at pH 5.5 (SFH). The boxes were then inoculated with 10ml of 10^9 SE and CP and allowed to equilibrate for 1 hour. For each treatment 102mL of water (C), 102g of a commercial SB product (SB), 102mL of SFL, or 102mL of SFH was added. NH_3 measurements were performed before litter was inoculated (D0), then on D1 and D4. After NH_3 measurements litter samples were collected for enumeration of total aerobes and anaerobes, Salmonella and *C. perfringens*, pH level, and moisture %. Samples were serially diluted in PBS, then spread plated onto Tryptose Sulfite Cycloserine Agar and Xylose Lysine Tergitol 4 Agar and spot dropped on Anaerobic and Plate Count Agar for *C. perfringens*, Salmonella, total anaerobe and total aerobe, respectively. pH was determined by adding 45ml of DI-H2O to 5 g of litter and analyzing 1 hour later. Moisture was determined by drying 10g litter at 80C for 24 hours, the sample was then weighed, and % moisture calculated.

Data was analyzed using a single factor ANOVA, if significant (P<0.05), means were separated using Tukey HSD. No statistical significance was found in enumeration or moisture; nor in C vs SFH. NH_3 and pH showed significance between SB(NH_3: 3.152 ppm D1, 5.768 ppm D4. pH: 6.16 D1, 6.26 D4) and the other treatments, as well as significance for SFL (NH_3: 5.66 ppm D1, 15.66 ppm D4. pH: 7.5 D1, 8.18 D4) vs C and SFH. SB vs SFL showed no significance on D1 NH_3 but was significant on D4; for pH there was significance on D1 but not D4. Results may indicate a low pH SFmay be an acceptable substitute for SB.

Key Words: Litter, Amendments, Sodium Bisulfate, Sodium Formate, Ammonia

P265, **Defining broiler house influence on the gastrointestinal microbiome** Anh Do*GS, Gissel Pedraza, Tricia Van Laar, Katy Tarrant *California State University, Fresno*

The beneficial utilization of probiotics and prebiotics in poultry diets to influence performance traits and maintain flock health via precise bacterial community formation in the bird's intestinal tract has been the main topic of interest of numerous studies. Currently, producers in the field have been aiming to replicate the findings from these studies by applying the same formulation of prebiotics or probiotics to the diet for all flocks within the same region in an umbrella-treatment fashion. However, considering the drastic effect seasonal barn environments have on microbial diversity, it is crucial to consider unique application plans for each house in order to optimize efficacy and avoid negligible benefits from the use of costly additives, which this year-long study focuses on. Across three seasons, five

birds from each of four commercial broiler houses on the same complex were selected for testing. Broilers originated from the same breeder source and received consistent industry-standard diets. Birds were euthanized at day 35 and their ceca collected. In addition, five litter samples from each of these houses were also collected. Whole genomic DNA was extracted from all samples and the 16S rRNA gene was amplified and sequenced. Ceca samples had higher levels of diversity than litter samples as measured by observed operational taxonomic units (OTUs) (163.6 ± 23.7 and 83.6 ± 26.6, respectively $P < 0.001$) and Shannon's Diversity Index (5.9 ± 0.4 and 4.8 ± 0.4, respectively $P < 0.001$). The ceca community was significantly different from the litter community as well by weighted UniFrac ($P = 0.001$) and Bray-Curtis ($P = 0.001$) analysis. Sample diversity was not significantly different in ceca or litter samples between each house. There were no seasonal differences. As the microbial communities did not differ between house, pre- or probiotic applications do not need to be considered house specific.

Key Words: probiotics, prebiotics, microbiome, microbial, sequencing

P266, **Comparing broiler performance and paw quality between a deep carbonaceous litter system and a conventional litter system.** Garret Ashabranner*GS, Ian Brock, Joey Bray *Stephen F. Austin State University*

Poultry litter is primarily comprised of a carbon source, fecal matter and the naturally present microflora responsible for decomposition. The poultry litter management system known as the deep litter system, refers to a heavily carbonaceous litter. The premise of this method is that for every application of nitrogen excreted from the birds, an application of carbon is added to combat it, forming a compost pile like effect. Conversely, a conventional litter system uses old litter with minimal applications of carbon. This study observed bird performance and paw quality to the effects of both litter systems. Birds were weighed individually to determine average live weight at day 49. Paw quality scores were examined using the Welfare Quality® Consortium protocol (2009). Feed conversion ratio was calculated to determine the gain in weight per pound of feed fed. This study consisted of two, 100 ft.2 floor-pens stocked at a density of 1 bird/ft2 for a total of 100 birds/pen. One pen represented a conventional litter method and the other representing a deep carbonaceous bedding. After each flock both pens were de-caked and additional carbon material was added to the deep-litter pen only. This study consist of three consecutive flocks. All data was analyzed through SAS 9.4 (2013) utilizing a One-way ANOVA and Proc GLM while separating means using LS Means ($a<0.05$). Comparing the average live weight of treatments within flock one, no significance was shown through a P-value of 0.1791. However, there was a significant difference in flock two ($P = 0.0019$) and flock three ($P = 0.0002$) with the birds reared on the deep-litter system have significantly higher live weights. Feed conversion showed no significant difference among treatment during the study ($P = 0.3085$). When analyzing paw quality scores between treatments for flock one, there was no significant difference ($P = 0.2215$). Birds reared on the deep litter system had a significantly lower paw quality score when compared to birds reared on the conventional litter after flocks two ($P = 0.0009$) and three ($P = 0.0041$). The results suggest a deep carbonaceous bedding shows compensatory growth, while improving paw quality, through the maturation of the litter. This mimics similar effects observed by Kennard et al., 1959.

Key Words: Broiler, Litter, Performance, Paw quality, Carbon

P267, **Impact of feed change timing and withdrawal times on intestinal pH and performance** Garrett Powell*GS, Ariel Bergeron, Daniel De Leon, Christopher Eagleson, Audrey McElroy *Texas A&M University*

When disruption occurs within the intestinal tract consequences may arise therefore it is important to have a complete understanding. The objective of this study was to evaluate the impact of different feed change days and withdrawal times on intestinal pH and performance.In this trial 9 treatments were replicated 4 times within 36 floor pens, with a total of 900 Cobb 500 male broilers. Treatments varied in feed change day (d14, d17, d21) and time of feed withdrawal (0 hrs, 3 hrs, 6 hrs). Birds and feed were weighed on d14 and d28; FI and FCR were calculated. Mortality was recorded daily and pH of small intestine was measured at feed changes. Data was analyzed using a 3x3 factorial arrangement in a randomized complete block design. Means were deemed significantly different at $P\leq0.05$ and separated by Tukey's HSD. Feed withdrawal time from 0 hrs to 6 hrs caused pH in duodenum ($P=0.0006$) to shift to neutral and pH in jejunum ($P=0.0416$) to be more acidic. Feed change day affected pH of the ileum ($P=0.0379$) with d17 feed change reducing pH compared to d21. In the duodenum ($P=0.0079$) the affect was reversed with d21 reducing pH compared to d17. Feed change day affected FI from d14 to d28 ($P=0.0279$) with birds changing at d17 and d21 having increased FI. Throughout the trial, there was an interaction of feed change day and withdrawal time ($P=0.0086$) with birds withdrawn from feed for 3 hrs and 6 hrs at d21 having an increased FI compared to feed change at d14 with 6 hrs withdrawal. Feed change day affected FCR from d14 to d28 ($P<0.001$) and d0 to d28 ($P=0.0004$) with birds changed at d14 having a reduced FCR. Feed withdrawal time affected FCR for d14 to d28 ($P=0.0107$) and d0 to d28 ($P=0.0154$) with 3 hrs withdrawal time increasing FCR. The withdraw time and day of feed change may negatively affect the pH of the small intestine and bird performance. Integrators should consider feed change day and withdrawal time as it may lead to disturbance of the small intestine and a loss in performance which may hinders profits.

Key Words: withdrawal time, feed change day, intestinal pH, performance

P268, **Evaporative water loss from litter at varying inclusion rates of biochar and windspeeds** Matthew Rowland*UG, John Linhoss *Mississippi State University*

Improving the water holding capacity of poultry litter could help offset some of the negative production impacts associated with elevated litter moisture, such as increased ammonia levels, poor footpad scores, and increased pathogenic microbial populations. Biochar (BC), charcoal that is used for agricultural purposes, used as a litter amendment has been shown to improve water holding capacity in pine shavings (PS), but how it influences the ability of water to evaporate from litter is unclear. Evaporative water loss for BC and PS mixtures was measured using custom wind tunnels at air velocities of 0, 1.3, 2.5, and 3.6 m/s for 100 hours. BC was included in clean pine shavings at inclusion rates of 0, 10, 20, and 100% by weight and samples were wetted to initial moisture contents (MC) of 25 or 50%. Treatments were arranged in a 2×4×4 factorial design with main effects of initial MC, BC inclusion rate, and air velocity and represented by three replicate samples (24 per wind tunnel, 96 total). There was a significant main effect of air velocity ($P<0.001$), BC inclusion rate ($P<0.001$), and initial MC ($P<0.001$), on evaporative water loss. BC at inclusion rates of 10 and 20% did not significantly affect evaporative water loss when compared to clean PS. Evaporative water loss at air velocities of 2.5 and 3.6 m/s was significantly higher than the 0 and 1.3 m/s treatments ($P<0.05$). There were significant BC inclusion rate × windspeed × initial moisture content interaction effects ($P < 0.001$). At 25 and 50% initial MC, evaporative water loss was highest at a BC inclusion rate of 100% and 2.5 m/s windspeed. The ability of BC to improve poultry litter water holding capacity while not adversely affecting evaporative water loss indicates that BC shows promise as a poultry litter amendment.

Key Words: Biochar, Evaporative rate, charcoal

Environment, Management and Animal Well-Being, Production Impacts on the Environment

P269, **Water conservation and production benefits of sprinkling broilers** Jonathan Moon*[1], Jan DuBien[1], Andrew Brown[1], Yi Liang[2], Tom Tabler[1] [1] *Mississippi State University,* [2] *University of Arkansas*

Maintaining broiler comfort during hot, humid weather is critical to weight gain, FCR, flock welfare and livability. The purpose of the current study was to determine effectiveness of sprinkler technology to improve broiler performance and water conservation efforts during hot weather. During a 63 d summer flock (June-August 2019), a commercial sprinkler system (SS) was used in combination with a conventional evaporative cool cell system (CC) in two commercial broiler houses (42 x 400 ft; 13 x 122 m) each housing 16,016 Cobb 700 straight run broilers at Mississippi State University. Two lines of overhead sprinklers above the feed lines spaced evenly 20 ft (6 m) apart and 7ft (2.1 m) above the litter surface were intermittently operated to apply controlled volumes of large water droplets onto the birds. Previously, SS and CC were used in combination at increasingly higher CC set point temps ranging from 82°F (28°C; 2014) to 86°F (30°C; 2018-2019) and finally, 90°F (32°C; 2019). SS was responsible for an increasing amount of cooling as CC set point increased. During the June-August 2019 flock, sprinklers were initiated at 23 d of age. CC initiation was delayed until 45 d and allowed only when house temperature reached 90°F (32°C). Current sprinkler research is based off recent analysis of 1995-2005 University of Arkansas experimental sprinkler data analyzed as a Randomized Complete Block Design indicating a significant reduction in cooling water use (P=0.003) and average daily mortality from d 35 to harvest (P=0.035) for SS vs. CC. Pay per lb increased for SS vs. CC cooling (P=0.064). In the AR study, no differences were found for water intake, FCR, or avg wt. (P>0.05). While more research is needed, our data suggest that 63 d old broilers can maintain performance at 90°F (32°C) house temp and ~65% house humidity with reduced cooling water use when SS is the first line of cooling defense. Maintaining performance at 90°F (32°C) is likely possible only because of lower house humidity levels that SS allows vs. CC; thus assisting in respiratory evaporation. Future research should investigate feasibility of using SS alone to cool broilers and the effects on house environment (temperature, wind speed, and humidity) as well as flock welfare and water conservation.

Key Words: Broiler, Sprinkler, Cooling, Heat stress, Water usage

P270, **Pre-compost carcass reduction and mixing as a method of laying hen carcass preparation for composting during a disease outbreak** Abigail van der Swaagh*[UG], Richard Fulton, Dale Rozeboom, Melissa Millerick-May, Zac Williams *Michigan State University*

During a catastrophic disease outbreak, poultry producers need to a safe, effective and rapid means of carcass disposal. Three methods used previously include landfill, on-site burial and windrow composting of carcasses. The current USDA approved whole bird layered compost method is time consuming and labor-intensive when handling thousands of carcasses. Grinding carcasses and mixing with a carbon source has been attempted but met with concern that virus particles would be dispersed during mechanical deconstruction of carcasses. This study aimed to determine potential for pathogen dispersion during pre-compost carcass grinding and carbon mixing via mechanical mixer (ReMix). Hens were vaccinated 72 hours prior to compost with 3x dose New Castle Disease Virus (B1 Type, Lasota Strain, Live Virus) via ocular route. Approximately 800 Single Comb White Leghorn hens, weighing an average of 1.6 kg each, were used for each replication with three replications per treatment. Treatments were USDA layered method, ReMix with open top and ReMix with cover. Duration of each ReMix treatment was 10 minutes, estimated to be necessary length of time to assure carcass deconstruction. Compost windrows were formed from carcasses, dried laying hen manure, and pine shavings as carbon source with an approximate 2:1 C:N ratio. To verify shedding of virus, tracheal swabs were taken before catching and euthanasia. To determine the presence of and dispersion of virus in air, portable BGI high-volume air samplers were placed in the following locations: poultry housing room, immediately adjacent to carcass pile during loadout, one sampler on each side of the ReMix, and six samplers placed 20 feet from implements during windrow formation, two on each long side and one each end. Air samplers were set to collect TSP per EPA reference methods. Air sampling began a minimum15 minutes before the initiation of each trial and concluded a minimum of 15 minutes after the conclusion. Newcastle virus was captured during loadout in 1 of 9 trials but was not detected during mechanical carcass reduction or during the formation of nine windrows. Results of our preliminary investigation indicate mechanical destruction of carcasses may be a viable option for handling mass mortality during a virus outbreak.

Key Words: Compost, Mass mortality, Carcass reduction, Virus dispersion, Laying hens

Metabolism and Nutrition, Amino Acids

P271, **Effects of varying levels of DL-methionine and TSAA on [13C] Met and [13C]Cys oxidation** Jordan Weil*[1], Pramir Maharjan[1], Antonio Beitia[1], Katie Hilton[1], Nawin Suesuttajit[1], Victor Naranjo[2], Craig Coon[1] [1] *University of Arkansas,* [2] *Evonik Nutrition and Care*

Total sulfur amino acids (TSAA) such as methionine (Met) and cysteine (Cys) are involved in not only protein synthesis, but also cellular metabolism. Considering so, Met, Cys, and corresponding metabolites play an important role in tissue building, muscle repair, and the reduction of oxidative stress. In two experiments, 2400 Cobb 500 chicks were reared to determine the rate of CO_2 produced through stable isotopes and indirect calorimetry as well as the oxidation of [1-^{13}C]Met and [1-^{13}C]Cys. Birds were fed grower diets containing varying levels of TSAA in both experiments (Evonik AMINOChick® 2.0). At 21d, birds were selected for either control, intravenous (IV) or intragastric (IG) infusion of [1-^{13}C]Met (Exp 1), or [1-^{13}C]Cys (Exp 2), followed by sampling at intervals ranging from 5-420 min post-infusion. Blood was collected and $^{13}CO_2$ enrichment was determined by isotope ratio mass spectrometry (IRMS). Small intestine, pancreas, kidney, and liver tissues were harvested at each of the time periods in order to determine the amino acid enrichment over time. Samples were analyzed using a gas chromatography-mass spectrometer (GC-MS) and high pressure liquid chromatography (HPLC). Results were analyzed using JMP Pro 13.1. Data gathered from the experiments showed that varying intakes of TSAA results in poorer feed conversions when feeding a deficiency in regards to the requirement. Additionally, a deficiency of TSAA resulted in a greater loss of Met label as $^{13}CO_2$ ($p<0.05$). Comparison of $^{13}CO_2$ showed differences in IG and IV infusions ($p<0.05$), suggesting differences in parenteral and enteral uptake of nutrients. Loss of [1-^{13}C]Met through $^{13}CO_2$ in birds fed adequate TSAA increased (18 vs 11%; $p<0.05$), as determined through IRMS. Diets deficient in Cys showed differences in IG and IV infusions and resulted in a decrease in glutathione production in the liver ($p<0.05$). Understanding the TSAA and glutathione pathways is increasingly important as the poultry industry moves towards

complete ABF production practices. Ultimately, the loss of the Met and Cys labels to $^{13}CO_2$ proves worthy for understanding TSAA utilization and metabolism, considering greater $^{13}CO_2$ production from [^{13}C]Met may signify increased Cys synthesis.

Key Words: methionine, amino acids, glutathione, stable isotopes, broilers

P272, **Gene expression of fatty acid binding proteins in abdominal and intramuscular fat tissues of Thai native crossbred chickens** Supanon Tunim*[1], Yupin Phasuk[1], Monchai Duangjinda[1], Samuel Aggrey[2] *[1] 1Department of Animal Science, Faculty of Agriculture, Khon Kaen University, [2] Department of Poultry Science, University of Georgia*

Animal based diets generally have higher fat content compared with plant based diets. From an animal breeding viewpoint, excessive fat deposition in farm animals especially poultry should be selected against to respond to the desires of healthy consumers and also improves feed efficiency. Compared to exotic breeds, Thai native chickens have been found to have low fat and excellent meat quality. The production Thai native chicken is limited by slow growth rate. The production of Thai native chicken on a commercial scale is usually undertaken through crossbreeding of the Thai native chickens with exotic breeds to increase growth efficiency, but the exotic chickens also have high amounts of abdominal (ABF) and intramuscular fat (IF). We conducted this experiment to study the relationship between the mRNA expressions of abdominal fatty acid binding protein (A-FABP) and heart (H-FABP) in Thai native chicken crossbreds and evaluate the level of exotic inclusion in native Thai chicken that will improve growth and maintain its relatively low carcass fat. The mRNA expression of A-FABP) and H-FABP were evaluated at 6, 8, 10 and 12 weeks of age in 4 chicken breeds using the delta delta Ct method using RNA extracted from ABF and IF from the Pectoralis major muscle. There breeds were 100% Chee breed (CH) (100% Thai native chicken background: 0% broiler background), CH male and broiler female (KM1) (50% Thai native chicken background: 50% broiler background), broiler male and KM1 female (KM2) (25% Thai native chicken background: 75% broiler background), and broiler (BR) (0% Thai native chicken background: 100% broiler background). The BR breed was only evaluated at 6 weeks of age. At week 6, the CH breed has a significantly lower ($P<0.05$) A-FABP expression in ABF and IF compared with the other breeds. At 8-12 weeks, the KM2 groups showed significant upregulation ($P<0.05$) of A-FABP in both ABF and IF compared to the CH and KM1 groups. The expression of H-FABP did not follow any consistent pattern in both ABF and IF across the different ages. The results show that some level of crossbreeding of CH chicken can be done with exotic chicken to improve growth rate and maintain the low ABF and IF content of the meat.

Key Words: Thai native chickens, abdominal fat, intramuscular fat, FABPs, gene expression

P273, **Effects of varying digestible valine to lysine ratios on growth performance, meat yield, and return over feed cost of broilers from 1 to 33 d of age** Ruben Kriseldi*[1GS], Paul Tillman[2], Kevin Touchette[3], William Dozier, III[1] *[1] Auburn University, [2] Poultry Technical Nutrition Services, [3] Ajinomoto Animal Nutrition North America Inc.*

An experiment was conducted to evaluate effects of varying digestible Val to Lys ratio (dVal:dLys) on growth performance and meat yield of broilers from 1 to 33 d of age. A total of 1,500 Yield Plus × Ross 708 was placed in 60-floor pens (25 birds/pen). Each pen was provided with 1 of 3 dietary treatments, which were formulated to contain dVal:dLys at either 0.74, 0.78, or 0.82. Diets were fed during starter (1 to 14 d), grower (15 to 25 d), and finisher (26 to 33 d) periods. Feed and birds were weighed at 1, 14, 25, and 33 d of age to determine BW gain, feed intake, and feed conversion ratio (FCR). At 34 d of age, 10 birds/pen were processed to determine carcass characteristics. The return over feed cost (ROFC) for total debone products was calculated based on L-Val price of $6.30/kg, whereas prices for debone products were obtained from Monthly Southern States Broiler Parts of USDA Economics, Statistics, and Market Information System on October 31st, 2019. From 1 to 14 d of age, FCR of broilers provided diets formulated to contain dVal:dLys of 0.78 (1.158) and 0.82 (1.159) was approximately 2 points lower ($P < 0.05$) than those provided diets with 0.74 (1.178) dVal:dLys. Similarly, from 1 to 25 d of age, broilers fed diets containing 0.78 and 0.82 dVal:dLys had 1.4 (1.256) and 1.7 (1.253) points lower ($P < 0.05$) FCR than those consuming diets with 0.74 (1.270) dVal:dLys. From 1 to 33 d of age, FCR of broilers did not differ ($P > 0.05$) between those provided diets with dVal:dLys of 0.74 and 0.82. In contrast, broilers fed diets formulated to contain dVal:dLys of 0.78 had FCR that was 1.8 points lower (1.372 vs. 1.390; $P < 0.05$) than birds fed diets with 0.74 dVal:dLys. Carcass characteristics of broilers were similar ($P > 0.05$) among treatments, except the wings of broilers fed the 0.82 dVal:dLys diets were 5 g heavier (173 vs. 168 g; $P < 0.05$) than those fed the 0.74 dVal:dLys diets. Feeding broilers with diets formulated to contain dVal:dLys of 0.78 ($1.71/bird) and 0.82 ($1.70/bird) provided ROFC of $0.03 and $0.02/bird, respectively, than feeding the 0.74 ($1.68/bird) dVal:dLys diets. Therefore, optimum growth performance and ROFC of broilers may be obtained by formulating diets to 0.78 dVal:dLys during a 33-d production period.

Key Words: broiler, valine, return/feed cost

P274, **Effects of lysine biomass supplementation on growth performance and clinical indicators in broilers** Julianna Jespersen*[1GS], Susann Richert[2], Maci Oelschlager[1], Ryan Dilger[1] *[1] University of Illinois, [2] Evonik Nutrition & Care GmbH*

The use of microbial fermentation to produce crystalline amino acids may also provide a cost-effective additive for maintaining growth and health of broilers. In this study, we investigated the effects of feeding Biolys®77, a fermentation biomass product with a minimum purity of 54.6% L-lysine, on growth performance, organ growth, and clinical outcomes of broilers. Beginning at 2 d post-hatch, a total of 216 Ross 308 chicks were housed in cages and randomly assigned to 1 of 3 dietary treatments each fed to 12 replicate cages of 6 birds. Practical corn-soybean meal-based dietary treatments included: negative control (NC; no AA supplementation, 1.01% SID Lys starter and 0.86% SID Lys grower), NC + 0.23% L-Lys HCl (positive control; PC), and the NC supplemented with 0.30% Biolys®77. Feed and water were provided ad libitum throughout the study. Individual bird and feeder weights were recorded on study d 0, 10, 21, and 35. At study conclusion, birds from each treatment group were randomly selected to permit the collection of blood and organs. The PC and the 0.30% Biolys®77 diets elicited similar body weights at all time-points, where body weights were higher ($P < 0.05$) than the NC on d 21 and 35. Average weight gain for the NC was lower ($P < 0.001$) at all time-points, except d 0-10, when compared with the PC and 0.30% Biolys®77 groups. The PC and 0.30% Biolys®77 groups had similar FCR at all time-points except d 10-21, when the 0.30% Biolys®77 treatment had a lower ($P < 0.001$; i.e., better) FCR than the PC. The 0.30% Biolys®77 treatment had increased ($P < 0.001$) relative weights of the spleen and ileum when compared with the PC and NC groups. The heterophil proportion was higher ($P < 0.001$) and the lymphocyte proportion was lower ($P < 0.001$) in the 0.30% Biolys®77 treatment when compared with PC and NC groups. The 0.30% Biolys®77 treatment elicited results similar to the PC treatment for circulating concentrations of albumin, calcium, AST, CPK, and GLDH as clinical indicators. Collectively, these results suggest that addition of 0.30% Biolys®77 restored growth performance when added to a Lys-deficient practical diet and elicited results identical to the Lys-adequate PC diet with no negative effects on clinical outcomes.

Key Words: lysine, broiler, biomass, performance

Metabolism and Nutrition, Enzymes, Feed Additives

P275, **Strategies to Reduce Colonization of Salmonella Enteritidis in Layers** John Padgett*[1,8GS], Paul Price[1,2], Thomas Gaydos[3], Roy Berghaus[4], Jennie Baxter[5], Charles Hofacre[5], Michael Sims[6], Ken Macklin[7] [1] *Phileo by Lesaffre,* [2] *Clemson University,* [3] *Gaydos Technical Services,* [4] *University of Georgia, College of Veterinary Medicine,* [5] *Southern Poultry Research Group, Inc.,* [6] *Virginia Diversified Research,* [7] *Auburn University,* [8] *Texas A&M University*

Salmonella Enteritidis is a significant foodborne pathogen that can often be traced back to poultry and poultry products. Feed ingredients such as *Bacillus* probiotics and parietal yeast fractions have gained in interest to reduce food safety issues. Consequently, 2 in vivo trials were setup to evaluate the ability of these products to reduce *Salmonella* Enteritidis colonization in the laying pullets and hens. In the first trial, 60 Hy-Line Brown pullets aged 9 weeks were randomly distributed to individual cages in 15 replicate blocks of 4 pens. The treatments were control, 500g/ton yeast parietal fraction , 500g/ton *Bacillus* spp probiotic and 1kg/ton yeast culture. On day 3 of the trial all birds were orally administered $3x10^7$ CFU of a nalidixic acid resistant *Salmonella* Enteritidis. On day 10, seven days after inoculation all birds were humanely euthanized, and the ceca aseptically removed for CFU determination. In the 2nd trial, 60 Hy-Line hens aged 56 weeks were placed in individual cages and fed a mash diet containing one of the following treatments, control, 500g/ton *Bacillus* spp. probiotic, 500g/ton yeast parietal fraction or a combination of yeast parietal fraction and *Bacillus* spp. probiotic. At 60 weeks of age all hens were challenged orally with 7×10^7 CFU/bird of *Salmonella* Enteritidis. At 61 weeks of age, birds were humanely euthanized, and the ceca aseptically removed and cultured for *Salmonella* Enteritidis prevalence and number by the Most Probable Number (MPN) method. Results of the first trial show that the mean quantity of *Salmonella* Enteritidis detected in the ceca expressed in $\log_{10}$ MPN/g was 2.52 in the control, 2.49 in the yeast parietal fraction treatment, 1.73 in the probiotic treatment, and 1.66 in the yeast culture treatment. In the second study, the control birds had 3.35 $\log_{10}$ MPN/g of *Salmonella* Enteritidis in the ceca. The Probiotic group had 1.90 MPN/g, a reduction of 1.44 ($p<0.05$) and the yeast cell wall group had 1.84MPN/g a reduction of 1.51 ($p<0.05$). The combination had 2.56 MPN/g a numerical reduction of 0.79 ($p=0.14$). These studies demonstrate the ability of *Bacillus* spp. probiotic and the yeast parietal fractions or cultures to reduce the load of *Salmonella* Enteritidis in layer ceca.

Key Words: Salmonella, Layer, probiotic, yeast, feed

P276, **Effects of Curcumin on Salmonella enteritidis colonization in the liver-gall bladder, spleen, ovary, ceca, and fecal shedding in laying hens under heat stress.** Sudhir Yadav*[GS], Sanjay Kumar, Woo Kim *The University of Georgia*

The aim of this study was to evaluate the effect of curcumin on *Salmonella enteritidis* translocation in different organs of laying hens kept under heat stress condition. A total of 50 laying hens (18 weeks old) were randomly distributed individually per cage with 10 replicates per treatment. The five treatment groups were control (corn-SBM based basal diet), control + 0.05% Curcumin, control + 0.1% Curcumin, control + 0.2% Curcumin, and control + 0.4% Curcumin. The trial was conducted for 14 days under heat stress (32-35°C), where temperature and humidity were monitored using data loggers. All the hens were challenged orally with 10^7 CFU/ml Nalidixic acid-resistant *Salmonella enteritidis* (NAL^R SE) on Day 7. At day 0, 3 and 7 post-infection, all hens were screened for *Salmonella enteritidis* in fecal shedding. At d 14 (7 dpi), BW, BWG, and FI were measured, and organ samples including liver-gall bladder, spleen, and ovary were collected for *Salmonella enteritidis* growth on the plates. Cecal samples were plated, and log CFU/ml count was recorded. One-way ANOVA was performed, and means were compared by Tukey's test ($P<0.05$) using SAS. There were no significant differences between treatments during the experimental period of 14 days although numerically control + 0.2% Curcumin treatment group has the highest BW and BWG and lowest FI compared to the control and other treatments. The cecal S*almonella* count was lowest in the highest curcumin diet-fed birds. The fecal shedding data showed that there were no positive birds on day 0 post-infection, whereas on day 3 and 7 post-infection *Salmonella enteritidis* positive birds remained consistent in the control group and increased in other treatments except in group fed 0.2% Curcumin; it decreased over time. Spleen and liver *Salmonella* translocation showed fewer positive birds in control and 0.2% Curcumin fed birds. For the translocation in the ovary, the control group did not have any positive birds, whereas 0.1% Curcumin fed group had the highest number of positive birds. In overall, the birds fed 0.2% curcumin had a beneficial role in growth performance along with decreasing *Salmonella* translocation in different organs.

Key Words: Curcumin, Salmonella enteritidis, Heat stress, layers

P277, **The Effects of two corn-expressed phytases on broiler growth performance and tibia mineralization** Victoria Ayres*[1GS], Morgan Basye[1], Timothy Boltz[1], Angela Lamp[1], Philip Lessard[2], Michael Lanahan[2], Joseph Moritz[1] [1] *West Virginia University,* [2] *Agrivida Inc.*

The current study hypothesized that phytase activity concentration in grain effects mixer homogeneity, thermal stability post pelleting, and efficacy regarding growth performance and tibia ash of broiler chickens. The objective of this study was to examine the effects of two corn-expressed phytases, which differ in concentration (3,000 FTU/g or 9,677 FTU/g) of the enzyme within the grain, on mixer homogeneity, broiler performance, and tibia mineralization. Dietary treatments included a positive control (PC), with recommended levels of calcium and phosphorus, and a negative control (NC), lower in calcium and non-phytate phosphorous. Six additional diets were also batched containing corn-expressed phytase at high or low grain concentrations formulated to either 3,000, 6,000, or 9,000 FTU/kg added to the NC. Mixer coefficient of variation (CV) based on enzyme activity did not differ among treatments. Prior to pelleting, dietary treatments were conditioned at 70°C for 15s. Enzyme recovery post pelleting did not differ among treatments. Diets were fed as crumbles for the starter and grower phases and as pellets for the finisher phase to Hubbard x Ross 708 (n=1,632) male broiler chicks for 39d. Performance metrics were examined on days 10, 21, and 39. On d21, 5 birds/pen were euthanized via cervical dislocation, and the left tibiae were excised to determine tibia mineralization. Treatments were arranged in a 2 (phytase type) x 3 (phytase inclusion) factorial in a randomized complete block design. Overall d1-39 broiler performance demonstrated that broilers fed the NC treatment had increased feed conversion ratio (FCR) compared to all other treatments ($P<0.05$). Broilers provided PC and NC also produced expected differences in tibia ash when measured as a percentage or as mg/bird ($P<0.05$). While there were no differences between phytase products, birds that were fed a phytase with a 9,000 FTU/kg inclusion had a higher tibia ash/bird and tibia ash percentage ($P<0.05$). This study demonstrates that corn-expressed phytases can improve d21 tibia mineralization and overall d39 FCR, regardless of concentration expressed in the grain.

Key Words: phytase, recombinant, broiler, tibia mineralization, mixer homogeneity

P278, **The effect of NSPase enzymes in an energy deficient diet on 0 to 38 d broiler performance** Timothy Boltz*[GS], Victoria Ayres, Kari Harding, Angela Lamp, Joseph Moritz *West Virginia University*

Use of by-products, such as corn distillers dried grains with solubles (DDGS), in feed can result in dietary non-starch polysaccharides (NSP) that limit broiler performance. This problem may be remedied by using exogenous enzymes. The current study hypothesized that the use of NSPase blends would improve 38d broiler performance in energy deficient diets that contain DDGS. The study objectives were to determine the

effects of four different NSPase blends on 38d broiler feed intake (FI), live weight gain (LWG), and feed conversion ratio (FCR). In addition, broilers fed treatments containing NSPase blends and NC were used to calculate 40d digestible amino acid concentration and nitrogen corrected apparent metabolizable energy (ME). A positive control (PC) and negative control (NC) diet, both containing DDGS, were formulated so that the NC was 132 kcal/kg less in ME than the PC. One of four NSPase blends (A-D) was added to the NC diet. Diets were conditioned at 85°C for 30s, extruded through a 4.8 x 38 mm pellet die, and fed as crumbles (starter and grower) or pellets (finisher). Twelve replicate pens of 23 Hubbard x Ross 708 cockerels consumed one of seven dietary treatments for 38d. Data were analyzed using ANOVA and means associated with significant effects were separated using a Fisher's LSD. By design, broilers fed PC produced expected increased FI, LWG and decreased FCR compared to NC fed broilers (P<0.05). Feed conversion ratio and LWG were similar between the PC and dietary treatments containing the NSPase blends through the 0 to 38d period (P>0.05). NSPase blends B and C demonstrated increased nitrogen corrected apparent ME relative to diets containing NSPase blend D and the NC (P<0.05). Consistent NSPase blend effects on digestible amino acid concentration were not apparent. This study demonstrates that the use of NSPase enzymes in diets deficient in ME can improve broiler performance to levels comparable to a diet with an adequate ME.

Key Words: NSPase, Enzymes, Broilers, Energy

P279, **Effects of conditioning time and temperature on pellet manufacture variables, mixer-added muramidase activity, and broiler performance** Kenzie George*[1UG], Angela Lamp [1], Nelson Ward [2], Tina Wang [2], Joseph Moritz [1] [1] *West Virginia University,* [2] *DSM Nutritional Products Inc.*

Determining the efficacy of mixer-added enzymes post pelleting is essential for commercial implementation. A microbial muramidase enzyme (BalanciusTM) was developed to improve gastrointestinal functionality by hydrolyzing peptidoglycans from dead bacterial cell debris in the intestinal tract. The objective of this study was to evaluate feed manufacturing variables, enzyme activity and broiler performance of this muramidase in pelleted broiler feed. Dietary treatments included a nutrient adequate corn and soybean meal basal diet with and without muramidase at 25,000 LSU(F)/kg. The basal diet was conditioned at 88°C for 30 and 60 seconds. The diet containing the muramidase was conditioned at 30 and 60 seconds and at 77, 82, and 88°C. Treatments were arranged in a 2 (conditioning time) x 3 (conditioning temperature) factorial with two controls in a randomized complete block design and replicated 3 times across 3 days of feed manufacture. Increasing conditioning temperature decreased pellet mill motor load (P<0.05), likely due to the lubricating effects of increased steam to achieve higher conditioning temperature. Subsequently, muramidase activity decreased at 77oC compared to 82 and 88oC conditioning, perhaps due to increased frictional force within the pellet die at the lower conditioning temperature. The 8 dietary treatments were fed as crumbles to 12 replications of 10 male Hubbard x Ross 708 broiler chicks for 21 days. Twenty-one day performance data did not differ among treatments analyzed as a 2 x 3 factorial (P>0.05). However, when muramidase treatments were compared to their respective controls, feed conversion ratio was decreased and live weight gain increased at 30 sec conditioning (P<0.05), but not 60 sec conditioning (P>0.05). These results may be attributed to higher analyzed muramidase activity for the 30 sec 88oC treatment compared to 60 sec 88oC treatments (P=0.07). Muramidase was shown to improve performance post pelleting when added to a nutrient adequate control.

Key Words: muramidase, conditioning time, conditioning temperature, pellet manufacture, broiler

P280, **Interactive effects of dietary levels of fiber, protein and multi-enzyme supplementation on growth performance, nutrient utilization and short-chain fatty acid profile in broilers fed corn-soybean meal diets** Yang Lin*[GS], Oluyinka Olukosi *University of Georgia*

A total of 440 Cobb 500 male broiler chicks were used in a 21-day experiment to study how a multi-enzyme influence growth performance, nutrient utilization and short-chain fatty acid (SCFA) profile. Birds were allocated to 8 treatments in a randomized complete block design. Each treatment had 11 replicates with 5 birds per replicate cage. The 8 treatments were based on 4 corn-soybean meal basal diets with different levels of protein and fiber, namely: LFHP (low fiber and high protein), LFLP (low fiber and low protein), HFHP (high fiber and high protein), and HFLP (high fiber and low protein). Each of the basal diets were supplemented with or without multi-enzyme (xylanase and protease). Birds and feed were weighed on days 0 and 21. Excreta, jejunal and ileal digesta, and caeca contents were collected from 6 randomly selected replicates per treatment at the end of the experiment. Weight gain, feed intake and gain: feed were greater ($P < 0.05$) in birds receiving high protein (HP) diets but enzyme supplementation had no effect. The multi-enzyme increased ($P < 0.01$) AME and crude protein and DM retention. Birds receiving the HP diets had greater ($P < 0.05$) AME levels and DM retention. Broilers fed higher fiber had lower ($P < 0.01$) DM retention and AME. There was significant ($P < 0.01$) fiber × protein interaction on nutrient utilization; DM retention, N digestibility, and AME were greater in LFHP; however, the reverse was observed for HFHP diets. In addition, there was significant ($P < 0.05$) protein × enzyme interaction for DM retention and N digestibility. The improvements in N digestibility and DM retention due to enzyme addition was lower in HP diets. Caeca acetate content was lower ($P < 0.05$) in enzyme-supplemented diets. Weight gain, GF and FBW were significantly positively correlated with acetate. In addition, the CP retention was positively correlated with propionate and valerate whereas AME was negatively correlated with acetate but positively correlated with propionate, isobutyrate and valerate. In conclusion, the HPLF diet had greater positive effects on nutrient utilization in the broiler, and the nutrient utilization responses to multi-enzyme were more sensitive to low protein diets.

Key Words: Xylanase, Protease, Fiber, Protein, Broilers

P281, **Dietary essential oil feed additive (Biolex™) modulated growth performance, short chain fatty acids and intestinal histomorphology with no effect on immune organ or gastrointestinal tract of broiler chickens** Mohammad Pilevar*[1GS], Alamanda Calvert [2], Nathan Horn [2], Revathi Shanmugasundaram [1], Oluyinka Olukosi [1] [1] *University of Georgia,* [2] *Biomatrix International*

A 42-day study was carried out to evaluate the growth performance, immune organ weight, digestive organ weight, ceca short chain fatty acids (SCFA) profile, and ileum histomorphology responses of broiler chickens fed a blended essential oil, prebiotic, and acidifier product (Biolex™). A total of 540 broiler chicks (Cobb 500) were allocated into three treatments, each with 12 replicates and 15 birds/replicate pen in a randomized complete block design. The three corn-soybean meal dietary treatments were supplemented with 0.0 (Control), 0.5 or 1.0 g/kg of Biolex™ for 42 days. Body weight and feed were recorded on day 0, 14, 28 and 42 of the experiment. On day 42, three birds per pen with body weight closest to the pen average weight were selected and euthanized. Spleen, bursa of Fabricius, gizzard, pancreas, duodenum, jejunum, and ileum were weighed and also expressed relative to body weight of individual bird. The mid-section of ileum was collected for histomorphology measurements. The ceca content from two birds per pen were used to determine the SCFA content. Body weight gain from day 14 to 42 was greater ($P < 0.05$) in birds fed the 1.0 g per kg premix compared with the control. Total body weight gain and day 42 body weight increased linearly ($P < 0.05$) with Biolex™ supplementation. There were no treatment effects on absolute or relative digestive or immune organ weights on day 42. The propionate content in the ceca tended to be greater ($P = 0.072$) in birds fed 0.5 g/kg Biolex™

compared to other treatments. Surface area (μm^2) of villi in the ileum at day 42 increased linearly ($P < 0.05$) with Biolex™ supplementation. There were no differences among treatments for other morphological parameters measured in ileum. In conclusion, the data indicate improvements in overall performance of birds receiving diets supplemented with Biolex™. Part of this improvement in growth performance could have resulted from the observed increase in villi surface area which likely enabled enhanced nutrient utilization in the broiler chickens.

Key Words: Broilers, essential oil, short-chain fatty acid, prebiotic, growth performance

P282, **Effects of feeding broiler chickens non-viable lactobacillus fermentation biomass and top-dressed trace minerals without or with ionophore** Munene Kithama[*1GS], Elijah Kiarie[1], Parry Bast[2] [1] *University of Guelph,* [2] *Bio-Ag Consultants & Distributors Inc*

We evaluated effects of non-viable lactobacillus fermentation biomass (LAB) and trace minerals (TM) fed singly or combined without or with ionophore (ION). Control (C) diet was formulated (starter; d 0-24 and finisher; d 25-35) with the trace mineral premix containing basal 60, 10, 70 and 70 g/kg of Fe, Cu, Zn and Mn, respectively. An pre-mixture of C diet was top-dressed with either LAB (1 kg/ton) or TM (1.2 kg/ton; 3, 2.4, 6 and 8 g/kg of trace mineral premix of Fe, Cu, Zn and Mn, respectively) or combination (LAB+TM, 2kg/ton). Diets were fed without or with ION (Salinomycin Na, 25 mg/kg) creating a 4 x 2 factorial arrangement. A total of 1,008 d old Ross 708 male chicks were placed in cages (14 birds/cage) based on BW and allocated to treatments (n=8). Feed intake (FI) and BW were monitored by phase, and on d 24 and 35 samples of birds were bled for plasma and necropsied for organ (d 24) and breast weight (d 35) respectively. There was interaction (P≤0.04) between additives and ION on starter BWG; in absence of ION, LAB+TM birds had higher BWG (1.02 vs. 0.96 kg; P=0.04) relative to C whilst LAB and TM birds had similar BWG (P>0.05) to C. Birds fed ION had better (P=0.004) FCR than non-ION birds in finisher phase. There were no treatment effects (P>0.05) on overall (d 0-35) FI, BW and FCR and breast weight. However, birds fed LAB had heavier (239 vs. 228 g/kg BW; P=0.07) breast relative to C birds. There was interaction between additives and ION on gizzard and small intestine weight. In the presence of ION, birds fed LAB showed heavier gizzard (18.2 vs. 15.3 g/kg BW) and lighter small intestine (25.1 vs. 29.8 g/kg BW) than birds fed C. Birds fed ION had lighter (P=0.046) ceca than non-ION birds. Interaction between additives and ION produced heavier liver (P=0.01) than birds fed C. There was interaction (P≤0.06) between additives and ION on plasma concentration of aspartate transaminase (AST) and lactate dehydrogenase (LDH) in starter phase; birds fed LAB+TM showed high concentration of AST (P=0.06) and LDH (P=0.02) than C birds in absence of ION. In conclusion, LAB improved growth performance in the starter phase, attributable to effects on gut physiology and metabolism; these effects were influenced by ionophore but not additional trace minerals.

Key Words: non-viable lactobacillus,, Ionophore, plasma biochemical profiles, trace minerals, growth performance

P283, **Azomite: an in vitro analysis of the binding affinity for aflatoxin B1, and fumonisin B1: individually and combined.** O. Tejeda[*1GS], S. Hassan[2], M. Jones[3], J. Ferrel[3], J. Fowler[1] [1] *University of Georgia Poultry Science Dept.,* [2] *University of Georgia Laboratory for Environmental Analysis,* [3] *Azomite Mineral Products, Inc.*

Mycotoxin contamination of feedstuff grains is a perennial worldwide concern, especially in tropical countries where humidity stimulates the development of fungi. AZOMITE®is a volcanic hydrated sodium calcium aluminosilicate (HSCAS)), described as a dacitic (rhyolitic) tuff breccia. HSCAS have been shown to improve feed manufacturing parameters such as flowability, pellet quality, and pellet throughput and have also been shown to adsorb certain mycotoxins (especially aflatoxin) with a relatively high affinity. Many factors can affect the binding efficiency of the HSCAS, one of the most critical is particle size surface area. Therefore, our objective was to determine the binding affinity of two grades of AZOMITE. The experiment consisted of titrated amounts of aflatoxin B1 (AFB1), and fumonisin B1 (FUMB1) in a suspension of 0.1% Azomite using two material grades, feed-grit and micronized. Mycotoxins were exposed to the solution for 24 hours to reach an equilibrium concentration. Mycotoxin concentrations used were 0, 1.125, 2.25, 4.5, 9, 18, and 36 ppm for AFB1and 0, 11.25, 22.5, 45, 90, 180 and 360 ppm for FUMB1. After mixing, mycotoxin concentrations of the supernatant were analyzed via High-Performance Liquid Chromatography (in duplicate) to determine the amount of unadsorbed toxin. The data were fit to a regression model to evaluate binding affinity and capacity. Affinity was determined by the slope of the ascending portion of the curve, and capacity was determined by the break-point of the model. The results showed a higher adsorption rate for AFB1 than FMB1 for both grades of AZOMITE. FUMB1 represented 1% of binding compared to AFB1. Micronized had approximately 55% higher adsorption capacity for AFB1 than feed-grit when presented in solution by itself. When FUMB1 was present in solution, the micronized grade had 3 times higher adsorption of AFB1compared to feed-grit. With feed-grit, the presence of AFB1 reduced the adsorption capacity for FUMB1 by approximately 20%. In the presence of FUMB1 there was an increased affinity for AFB1. This data suggests that the adsorption capacity of AZOMITEis higher when provided in smaller grade particle sizes.

Key Words: aflatoxin, fumonisin, AZOMITE, in vitro

P284, **Effect of Multi-Carbohydrase Enzymes on Broiler Nitrogen-Corrects Apparent Metabolizable Energy (AMEn) of Pelleted Feed** Michaela Braun[*1GS], Courtney Truelock[1], Kara Dunmire[1], Charles Stark[1], Sara Llamas Moya[2], Chad Paulk[1] [1] *Kansas State University,* [2] *Kerry*

The objective of this study was to evaluate the effect of multi-carbohydrase inclusion on growth performance, AME and AMEn in broilers. A total of 600 one-day old, male (Cobb 500; initial BW 41 g) broiler chicks were housed in 6 Petersime batteries and used in an 18-d study. Birds were randomly assigned to battery cages with 5 birds per cage. Cages were then randomly allotted to 1 of 8 treatments within location block and balanced by BW with 15 replicates per treatment. All diets were manufactured on a 1 ton 30-horsepower pellet mill (1012-2 HD Master Model, California Pellet Mill) and conditioned to approximately 82°C. Treatment diets consisted of a control and the control plus 1 of 7 enzymes (200 g/tonne). The control was composed of maize, soybean meal, DDGS, soy oil and mineral-vitamin premix and was formulated to provide 2937 kcal/kg for the starter and 2988 kcal/kg for the grower and meet birds nutrient recommendations. Dietary treatments were fed in 2 phases with a starter crumble offered from d 1-8 and grower pellet from d 9-18. On d 16, 17, and 18 fecal samples were collected and pooled samples were analyzed for calculation of AME and AMEn. Calculations for AME and AMEn were determined using the indirect method and titanium dioxide served as the indigestible marker. Data were analyzed using the MIXED procedure in SAS with pen as the experimental unit and pen location as the blocking factor. Analysis was performed using Dunnett's multiple comparisons for all parameters. For the starter, grower, and overall results, there was no evidence for differences ($P > 0.206$) in ADG, FCR, or ADFI. There was an overall effect for ($P < 0.026$) AME and AMEn. The diet containing enzyme 1 had a 150 kcal/kg and 151 kcal/kg increase in AME and AMEn respectively when compared to the control diet. However, diets containing the other enzymes had similar AME and AMEn compared to the control diet. In conclusion, broilers fed diets with added enzymes had similar growth performance to those fed the control. Diets containing enzyme 1 had an 150 kcal/kg and 151 kcal/kg increase in AME and AMEn compared to the control diet.

Key Words: Carbohydrase, Metabolizable Energy, Broiler

P285, **Effect of Multi-Carbohydrase Enzymes on Broiler Growth Performance** Michaela Braun*[1], Courtney Truelock [1], Kara Dunmire [1], Charles Stark [1], Sara Llamas Moya [2], Chad Paulk [1] *[1] Kansas State University, [2] Kerry*

The objective of this experiment was to evaluate the effect of multi-carbohydrase inclusion on the performance of broilers fed diets with decreased energy density. A total of 1,260 one-day old, male (Cobb 500; initial BW 44g) broiler chickens were used in a 42-d study. Birds were randomly assigned to floor pens with 15 birds per pen. Pens were then randomly assigned to 1 of 6 treatments within location block and balanced by BW with 14 replications per treatment. The starter diet was offered as a crumble (d 0 to 10) and the grower (d 10 to 21) and finisher (d 21 to 42) as a pellet. All diets were conditioned to approximately 86°C and pellets were screened to prevent differences in pellet quality. Treatment diets consisted of a positive control (PC), negative control (NC), and the NC with 1 of 4 enzymes. The NC was formulated to have decreased energy density (111 kcal/kg less metabolizable energy [ME]). Enzymes A, B, C, and D were added to provide 200, 200, 200, and 100 g/tonne respectively. From d 0 to 10, no differences were observed for FCR or ADFI. Broilers fed the NC and enzyme B and D had increased ($P < 0.015$) d 10 BW compared to those fed all other dietary treatments. From d 10 to 21, there were no differences for ADG or FCR. Broilers fed the PC had decreased ($P < 0.040$) ADFI compared to those fed other dietary treatments. There was no evidence for differences in the d 21 BW. From d 21 to 42, there was an overall treatment effect for ADFI ($P < 0.044$). Broilers fed the NC had increased ADFI compared to those fed PC, with those fed diets containing enzyme A, B, C, or D being intermediate. From d 0 to 42, no differences were detected for ADG or ADFI. Broilers fed the PC diet (1.50) had an improved ($P < 0.01$) FCR compared to for those fed the NC and diets containing enzymes A, B, or C (1.53, 1.54, 1.53, and 1.53, respectively). There were no significant differences in the calculated Productivity Index. In conclusion, broilers fed diets containing with increased (111 kcal/kg) ME had improved FCR. Adding exogenous enzymes to the NC pelleted at 86°C did not improve growth performance.

Key Words: Carbohydrase, Growth, Broiler

P286, **Effects of probiotics as an antibiotic substitute on growth performance, serum biochemical parameters, intestinal morphology and barrier function of broilers** Shenfei Long*, Tengfei He, Di Wu, Xi Wang, Xiaoman Wei, Xiangshu Piao *China Agricultural University*

The aim of this study was to investigate the effects of a combination of probiotics (*Bacillus subtilis* 5×10^9 CFU/g, *Bacillus licheniformis* 2.5×10^{10} CFU/g, and *Saccharomyces cerevisiae* 1×10^9 CFU/g) as an antibiotic substitute on growth performance, serum biochemical parameters, intestinal morphology and expression of tight junction proteins in intestinal mucosa of broilers. A total of 168 Arbor Acres broilers (45.04 ± 0.92 g) were randomly divided into 3 treatments, 7 replicate pens per treatment, 8 broilers per pen. The experiment included phase 1 (d 1 to 21) and 2 (d 22 to 42). The dietary treatments contained a corn soybean meal-based diet (control group, CON); an antibiotic group (basal diet + 75 mg/kg chlortetracycline; CTC), and a probiotics group [basal diet + probiotics (500 mg/kg in phase 1 and 300 mg/kg in phase 2; PB]. The results showed broilers fed PB showed improved ($P < 0.05$) feed conversion ratio (FCR) in phase 1 and increased ($P < 0.05$) average daily gain (ADG) in phase 2 compared with CON. In overall (d 1 to 42), broilers fed PB had improved ADG and FCR compared with CON ($P < 0.05$). The apparent total tract digestibility (ATTD) of the dry matter and organic matter was increased ($P < 0.05$) in broilers fed PB and CTC compared with CON. In addition, broilers fed PB showed increased ($P < 0.05$) ATTD of gross energy and tended to have increased ($P = 0.06$) ATTD of crude protein compared with CON. Broilers fed CTC and PB showed increased ($P < 0.05$) serum total antioxidant capacity concentrations in phase 1 compared with CON. Broilers fed PB tended to show increased level of IgM ($P = 0.07$) in phase 1, and IgA in phase 2 in comparison with CON. The duodenal villus height to crypt depth ratio was increased ($P = 0.05$) in broilers fed CTC and PB compared with CON. Moreover, broilers fed CTC and PB showed higher ($P < 0.05$) mRNA expression of zonula occludens-1 in jejunum than CON. In conclusion, dietary supplementation with probiotics can replace chlortetracycline on improving the growth performance, nutrient digestibility, serum antioxidant capacity, jejunal mucosal barrier function and intestinal morphology of broilers.

Key Words: antibiotics, broiler, growth performance, intestinal health, probiotics

P287, **Effects of varying phytase enzyme and total activity on phytate degradation and nutrient digestibility of young male Ross x Ross 708 broilers** Courtney Ennis*[1], Curran Gehring [2], Mike Bedford [3], Craig Wyatt [3], Kelley Wamsley [1] *[1] Mississippi State University, [2] Tucker Milling, [3] AB Vista*

Previous research in our lab investigated the use of 3 phytases (A, B and C) due to their unique characteristics relating to their sequence of IP6 hydrolysis. These data revealed some d 14 performance and tibia ash benefits with use of these combined phytase inclusion strategies (PIS). The current study was conducted to explain possible synergistic effects between PIS via lower inositol ester analysis and nutrient digestibility. This study utilized 7 PIS (A, B, C, A+B, A+C, B+C, or A+B+C) x 2 phytase activity levels (PA; 120 or 240 FTU/kg) in a factorial arrangement with randomized complete block design. Basal diets were antibiotic-free, all-vegetable with Ca and available P at 0.4 and 0.2%, respectively. On d 0, Ross x Ross 708 male chicks were randomly allocated into 140 raised wire cages (12 birds/cage; 10 replications/treatment). Ileal digesta was collected on d 14. Interactions occurred between PIS and PA for ileal proportions of IP4, IP5, and IP6 (P=0.014, P=0.004, and P=0.039, respectively). Higher ileal concentrations of IP4 were found when feeding 240 FTU/kg, regardless of PIS. Singular application of B and C had similar high IP5 concentration at both 120 and 240 FTU/kg, indicating reduced IP hydrolysis. Feeding A+B+C at 240 FTU/kg increased IP5. Birds fed C alone at 120 FTU/kg had more IP6 than those fed B, B+C, and A+B+C at 120 FTU/kg, as well as A, B, C, A+C, and A+B at 240 FTU/kg. Feeding higher PA of C alone resulted in decreased IP6 similar to all other PIS, as compared to those fed C at 120 FTU/kg. Interactions were found for Ca, P, lysine, threonine, valine and arginine digestibility (P<0.05). Birds fed B alone had improved Ca digestibility with increased PA, while those fed A alone and A+C had reduced digestibility at 240 FTU/kg. Feeding B and B+C increased P digestibility at higher PA. Birds fed B, B+C, and A+B+C demonstrated improved amino acid digestibility with increased PA, while those fed C and A+C exhibited a reduction. These data provide a mode of action for the observed performance data and assumed synergistic effects of combined use ofA, B, and C tested at these activity levels. Further research should investigate the effects of feeding phytase combinations with various diet specifications, as well as throughout an entire grow-out.

Key Words: phytase, exogenous feed enzyme, digestibility, inositol phosphate esters

P289, **Refined functional carbohydrates reduce cecal Salmonella reading load in a challenge trial compared to turkey hens fed a control diet** Sangita Jalukar*[1], Dan Karunakaran [1], Dan Moore [2], Sam Hendrix [2], Kari Kuykendall [2] *[1] Arm and Hammer Animal and Food Production, [2] Colorado Quality Research Inc.*

Refined functional carbohydrates (RFCs) are produced by enzymatic hydrolysis of the cell wall of *Saccharomyces cerevisiae* yeast. They are an all-natural feed ingredient with Generally Recognized as Safe (GRAS) status. RFCs have been demonstrated to have activity against gram negative bacteria, including *Salmonella*. This study was conducted to determine the effect of RFCs supplementation on cecal colonization in turkey hens challenged with *Salmonella reading*. Day-old poults were allotted with 22 poults/pen, 8 pens/treatment from day1-84 days. Treatments fed were, Control or RFC (CELMANAX), 0.5 kg/MT. Birds were chal-

lenged on d7 with two field strains of *Salmonella reading*, 1.0X10^6 CFU/bird. Performance and *S. reading* load in the ceca (6 birds/pen) was measured on D 84. Data were analyzed statistically, with significance noted at P<0.05. Results: The percentage of hens testing negative for *S. reading* was 75% in RFC group compared to 31.7% in the control fed hens (p<0.05). Birds fed RFC had average *S. reading* loads that were 1.77 logs less than the control fed hens (p<0.001). No statistically significant differences were noted for ending BW or feed intake between treatments. Feed conversion ratio (FCR) was similar between turkeys fed control or RFC. Conclusions: RFC supplementation in turkey diets could help reduce *S. reading* infection while maintaining performance.

Key Words: Salmonella reading, turkey, yeast

P290, **Effects of a worm compost leachate as source of humic substances in starter broiler chickens challenged with a vaccine against coccidiosis** Maria Angeles*[1], Sergio Gomez-Rosales [1], Yair Lopez-Garcia [1], Alejandra Dominguez-Negrete [2] *[1] National Center of disciplinary Research in Animal Physiology and Genetics, INIFAP, [2] Faculty of Natural Sciences, Autonomous University of Queretaro*

The objective of the study was to evaluate the growth performance, breast yield, tibia measurements and oocyst excretion in broilers chicks added with increasing levels of a worm compost leachate (WCL) in the drinking water and given a 10× live coccidiosis vaccine (COCV) at one d of age. Ross male broilers (n=180) were allocated in groups of two in holding cages from 1-21 d of age and were randomly assigned to three levels of the WCL (0, 15 and 30%) in the drinking water. Within each treatment, half of the chicks were inoculated with a COCV using a 10x dose of the commercial recommendation. The WCL was collected from a worm compost and contained 0.26 and 0.37% of humic and fulvic acids. The growth performance from 1-7, 1-14 and 1-21 d of age was registered. At 7, 14 and 21 d, excreta were collected for oocyst counting and 10 chicks from each treatment were killed to get the breast yield and tibia measurements. The data was subjected to ANOVA and orthogonal contrasts. In the period from 1-7 d, the FCR was lower in COCV challenged chicks with 0% addition of WCL in the drinking water, while in the rest of the treatments the FCR was similar (Interaction between WCL and COCV, P <0.05). The breast yield was higher in chicks receiving 30% of WCL. From 1-14 d and from 1-21d, the FCR was similar in non-COCV challenged chicks, but it was higher in COCV challenged chicks supplemented with increasing WCL (Interaction between WCL and COCV, P <0.01). From 1-21 d, the breast yield was higher in non-COCV challenged chicks with increasing levels of WCL; but in COCV challenged birds the breast yield diminished with increasing additions of WCL (Interaction between WCL and COCV, P <0.01). The dry weight and ashes of the tibia were similar in non-COCV challenged chicks, but these variables decreased in COCV challenged chicks with increasing WCL in the water (Interaction between WCL and COCV, P <0.01). At 7, 14 and 21 d, the oocyst excretion was higher (P <0.05) in COCV challenged chicks. In conclusion, the addition of WCL in COCV challenged chicks did not ameliorated the deleterious effects observed in the productive and tibia variables, nor reduced the oocyst excretion; while at 21 d the addition of the WCL improved the breast yield only in non-VCOC challenged chicks.

Key Words: Chicks, Worm compost leachate, Production, Tibia, Oocysts

P291, **The effects of oregano essential oil on pullet rearing** Emma Lund*[1], Wendy Wakeman [1], Ken Anderson [2] *[1] Anpario, [2] North Carolina State University*

Oregano (*Origanum vulgare*) essential oil (OEO) contains over 100 compounds with active properties. Four key compounds are carvacrol, thymol, ρ-cymene, and γ-terpinene. These compounds have been shown to have antibacterial, anti-inflammatory, and antioxidant properties. Oregano essential oil has been shown to be beneficial for gut health, and subsequent growth and production performance in poultry.

Aims: The purpose of this research was to investigate the effects of feeding an oregano essential oil throughout rearing on growth and body composition of Dekalb White pullets.

Methods: The study was conducted at North Carolina State University. Chicks of high health entered the study at day 1 of age and were allocated equally to one of two groups: basal diet (Control); or basal diet plus 300g/t OEO provided in the feed (5% OEO on an inert carrier (Orego-Stim® (OS), Anpario plc, UK). Pullets were housed indoors in rearing cages of 26 pullets per replicate, with 18 replicates per group (468 pullets per group) until 16 weeks of age. Space allocation was 48 inches^2 per pullet throughout rearing. Pullets were reared to meet the breed guidelines for body weight. Water was supplied *Ad libitum*. Body weight and feed consumption were recorded bi-weekly, and a subset of pullets were analysed for body composition at the end of rearing. Statistical analysis (Student's t-test) was performed using JMP software (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989-2019).

Results: There were no significant differences in body weight gain and feed intake. Shank length was significantly greater in OEO pullets compared to control pullets (97.06mm vs 95.44mm, p<0.05). Rear quarter percentage was significantly greater in OEO pullets versus control pullets (31.82% vs 31.03%, p<0.05). Pullets supplemented with OEO had an overall 0.77% more uniform body weight gain compared with control pullets.

Conclusions: Inclusion of an oregano essential oil in the pullet diet improved flock body weight uniformity in rearing, and had a positive effect on reproductive fitness, in terms of rear quarter and shank length. Improved flock uniformity, and individual reproductive fitness is expected to result in more consistent egg sizes and improved lifetime laying performance.

Key Words: Oregano, Origanum vulgare, Essential Oil, Pullet rearing

P292, **In vitro evaluation of the prebiotic properties of Lactobacillus fermentation product (Lumensa)** Sean Yang*[1], Jung Mun Yang [2], Elizabeth Wozniak [1] *[1] Cytozyme Laboratories, Inc., [2] Utah State University*

Lumensa™ for Poultry (LP; AAFCO 36.11 Dried *Lactobacillus acidophilus* Fermentation Product) applied to antibiotic-free diets has been shown to improve performance, reduce pathogenic bacteria and increase *Firmicutes* and *Bacteroidetes* ratio in the cecum of broiler chickens exposed to heat stress. The objective of this study was to evaluate the prebiotic effect and heat stability of LP. LP was evaluated for bacterial growth performance of *Lactobacillus* sp. compared with non-treated control, fructooligosaccharides (FOS) and yeast extracts (YE) treatments at 37°C under anaerobic condition (5.6% CO_2). Supplementation of LP (200 and 400 ppm) significantly increased cell density (OD_{650nm}, P < 0.01), bacterial counts (P < 0.01) and pH change (P < 0.01) after 24 h culture of *Lactobacillus* sp. at 37°C under anaerobic conditions compared with control, FOS (1,000~5,000 ppm) and YE (200~2,000 ppm) treatments. To evaluate the heat stability of LP, LP was exposed to 90 and 95°C for 2~30 min. Heat-treated LP were analyzed Trolox equivalent antioxidant capacity (TEAC) and biological activity (BA) using *Lactobacillus acidophilus* (ATCC 43121) compared to non-heat-treated control to assess the heat stability of LP. The TEAC and BA based on the growth of *L. acidophilus* were not affected by heating temperature and duration of treatment on LP (P > 0.05). In conclusion, Lumensa increased the growth of *Lactobacillus* sp. under anaerobic conditions and showed high thermal stability.

Key Words: heat stability, Lactobacillus sp., Lumensa

P293, **Study of peptidoglycan in broilers gastrointestinal tract and the effect of a novel muramidase in bacterial debris hydrolysis** Jacqueline Chimilovski[*1], Mikkel Klausen [2], Carsten Frederiksen [3], Yun-Ting Wang [4], April Levy [4] *[1] Novozymes Latin America Ltda, [2] Novozymes A/S, [3] Novozymes, [4] DSM Nutritional Products North America*

Microbiota in the gastrointestinal tract of animals lives in homeostatic equilibrium with the host. Natural death and cell turnover of the microbiota causes the release of cell components or bacterial cell debris to the gut environment that could interact with the intestinal wall and impact gastrointestinal functionality. Innovative tools to study bacterial waste are needed to understand how dead bacteria and cell debris can impact *in vivo* performance.

A major part of the bacterial biomass is peptidoglycan, a complex polymer forming the bacterial cell wall. It is built from long glycan strands held together by short peptide bridges. The glycan strand is composed of the β-(1,4)-linked sugars Nacetylglucosamine and Nacetylmuramic acid, the latter of which is uniquely found in peptidoglycans and, therefore, can be used as a bacterial waste biomarker.

We have adapted a method used previously to estimate bacterial biomass in soil samples to study bacterial waste peptidoglycan in animals' intestine content through the quantification of muramic acid. Soluble and total peptidoglycan were measured and used to calculate percent soluble peptidoglycan of total. A novel microbial muramidase has recently been described in literature, which is capable to selectively hydrolyze peptidoglycan from bacterial waste, thereby improving animal performance. Here we present data using this novel method to study muramidase peptidoglycan hydrolysis in 707 broilers digesta samples (crop sampled from 1 trial, jejunum from 9 trials, Ileum from 1 trial, caecum from 2 trials and excreta from 1 trial). All trials presented significant statistical muramidase effect on peptidoglycan hydrolysis compared to control when considering increase in percentage of soluble peptidoglycan (Turkey-Kramer HSD all pair comparison, p level = 0.05, SAS JMP®, Version 14). Percent soluble peptidoglycan on average increased upon muramidase supplementation: from 37% to 46% in crop samples, from 27% to 55% in jejunum samples, from 19% to 54% in ileum samples, from 8% to 14% in caecum samples and from 14% to 23% in excreta samples

Key Words: Enzyme, Peptidoglycan, Microbiota, Muramic acid, Muramidase

P294, **All-Lac® XCL 5X + Actigen® alleviates stress and promotes growth performance traits of broiler chicks** Lizza Macalintal, Anthony Pescatore[*], Tuoying Ao, Michael Ford, Karl Dawson, Ronan Power *Alltech-University of Kentucky Nutrition Research Alliance*

Alternatives to antimicrobial usage are changing the husbandry practices in the poultry industry. The poultry operations are gearing towards antibiotic-free farming in response to consumer demands and the changing market dynamics. Possible alternatives include pro-/pre-/synbiotic, essential oils, organic acids, and enzymes among others. Herein, two independent studies were conducted to investigate the effect of a probiotic, All-Lac® XCL 5X (lactic acid-based supplement, Alltech, Inc) and a yeast cell wall-based product, Actigen® (Alltech, Inc) on stress and growth performance of broiler chicks. Stressors in these studies were defined as stress due to bird handling (catching and restraining) and oral gavage technique. Identical experimental design was used in 2 experiments: 2 treatments with 10 replicate cages with 6 chicks/cage. In trial 1, birds were given a corn-soybean meal basal diet with or without All-Lac®. In trial 2, birds were fed corn-soybean meal basal diets with or without All-Lac® + dietary Actigen® (400g/T). In both experiments, All-Lac® (min. 250 billion CFU/g) was administered via oral gavage at a rate of 0.3ml/bird on d5-8 and 12-16. Fresh feed and water were provided ad libitum. Data were analyzed by one-way ANOVA using a randomized complete block design. In Trial 1, overall BW gain was significantly higher in birds that received All-Lac® gavage compared to control birds (P=0.02) by d21. Feed intake was higher (P<0.05) for the All-Lac® birds on d 7 but not 14 or 21. Feed to gain ratio was not different between treatments throughout the feeding trial. In Trial 2, weight gain of broilers provided with All-Lac®+ Actigen® supplementation was higher (P<0.05) on d7 (159.42 vs 147.92g) and 21 (868.38 vs 825.53g) compared to the basal diet-fed control birds. Feeding All-Lac® + Actigen® resulted in birds that converted feed to body mass more efficiently compared to non-supplemented birds (P<0.05) without affecting feed intake. Taken together, these results suggest that All-Lac® alleviated stress while supporting growth performance traits, however, in combination with dietary Actigen, better feed efficiency was achieved.

Key Words: probiotic, lactobacillus, yeast cell wall, stress, broiler

P295, **Temporal Survey of Salmonella serovars within a broiler complex using CRISPR-typing** Jodi Delago[*], Samantha Anderson, Ellen Davis, Alexandra Smith, Rebecca Wujek, Claire Podvin, Lindsay Leonard, Navee Thao, Lukus Wardecke, Thomas Rehberger *Arm & Hammer Animal and Food Production*

Salmonella is a leading cause of bacterial foodborne illness in the US. It is frequently carried by poultry and other food animals, often with no apparent symptoms. Understanding the serovar patterns of *Salmonella* carriage in birds and its spread and persistence in the pre-harvest production environment is crucial to developing effective controls measures. To this end we collected gastrointestinal tract (GIT) samples from breeder hens (n=87) and broiler chickens (n=112), as well as broiler house litter samples (n=48 from 10 different houses) and transport cage swabs (n=91) from a single broiler complex at 5 different time points over a 1-year period. Samples were enriched overnight in Tetrathionate broth (TT), followed by a second overnight enrichment in TT, and plated on XLT4 to determine *Salmonella* prevalence and to recover isolates. Prevalence data were analyzed using the GLM procedure of JMP and Least Squares Means were separated using Tukey HSD. *Salmonella* prevalence was greater in litter and transfer cage swabs than in broilers and breeders (p <0.05). Broilers had a greater prevalence of *Salmonella* than breeder hens (p <0.05). Up to five isolates were selected from positive samples. The CRISPR-2 region was amplified from each isolate and sequenced. Sequences were compared to a database of CRISPR-2 sequences of known *Salmonella* serovars to determine the presumptive serovar of the recovered isolates. Of the 802 isolates that were CRISPR typed, 485 were identified as CRISPR-type Typhimurium, 181 were Kentucky, and 114 were Enteritidis. Typhimurium and Kentucky were recovered at every time point and from every sample type. Enteritidis was also found in every sample type and was recovered at all timepoints except for the initial sampling. Two genetically distinct populations of Enteritidis, each having a different CRISPR sequence, were found within this complex. Other serovars that were observed in 5 or fewer total isolates were Infantis, Newport, Braenderup, Ouakam, and Cerro. The results of this survey suggest that *Salmonella* Typhimurium and Kentucky are commonly present at this complex and persisted over time. They were identified in the litter environment where they may serve as a reservoir to inoculate subsequent flocks. Furthermore, the recovery of the same *Salmonella* CRISPR types in transport cage swabs suggests that these birds are shedding *Salmonella* in transit to the plant and have the potential to contaminate the post-harvest environment.

Key Words: Salmonella, CRISPR-typing

P296, **Evaluating the response of broiler chickens fed various dietary levels of fermented seaweed blend** Mohsen Mohammadigheisar [1], Munene Kithama [1], Parry Bast [2], Elijah Kiarie[*1] *[1] University of Guelph, [2] Bio-Ag Consultants and Distributors Inc.*

Consumer and regulatory pressures on the use of antibiotics growth promoters have motivated researchers to find alternatives. We used 560 Ross 708 male broiler chickens to evaluate dose response of fermented seaweed blend (FSW) on growth, breast yield, organs weight, and plasma biochem-

ical profile. The FSW was a mixture of green, brown and red seaweed species fermented with lactobacillus culture (1.5 x 109 cfu/g), dried and ground into powder. Diets were: antibiotic free corn soy-bean meal diet with 0, 0.5, 1 or 2% FSW and a positive control (PC; 20mg/kg of avilamycin, 40mg/kg narasin and 40mg/kg of nicarbazin) fed in phase feeding program (starter/grower; d 0-24 and finisher; d 25-35). Diets were allocated to 8 replicate cages (14 birds/pen). Feed intake (FI) and BW was monitored by phase, and on d 24 and 35 samples of birds were bled for plasma and necropsied for organ weights and breast yield (d 35 birds only). In phase 1, birds fed PC diet had higher (P<0.05) BW and BWG than 0 and 2% FSW birds whilst birds fed PC and 0.5% FSW had 6.1 and 3.1 better FCR than birds fed 0% FSW. Quadratic increase (P<0.01) in BWG was observed for FSW in phase 1, peaking at 0.5 and 1% FSW. Birds fed PC, 0 and 1% FSW had higher (P=0.04) d 35 BW than for birds fed 0.5% FSW, however, there was no overall (d 0-35) diet effects (P>0.10) on FI and FCR. Supplemental FSW increased liver weight linearly and quadratically (P<0.01), specifically, birds fed 0.5, 1 and 2% FSW had 15.4, 21.5, and 17.6%, respectively heavier liver than for birds fed 0% FSW. Bids fed PC exhibited lighter (P<0.01) small intestine weight relative to the other birds and larger (P=0.03) bursa than birds fed 0.5% FSW whilst the weight of these organs was cubically increased (P≤0.03) by FSW. A quadratic effect (P=0.05) was observed on d 35 breast yield with birds fed 0.5% FSW showing the highest value. The FWS increased plasma protein and glucose linearly (P≤0.03) and lipase non-linearly (P<0.01) on d 24 and amylase linearly (P=0.03) on d 35. In summary, data indicated FSW at 0.5 and 1% inclusion supported growth performance to same extent of antimicrobials and coccidiostat. The effects of FSW on the liver, breast yield and plasma profile suggested metabolic effects that warranted further investigations.

Key Words: Broiler, growth performance, gut health, fermented seaweed blend,, plasma biochemical profile

P297, **Evaluation of a standardized blend of bioactives supplementation to support turkeys facing a coccidiosis challenge** Elisa Arnaud [1], Brian Glover [2], Catherine Ionescu [1], Jennifer Maurin*[1] *[1] Pancosma S.A., [2] Archer Daniels Midland Company*

Coccidiosis control in turkeys is a challenge in antibiotic free programs negating the use of ionophores as anti-coccidial. The objective of the study was to determine the value of a standardized encapsulated blend of bioactives (garlic tincture and eugenol from cloves) on turkeys infected with coccidiosis. A 42-d pen trial was conducted with 450 turkeys (hens, at hatch), using 3 dietary treatments, 6 pens/ treatments and 25 turkeys/pen to evaluate performance. Data were analyzed using ANOVA and Tukey's test (significant if *P*<=0.05) for means' comparisons on R software. The coccidiosis challenge was applied to all individual hens except a basal treatment UC (for Uninfected Control) challenging birds with a blend of *Eimeria* species (320,000 oocysts/ml) with major species that generally infect turkeys. Infectious dose was administrated on placement *via* oral gavage (0.5 ml/bird). Dietary treatments consisted of basal diets (Uninfected Control, UC) void of coccidiostats and antibiotics, basal diets with coccidiosis challenge (Infected Control, IC) and IC supplemented with bioactives (XT, XTRACT Shield at 150 g/ ston). Live performance was measured per pen at day 21 and 42. Lesion scores and oocysts counts were determined at 20, 21, 22, 35 and 42 days.

A significant difference (*P* =0.002) was observed from 0 to 42 days between UC and IC for weight gain (2.309 *vs* 2.033 kg), showing that the challenge was successful. XT significantly alleviated the negative effect of coccidiosis infection on weight gain from 0 to 21 days and 0 to 42 days *vs* IC (respectively 0.568 *vs* 0.495, *P*<0.001, and 2.226 *vs* 2.033 kg, *P*=0.002). XT improved feed efficacy compared to IC (1.67 *vs* 1.73). No differences between treatments were observed on lesion scores and oocysts excretion.

This trial showed that standardized bioactives have a potential to support bird robustness to deal with an Apicomplexa parasite challenge. By alleviating subclinical effects of coccidiosis, turkey performance can be improved.

Key Words: eugenol, garlic, plant-extract, Apicomplexa, performance

P298, **Performance efficacy of Victus® Broiler is supported by identification of enzyme components by proteomics** Nelson Ward*[1], April Levy [1], Eduardo Della Pia [2], Jacqueline Chimilovski [2] *[1] DSM Nutritional Products, [2] Novozymes A/S*

Victus® Broiler is a strategic enzyme composite targeting the physiological life stages of the bird, as well as ingredient composition of the diet. This enzyme assembly includes multiple carbohydrases with key debranching enzymes that dismantle non-starch polysaccharides (NSPs) in corn and soybean meal (SBM), as well as protease during the early growth stage and phytase in all phases. Protein analysis by proteomics was employed for a detailed proteome determination of this product. With appropriate modifications, enzyme families were grouped according to van den Brink and de Vries (2011).

Mass Spectrophotometry confirmed the presence of numerous enzyme activities. Carbohydrases included six (6) different β-1,4-endo-xylanases and β-1,4-xylosidase for arabinoxylan degradation and β-glucanase (β-1,3(4)-endoglucanase) for β-glucan degradation. Included were enzymes critical for cellulose degradation (β-1,4-endoglucanase (cellulase), cellulohydrolase and β-1,4-glucosidase), as well as other enzymes to disassemble the tightly interwoven NSPs in cell walls. Also revealed were enzymes associated with the deconstruction of pectins, xyloglucan and galactomannan in SBM. Additionally, a wide range of debranching enzymes for both hemicelluloses and pectins were identified, all playing key roles to remove substituents from the primary NSPs to facilitate efficacy by main chain carbohydrases. An ongoing tabulation of 21 North American broiler trials determined the Victus enzyme composite increased body weight by 0.4% over existing commercial enzyme combinations and programs, while improving feed conversion ratio (FCR) by 1.2% with a 2.9% reduction in feed costs per lb live weight. The FCR in the control and Victus® groups was correlated (P<0.05) such that the beneficial effect of Victus® on FCR was more pronounced in groups with higher inherent FCR implying advantages in flock uniformity. Mortality was unaffected.

In summary, proteomics confirmed the presence of strategic enzymes in Victus® Broiler with key roles to recover more energy, amino acids, P, Ca, and other components from commercial corn/SBM broiler feeds. Likewise, commercial broiler performance results are suggestive of a link between enzyme composition with live performance and lower production costs.

Key Words: Proteomic analysis, enzymes, debranching enzymes, broiler, Victus

P299, **In vitro evaluation of antimicrobial activity of short- and medium-chain fatty acid salts and their combinations against Campylobacter jejuni** Cinta Sol*[1], Mónica Puyalto [1], Bernat Canal [1], Manuel Gomez [2], Ana Carvajal [2], Juan Mallo [1] *[1] NOREL, SA, [2] Department of Animal Health, Faculty of Veterinary Medicine, Universidad de León*

Organic acid and their salts are feed additives used as alternatives to antibiotics for health and growth promotion in animal production. This was a preliminary study with the aim of investigating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different organic acid salts against six strains of *Campylobacter jejuni* (1 from collection and 5 field isolates). The products tested were sodium butyrate protected with sodium salt of palm fatty acids distillate (BUT), sodium salt of coconut fatty acids distillate (DIC) and their combination, sodium butyrate protected with sodium salt of coconut fatty acids distillate (70:30 ratio, DIC+). For each product and each strain, MIC was deter-

mined by the broth microdilution method using 96-well microplates and brain heart infusion (BHI) adjusted at pH 6.0. MBC was also determined by subculturing from wells without evident bacterial growth onto fastidious anaerobe agar (FAA). The value that inhibited and killed 50% of the tested isolates was calculated (MIC50 and MBC50, respectively). The values of MIC50 showed that DIC was the most effective bacteriostatic product (32 ppm) against *C. jejuni* followed by DIC+ (256 ppm) and BUT (3,000 ppm). Regarding MBC50, the trend was similar, DIC was the most effective (96 ppm) followed by DIC+ (256 ppm) and BUT (3,000 ppm). It is well known that butyric acid, a short-chain fatty acid has strong antimicrobial activity against Gram-negative bacteria. In contrast, coconut fatty acids distillate is a medium-chain fatty acid source rich in lauric acid, which has strong antimicrobial activity against Gram-positive bacteria. Both products are generally available as salts to facilitate the application in feed. In this study, the results showed that DIC was the most effective product against *C. jejuni*, followed by DIC+ and BUT. The activity showed by DIC could be a consequence of its natural origin, with a complex composition including not only lauric acid (C12) but also caproic (C6), caprylic (C8) and capric acids (C10), all of the products with activity reported against Gram-negative bacteria.

Further studies would be required to better understand these interactions as well as *in vivo* studies to verify their effects under field conditions.

Key Words: sodium butyrate, medium-chain fatty acids, Campylobacter jejuni

P300, **Impact of coffee weed (senna occidentalis) leaf aqueous extract on the blood profile of broiler chickens.** Sylvester Okosun, Dick Obasoyo, Stanley Omoikhoje*, Eromosele Ehebha *Ambrose Alli University*

The feeding trial was conducted for eight weeks to test the effect of coffee weed (Senna occidentalis) leaf aqueous extract on the haematology and serum chemistry of one hundred and twenty day-old Aborican broiler chickens. Each treatment group contained three replicates with eight birds per replicate to make a total of twenty four chicks per treatment group. The birds were assigned to five dosages (0, 25, 50, 75 and 100ml) of coffee weed leaf aqueous extract (CWLAE) designated as T1, T2, T3, T4 and T5. The haematological traits showed that packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and lymphocytes were ($P < 0.05$) affected by the coffee weed leaf aqueous extracts (CWLAE) but mean corpuscular haemoglobin concentration (MCHC), red disc width (RDW), platelets and neutrophils were ($P<0.05$) similar. Data on the serum biochemical indices of broiler chickens revealed that total protein, albumin and globulin values were ($P<0.05$) affected by the treatments but creatinine, urea, cholesterol, SGOT and SGPT values were not. It can therefore be inferred that the inclusion of coffee weed leaf aqueous extract up to 100ml in drinking water did not have any adverse effect on the blood profile of broiler chickens.

Key Words: Broilers, haematology,, probiotics, serum chemistry, water

P301, **Antibacterial activity of an essential oil combination against different enteric bacterial pathogens** Bernat Canal*[1], Luis Mesas [1], Cinta Sol [1], Monica Puyalto [1], Ana Carvajal [2], Manuel Gomez [2], Juan Jose Mallo [1] *[1] Norel S.A., [2] Universidad de Leon*

The non-therapeutic use of antimicrobial agents (AGPs) has been a common strategy to improve animal production. Their effects include the reduction/prevention of enteric diseases by promoting gut health and, particularly in poultry production, the control of necrotic enteritis caused by Clostridium perfringens. The focus is on developing alternative products to AGPs and, in this context, a key aspect is whether a broad range of bacterial pathogens can be equally susceptible to the same product and concentration. This trial aimed to evaluate the in vitro antibacterial activity of a combination of essential oils (EO – EO FIT® Poultry, Norel) against common bacterial pathogens present in poultry production. The enteric pathogens selected were Salmonella enterica ssp. enterica, Escherichia coli, C. perfringens and Campylobacter jejuni. Lactobacillus fermentum, as a common beneficial intestinal bacteria, was also included. The minimum concentration which inhibits/kills 50%/90% (MIC50/90/MBC50/90, respectively) of the isolates tested was calculated for each bacterial species by the broth microdilution method, using 96-well microplates with broth culture adjusted at pH 6. MBC was determined by subculturing aliquots from wells without evident bacterial growth. The results showed that the lowest MIC50/90 were obtained for C. jejuni (18.8/18.8 ppm) while S. enterica ssp. enterica (150/300 ppm), C. perfringens (150/150 ppm) and E. coli (300/300 ppm) required higher concentrations, being L. fermentum (600/1,200 ppm) the most resistant. Similar findings were observed for the MBC50/90, C. jejuni (18.8/75 ppm) was the most susceptible pathogen followed by C. perfringens (150/150 ppm), S. enterica ssp. enterica (300/300 ppm) and E. coli (300/300 ppm). Thus, the combination showed bactericidal activity at higher concentration for L. fermentum (1,200/2,400 ppm). It can be concluded that C. jejuni was particularly susceptible to this combination of EO while L. fermentum required the highest concentrations. These results suggested that a concentration of 300 ppm of these EO would be able to reach the MBC50/90 of all bacterial pathogens tested. However, further studies are necessary to better understand the effects on microbial population of these EO under in vivo conditions.

Key Words: Essential oils, Antibacterial effect, Synergy

P302, **Proteomic analysis of a multi-enzyme carbohydrase product and in vitro characterization of its activity on cereals and oil seeds** Jacqueline Chimilovski*[1], Eduardo Della Pia [2], Adam Smith [3], Philippos Fidiarakis [4] *[1] Novozymes Latin America Ltda, [2] Novozymes A/S, [3] DSM Nutritional Products, [4] DSM Nutritional Products AG*

MultiGrain® (DSM Nutritional Products, Basel, Switzerland) is a carbohydrase multi-enzyme product produced by *Trichoderma reesei*. This fungal source of MultiGrain was previously recognized as an exceptional producer of carbohydrases that synergistically degrade fiber (*Herpoel-Gimbert et al., 2008*). Advances in protein analysis by proteomics have allowed us to make a detailed proteome analysis of this multi-enzyme product. The proteome analysis using a Thermo Orbitrap Velos Pro LC/MS instrument has given new insights into the plant polysaccharide enzyme degradation potential of the *T. reesei* product. The enzyme proteome of the *T. reesei* fermentation product targeting plant polysaccharides was compared to the *in silico* predicted corresponding *T. reesei* secretome. The secretome was predicted from the *T. reesei* genome sequence using the SignalP 4.1 software solution. MS analysis indicated that the product contains a variety of different activities including different endo-xylanases and b-glucanases, xyloglucanase, and several other cellulases and debranching enzymes. The enzyme composition identified by proteomics was confirmed by (i) measurements of non-starch polysaccharides solubilized by the action of the enzyme product, (ii) viscosity measurements on wheat, rye arabinoxylan and b-glucan incubated with the carbohydrase and (iii) fluorescence and confocal microscopy images of substrates degraded by the commercial product. The data show that the multi-enzyme product can solubilize the complex cell walls fiber structure of both cereals such as wheat, barley, rye, oat, rice and protein rich plants such as canola and sunflower. The results provide evidence that Multigrain® can help releasing nutrients from their encapsulation in cereal cell wall structures by the synergistic effect of all these enzymatic activities and thereby have a positive impact on nutrient digestibility and explain the positive effect of its supplementation on livestock performance.

Key Words: Enzyme, Carbohydrase, Proteomic, Fiber, Polyssacharide

P303, **Titration of spray-dried plasma in broiler starter feed budget programs on early broiler performance** J Campbell[*1], R Gonzalez-Esquerra[1], J Polo[1], M Schwartz[2] [1] *APC, Inc.,* [2] *Schwartz Consulting Services*

Spray-dried plasma (SDP) has provided many benefits for improved performance during both production and challenge conditions in poultry and other livestock species. The objective was to determine if feeding titrated levels of SDP in either two starter feed budget programs would impact early broiler performance and mortality exposed to challenge model utilizing 10X cocci vaccination. The experimental design consisted of 6 treatments of titrated levels of plasma of 0, 1, or 2% SDP formulated into the starter feed budget fed from d 0 to 13 (454 g budget) or d 0 to 17 (681 g budget). Common grower and finisher diets were fed following the starter periods. Diets were corn/SBM-based and formulated with nutrient density like commercial practice in North America. All diets had similar nutrient profiles. Broilers were followed to d 35 of age. At placement, broilers were challenged with 10 X cocci vaccination via oral gavage of a one mL solution. The broilers were housed in cages with paper under them till d 16 to allow for natural cycling of coccidiosis vaccine. The broilers were housed as 8 or 9 birds per pen (0.046 m^2/bird density) with 10 pens per treatment and performance parameters measured. Data were analyzed as 2 x 3 factorial with linear contrast from d 0-13 and significance determined. From d 0 to 13, as plasma inclusion increased from 0 to 2% in the starter feed, BW (0.43, 0.45 and 0.46 kg) and adjusted feed conversion (1.218, 1.190, and 1.148) improved linearly ($P<0.06$) for 0, 1, and 2% inclusion level of SDP respectively. The level of challenge was higher than expected with an overall mortality of 18% across treatments with 6% occurring in the last phase, consequently variation was higher than normal. From d 0-35, BW (2.13, 2.14, 2.19, 2.14, 2.20, 2.21 kg) were numerically improved for 454 g budget for 0, 1, and 2% SDP and 681 g budget for 0, 1, and 2% SDP, respectively. No differences in feed conversion was observed due to the unusual variation. In conclusion, these results indicate that increasing levels of SDP up to 2% in the starter feed positively impacted early broiler performance by improving gain and feed conversion linearly and numerically improved overall BW to d 35 under high challenge.

Key Words: spray-dried plasma, broilers, challenge, poultry

P304, **The effects of refined functional carbohydrates (RFCs) supplemented to laying hens on egg production and mortality under commercial conditions** Sangita Jalukar[*1], Dan Karunakaran[1], Jill Nezworski[2] [1] *Arm and Hammer Animal and Food Production,* [2] *Blue House Veterinary LLC*

Enzymatic hydrolysis of yeast produces refined functional carbohydrates (RFC) which have been shown to improve poultry performance and reduce *Salmonella* prevalence. This study was conducted to evaluate the effect of RFC supplementation until 60 weeks of age to laying hens on egg production, mortality and *Salmonella* prevalence under commercial conditions. A total of eight commercial flocks were selected, assigned to two treatments and were matched for placement date. Day-old White Leghorn type layer chicks were fed a non-medicated commercial pullet and layer diet in mash form supplemented with CELMANAX™ (Arm and Hammer Animal and Food Production, Princeton, NJ), 0 or 500 g/MT until 60 weeks of age. Each commercial flock consisted of approximately 90,000 birds housed in stacked deck belted cage systems. Mortality, egg production, and feed intake data was collected weekly. Eggs per hen housed was calculated as is and standardized for difference in age at light stimulation. For *Salmonella* testing, samples were collected using NPIP and FDA environmental sampling procedures and cultured as per NPIP culture methods. Environmental samples were collected at a rate of two swabs per row resulting in 8-14 samples per time point. Results from four control and four RFC houses were combined and means calculated for each parameter. RFC supplementation reduced cumulative mortality at 60 weeks of age from 7.4 % in control to 5.1 % in hens fed RFC. Average eggs per hen housed (EHH) were 257.2 for control and 260.8 for RFC treatments. Feed intake per 100 eggs produced was similar between treatments. Environmental *Salmonella* prevalence at 45 weeks of age decreased from 33.7% in control to 20.0% for RFC houses. In conclusion, refined functional carbohydrates improved egg production parameters and reduced mortality while reducing but not eliminating environmental *Salmonella* prevalence in commercial layers.

Key Words: Layers, Yeast, Salmonella, egg production

P305, **Addition of an extract of humic substances in broiler chickens subjected to intermitting feed restriction** Alejandra Domínguez-Negrete[1], Sergio Gomez-Rosales[*2], Maria Angeles[2], Luis Lopez-Hernandez[2], Tercia Reis de Souza[1], Yair Lopez-Garcia[2], Anai Zavala-Franco[3], Guillermo Tellez-Isaias[4] [1] *Faculty of Natural Sciences, Autonomous University of Queretaro,* [2] *National Center of disciplinary Research in Animal Physiology and Genetics, INIFAP,* [3] *Center for Research and Advanced Studies of the National Polytechnic Institute, CINVESTAV-IPN,* [4] *Department of Poultry Science, University of Arkansas*

The objective of the study was to evaluate the growth performance, tibia measurements, dietary components balance, breast meat quality and antioxidant indicators and microbiological status of broiler fed an extract of humic substances (EHS) under ad libitum (ADLIB) or feed restriction (REST) to induce intestinal inflammation. Individually caged broilers (n= 180, from 14-35 d of age) were assigned to a factorial arrangement of 3 dietary treatments: 1) Positive control with bacitracin methylene disalicylate (BMD) and salinomycin; 2) Negative control without BMD nor salinomycin, and 3) Same as negative control but with 0.25% EHS in the feed, and 2 feeding regimens 1) ADLIB or REST for 24 h on d 1, 7 and 14 of the study. The EHS was obtained from a worm compost of sheep manure using an alkaline solution. The EHS was dried up in stove and ground using a 1 mm sieve before adding it to the feed. The characterization of the EHS showed that the concentration of HS and the aromaticity index were 69.1% and 53.8%, respectively. Results were subjected to ANOVA. There was no interaction between the dietary treatment and feeding regimen in any of the variable responses evaluated. The growth performance, tibia measurements, the balance of dietary components and the antioxidant status of the breast meat were similar among dietary treatments. Positive control and EHS-fed broilers showed higher carcass yield ($P < 0.05$) and lower oocyst excretion ($P < 0.01$) compared to negative control birds. Lactic acid bacteria (LAB) and Clostridium perfringens (CLOS) were higher in negative control and EHS-fed broilers compared to positive control ($P < 0.01$). The final body weight, FI and WG, as well as the legs and thighs weight and the breast and carcass weight and yield were lower ($P < 0.01$) in REST broilers compared to ADLIB broilers. Lower pH and higher superficial L and water loss by centrifugation ($P < 0.05$) in breast meat of REST broilers were also observed. Higher carcass yield could be due to lower CLOS and oocyst excretion in positive control but to higher LAC and lower oocyst excretion in EHS-fed broilers. Feeding regimen had several significant effects on broilers growth and meat quality. In conclusion, EHS could replace BMD and salinomycin in broiler chickens.

Key Words: Broiler chickens, Humic substances, Productivity, Lactic acid bacteria, Eimeria oocysts

P306, **Production benefits of applying substrate-based matrices for combined xylanase, amylase, and protease with Buttiauxella phytase in broilers** Julien Kanarek[*1], Abiodun Bello[1], Eric Sobotik[2], Gregory Archer[2], Yueming Dersjant-Li[1], Rachael Hardy[1], Katie Waller[1], Leon Marchal[1] [1] *DuPont Animal Nutrition, DuPont Nutrition & Biosciences,* [2] *Texas A&M University*

This study evaluated the substrate-based matrices for combination of a *Buttiauxella* phytase (Axtra® PHY) with an enzyme mixture (Axtra® XAP: xylanase, amylase, and protease [XAP]) in broilers from 1 to 42d. Five diets were fed as crumble and pellet in 4 dietary phases. The diets included a nutrient-adequate positive control (PC) and 2 negative controls: NC1 = PC reduced by 74 kcal/kg ME, 0.03-0.04% dig AA, 0.2% Ca, 0.19%

avP, and 0.04% Na (with 0.3% phytate P and 12-12.5% total NSP) and NC2 = PC reduced by 156 kcal/kg ME and 0.05-0.06% dig AA, 0.2% Ca, 0.19% avP, and 0.04% Na (with 0.3% phytate P and 13-13.5% total NSP). Other diets were NC1 plus the phytase at 1000 FTU/kg (NC1+PHY) and NC2 plus the phytase at 1000 FTU/kg and XAP (2000 U/kg xylanase, 200 U/kg amylase and 4000 U/kg protease; NC2+PHY+XAP). Male Ross 308 broilers (n=1,200) were randomized to 60 floor pens (12 replications/treatment). The diets contained corn, wheat, soybean meal, rapeseed meal, wheat bran, and soy hulls and were fed *ad-libitum.* Feed intake and BW of birds from 0-42d were measured per pen per phase and BW-corrected FCR (FCRc) was calculated. Data were analyzed using Proc. GLM of JMP 14.1. Compared to PC (1.69), the d42 FCRc was increased ($P<0.05$) by NC1 (1.83) and NC2 (1.88) and was maintained by phytase (1.70) and phytase+XAP (1.72) inclusion. The 42d BW was decreased by NC1 ($P< 0.05$) and numerically by NC2 vs the PC diet and the use of phytase and phytase + XAP alleviated the adverse effect, with similar effect on feed intake. Eviscerated carcass weight was decreased ($P<0.05$) by NC1 (-130 g) and by NC2 (-142 g) vs PC and was alleviated by NC1+PHY (+206 g) and by NC2+PHY+XAP (+195 g). Similarly, the dietary phytase and phytase+XAP inclusion increased breast weight vs respective NC to same level as PC. Also, the 42d bone breaking strength and ash content were also improved by NC2+PHY+XAP vs NC2. The NC1+PHY and the NC2+PHY+XAP reduced the feed cost/kg BWG by 5.4 and 6.3% vs PC, respectively, leading to estimated net saving of $15.7 and $18.2 per ton of feed. These findings confirmed that using a substrate-based matrices of XAP in combination with *Buttiauxella* phytase decreased feed cost and maintained growth performance, carcass yield and bone quality of broilers through 42d.

Key Words: broilers, Buttiauxella sp. phytase, xylanase amylase protease, growth performance, carcass yield

P307, **Efficacy of NSPase supplementation on performance of broilers** Anouk Lanckriet [1], Natalia Soares [1], Nikolay Outchkourov [1], Jaime Sánchez* [2], Carlos Millán [2], Marta Gracia [2] *[1] Huvepharma NV, Uitbreidingstraat 20, B-2600, [2] Imasde Agroalimantaria, S.L., C/ Nápoles 3, 28224 Pozuelo de Alarcón*

A trial was conducted in order to determine the effect of supplementing a combination of NSPases on broiler performance. The NSPase enzyme mixture contained endo-1,4-beta xylanase and endo-1,4-beta glucanase. A total of 660 one-day-old Ross 308 broilers, half males and half females were placed in 30 floor pens (15 per treatment), with 22 broilers (11 males and 11 females) per pen. There were two treatments, T1: Control; T2: NSPase supplemented to the diet at 500 g/t (providing 1500 EPU/kg of xylanase and 100 CU/kg of 1,4-beta-glucanase). Starter diets were offered to the birds from day-old until 14 days of age, grower diets from 14 to 28 days and finisher diets from 28 to 42 days. The diets (mash) and water were ad libitum available. Diets were mainly based on wheat, barley and soybean meal. Measurements of BW and feed intake were recorded by pen at 14, 28 and 42d. Data were analysed as a randomized design by GLM of SPSS v. 24.0 with treatment as main effect. In the starter phase (0-14d), NSPase enzymes tended to improve FCR (1.35 *vs* 1.32 feed/gain, for T1 *vs* T2; P = 0.057). In the grower phase (14-28d), NSPases increased ADG significantly (63.9 *vs* 66.8 g/d, for T1 *vs* T2; P = 0.019) and improved FCR over the basal diet (1.47 *vs* 1.41 feed/gain, for T1 *vs* T2; P = 0.002). In the finisher phase (28-42d), broilers receiving NSPases exhibited improved FCR and ADG, a near-significant trend (2.05 *vs* 1.98 feed/gain, for T1 *vs* T2; P = 0.075). Overall for the whole fattening period (0-42d), NSPases supplemented broilers exhibited significantly improved ADG (52.1 *vs* 54.4 g/d, for T1 *vs* T2; P = 0.013), significantly lower feed conversion (1.71 *vs* 1.65 feed/gain, for T1 *vs* T2; P < 0.001) and significantly increased EPEF (298 *vs* 320, for T1 *vs* T2; P = 0.005) than the control chickens. It is concluded that the tested xylanase/glucanase enzyme combination significantly improved zootechnical performance of broilers during the whole fattening period by means of increased growth (+4.4%), reduced feed conversion (-3.5%) and improved EPEF (+7.4%).

Key Words: xylanase, glucanase, broiler chickens, performance, feed conversion

P308, **Effect of polyphenolic plant bioactive compounds on dry matter digestibility, standardized ileal amino acid digestibility and excreta metabolizable energy in broiler chickens fed a corn-soybean meal diet** P. Utterback*[1], M. Fernández Miyakawa [2], E. Pierson [3], C. Parsons [1] *[1] University of Illinois at Urbana-Champaign, [2] INTA, CONICET, [3] The Pierson Consulting Group*

A 21-day (Da) chick trial using Ross® 308 male broiler chicks (300 total) was conducted to determine the effect of adding a blend of polyphenolic based plant compounds extracted from the woods of the Italian chestnut and Argentine quebracho trees (Silvafeed® Nutri P – NP) on various response variables. The corn-soybean (C/S) meal-based diet was formulated to meet, or, exceed, recommended Ross® 308 Broiler Chicken requirements. All chicks were subjected to fasting stress (~48 hrs.) prior to placement. Twenty replicate groups (5 chicks/group) were placed on each of 3 dietary (Di) treatments: Di1 = Non-supplemented control; Di2 = Di1+0.05% NP; or, Di3 = Di1+0.10% NP. Diets were formulated for two periods (P) of growth: starter (SP: 00 to 07 d of age) and grower (GP: 08 to 21 d of age). On Da07, weight gain, feed consumption, feed efficiency and dry matter digestibility were determined for the first week. At the end of the trial, growth performance of the chicks was again determined, and, excreta collected for AMEn determination. In addition, all chicks were euthanized and ileal digesta were collected for standardized amino acid digestibility (SIAAD) determination. During both the SP and GP, no significant differences (P > 0.05) among diets were found in weight gain, feed consumption, or feed efficiency. There was no significant effect of diet on dry matter digestibility (DMD) during the SP. However, DMD was significantly increased (P < 0.05) by addition of NP during the GP (Di1: 73.7%c.; Di2: 74.6%b; Di3: 75.6%a). Similarly, MEn values at 21 d were significantly increased at each level of NP (Di1: 3,136c.; Di2: 3,185b; Di3: 3,267a, kcal/kg DM, respectively). SIAAD of almost all amino acids was significantly increased by addition of 0.05% NP. Addition of NP to C/S diets fed to broiler chicks to 21 days of age increased DMD, AMEn and standardized ileal amino acid digestibility.

Key Words: polyphenolic plant extracts, dry matter digestibility, metabolizable energy, amino acid digestibility, broiler chicken

P309, **Effects of higher doses of microbial phytase on performance and bone ash in broilers fed moderately deficient non-phytate phosphorus using corn-soybean meal-based diets** Megharaja.manangi@novusint.com Manangi*, Eric Myers, Vivek Kuttappan, Juxing Chen, Deana Hancock, Mercedes Vazquez-Anon *Novus International, Inc.*

A 41-d floor pen experiment was conducted to evaluate the effects of supplementing higher doses (up to 3 times the standard dose of 500U) of phytase (CIBENZA® PHYTAVERSE®, Novus International, Inc.) in broiler chicks fed non-phytate phosphorus (NPP) deficient corn-SBM based diets. A total of 1584 Ross 308 male broiler chicks were assigned to 6 treatments with 12 pens/treatment and 22 chicks/pen. Treatments consisted of T1 with reduced levels of NPP and no added phytase (Neg Ctrl, NC); T2 to T5 with 500, 750, 1000 and 1500U of phytase/kg diet added to T1, respectively; T6 with industry levels of NPP (Pos Ctrl, PC). For starter, grower and finisher diets, the PC NPP levels were maintained at 0.48, 0.45 and 0.42%, respectively, and PC Ca levels at 0.93, 0.86 and 0.80%, respectively. The NC NPP and Ca for all three phases was reduced by 0.20% and 0.15%, respectively, from the PC. The study was carried out as RCBD and the data were analyzed using 1-way ANOVA. Significance was tested at P≤0.05. D27 results indicated that NC had reduced (P≤0.05) gain (1.37 vs. 1.62 kg/bird), and FI (1.83 vs. 2.14 kg/bird) but not FCR (1.335 vs. 1.322; P≥0.05) compared to PC. Adding phytase at 500U or more improved gain

and FCR over NC (P<0.05) and comparable (P>0.05) to or better (P<0.05) than the PC. Only the group fed 1500U had higher (P<0.05) gain (56g more) along with a 2.3-point improvement (P<0.05) in FCR compared to PC. D41 results indicated that the NC had reduced (P≤0.05) gain (2.84 vs. 3.26 kg/bird), FI (4.30 vs. 4.87 kg/bird), FCR (1.513 vs. 1.495), mortality (4.93 vs. 1.44%) and % ash (44.84 vs. 46.61) compared to PC. The responses for gain, FI and FCR were similar (P≥0.05) for all other doses including PC, and higher (P≤0.05) compared to NC suggesting 500U/kg diet could compensate for the deficiency caused by the NPP reduction. For mortality and % ash, 750U and higher were different from NC (P<0.05) suggesting the need for higher doses beyond 500U to compensate the deficiency of 0.2% NPP. In conclusion, a) up to 27 days, 1500U not only compensated 0.2% NPP reduction but also improved gain and FCR beyond PC; and b) up to 41 days, though 500U was enough to improve gain and FCR, 750U and above were needed for % ash and mortality improvements beyond NC and comparable to PC.

Key Words: Phytase, Broiler, Bone ash

P310, **Supplementation of essential oil combination improves performance in broilers** Basharat Syed [1], Jutta Kesselring [1], Jaime Sánchez* [2], Daniel Erdozain [2], Marta Gracia [2] [1] *BIOMIN Holding GmbH, Erber Campus 1, 3131,* [2] *Imasde Agroalimentaria, S.L., C/ Nápoles 3, 28224 Pozuelo de Alarcón*

A trial was conducted in order to determine the effect of supplementing a premix containing a combination of essential oil components on broiler performance and digestibility. The premix contained essential oil components of oregano, caraway and menthol. A total of 880 one-day-old Ross 308 male broilers were placed in 40 floor pens (20 per treatment), with 22 broilers per pen. There were two treatments, T1: Control; T2: Premix supplemented to the diet at 2 kg/t. Starter diets were offered to the birds from day-old until 14 days of age, grower diets from 14 to 28d and finisher diets from 28 to 42d. The diets (mash) and water were ad libitum available. Diets were mainly based on corn and soybean meal. Finisher diets included titanium dioxide as inert marker. At the end of trial (42d), 7 broilers per pen were euthanized and ileal content collected to measure protein apparent ileal digestibility. In addition, measurements of BW and feed intake were recorded by pen at 14, 35 and 42d. Data were analysed as a randomized design by GLM of SPSS v. 24.0 with treatment as main effect and initial BW used as a covariate. In the starter phase (0 to14d), chickens receiving the Premix grew more (+3.6%, 25.0 *vs* 25.9 g/d, for T1 *vs* T2; P = 0.0104) and exhibited better feed conversion ratio (-2.9%, 1.38 *vs* 1.34 feed/gain, for T1 *vs* T2; P = 0.0009) than control broilers. No significant differences in performance were observed between treatments from 14 to 35d. During the last week of trial (35 to 42d), there was a trend towards a lower feed conversion ratio with Premix supplementation (2.01 *vs* 1.95 feed/gain, for T1 *vs* T2; P = 0.0944). During the whole period (0 to 42d), Premix supplemented broilers exhibited better feed conversion ratio (1.62 *vs* 1.60 feed/gain, for T1 *vs* T2; P = 0.0176) than control broilers. In addition, protein apparent ileal digestibility at 42d was increased by 3.9% when Premix was included into the diet (71.5 *vs* 74.3%, for T1 *vs* T2; P = 0.0428). It is concluded that the tested combination of essential oil components improved zootechnical performance of broilers during the whole period by improving protein ileal digestibility and feed conversion ratio.

Key Words: Oregano, Caraway, menthol, digestibility, feed conversion ratio

P311, **Digestibility of ingredients with and without the use of enzymatic complex in broilers feed** Marcia Sakamoto [1], Adriana Nascimento* [2], Paulo Pozza [1], Alice Murakami [1] [1] *Universidade Estadual de Maringa,* [2] *Alltech do Brasil*

The objective of this trial was to evaluate the total and ileal digestibility of soybean meal, meat bone meal, corn and wheat bran, with and without Allzyme SSF® E+C (EC) supplementation, in broilers fed from 17 to 30 days old. The trial design was a factorial scheme 4x2+2 (4 ingredients x with and without enzyme complex) + 2 reference diets (with and without EC), with 6 replications and 4 birds each (n = 24 birds / treatment). The EC was included in feed at 200 g / ton of feed. Soybean meal, meat bone meal and wheat bran replaced 20% and corn replaced 40% of the reference diet for individual ingredient testing. The experimental period included 5 days of adaptation, followed by 5 days of excreta collection. Marker (ferric oxide -1%) was used at the beginning and end of the excreta collection. An additional 3 days of feeding with 0.5% chromic oxide as an indicator for the ileal collection followed. Apparent metabolizable energy (AME), apparent metabolizable energy corrected for nitrogen (AMEn) and total and ileal digestibility of calcium and phosphorus were determined. The averages of each parameter were compared by orthogonal contrast between diets with or without EC for each feed. The AME and AMEn, without and with the EC, were respectively: 2,282 and 2,368; 1,970 and 1,981 kcal / kg for soybean meal; 1,920 and 1925; 1,627 and 1,662 kcal / kg for meat and bone meal; 2,939 and 3,035; 2,880 and 2,949 kcal / kg for corn; 1,769 and 1791; 1,618 and 1,623 kcal / kg for wheat bran. The addition of EC increased AME of soybean meal and corn by 3%, meat meal by 2.6%, and wheat bran by 1.2% (P>0.05). Similarly, the total and ileal digestibility coefficients of Ca and P were improved with enzyme supplementation for all ingredients. For calcium digestibility, the highest increases in total digestibility with EC were for meat and bone meal of 12.1% and wheat bran of 12.6% (P>0.05). The meat and bone meal also showed an increase (P<0.05) of 27.8% in phosphorus ileal digestibility with EC. For corn and soybean meal, the phosphorus digestibility was improved in both total and ileal analyses. In this study the enzyme complex improved metabolizable energy as well as the digestibility coefficients of Ca and P of ingredients.

Key Words: calcium, digestibility, phosphorus, metabolizable energy

P312, **Assessing the beneficial effects of an organic acids based formulation for broiler performance and gut function** Roger Berrios Arauz*[1], Konstantinos Mountzouris [2], Irida Palamidi [2], Michaela Mohnl [1], Nataliya Roth [1] [1] *Biomin Holding GmbH,* [2] *Agricultural University of Athens*

Assessing the findings of studies on the application of an organic acid-based feed additive formulation clarify the beneficial effects on growth performance, nutrient digestibility, digestive enzymes, microbiota composition and microbial glycolytic enzyme activity in broilers. The studies evaluated the supplementation of broiler feed with an organic acid-based feed additive (FA), avilamycin (AV) or a combination of FA and AV. Therefore, FA and AV were used as main factors on a 2x2 factorial study design. Consequently, 544 day-old male broilers were allocated in four treatments: Control (C), 1g FA/kg diet, 2.5mg AV/kg diet and combination of FA and AV (FAAV) with eight replicates during 42 days. All data were tested for normality using the Kolmogorov-Smirnov test. Data were analyzed with the general linear model - general factorial ANOVA. Treatment means were compared by Tukey HSD test using SPSS version 8 (SPSS, Chicago, IL, USA). The following parameters were improved significantly (P ≤ 0.05) when compared with C. Body weight gain was improved by FA and AV addition (P = 0.002 and P = 0.035 respectively). Feed conversion was improved (P = 0.014) by AV addition. Results in nutrient digestibility showed improvement of dry matter, organic matter digestibility and energy retention (P ≤ 0.05) by FA and AV inclusion. Additionally, AV addition improved (P ≤ 0.05) ether extract and crude protein digestibility. Pancreatic trypsin was lower (P ≤ 0.05) for FA and AV addition. Clostridium perfringens subgroup was reduced (P ≤ 0.05) in ileal digesta by AV inclusion. FA inclusion increased (P ≤ 0.05) levels of Clostridium coccoides subgroup, Clostridium leptum subgroup in cecum digesta. FA stimulated (P ≤ 0.05) the activity of microbial glycolytic enzymes (α-glucosidase, β-glucosidase, α-galactosidase, β-glucuronidase) in ileal digesta. In conclusion, the findings provide supportive evidence that FA supplementation improves broiler growth performance by modulating the activity of microbial glycolytic enzymes in ileal digesta and microbi-

ota composition in the cecum, which ultimately leads to an improvement in nutrient digestibility.

Key Words: Acidifier, Microbiota, Digestibility, Microbial glycolytic enzymes, Digestive enzymes

Metabolism and Nutrition, General Nutrition

P313, **Effect of almond hull as an alternative feed ingredient on laying hen performance, egg quality, and body composition** Jinquan Wang*GS, Fanbin Kong, Woo Kim *University of Georgia*

The objective of this study was to evaluate two varieties of almond hulls (Non-pareil: NP; Carmel: CA) as alternative feed ingredients for laying hens. The TMEn values from NP and CA were 1,624 and 1,514 kcal/kg which were previously reported in PSA 2019. A total of 100 24wk-old Hyline W-36 layers were randomly allocated to five experimental treatments with five replicates as four birds each for eight weeks with a completely randomized design. Treatments consisted of a control diet (T1) containing corn and soybean meal; T2 and T3 were formulated to contain 7.5 and 15% of NP; and T4 and T5 were formulated to contain 7.5 and 15% of CA. Egg production was recorded daily for hen day production. Egg quality was measured on wk28 and wk32. Egg yolk color was measured by a colorimeter at wk32. Two birds from each replicate were sacrificed for dual energy X-ray scanning at the end of the trial. Data of T2 and T3 (NP) with control and T4 and T5 (CA) with control were subjected to statistical analysis with SAS using one-way ANOVA in GLM separately. The significance level was set at $p<0.05$. Means were separated using DMRT. For NP hulls, inclusion at 7.5% showed a lower body weight compared to control group only at wk28. All treatments maintained their peak egg production (> 90%) and no significant difference in treatments. For the egg quality such as egg weight, specific gravity, haugh unit, yolk weight, shell weight, and shell thickness, there was no significant difference at wk28 and 32. However, at wk32, the diet containing 15% of NP hulls increased the red and decreased yellow color in yolk. Supplementation of NP at 7.5 and 15% decreased body fat percentage and fat mass compared to the control group. For CA hulls, inclusion at 7.5 and 15% decreased body weight at wk28. Inclusion of CA hulls at 15% decreased the bodyweight at wk32 as well. There was no significant difference on hen day production and egg quality across the treatments during the whole period. Inclusion of CA hulls at 15% decreased yellow color in yolk at wk32. Supplementation of CA at 7.5 and 15% decreased body fat percentage and fat mass compared to control group. In summary, inclusion of NP or CA hulls up to 15% had no negative effect on laying hen egg production and egg quality.

Key Words: Almond hull, Alternative ingredient, Laying hen, Performance, Body composition

P314, **Feed efficiency and gastrointestinal and immunological organ development of fast versus slow growing broilers** Maci Oelschlager*GS, Zeyi Moo, Brad Gorenz, Ryan Dilger, Roderick Mackie, Alexandra Smith *North Carolina State University, Prestage Department of Poultry Science*

Broiler growth rate remains an important topic in the poultry industry, though causative relationships between feed efficiency and organ development remain unknown. The objective of this study was to investigate the difference in feed efficiency and gastrointestinal and immunological organ development of fast versus slow growing broilers. As such, 300 day-old Ross 308 male chicks were housed in battery cages with free access to water and a common practical diet. Based on body weight gain from study d 0-11, 20 birds closest to each of the 90th and 10th percentiles were selected. Within each growth rate (fast vs. slow), 10 birds were euthanized to permit organ collection at d 11 while the remaining 10 birds in each group were individually housed until study d 25 and then euthanized. At each time-point, lengths of the duodenum, jejunum, ileum, ceca, and colon were recorded, and weights were collected for the duodenum, jejunum, ileum, ceca, colon, liver, spleen, Bursa, cecal tonsils, crop, gizzard, proventriculus, and pancreas. As expected based on the selection criteria at day 11, fast-growing birds exhibited higher ($P < 0.01$) BW on d 11 and 25, and higher BW gain ($P < 0.01$) compared to the slow-growing birds. Feed efficiency was improved with a lower FCR ($P < 0.05$) from d 11-25 in the heavier birds. Additionally, fast-growing birds had increased ($P < 0.01$) raw length of the ceca at d 11 and decreased ($P < 0.01$) relative length of the duodenum, ileum, ceca, and colon at d 11 and 25 compared with slow-growing birds. Fast-growing birds also exhibited higher ($P < 0.05$) raw weights of the jejunum, colon, liver, gizzard, proventriculus at d 11 and 25 compared with slow-growing birds. Finally, fast-growing birds had decreased ($P < 0.05$) relative weights of the duodenum and liver when compared with slow-growing birds. This data shows that fast birds had improved feed efficiency, while demonstrating overall decreased relative organ weights and lengths in comparison to slow growing birds,. Taken together, these results indicate significant relationships between feed efficiency and lengths and weights of gastrointestinal and immune-related organs in birds differing in growth rate. Further research will investigate host microbe interactions of these growth phenotypes.

Key Words: broiler, feed efficiency, gastrointestinal, immunological, nutrition

P315, **Adaptation length and assay feedstuff substitution levels influenced metabolizable energy value of soybean meal calculated by the difference and regression methods in a method-dependent manner** Shravani Veluri*GS, Oluyinka Olukosi *Department of Poultry Science, University of Georgia*

A total of 72 male broilers were used in the study to investigate the effect of adaptation length and substitution levels on AME of soybean meal (SBM) determined by both the difference and regression methods. At 14 days old, the broilers were allocated to 4 treatments with 6 replicates of 3 birds per replicate cage. The experimental diets were fed for either 4 days or 7 days as adaptation lengths, and excreta were collected on days 20 and 21. Within each adaptation period, 4 diets comprising one basal and 3 test diets in which SBM substituted the energy yielding components of the basal diets at 150, 300, or 450 g/kg were fed, corresponding to levels 1, 2 or 3, respectively. The AME of SBM was calculated using the difference method at each substitution levels. In addition, AME of SBM was calculated by the regression method using four different combinations of the substitution levels (i.e. 0, 1 and 2; 0, 1 and 3; 0, 2 and 3; or 0, 1, 2 and 3). The data for AME determined by the regression method were analyzed as 2×4 factorial for the influence of adaptation length (2) and combination of substitution levels (4). There was no adaptation × substitution level interaction for AME determined by both regression and difference methods. For AME determined by the difference method at different substitution levels, 150 g/kg gave lower AME values ($P < 0.01$) than that determined at 300 and 450 g/kg substitution levels but adaptation length had no effect. For AME determined by the regression method, substitution levels had no effect on AME of SBM but AME was greater ($P < 0.05$) in birds with 4-day adaptation compared with 7-day adaptation length. AME values determined by the two methods were also statistically compared. There was significant method × adaptation length interaction ($P < 0.05$) for SBM metabolizable energy. The AME determined by regression method after 4-day adaptation length was greater ($P < 0.10$) than that determined after 7-day adaptation length, but there was no difference in AME for SBM

determined by the difference method at different adaptation lengths. It was concluded that AME of SBM was more reliably determined at substitution levels greater than 150 g/kg, and that the regression method was more robust for determination of AME for SBM.

Key Words: Soybean meal, adaptation length, substitution level, difference method, regression method

P316, **Determination of daily dietary energy utilization for maximal protein deposition in fast-growing male broilers from 3 to 17 days of age** Nawin Suesuttajit*GS, Antonio Beitia, Jordan Weil, Pramir Maharjan, Diego Martinez, Katie Hilton, Craig Coon *University of Arkansas, Center of Excellence for Poultry Science*

Fast-growing broilers have the potential to gain body protein quickly through nutrient intake during starter and grower periods. However, protein deposition requires adequate calorie intake in combination with digestible amino acids for maximum accretion. Research is needed to understand when and how much additional energy intake is needed for maximum protein deposition in order to better design feeding programs for feeding phases. Two studies were conducted to determine the daily dietary energy utilization for protein deposition by using dual energy x-ray absorptiometry (DEXA). In Exp1, a total of 1440 fast-growing male broilers were randomly assigned to 48 floor pens and fed one of two test diets from d3 to d10 throughout an 8-day experimental period (8 trts; 6 reps). Each treatment consisted of feeding one diet (Diet 1) for a set period of days and then switching the diet to a higher energy diet (Diet 2). Diet 1 was formulated to Cobb500 starter recommendations while the Diet 2 contained 50 extra kcals ME/kg and a constant protein to energy ratio, similar to the ratio in Diet 1. At d10, broilers were scanned to determine the dietary energy intake needed for maximum protein deposition. In Exp2, a total of 1440 fast-growing male broilers were randomly *assigned* to 48 floor pens and fed one of two test diets from d10 to d17. As in Exp1, treatments contained two diets that were switched on different days. Diet 1 was formulated to Cobb500 grower recommendations and Diet 2 contained an extra 50kcals ME/kg and the same protein to energy ratio as contained in Diet 1. At d17, broilers were examined to determine the dietary energy intake needed for maximum protein deposition. Data was analyzed using JMP Pro 14 (SAS Institute, 2018). Exp1 showed lean mass and protein mass increased when the diet was switched a higher energy containing diet on d6 *(P<0.05)*. Exp2 determined that protein mass increased in the treatments that had diet changes on d11 and d16 *(P<0.05)*. These studies indicate that the increase in the daily intake of calories at certain days in starter feeding phase may be necessary to gain more protein mass with standard diets. Thus, protein deposition may require additional calorie densities and intakes in order to sustain the protein gain advantage.

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

P317, **Performance and economic analysis of differing feed schedules fed to modern commercial broilers** Garrett Mullenix*GS, Michael Kidd *University of Arkansas*

The objective of this study was to determine how changes in feed schedule programs impact live performance, processing yields, and economic analysis of broilers. Two experiments were conducted where 384 Cobb 500 fast feather commercial broiler chicks were sexed and placed in 32 pens (n=12 birds/pen). The feeding schedule programs differed in feed amounts fed in a four-phase feeding program with feed program 1 having less starter, finisher, and withdrawal 2, but more withdrawal 1 feed. Feeding program average cost was $228.14/ton for program 1 and $229.20/ton for program 2, which had more withdrawal 1. All diets were formulated to the meet the recommendations of the primary breeder. The broilers were grown out to 41 d for live performance and processing measurements. A 2 x 2 factorial design was used with gender and feed program as the factors. Male broilers were ($P < 0.001$) heavier and had better feed conversion ($P = 0.046$; 1.58), than females (1.63). Female broilers had higher tender and total breast yields ($P < 0.001$ and $P = 0.014$, respectively) while incurring less ($P < 0.001$) incidence of woody breast, 19.1% vs 42.1%. Feed cost per pound was higher ($P = 0.028$) for feed program 2, yet no performance parameters or processing yields were expressively affected.

Key Words: broilers, feed schedule, economic analysis, myopathy

P318, **Evaluation of EMOH water treatment for broiler chickens as measured by production traits** Ariel Diggan*1GS, Cirenio Hisasaga [1], Anh Do [1], Rodrigo Lopez [1], Jennine Ochoa [2], Katy Tarrant [1] *[1] California State University, Fresno, [2] University of California, Davis - California Animal Health and Food Safety Laboratory System*

Previous studies have indicated that oxygenated drinking water may improve performance traits, fat to body weight ratio, antioxidant effects, and metabolic and immune factors in the blood. Additionally, the use of hydrogen peroxide as a water line flushing agent or dilute water treatment has shown promise in many applications, within and outside of poultry production. The Electron, Magnetics, Oxygen and Hydrogen (EMOH) water treatment system, although initially intended for agricultural irrigation uses, might have the potential to match or exceed the benefits of the methods listed above. EMOH combines water oxygenation with low, consistent levels of hydrogen peroxide. In addition, water cluster size is decreased, aiding in faster and easier absorption into the body. The purpose of this study was to examine the effects of EMOH treated drinking water consumption on performance traits in broilers. The study consisted of two flocks each of 504 Ross 708 broilers. For each flock, 36 test pens containing 14 birds were arranged into four rows of 9. Each row encapsulated an independent water line, and was hand fed. Lines were alternatively designated treatment (EMOH from the same device) and control (non-treated water). Efficacy and consistency of dissolved oxygen was monitored throughout both flocks by the manufacturer. Dissolved oxygen levels averaged 108% for treatment lines, and 85% for control. Feed and water were provided ad libitum during the growout period lasting 42 days in Flock 1 and 49 days in Flock 2. Body Weight (BW) was taken at the completion of the trial, as well as FCR. Means were compared using Tukey's HSD in JMP with significance determined at $P \leq 0.05$. Treatment versus control BW was not significantly different in Flock 1 (at 2.03 kg versus 2.00 kg; $P = 0.17$). Flock 1 FCR approached significance at 1.703 in treatment birds and 1.801 in control birds ($P < 0.06$). Flock 2 BW was significantly larger in the treatment birds (2.62 kg versus 2.52 kg; $P = 0.026$) group, and FCR was not significantly different compared to control birds (1.955 versus 1.998). To conclude, EMOH treatment has the potential to improve BW and FCR in broilers as addition to organic and no antibiotics ever houses.

Key Words: oxygenated water, dissolved oxygen, broiler, performance

P319, **Choice feeding plant-based protein and animal byproducts to turkeys** Liam Carlson*GS, Jeffre Firman *University Of Missouri*

Utilizing by-products of animals has been around for centuries in various ways. Today, we utilize by-product for many commodities, including animal feed. The rendering industry produces an annual volume of 11.2 billion pounds of animal derived proteins and 10.9 billion pounds of rendered fat in the United States. Animal welfare groups claim it is not a natural behavior for poultry to eat animal protein and are pushing for animals to be produced on vegetarian diets. The purpose of this study was to allow turkeys to choose feed from both individual plant-based protein sources and animal-based protein sources to determine what they would consume if allowed to manage their own diet. 166 2-week-old turkeys were housed in in groups of 20 (8 pens), or individually (6 pens). Each pen was given water and feed ad-libitum and given corn, vitamin/mineral premix, soybean meal, poultry by-product meal, bovine meat and bone meal, and bovine blood meal. All feed sources were put in individual feeders and randomized in each pen. Birds were given 16 hours of light and temperature

was recorded daily. Feed intake was recorded in the morning and feeders were randomized each recording period over the 12 day experiment. Results showed that turkeys housed in groups ate up to 9% of their total diet in animal by-product on average. Individual pens showed variability, consuming between 1% and 33% of their total diet in animal by-products. Statistical analysis was performed on rank transformed data using SAS PROC GLM to meet ANOVA assumptions and showed turkeys choice in eating the three animal-byproducts in amounts that statistically differed from one another at a p-value of <.0001, supporting that they did select animal by-product feeds when given a choice. These results show that when given the choice between a plant-based protein source or an animal by-product protein source, turkeys will consume animal by-products.

Key Words: Turkey, By-product, Choice Feeding, Nutrition, Rendering

P320, **Color of feed effects on performance of high growth rate broilers** Joseph Gulizia*UG, Kevin Downs *Middle Tennessee State University*

This research study was conducted to determine color of feed effects on average body weight, body weight gain, cumulative feed consumption, and adjusted feed conversion of high growth rate broilers. Treated diets were colored using food grade powdered dye. Four treatments [control (complete broiler starter diet), red (#bb5a58 hex color code), green (#739a2d hex color code), and blue (#4a9c9d hex color code)] with 4 replicates (60 birds/treatment) were fed to 240 male Cobb 500 broilers over a 21 d battery cage grow out. Bird weights and feed consumption data were collected on 7 d intervals. Data were analyzed as a completely randomized design with battery cage representing the experimental unit using the ANOVA procedure. Overall, there were no significant treatment effects for average body weight at d 7 (P=0.6060), 14 (P=0.8135), and 21 (P=0.4480); body weight gain between d 0 to 7 (P=0.6953), 0 to 14 (P=0.7791), and 0 to 21 (P=0.6235); and cumulative feed consumption between d 0 to 7 (P=0.9661), 0 to 14 (P=0.7965), and 0 to 21 (P=0.8956). There were no significant treatment effects for adjusted feed conversion between d 0 to 7 (P=0.7377). However, there tended to be treatment differences for adjusted feed conversion between d 0 to 14 (P=0.0576) and a significant effect between d 0 to 21 (P=0.0061). Birds on control tended to have a lower adjusted feed conversion than both red and green treatments between d 0 to 14. Adjusted feed conversion between d 0 to 21 for control (1.23) was significantly less than red (1.27; P=0.0008), green (1.26; P=0.0093), and blue (1.25; P=0.0556). Additionally, there were no observed treatment differences for mortality anytime during the 21 d grow out (P>0.05). These results are indicative that inherent stimulatory color spectral effects for red, green, and blue did not influence most high growth rate broiler performance parameters. Therefore, based on the results of this study there appears limited economic value in coloring commercial broiler feed red, green, or blue as a method to increase feed consumption.

Key Words: Broiler, Growth, Performance, Feed Color

P321, **Comparison of Two Methods for Extraction of Lipid from Broiler Tibiae in the Procedure to Analyze Bone Ash** Austin Silva*UG, Alex Rosario, Ariel Bergeron, Christopher Eagleson, Kimberley Gardner, Audrey McElroy *Texas A&M University*

Routine methodology for lipid extraction from broiler tibiae prior to ashing involves a process of heating the bones in petroleum ether in a Soxhlet apparatus, which poses a safety hazard due to the highly flammable ether. The current study was conducted to compare an alternative, and perhaps safer method, of lipid extraction from the tibiae. Left and right tibiae were removed from 80 d23 and 80 d55 broilers. Left tibiae from d23 and d55 were used in extraction Method 1 and right tibiae were used in extraction Method 2. For the traditional Method 1, using a Soxhlet apparatus, the tibiae were defatted in 4 L of heated petroleum ether for 16 h or until the ether was clear. For the alternative Method 2, the tibiae were placed on a metal rack in a 5-gallon bucket. A volume of 5 L of petroleum ether was poured into the bucket to fully cover the tibiae. The bucket was sealed, and tibiae soaked for 48 h. Tibiae from both methods were removed from their respective apparatus and placed in a fume hood for 24 h. Bone ash was completed on tibiae from methods to acquire a percent tibia bone ash. The data was analyzed using a paired t-test on the percent bone ash from the left tibiae of Method 1 and 2 and the right tibiae from Method 1 and 2. The tibiae subjected to Method 1 showed a similar percent ash to scientifically published ranges. Whereas, Method 2 showed a percent ash that was significantly lower, suggesting the levels of organic matter was higher in the right tibiae (P<.0001). Results suggested Method 1 to be a procedure that was more effective to defat tibiae compared to Method 2.

Key Words: lipid extraction, bone ash

P322, **Characterization of wheat midds from different wheat varieties.** Rachel Kort*UG, Caitlin Evans, Haley Wecker, Charles Stark, Chad Paulk *Kansas State University*

Wheat midds are the combination of wheat middlings and screenings in the flour milling process. However, wheat midd composition may vary depending on the variety of wheat milled. Therefore, the objective of this study was to determine if wheat midds from multiple wheat varieties differed in particle geometric mean diameter (d_{gw}), geometric standard deviation (S_{gw}), and flowability measurements. Wheat midds (durum [n=6], hard red winter [HRW, n=3], and soft red winter [SRW, n=3]) were sourced from flour mills in different regions of the U.S. Samples were collected prior to shipment to a feed mill. Samples were analyzed for particle size with the inclusion of sieving agent according to ASAE standard S319.2. Flowability was characterized by measuring angle of repose (AoR), bulk density, and tap density. These measurements were then used to calculate compressibility and Hausner ratio. Data were analyzed as a completely randomized design using PROC GLIMMIX procedure of SAS 9.4 (Cary, NC). Particle size d_{gw} and S_{gw} differed (P< 0.01) between wheat middling varieties. Durum, HRW, and SRW d_{gw} were 315, 406, and 581 µm and S_{gw} were 2.13, 2.23, and 2.64, respectively. For flowability characteristics, wheat middling sources differed (P< 0.01) in bulk density, tap density, and AoR. Durum, HRW, and SRW bulk densities were 0.311, 0.323, and 0.273 g/mL, tap densities were 0.423, 0.440, 0.380 g/mL, and AoRwere 49.7, 47.3, and 43.5°, respectively. However, there was no evidence for differences in compressibility and Hausner ratio between wheat midds. All samples were analyzed for critical orifice diameter; however, all samples failed to flow through a 34 mm opening. In conclusion, wheat midd particle size decreased from SRW, HRW, to Durum. This resulted in SRW having the best flowability characteristics and durum having the worst. Based on AoR, SRW was classified as passable flow (41-45°) and HRW and Durum as poor (46-55°). However, all samples were classified as poor based on the compressibility index (26-31) and Hausner ratio (1.35-1.45). Based on the samples collected herein, wheat midd variety is confounded with particle size. Further research is needed to determine if wheat variety influences flowability when ground to a common particle size.

Key Words: wheat midds, density, particle size, angle of repose

P323, **Effect of storage environment on the flowability of crystalline tryptophan and lysine** Danielle Funk*UG, Caitlin Evans, Haley Wecker, Charles Stark, Chad Paulk *Kansas State University*

The objective of this experiment was to evaluate the effect of storage environment on the flowability of crystalline Trp, Lys, and a Lys:Trp mix. Treatments were arranged as a 3 × 4 factorial in a completely randomized design with 3 AA treaments (L-Trp, L-Lys, 10:1 mixture of L-Lys:L-Trp) and 4 storage conditions (17°C and 38% RH, 40°C and 77% RH, 2°C and 40% RH, and -18°C and 41% RH). Samples were stored at the designated condition for 48 hr and then analyzed for angle of repose (AoR), critical orifice diameter (CoD), bulk density (BD) and tap density (TD). Measurements were then used to calculate the compressibility index (CI) and composite flow index (CFI). Samples were stored in 3 separate trilayer paper bags to provide 3 replicates per treatment. There was an AA

× storage interaction ($P < 0.04$) for AoR and compressibility. Lys stored at -18°C had poorer AoR (27.5°) compared to Lys stored at 17°C (20.8°), with Lys stored at 40°C and 2°C being intermediate (23.5 and 25.2°, respectively). Storage condition did not affect AoR of Trp and the Lys:Trp mix. When stored at 17°C, Lys had improved AoR compared to Trp, with Lys:Trp being intermediate (20.8, 30.7, and 26.6°, respectively). For the remaining storage conditions, AoR was similar for Lys, Trp, and Lys:Trp. Trp had greater ($P < 0.01$) CoD compared to Lys and Lys:Trp (25, 6, and 6 mm, respectively). All samples stored at 40°C had poorer ($P < 0.02$) CoD compared to all samples stored at 17°C and 2°C (14, 12, and 12 mm, respectively). Lys had improved ($P < 0.01$) CFI compared to Lys:Trp. However, both Lys and Lys:Trp (95.2 and 93.0, respectively) had improved ($P < 0.01$) CFI compared to Trp (70.7). In conclusion, the storage condition of AA did not influence CFI. However, creating a 10:1 Lys:Trp mixture resulted in a similar CFI as Lys and improved the CFI by 22.3 compared to Trp.

Key Words: L-Tryptophan, L-Lysine, amino acid, flowability

P324, **Effect of Enogen® feed corn on pelleting characteristics of poultry diets** Courtney Truelock*, Adrian Baker, Chad Paulk, James Drouillard *Kansas State University*

This experiment was designed to evaluate effects of corn type and conditioner retention time on pelleting characteristics of poultry diets. Treatments were arranged as a 2 × 2 factorial of corn source (conventional [CON] and Enogen® feed corn [EFC, Syngenta Seeds, LLC]) and conditioner retention time (30 and 80 s). Diets were steam-conditioned (Wenger twin staff pre-conditioner, Model 150) for 30 or 80 s at 75ºC and pelleted (1012-2 HD Master Model, California Pellet Mill) with a 4 × 32 mm pellet die. All treatments were manufactured as starter, grower, and finisher poultry diets. Diet phases were pelleted on 3 separate days, representing 3 replications. Pellet mill amperage was recorded at 5-s intervals throughout each processing run and used to calculate energy consumption. Conditioned mash and cooled pellet samples were analyzed for available starch and pellet durability index (PDI) was measured for cooled pellet samples. Data were analyzed using the GLIMMIX procedure in SAS 9.4, with corn type, conditioner retention time, and their interaction as fixed effects, phase as the random effect, and pelleting run as the experimental unit. There was no evidence ($P > 0.23$) for a corn type × conditioner retention time interaction for any of the pelleting responses observed in this study. Available starch in cooled pellets was greater ($P = 0.01$) in EFC diets than for CON (6.1 and 5.4%, respectively). There was no evidence for differences ($P > 0.30$) for PDI and energy consumption between treatments. For the CON 30s, CON 80s, EFC 30s, and EFC 80s the average PDI was 93, 90, 92, and 93% for starter diets, 88, 89, 88, and 89% for grower diets, and 73, 77, 74, and 70% for finisher diets, respectively. For the CON 30s, CON 80s, EFC 30s, and EFC 80s, mean energy consumption values were 22.2, 22.6, 22.6 and 22.1 kWh/ton for starter diets, 22.1, 21.2, 21.7, and 22.3 kWh/ton for grower diets, and 22.0, 22.0, 22.1, and 22.1 kWh/ton for finisher diets, respectively. In conclusion, pelleting of EFC resulted in greater available starch in cooled pellets compared to CON with all other pelleting parameters remaining consistent between the two corn types, suggesting a greater benefit of pelleting EFC compared to CON, with no sacrifice in throughput.

Key Words: Energy consumption, Enogen feed corn, pelleting, retention time, starch availability

P325, **Effect of die speed on pellet quality and xylanase stability** Caitlin Evans*, Marut Saensukjaroenphon, Chad Paulk, Charles Stark *Kansas State University*

The objective of this experiment was to determine the effect of pellet mill model (PM), die compression (L:D), and die retention time (DRT) on phytase stability (PS) and pellet quality. Treatments were arranged as a completely randomized design. Diets were pelleted using either a CPM 1012-2 HD Master Model or a 3016-4 Model equipped with a 4.8 × 50 mm (10.6 L:D), a 4.8 × 31 mm (6.6 L:D) or a 4.8 × 44 mm (9.3 L:D) with approximately 30 sec conditioning retention time at 85°C with a designated production rate. These processing conditions were used to create the following DRT treatments: 10.6 L:D with 4.3 sec DRT, 10.6 L:D with 2.9 sec DRT, 9.3 L:D with 1.7 sec DRT, 9.3 L:D with 1.1 sec DRT, 6.6 L:D with 2.6 sec DRT, and 6.6 L:D with 1.6 sec DRT. The PM were run 3 separate times to provide 3 replicates per treatment. There was an overall effect ($P<0.001$) of treatment on PS in cooled pellets. Phytase was more stable regardless of DRT when diets were manufactured with the 1012 PM using the 6.6 L:D die compared to the 10.6 L:D die ($P<0.05$). The hot pellet temperature (HPT) of 10.6 L:D die was 91–99°C, while 6.6 L:D die was 84–87°C; however, the PS was similar between the feed pelleted with 1012 PM equipped with 6.6 L:D die and the 3016 PM equipped with 9.3 L:D regardless of DRT ($P>0.05$). The HPT of feed pelleted with the 1012 PM equipped with 6.6 L:D die was 84–87°C, while the feed pelleted with the 3016 PM equipped with 9.3 L:D die was 82–84°C. As the die L:D increased (quadratic, $P<0.001$) PS decreased. Therefore, PM or DRT did not affect PS when the HPT was less than 87°C. Pellet quality increased (linear, $P<0.001$ for standard pellet durability index (PDI) or quadratic, $P<0.001$ for modified PDI) as die L:D increased. The die L:D had greater effects on both PDI methods than the DRT; however, increased DRT improved ($P<0.05$) pellet quality when the feed was pelleted with 6.6 L:D, but not when pelleted using the 9.3 or 10.6 L:D. In conclusion, the phytase that was produced by *Trichoderma reesei* strain could tolerate HPT up to 87°C, regardless of PM, L:D and DRT. However, PS was dramatically reduced when HPT ranged from 91–99°C. Therefore, HPT should be measured to monitor PS. Increasing L:D had the greatest effect on improving pellet quality.

Key Words: die speed, xylanase, pellet quality

P326, **Effect of feed form, corn particle size and extrusion of corn on broiler performance** Marut Saensukjaroenphon, Caitlin Evans*, Haley Wecker, Chad Paulk, Charles Stark *Kansas State University*

The objective of this study was to determine the effect of corn particle size, extrusion processing of corn and feed form on the growth performance of 21 d old broiler chicks. Male broiler chicks (270, Cobb500) were randomly allotted to dietary treatments and placed with 5 birds per battery cage, totaling 8 replicates per treatment. There was a total of 8 dietary treatments consisting of six crumble diets with 400, 800 or 1200 µm raw corn (CRC) or extruded corn (CEC) and two mash diets with 400 or 800 µm extruded corn (MEC). At d 21, the gizzard (GW) and pancreas weights (PW) were collected from 2 birds of average weight from each cage. Data were analyzed using the GLIMMIX procedure of SAS. To evaluate the effect of corn type on growth performance, treatments were analyzed as a 2 × 3 factorial of corn processing technique (raw corn and extruded corn) and corn particle size (400, 800 and 1200 µm). There was an interaction between corn type and corn particle size on relative GW and PW, but not BW, ADFI, or FCR from d 0 to 21 ($P>0.05$). At 21 d, the CRC diets had greater BW (860 vs. 788 g) and ADFI (51 vs. 46 g) compared to CEC diets, though FCR was similar (1.31 vs. 1.32). Particle size did not affect BW or ADFI, however, FCR increased linearly (1.30, 1.32, and 1.33) as particle size increased (400, 800, 1200 µm). To evaluate the effect of feed form on growth performance, treatments were analyzed as a 2 × 2 factorial of feed form (mash and crumble feed) and corn particle size (400 and 800 µm). There were no interactions between corn particle size and feed form. At d 21, the CEC diet had greater BW (792 vs. 707 g) and greater ADFI (46 vs. 42 g) as compared to the MEC diet, however, FCR remained similar (1.32 vs. 1.34). GW (1.42 vs 1.76%) and PW (0.24 vs 0.28%) were decreased by CEC diets compared to MEC. There was no difference in BW, ADFI, FCR or PW at d 21 ($P>0.05$) between diets containing 400 or 800 µm corn regardless of feed form. The GW was greater for the extruded corn diet containing 800 µm versus 400 µm corn (1.67 vs 1.50%). This study indicates that replacing ground corn with extruded corn does

not improve growth performance during the starter period. However, increasing corn particle size does improve gizzard development, but may increase FCR in chicks.

Key Words: ground corn, extruded corn, pelleting, starch

P327, **Effect of cooling method, sample preparation, storage condition and storage time on phytase activity of a corn-soy diet** Caitlin Evans*[1], Marut Saensukjaroenphon[1], Cassandra Jones[1], Craig Wyatt[2], Chad Paulk[1], Charles Stark[1] *[1] Kansas State University, [2] AB Vista*

The objective of this experiment was to determine the effect of cooling method, sample preparation, storage condition and storage time on phytase stability. In experiment 1, treatments were arranged in 2 x 2 factorial of sample preparation (none and freeze-dried) and storage condition (ambient storage and freezer storage). In experiment 2, treatments were arranged in a 2 × 3 factorial of cooling method (counterflow cooler and freezer) and sample preparation (non-dried and freezer storage (NDFS), freeze-dried and freezer storage (FDFS), freeze-dried and ambient storage (FDAS)). In experiment 3, treatments were arranged in a 5 × 3 × 2 factorial of cooling method (none, heat diffusion (HD), fan cooler (FC), counterflow cooler (CF) and freezer (F)), storage condition (ziploc®/ambient (ZA), ziploc®/frozen (ZF) and vacuum/frozen (VF)) and storage time (1 wk and 3 wk). There were 3 replicates per treatment. Data were analyzed using the GLIMMIX procedure of SAS. For experiment 1, there was no interaction between drying process and storage condition for mash samples collected from the mixer (P<0.122). The sample drying process (none and freeze-dried; 921 and 862 FTU/kg) and storage condition (ambient and freezer; 903 and 879 FTU/kg) did not impact phytase activity (P>0.539). For experiment 2, there was no interaction between the cooling method and sample preparation for phytase stability of conditioned mash samples (P<0.144). Cooling method (counterflow and freezer; 76 and 80%) and sample preparation (NDFS, FDFS, and FDAS; 77, 81, and 78%, respectively) did not affect the phytase stability (P>0.686). For experiment 3, there were no three-way or two-way interactions among cooling method, storage condition and storage time (P>0.686). The cooling method (FC, F, HD, CF, none; 48, 51, 55, 54, and 47%, respectively), storage condition (ZA, ZF, and VF; 51, 49, and 53%, respectively) and storage time (1 and 3 wk; 53 and 49%) did not impact phytase stability of pellets (P>0.348). Therefore, freeze-drying, vacuum sealing and freezing were not required when the feed samples were analyzed within 3 weeks of production. Samples, however, should be dried prior to sending to the lab to prevent mold growth.

Key Words: storage condition, phytase stability, sample preparation, enzyme analysis

P328, **Corn kernel hardness, drying temperature and storage time affected the particle size geometric mean and standard deviation in roller and hammer milling** Hernan Cordova-Noboa*[1], Edgar Oviedo-Rondón[1], Andres Ortiz[1,2], Yilmar Matta[1,2], Juan Martinez[1,2], Juan Hoyos[1,2], Jonatan Yanquen[1,2], Adam Fahrenholz[1], Daniela Buitrago[1,2], Ivan Ospina[1], Miguel Chico[1], Viviana San Martin[1], Lina Peñuela[1,2] *[1] North Carolina State University, Prestage Department of Poultry Science, [2] Universidad del Tolima, Facultad de Medicina Veterinaria y Zootecnia*

Corn kernel hardness and drying temperature affect mean particle size and its distribution subsequent to grinding. Experiments also aimed to evaluate the effect of storage post-harvest. The effects of grain drying temperatures (35, 80 and 120°C) for two corn hybrids, varying in kernel hardness (average and hard), on particle size geometric mean (dgw) and standard deviation (Sgw) post grinding were evaluated one wk post-harvest and after 30 wk of storage. For roller-milling (RM), 4 combinations of roller gap settings were studied (0-0, 25-20, 30-30, and 35-35), whereas for hammermilling (HM), 3 rotor speeds were studied (900, 2,400 and 3,600 rpm), in each case grinding through a 12-12 screen combination. In addition, treatments in the HM were also investigated at 12, 17, and 20 wk of storage. For all tests, 3 samples of ground corn per treatment combination were evaluated by the sieving method. Data were analyzed in a completely randomized design and mean separation was done using Tukey's test. After one wk post-harvest, the combination of RM, harder corn, and 35°C drying resulted in higher ($P < 0.001$) dgw as compared to similar corn dried at 80°C. The combination HM, hard kernel corn, and 120°C drying yielded larger ($P < 0.001$) particles than similar corn dried at 35 and 80°C. At 30 wk, results obtained by RM with 0-0 and 30-30 roller gap showed that hard kernel corn dried at 35°C yielded bigger ($P < 0.05$) particles than drying at 80°C. In addition, the Sgw in RM was increased ($P \leq 0.01$) when corn was dried at 80°C compared to 35°C. Moreover, when grinding finely in HM (dgw < 500 µm) the corn with average hardness dried at 80°C had higher ($P < 0.01$) dgw than at 35°C (422 vs. 408 µm). Storing corn for 20 and 30 wk decreased ($P < 0.05$) the particle size in HM and RM. Regression analysis for HM at 2,400 and 3,600 rpm showed that dgw was reduced ($P < 0.001$) as storage time increased independently of kernel hardness. Overall, grinding hard kernel corn yielded larger particles, and lower Sgw regardless of the grinding. In conclusion, kernel hardness and grain drying temperatures influenced the particle size and distribution subsequent to grinding in both the roller and hammer mill, and storage time reduced the particle size regardless of the grinding method.

Key Words: roller mill, hammermill, dgw, drying temperature, stored corn

P329, **Apparent metabolizable energy of Pequi oil in broilers** Jessica Cruvinel, Cassio Oura, Priscila Groff-Urayama, Pedro Trevisani, Jose Sartori, Tatiane Santos, Fernanda Lima-Krenchinski, Andrey Assunção, Julianna Batistioli, Érica Mello, Antonio Pezzato *São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science*

Pequi oil (Caryocar brasiliense) is a characteristic of the Brazilian Midwest (biome Cerrado) and this study was conducted to investigate the apparent metabolizable energy of pequi oil in broilers. A total of 80 one-day-old males cobb® 500 were distributed a completely randomized design in metabolism cages at 14 to 21 days with two treatments of 8 repetitions and 5 birds per experimental unit. The treatments were: reference diet based on corn and soybean meal, formulated using the chemical composition values of the feed and the nutritional requirements for broilers of regular-medium performance according to Rostagno et al. (2017), and test diet, addtion of 10% of pequi oil in reference diet. All cages will be equipped with a tray previously prepared and lined with plastic for the collection of excreta. Excreta collection involved an adaptation period of 5 days of adaptation the diets and 5 days of excreta collection (feces and urine) with control of feed intake. In order to measure the total food consumed and the total excreta produced during this time period. Unmarked excreta in the first collection and marked excreta in the last collection were discarded (marker as 1% ferric oxide). There were two daily collections 08:00 am and 4:30 pm and the excreta collected were packed in plastic bags, weighed and stored in the freezer. The excreta were thawed, and at ambient temperature weighed and homogenized for removal of a sample from each experimental unit and weighed (200-300g aliquot). Dried in a forced ventilation oven at 55ºC for 72h, in order to promote pre-drying and air dry matter determination. Subsequently the samples were ground in a knife mill (16 mesh sieve with 1mm sieves). The excreta and diets samples were sent to the bromatology laboratory for analysis of dry matter and crude energy. Based on the results of the analyzes, the value of metabolizable energy was calculated using the formulas proposed by Matterson et al. (1965). The crude energy of pequi oil was 9.219 Mcal/kg and the apparent metabolizable energy was 7.370 Mcal/kg. Acknowledgment: Fapesp proc. 2018/25363-2 and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) finance Code 001 for finacing this study.

Key Words: Caryocar brasiliense, Pequi

P330, **Ureatic activity and protein solubility in KOH of soybean meal samples obtained in poultry feed mills** Mihayr Jardim [1], Emmanuel Arnhold [2], Fabyola de Carvalho [2], Roberto Jardim Filho [3], Jose Stringhini[*2,4] *[1] Nutrial Insumos Agropecuarios, [2] Departamento de Zootecnia, EVZ/UFG, Brazil, [3] Sao Salvador Alimentos, [4] CNPq researcher*

The use of soybean meal in poultry diets can must be tested for antinutritional factors, which is easily identified with ureatic activity and protein solubility in KOH analysis. This research aimed to evaluate soybean meal quality measured by ureatic activity and KOH solubility in poultry feed mills. For this analysis, 283 samples were obtained from a broiler producing company located in the state of Goiás, Brazil. Soybean meal samples were categorized by the amount of KOH-soluble protein in 5 groups: < 75%; 75% to 80%; 80% to 85%; 85% to 90% and > 90% and 5 other categories regarding ureatic activity: 0; 0.01; 0.011-0.05; 0.051-0.1 and > 0.1. Descriptive statistics was used to evaluate results. Great variation was observed for the ureatic activity index and solubility in KOH. The percentage of samples obtained were classified in 5 categories of protein solubility in KOH (%): < 75%-3.18%; 75% to 80%-7.77%; 80% to 85%-52.64%; 85% to 90%-35.33%; > 90%-1.06%. For the categories of Ureatic activity index (Δ pH), the percentual values found for each of the 5 categories evaluated were, as follows: 0 – 15.2%; 0.01 – 14.12%; 0.011-0.05 – 39.92%; 0.051-0.1 – 17.67%; > 0.1 – 13.07%. For protein solubility in KOH, it was observed that 60.41% of the samples analyzed presented levels varying from 75 to 85% of protein solubility in KOH, that could be considered as a secure index to identify any change in soybean meal processing. But, it is necessary to pay attention to the 39.59% of these samples that the results were outside of the safe range. On the other hand, 87.0% of soybean meal samples had an ureatic activity index lower than 0.1 Δ pH, which can be considered adequate for chickens. But it is important to consider that 13.0% of these samples can be classified as low quality samples. Another value that draws attention is 10.25% of samples with ureatic activity value higher than 0.1 Δ pH and protein solubility greater than 85%. This number of samples exceeding 10% means the possibility of reducing the ingredient quality soybean meal and eventual hazards in broiler performance when this ingredient is added to broiler diets. However, it is important to investigate the possibility of errors in sampling or analysis to ensure effective quality control of the ingredients.

Key Words: diet, ingredient, processing, quality, soybean

P331, **Effect of dietary soy hulls addition on growth performance, digestive traits, and apparent ileal digestibility of nutrients in broiler chickens** Alper Kurul [1], Özcan Cengiz [2], Ahmet Pekel[*1] *[1] Istanbul University-Cerrahpasa, [2] Adnan Menderes University*

An experiment was conducted to evaluate the effects of dietary soy hulls (SH) addition on growth performance, digestive traits, and apparent ileal digestibility of dry matter, nitrogen and phosphorus in broiler chickens. Ross 308, male broilers (n = 224) were assigned to one of four diets including 0, 20, 40, or 60 g/kg SH, with 8 replications per treatment from 0 to 21 d of age in a completely randomized design. Soy hulls was added to corn-soybean meal-fish meal-corn gluten meal-based basal diet at the expense of sepiolite. Chromic oxide was used as an indigestible marker to determine apparent ileal digestibility. Data were subjected to orthogonal contrasts using general linear model procedure of SAS. Growth performance and small intestine weight and length, and gizzard content pH were not affected (P > 0.05) by increasing levels of dietary SH. Increasing the dietary SH level resulted in a linear increase (P < 0.01) in absolute empty gizzard weight. Relative gizzard weight was higher (P < 0.05) in birds fed on 60 g/kg SH diet compared to birds fed on 40 g/kg SH diet. Apparent ileal digestibility of nitrogen was not affected (P > 0.05) by dietary SH inclusion. However, apparent ileal digestibility of dry matter and phosphorus increased linearly (P < 0.01) with increasing dietary SH levels. In conclusion, the inclusion of SH in a corn-soybean meal-fish meal-corn gluten meal-based diet improved ileal digestibility of dry matter and phosphorus and increased gizzard weight without affecting relative weight and length of small intestine and growth performance of broilers at 21 d of age. Thus, these findings indicate that dietary SH inclusion up to 6% may be beneficial for improving phosphorus digestibility without adversely affecting growth performance of broilers at 21 d of age.

Key Words: apparent ileal digestibility, broilers, digestive traits, growth performance, soy hulls

P332, **Feeding Spray-Dried Plasma improves nutrient digestibility in young broilers** Javier Polo, Joy Campbell, Ricardo Gonzalez-Esquerra *APC, Inc.*

Spray-Dried Plasma (SDP) improves the digestive system's development and nutrient absorption when fed to weaned piglets which partially contributes to their growth and overall health. Parsons, et al., 2019 found that the amino acid digestibility of SDP was 95% vs 96% on average when assayed in 10 vs 21 d-old chickens, respectively. Beski et al., 2017 reported higher intestinal sucrase, maltase and alkaline phosphatase activities in 24 d old broilers fed 2% plasma for the first 5 or 10 days of life. This trial aims at assessing the effect of feeding SDP in the starter diet to broilers on dietary nutrient digestibility at 7 d of age and on overall performance. Two groups of unsexed 1-d old Ross 308 chicks were randomly assigned to 20 floor pens of 35 birds per pen. Birds were fed a control diet or a diet with 2% SDP from 0 to 10 d of age. SDP was formulated so that both diets had similar nutrient profile. From 10 to 42 d, both groups were fed the common diets. All diets were commercial-type and had infeed enzymes. Ileal digestibility was tested at 7 d of age using 5 g of TiO2/kg of diet as marker (Myers et al., 2004) and 1 bird/pen. The ileal digestibility of organic matter (70.1 vs 72.5%), dry matter (71.4 vs 74.0%) and crude protein (77.2 vs 80.5%) improved (P<0.001) in birds fed SDP vs the control diet, while that of ether extract was not different (93.0 vs 94.7, respectively). Liver weight was also higher (3.88 vs 4.30 as % of BW) in 7 d chicks fed SDP vs controls (P<0.05). BWG and feed efficiency were numerically higher by 1.4% in SDP birds (P>0.05) at that age. At 42 d of age, birds fed SDP had 2.35% lower intake than controls (P<0.05) with similar BWG. Feed conversion at 42d was not statistically different between the two groups (1.595 vs 1.573, respectively). However, the average performance of the chickens in this study at 42 d of age was superior to the Ross 308 2017 Guidelines (BW = 3.19 vs 2.92 kg and FCR = 1.584 vs 1.631, respectively) with a cold carcass yield of 76.9%. Feeding SDP in the starter diet improved nutrient digestibility in very young healthy broilers.

Key Words: Spray-Dried Plasma, Broilers, Early nutrition, Nutrient digestibility, gut health

P333, **Evaluation of three different proportions of fine particles in the feed and its effects on performance in broiler chickens.** Jimena Orozco[*1,2], Rebeca Zamora [1,2], Sergio Salazar [1], Emilio Cura [2], Humberto Ugalde [2], Marlon Menjivar [2] *[1] University of Costa Rica, [2] Cargill*

National industries that produce balanced feed for animals use high volumes of imported raw materials, which translates into high formulations costs. The importance of improving feeding practices in the poultry sector.

Pelletizing is the most widely used thermal process in the poultry industry. Using pellet in broiler diets has advantages in improvements in the digestibility of starches due to chemical changes during this process. Improvements in BW, FC and FCR in broiler (Calet 1965, Douglas et al. 1990).

The experiment was carried out in an experimental farm, 3150 one-day-old Cobb 500 mixed birds were used, weighted once a week. The treatments consisted of different percentages of fine particles (FP) in the feed, 0% FP, 15% FP and 30% FP. Different sizes of one-day-old chicks were used, small: with 30, medium 40 and large 55 weeks of age in breeders.

Different results of zootechnical parameters such as accumulated body weight (BW), feed consumption (FC) and feed conversion (FCR) at 7, 21 and 35 days of age. Chick size has a significant effect (P <0.0001) on the BW of birds at 7 days of age. The large chicken presented greater BW

than the medium and medium greater than the small one. At 21 days of age of the birds, were differences (P <0.0001) large-medium chick BW was greater than the small. No significant differences (P> 0.05) between the 3 levels of FP in the feed until 21 days, but at 35 days of age it has a difference. At 35 days of age, there are significant differences (P <0.0001). The highest BW obtained was the chickens that were fed with 0% FP. No differences were observed between the BW of chickens fed with 0% and 15% of FP, nor between those of 15% and 30% of FP. Significant difference (P = 0.014) was observed between FP levels of 0 and 30%. There were no differences between 0 and 15% of FP, nor between 15 and 30% of FP. According to the level of FP in the feed, the best conversion was the chicken that consumed feed with 0% FP.

Initial weight of the bird had an effect during all the period. Effect of the percentage of fine particles in the feed, 0% of fine particles in the feed obtained the best BW, FC and FCR. The highest carcass weights are observed in large and medium-sized chickens, and those with consumption of 0 and 15% FP.

Key Words: Pellet, Fine particles, Broilers

P334, **Novel formulations improve feed conversion in broilers** Stefan Buzoianu [1], Ava Firth [1], Jaime Sánchez[*] [2], Inmaculada Ramírez [2], Marta Gracia [2] [1] *Tonisity International, C/ Fitzwilliam place 16,* [2] *Imasde Agroalimentaria, S.L., C/ Nápoles 3, 28224 Pozuelo de Alarcón*

A trial was conducted in order to determine the effect of two novel formulations (Avian liquid and Avian dry mix) on broiler performance. The bioactives included in the Avian formulations have been shown previously to improve gut health, microbial profile and production performance in pigs. The trial was designed as a maximum dose-duration trial in order to optimize the chances of detecting the impact of the two formulations. A total of 216 one-day-old Ross 308 male broilers were placed in 72 cages (24 per treatment), with 3 broilers/cage initially, 1 bird/cage being euthanized for histology at day 11. There were three experimental treatments, T1: Control; T2: Avian liquid supplemented to water at a 1:100 dilution; and T3: Avian dry mix supplemented to the diet at 30 kg/t. T2 and T3 diet composition were adjusted for CP, Na, Cl, Ca, K and ash content of the Avian formulations. Measurements of BW, feed intake and water intake were recorded by pen at 11, 21 and 42d. Data were analysed as a randomized design by GLM of SPSS v. 24.0 with treatment as main effect followed by Tukey's mean separation test. In the first phase (0-11 d), Avian dry mix increased body weight and ADG significantly. Also, both Avian formulations improved FCR during phase 1 over the basal diet (1.19b, 1.12a and 1.10a feed/gain, for T1, T2 and T3, respectively; P = 0.0011). In phase 3 (21-42d), broilers receiving Avian liquid exhibited significantly improved FCR, with no reduction in ADG (1.62b, 1.55a and 1.64b feed/gain, for T1, T2 and T3, respectively; P = 0.0019). Overall for the whole fattening period, Avian liquid significantly improved FCR over the other two treatments (1.49b, 1.43a and 1.50b feed/gain, for T1, T2 and T3, respectively; P = 0.0036). No significant differences between treatments were found on ileal histology data at 11d (villus height, thickness, crypt depth, villus height/crypt depth or goblet cells), but this is being investigated further. It is concluded that the formulation Avian dry mix significantly improved BW, growth and FCR in young broilers. Moreover, the supplementation of drinking water with Avian liquid improved FCR in broilers for their overall productive life (0-42 days).The observed improvement in FCR saved 179 g of feed per bird compared to control.

Key Words: Avian dry mix, Avian liquid, broiler chickens, performance, feed conversion

P335, **Feeding Spray-Dried Plasma in the starter period improves performance early in life and at slaughter age in male turkeys** Krzysztof Kozlowski [1], Javier Polo [2], Joy Campbell [2], Ricardo Gonzalez-Esquerra[*] [2] [1] *University of Warmia and Mazury,* [2] *APC, Inc.*

Spray-dried plasma (SDP) modulates immunity improving overall health and performance in piglets and chickens. Chickens fed SDP from 0 to 5 or 0 to 10 days of age, had higher intestinal digestive enzyme activities at 24d of age (Beski et al., 2017) and improved nutrient digestibility at 7d old chickens has been reported.The effect of feeding SDP in the starter diet of turkeys on early and late performance was investigated. SDP at 0, 2 or 4% was formulated and fed from 0 to 4 wks of age in pelleted diets of similar nutrient profiles. A common dietary program was fed to all groups thereafter. Nutrient densities followed the genetic company's guidelines using wheat, corn and SBM previously analyzed by NIRS. Each treatment had 9 floor-pens per treatment of 10 m2 with 27 Hybrid Converter toms per pen randomly allocated at 1 d of age and followed to 20 wks. Clean pelleted straw was used as bedding material. At 4 wk of age, a 24 h period of stress was imposed by increasing room temperature until panting was evident (at about 35C) and reducing available floor space by 50% to simulate a level of stress caused during transportation. Pellet quality improved when using SDP so that diets with 2 and 4% SDP had 29.2 and 31.3% lower particle size material of < 1 mm, and 14.3 and 42.0 % higher particle size material of > 3.15 mm, respectively, vs the Control starter diet. At 2 wk of age, FCR improved linearly with SDP addition (P=0.005). At 6 wk of age, body weight gain (BWG) was optimized by feeding 2% SDP (P = 0.067). By 20 wk of age, the BW of the Control group was superior to that suggested by the Hybrid Converter Performance Goals (22.11 vs 21.70 kg, respectively). At 20 wk of age, the 2% SDP group had numerically lower FCR vs the Controls (P = 0.11;2.903 vs 2.835) and tended to increase the Production Efficiency Index (P=0.1;538.2 vs 515.7). A tendency for low mortality was observed at 20 wk of age feeding 2% SDP in the starter diet (5.32 vs 3.09% in the Control birds) (P = 0.11). Feeding SDP to young turkeys reduced SBM level by up to 7% and improved pellet quality in starter diets, improved nutrient utilization early in life, and BWG after simulated transportation stress. No significant differences between 2 and 4% SDP were found throughout the trial.

Key Words: Spray-Dried Plasma, turkeys, early feeding, early nutrition, toms

P336, **Evaluating the effect of two commercially available coccidia vaccines challenge on performance and intestinal integrity of 21-day-old broiler chickens** Isaac Oluseun Adejumo[*], Sunday A. Adedokun *Department of Animal and Food Science, University of Kentucky*

Commercially available coccidia vaccine have been used to create intestinal inflammation in broilers for nutritional studies. However, there is a dearth of information on the comparison of the effects of these vaccines on performance and gut health of broilers. This study compared the effects of two commercially available coccidia vaccines (Coccivac®B52 and D2, Merck Animal Health) challenge (CVC) on performance, gut health, and ileal nutrient and energy digestibility in broiler chickens. One hundred and sixty eight day-old broiler chicks were randomized to 3 treatments (control, B52-CVC, and D2-CVC) in a completely randomized design on d 14 with 7 replicate cages per treatment and 8 birds per cage. All birds were fed a standard corn soybean meal-based diets from d 0 to 14. The control birds were orally gavaged with 0.6 mL of distilled water while the B52 and D2-challenged birds were orally gavaged with 0.6 mL of distilled water containing 20x of their respective vaccine. Birds and feed were weighed on d 14 and 21. On d 21, birds were euthanized followed by samples collection. A section of the mid duodenum, jejunum, and ileum were removed for histology while one tibia and femur from one bird per cage were removed. Data were analyzed using the GLM procedure of SAS. Tukey's test was used to separate treatment means while specific

contrasts were used to compare B52 vs. control and B52 vs. D2 treatments. No significant effect of vaccine type was observed on tibia and femur ash. BW gain and feed intake were lower (P < 0.05) in the CVC birds compared to the control birds. The D2-challenged birds had lower (P < 0.05) BW gain (8.5%) and feed efficiency compared to B52-challenged birds. Ileal DM, N, energy, Ca, and amino acids digestibilities were lower (P < 0.05) in the CVC-birds compared to the control group. Duodenal and ileal crypt depths were lower (P < 0.05) in B52-challenged birds compared to D2-challenged birds while villi height was higher (P < 0.05) in B52 compared to D2-challenged birds. Results from this study showed that the D2-challenged birds had lower performance and deeper duodenum and ileum crypt depth compared to B52-challenged birds.

Key Words: broiler chicken, histology, ileal digestibility, amino acid, performance

P337, **EFFECT OF BIO-CHOLINE AS A REPLACEMENT TO SYNTHETIC CHOLINE ON QUALITY AND VALUE ADDITION IN EGGS** NIKAM G*, DHUMAL V, MAMDE S, NANDEDKAR V, GANGANE R, Khose K, Shivi MAINI *ASSISTANT PROFESSOR OF POULTRY SCIENCE,MAHARASHTRA ANIMAL AND FISHERY SCIENCES UNIVERSITY*

The experiment was conducted on 150 layer, randomly devided into three tratment with five replicate 50 birds in each.Treatment group A with synthetic choline chloride @ 500g/ton of feed. Treatment group B with Bio-Choline @ 375 gm/ton. The treatment group C with Bio-Choline @ 375 gm/ton + Herbal E @ 50g/ton of feed. Non significant findings were recorded with egg shell thickness with the herbal products. The results revealed improved shape index for eggs. There were significant differences (P<0.05) with the yolk index, albumin index. The supplementation of Bio-Choline @375g/ ton of feed alone or in combination with Herbal E @ 50g/ton of feed reduced the cholesterol level (35th and 45th) weeks. The results revealed lowered fat content in eggs with Bio-Choline @375g/ ton of feed alone or in combination with Herbal E@50g/ton of feed and also lowered saturated fatty acids in egg. Non significant influence was recorded for trans fatty acid content in eggs. The DHA content in eggs was higher with bio-choline and herbal vitamin E supplementation. The lower omega 6: omega 3 (n6:n3) ratios noticed for eggs from bio-choline and herbal vitamin E supplement groups.

Key Words: Bio-choline, DHA, Herbal E, trans fatty acids, layers

P338, **Early post-hatch feeding of resistant starch can influence cell-mediated immunity and gut microbiota diversity in broilers** Amit Singh*, Birendra Mishra, Rajesh Jha *University of Hawaii at Manoa*

This study evaluated the prebiotic potential and the effect of early feeding of two different types of resistant starch (RS): type 2 (RS2) and type 4 (RS4) during the early post-hatch period on the parameters of growth, cecal fermentation, histomorphometry, immunity and gut microbiota diversity of Cobb 500 broilers. A total of 300 day-old chicks were randomly assigned to 30 cages (10 birds/cage) and cages were equally allocated to 6 treatments (n =5). The treatments included i) no feed (NF; starved), ii) RS2, iii) RS4, iv) 50% commercial feed (CF) + 50% RS2, v) 50% CF + 50% RS4, and vi) CF (control). The treatment diets were fed for two days post-hatch (48 hrs. ± 2 hrs.), after which all birds were fed CF *ad libitum* till D21. The feed intake and body weight were recorded weekly to calculate average daily gain (ADG) and feed conversion ratio (FCR). On D7 and D21, ileal samples were collected for histomorphometric and immune-related gene expression analysis. Cecal digesta was collected for DNA extraction and genomic sequencing on D7 and D21, and SCFA determination on D21. At D21, there was no difference in SCFA and FCR among treatments (P >0.05), but the average daily feed intake and ADG were higher in CF+RS2, CF+RS4 and CF groups compared to NF, RS2 and RS4 groups (P <0.01). At D7, RS4+CF group had higher expression of CD3 (a T-cell marker) genes while RS4 and RS4+CF groups exhibited a reduced level of IL-4 compared to control (P <0.05), but these effects were not significant at D21. The villus height, crypt depth, and their ratio were not affected across treatments. At D7 RS2+CF had a higher (P <0.05) Shannon index than NF while it tended (P =0.083) to be lower on D21. At D7 RS4+CF had a higher (P <0.05) OTU than NF. At D7, RS2+CF exhibited a trend (P =0.059), whereas it had a significant difference (P <0.05) for Bray Curtis beta-diversity with the NF group at D21. At D7, RS2 had a significant (P <0.05) Unweighted UniFrac metrics for beta diversity with the NF group. The results indicate that the effect of early post-hatch feeding with RS on the immunity of chicks is transient and could be independent of the microbiota. However, it can have a prolonged effect on cecal microbiota diversity compared to early starved but not early fed broilers.

Key Words: broilers, gut-health, histology, immunity, resistant starch

P339, **Effects of exogenous inclusions non-starch polysaccharides in typical starter diet on performance, GI microbiota, nutrient digestibility, and net energy availability** Pramir Maharjan*, Katie Hilton, Antonio Guerra, Jordan Weil, Nawin Suesuttajit, Diego Patino Patr, Craig Coon *University of Arkansas*

A study was conducted to understand effects of feeding soy NSP exogenously added in typical starter diet in broiler chicks on performance, GI microbiota, nutrient digestibility and dietary net energy availability.Initially, the bulk extraction of Cell Wall Material (CWM) (non-starch polysaccharide levels (NSP) analyzed value~ 27% on DM basis) and soluble non-cellulosic polysaccharides (NCP) from SBM were performed to conduct *in-vivo*study. Two treatment diets were created: T1-common starter (Cobb 500 nutrient specs); and T2- starter +CWM (at 3 %) + 0.2 %, soluble NCP (via drinking water). Birds (Cobb 500) were reared using floor pens (n= 40 birds/treatment) until d 6 with common starter diet. Broiler chicks were then fed experimental diets from d 7 to d 14. Performance was measured at d 12. GI microbiota was assessed using 16s rRNA gene sequencing for ileal and cecal digesta before (d 6) and after feeding treatment diets (d 12). NCP and amino acid (AA) ileal digestibility were measured using TiO_2as indigestible marker in feed and ileal digesta. Dietary net energy (NE)were measured calculating heat increment HI (Fed heat production (HP) – Fasted HP) and subtracting it from metabolizable energy. Results showed that BW recorded on d12 were 342 g/b and 302 g/b whereas FCR was 0.96 and 1.1 respectively for T1 and T2 groups. T2 had a lower digestibility coefficient (P < 0.05) for all the AA analyzed (by 3-5 units). Total NCP digestibility was higher for T1 diet (by 7 units). Microbiota results showed ileal *Lactobacillusacidpiscis*went to higher abundance for TI and T2 from d 6 to d 14. Unclassified *Lactobacillus*and *E. coli*were higher in T2 (*P*<0.05). In cecal digesta*, E. coli*abundance went up (*P*<0.05) for T2, whereas *Enterococcus*abundance were higher in T1. Even though there were difference in body composition (BC) (protein: fat ratio was higher for T1 at d 12 and d 14), significant difference in HI was not observed between treatment groups, thus not differing in the dietary net energy (Kcal/kg of FI) value between treatment diets. Overall, NSP added to starter diet showed effects on performance, GI microbiota, nutrient digestibility, and overall BC composition of broilers.

Key Words: non-starch polysaccharides

Metabolism and Nutrition, Vitamins and Minerals

P340, **Increased breast meat yield of Ross 708 broilers in response to the in ovo injection of various forms of vitamin D3** Abdulmohsen Alqhtani*[1GS], Saman Fatemi [1], Katie Elliott [1], Ayoub Mousstaaid [1], Abiodun Bello [2], April Levy [3], David Peebles [1] [1] *Mississippi State University,* [2] *University of Alberta,* [3] *DSM Nutritional products*

Breast meat yield has been shown to increase by the use of supplemental dietary vitamin D_3 (D_3) or 25-hydroxyvitamin D_3 ($25OHD_3$) under commercial conditions. Therefore, the objective of this study was to determine effects of the *in ovo* administration of vitamin D_3 (D_3) and its metabolite, $25OHD_3$, on breast meat yield when broilers are fed commercial diets. Live embryonated Ross 708 broiler hatching eggs were randomly assigned to one of the following 5 *in ovo* injection treatments at 18 d of incubation (doi): 1) non-injected; 2) diluent; diluent containing either 3) 2.4 µg D_3, 4) 2.4 µg $25OHD_3$, or 5) 2.4 µg D_3 + 2.4 µg $25OHD_3$. A 50 µL solution volume was injected into each egg using an Inovoject multi-egg injector. At hatch, 18 male chicks were randomly assigned to each of 30 pens and birds were fed commercial diets for the starter (0-14 d of age (doa)), grower (15-28 doa) and finisher (29-42 doa) dietary phases. At 14 and 39 doa, one bird from each of 6 replicate cages in each treatment group (30 total birds) was weighed and then sampled for determination of the weights of their pectoralis major (P. major) and pectoralis minor (P. minor) muscles, and breast yield was calculated by adding the values of the P. major and P. minor muscles. At 43 doa, the remaining birds were processed and whole carcass, and P. major, P. minor, drumstick, thigh, and wing weights and yields (percentage of carcass weight) were determined. A randomized complete block experimental design was employed and all data were analyzed using a one-way ANOVA. At 39 and 43 doa, P. major (P_{d39}= 0.05; P_{d43}= 0.029) and breast yield (P_{d39}= 0.041; P_{d43}= 0.044) were increased in response to the *in ovo* injection of $25OHD_3$ alone in comparison to all other treatments. These results indicate the *in ovo* injection of 2.4 µg of $25OHD_3$ increased the breast meat yield of Ross 708 broilers.

Key Words: In ovo injection, Broilers, Breast meat yield, Commercial diets, 25-hydroxyvitamin D3

P341, **Rapid chick bioavailability study of highly concentrated (500,000 IU D3/kg) cholecalciferol sources** Kimberly Gardner*[GS], Christopher Bailey *Texas A&M University*

A total of 240 day-old Cobb-700 by-product males were distributed within two stainless steel 24-pen battery brooders and placed on a mash corn-soy broiler starter diet containing 0.75% calcium and 0.375% non-phytase phosphorus. A custom vitamin premix, completely devoid of vitamin D3, was utilized within the basal diet to minimize maternal stores of vitamin D3. At 10 days of age all birds were weighted and distributed among the 48 brooder pens so that each group of five birds weighed within ±20 g of each other. A randomized design was used in which any individual pen of broilers had a totally random choice of receiving any of the treatments. A total of 8 pens remained on the negative control diet with no access to vitamin D3 over an 18 day rearing period. All birds were depleted of vitamin D3 over the first 10 days of the study by feeding them the negative control basal mash. At 10-days of age birds were offered the test treatments until day-18 of the study. Treatments consisted of 2 concentrated sources of vitamin D3 (500,000 IU /g) fed supplemented at 125, 250, 500, and 1000 IU D3/kg. All birds were euthanized by cervical dislocation and both the left and right tibia were collected. Tibia breaking strength was determined for both fresh and fat-free dried bones using a texture analyzer and then correlated with bone ash. Data were analyzed as a 2 X 4 factorial with a separate negative control using the GLM Procedure of SPSS. There were differences ($p<0.05$) due to treatment levels of D3 fed with both breaking strength and bone ash increasing as dietary concentration increased. There were no differences in source for any of the variables measured. Source data were therefore combined and modeled for all birds using the NLIN procedure of SAS for both broken-line and quadratic fits. The 2 linear line model for bone ash broke at 284.8±35.8 IUD3/kg diet while the single slope quadratic model broke at 392.7±74.0 IUD3/kg diet. Fresh tibias showed a lower breaking strength (10.45±0.34 kg) when compared to the fat-free dried tibias (13.33±0.33 kg). The tibia breaking strength of the fresh tibias was highly correlated with total ash percent (r= 0.823). While the fat-free dried tibia breaking strength was poorly correlation with total ash percent (r=0.024).

Key Words: Cholecalciferol, Breaking strength, Bone ash, Broiler, Correlation

P342, **Effects of riboflavin and Bacillus Subtilis on blood components of male broiler with or without coccidial challenge** Sabin Poudel*[GS], Wei Zhai *Mississippi State University*

Probiotics, along with intestinal commensal bacteria, produce bile salt hydrolase (BSH). BSH hydrolyze conjugated bile, which depletes the emulsification capacity of bile salt and reduces lipid digestion and absorption. In this study, we hypothesized that the supplementation of extra riboflavin (as a BSH inhibitor and an antioxidant) and probiotic *Bacillus subtilis* in the broiler diet would minimize adverse effects of a coccidial challenge, reduce stress, and increase antioxidant enzyme production. The variables observed included blood Heterophil to Lymphocyte (H: L) ratio (a stress indicator), serum superoxide dismutase (SOD) (an antioxidant enzyme that removes superoxide to protect cells), and their relationship with the development of woody breast (WB). A total of 1,248 d-old Ross 708 male broiler were randomly assigned to 12 treatments in a 3 × 2 × 2 (riboflavin × *Bacillus subtilis* × cocci) arrangement, and each treatment consisted of 8 replicating blocks. Diets with three levels of riboflavin, (0.75, 6.6 (recommended), and 20 ppm), with or without *Bacillus subtilis* (1.1×10^8 CFU/Kg), were fed to the birds. On d 14, 20× doses of commercial cocci vaccine were orally gavaged to birds in the challenged groups. On d 35, one bird per pen was selected, and whole blood was collected. Data were analyzed using 3-way ANOVA and partial correlation in SAS 9.4. There was no difference in the H: L ratio among treatments. When birds were fed 6.6 ppm of riboflavin, cocci challenge lowered serum SOD levels (P = 0.0377). *Eimeria spp.* proliferation in the intestinal cells can increase the production of free radicals (superoxide). More SOD may have been consumed to neutralize the increased free radicals in challenged birds, which resulted in lower SOD. The H:L ratio was positively correlated with the moderate WB (P = 0.0451), which indicates that increased severity of WB might have induced more stress in birds or vice versa. In conclusion, riboflavin and *Bacillus subtilis* did not alter the stress induced by the coccidial challenge and development of WB. However, feeding different levels of riboflavin during coccidial challenge might affect birds' antioxidation enzyme production and utilization.

Key Words: Antioxidant, Bacillus subtilis, Broiler, Coccidiosis, Riboflavin

P343, **Assessment of novel water treatment equipment (H2Oelite) on broiler performance, tibia ash and mineral digestibility** Carley Rhoads*[1UG], Courtney Ennis [1], Andrew Brown [1], Pratima Adhikari [1], Tom Tabler [1], A. Elzind [2], Kelley Wamsely [1] [1] *Mississippi State University,* [2] *H2O Elite*

Various water treatment options exist to help poultry growers with their water quality; however, cost or application method may preclude their use. The H2Oelite device is new technology that magnetically treats the water and has been reported to lower water surface tension, thus improving both mineral solubility and hydration; in addition, scale and biofilm buildup inside contact surfaces of the treated water may be reduced. This may be a cost-effective water treatment option for growers, though research is needed. Water was sourced from a local grower and supplied via a holding tank. On demand, water entered the room via pressurized pump, then

treated via H2Oelite or not treated, then supplied to a group of cages as Randomized Complete Block Design. Location served as the block and a unit of 20 raised wire cages served as the experimental unit (4 replications/treatment). A total of 1,920 Ross x Ross 708 female broilers were equally allocated to 160 cages; 240 chicks/experimental unit. On d 20, feed and fecal samples were collected and analyzed for TiO2 to determine total gastrointestinal tract mineral digestibility. On d 21, birds were individually weighed and feed intake was recorded to determination of BW, BW gain, bird uniformity, feed intake/bird (FI), % mortality, and FCR. On d 21, 20 chicks per experimental unit were selected for tibia ash. No significant differences were found for any of the performance variables measured (P>0.05). However, 0-21 d % mortality demonstrated a trend (P=0.158) for reducing mortality when birds were provided treated water. However, chicks provided untreated water had increased % tibia ash (P=0.014). While not significant, total tract Ca digestibility data tended to increase with chicks provided untreated water (P=0.182). Though more research is needed, these data suggest that H2Oelite may help reduce 21 d mortality, possibly via biofilm reduction. Data also shows that digestibility of minerals (i.e. Ca) is changed via H2Oelite, however not enhanced, under these conditions. It is possible that the adequate nutritional plane of the diets may have played a role in these data. Future research should further investigate H2Oelite effects on biofilms, as well as effects in combination with diets varying in mineral content.

Key Words: water treatment, tibia ash, broiler performance, calcium digestibility

P344, **Effects of dietary Ca source and concentration, and vaccine dosage on d14 and d29 Broiler performance** Ariel Bergeron*GS, Christopher Eagleson, Garrett Powell, Audrey McElroy *Texas A&M University*

Due to the absence of antimicrobial drugs within the feed, a better understanding regarding the mitigation of NE is necessary. Recent research has identified that dietary Ca levels could potentially induce NE pathogenesis, but the mechanism is unknown. In theory, the amount and availability of Ca in the lower intestine can provide an enriched environment for C. perfringens bacteria, resulting in an increased proliferation of the bacteria and toxin activity. The current study was to assess broiler performance when fed diets that differed in Ca source and concentration, as well as subjected to differing coccidia vaccine levels. Treatments were arranged in a 2x2x2 factorial, that varied in vaccine level of a 1X or 2X dosage of commercial coccidia vaccine (Coccivac B52), and dietary treatment consisting of Ca sources Calmin or limestone that was fed at a concentration of 1.05% or 1.25% of the diet. Diets were fed to 10 replicate floor pen of 30 male Cobb 500 broilers. Data were analyzed using a 2x2x2 factorial arrangement in a randomized complete block design. Broilers dosed with 1X vaccine exhibited improved BWG at d14(P=0.0401). Ca source and concentration at d14 interacted to affect BWG, at 1.05% concentration broilers that consumed diets with limestone had improved BWG compared to diets of Calmin(P=0.001). Broilers dosed with 1X vaccine exhibited improved FC at d14(P=0.0001). Broilers provided diets with the Ca source of limestone had improved FC at d14(P=0.0001). Broilers provided diets with concentrations of 1.05% had improved FCR at d14(P=0.0093). Broilers dosed with 1X vaccine exhibited improved FC at d29(P<0.0001). Broilers provided diets with the Ca source of limestone had improved FC(P=0.0036). Ca source, Ca concentration and vaccine at d29 interacted to affect BWG(P=0.05). Birds provided limestone concentrations at 1.05% that received 1X or 2X dose of vaccine at d29 performed better than birds fed Calmin at concentrations of 1.05% or 1.25% with 1X or 2X dose of vaccine. This trial supports previous data indicating nutritionists should consider the interaction of Ca source, concentration, and vaccine to help mitigate the risk of NE through limiting the availability of excess Ca that could contribute to the occurrence of NE.

Key Words: Calcium, Necrotic Enteritis, Limestone, Coccidia Vaccine, Broiler

P345, **The Role of Dietary Calcium level and source with different protein sources on the development and pathogenesis of Necrotic Enteritis** Christopher Eagleson*[1], Zach Akers [1], Alex Rosario [1], Austin Silva [1], Chance Williams [2], Craig Wyatt [3], Dan Klamfoth [4], Audrey McElroy [1] *[1] Texas A&M University, [2] Wayne Farms LLC, [3] AB Vista, [4] Lhoist North America*

Previously we reported research indicating differences in calcium inclusion rate, source, and particle size impact on bird performance. To better understand the impact that other dietary strategies have on the pathogenesis of necrotic enteritis, two protein sources were explored. A randomized complete block design was conducted to determine the impact of Ca sources at 2 different inclusion rates with different protein sources on the pathogenesis of NE. The protein sources were all veg and MBM addition. A total of 4,320 d-old Ross male broilers were assigned to 8 dietary treatments fed in 3 phases (0-15 d, 15-23 d, and 23-35 d) each containing 12 replications. NE was allowed to occur naturally following mild intestinal response to day of age coccidia vaccination. Dietary treatments were: all veg with standard levels of Ca 1(trt1), all veg with low levels of Ca 1(trt2), all veg with standard levels of Ca 2(trt3), all veg with low levels of Ca 2(trt4), MBM with standard levels of Ca 1(trt5), MBM with low levels of Ca 1(trt6), MBM with standard levels of Ca 2(trt7), MBM with low levels of Ca 2(trt8). All data were subjected to an ANOVA using the GLM procedure. Means were deemed significantly different a P≤0.05 and separated using Tukey's HSD. A 2x2x2 factorial analysis was used to determine the impact of dietary Ca inclusion rate, Ca source and different protein sources. At d 15 there was a three-way interaction (P<0.05) with birds consuming trt 2 having a reduced BW compared to all other treatments except trt 5. At d 23, there was a protein X source interaction (P<0.05) with birds consuming the all veg with Ca 1 having reduced BW compared to the others. At d 35 there was no difference in the BW of the birds. During d 0-15 there was a protein X level interaction (P<0.05) with birds consuming standard Ca levels of the veg diet reducing the FCR. During d 0-23 there was a Ca inclusion rate (P<0.05) main effect with birds consuming standard levels having reduced FCR. There was no difference in FCR for d 15-23, d 23-35, and d 0-35. There was no difference in the livability throughout the trial. The data suggest that early on the Ca source and level interact with the protein source to impact the performance of the bird, but differences diminish as the bird age.

Key Words: Broiler, Necrotic Enteritis, Calcium

P346, **The impact of dietary vitamin E and selenium on body composition, intestinal integrity and immunity of broilers subjected to heat stress** Ali Calik*, Nima Khodambashi Emami, Mallory White, Rami Dalloul *Avian Immunobiology Laboratory, Department of Animal & Poultry Sciences, Virginia Tech*

High ambient temperature is one of the most common stressors in modern poultry production, resulting in poor performance and increased mortality. This study evaluated the effects of vitamin E (Vit E) and selenium (Se) supplementation on broiler body composition, mRNA abundance of heat shock proteins (HSPs), immunity markers, and nutrient transporters in jejunum tissues of broilers exposed to daily 4-h elevated temperature during d 28-35. A total of 640 Cobb500 male birds were randomly allocated to 32 floor pens in a 2 ´ 2 factorial arrangement that included heat stress (optimal and high heat) and dietary treatments (basal diet or Vit E/Se). Vit E and Se were added to the basal diet at the rate of 250 mg/kg and 1 mg/kg diet, respectively. For the heat stress (HS) groups, temperature was maintained at 35±1°C and applied once daily from 10 AM to 2 PM, then reduced again to 25±1°C for the remainder of the day. Body composition was measured by dual energy X-ray absorptiometry from two birds/pen. Jejunal samples were taken on d 28 and 35 (2 birds/pen) from birds of average pen weight for qPCR analysis. Data were subjected to a 2-way ANOVA using the GLM procedure of JMP (Pro13). Dietary Vit E/Se supplementation significantly increased carcass, tissue, lean weights and bone mineral content (g) on d 35 during HS challenge. HS significantly upregulated

HSP response on d 28 and d 35 in the jejunum. However, mRNA abundance of HSP70 and HSP90 was significantly downregulated with the Vit E/Se treatment on d 28 and 35. Expression levels of the nutrient transporters related genes NaPi, PepT1, SGLT1 were significantly upregulated by HS on d 28. However, dietary Vit E/Se administration had no effect on mRNA levels of NaPi, PepT1, SGLT1, SI, IAP ZO-1, MUC2, TTF2, TLR2, TLR4, IL-1b, IL-10, TNFa, iNOS, and IFNg on d 28 and d 35. In conclusion, dietary supplementation of Vit E and Se may help alleviate the negative effects of high ambient temperature and improve body composition by influencing heat shock proteins and modulating antioxidant status of heat-stressed broilers.

Key Words: Broiler, heat stress, heat shock proteins, immune response, Vitamin E Selenium

P347, **Investigation of replacing Vitamin E with EconomasE® in layer diet** Tuoying Ao*, Marquisha Paul, Lizza Macalintal, Mike Ford, Anthony Pescatore *Alltech-University of Kentucky Nutritional Research Alliance*

EconomasE® is a proprietary blend of ingredients that was designed as an antioxidant support pack to allow more efficient recycling of key antioxidants including vitamin E (VE). Previous study indicated that replacing Vitamin E with EconomasE® in broiler diet had equal or better effects on performance, meat quality and total antioxidant capacity of broiler chickens. Current study was designed to investigate the effect of replacing VE with EconomasE® in layer diet on the production performance and egg and shell quality of laying hens. Dietary treatments included: 1) corn-soy diet supplemented with 0.3 ppm Se as selenite and 35 IU/kg VE; 2) corn-soy diet supplemented with 200 g/Ton EconomasE®, 35 IU/kg VE and no Se; 3) corn-soy diet supplemented with 200 g/Ton EconomasE®, 21 IU/kg VE and no Se; 4) corn-soy diet supplemented with 200 g/Ton EconomasE®, 14 IU/kg VE and no Se; 5) corn-soy diet supplemented with 200 g/Ton EconomasE®, 7 IU/kg VE and no Se. Four hundred twenty Hy-Line W36 layers at 40 weeks of production were randomly assigned to each of five dietary treatments with 7 replicate units of 12 layers. The layers were raised in layer cages until 80 weeks using two birds per cage (25 x 41 cm) and photo-stimulated with 16L: 8D. Feed and water were provided *ad libitum*. No effects of dietary treatments on egg production, feed intake, feed conversion ratio and body weight were detected. No effects of dietary treatments on egg weight, Haugh unit, egg breaking strength, eggshell percentage and egg yolk color were noticed. These data suggest using EconomasE® to replace up to 80% VE and 100% Se in layer diet did not impact production performance and egg and shell quality of laying hens.

Key Words: Laying hens, Performance, Egg quality, Vitamin E

P348, **Effects of cholecalciferol and 25-hydroxycholecalciferol supplementation with different levels of dietary calcium on performance, egg quality and serum biochemistry of laying hens** Masud Rana, Chun Ik Lim, Kyeong Seon Ryu *Jeonbuk National University*

The objective of the study was to assess the effect of cholecalciferol (vitamin D_3) and 25-hydroxycholecalciferol 25-(OH)D_3 with dietary calcium (Ca) levels on performance, egg quality and serum biochemical traits of laying hens from 61 to 68 weeks of age. A total of 540 Hy-Line brown laying hens were randomly distributed into 9 treatments with 5 replicates and 12 hens in each. The experiment consisted of a 3×3 factorial design with 3 levels of Ca (3.5, 4.0 and 4.5%) and 3 levels of vitamin D_3 (3000, 2000 and 1000 IUkg^{-1} diet) with 0, 25 and 50 µgkg^{-1} of 25-(OH)D_3. Data were analyzed by analysis of variance using the GLM procedure of SAS (SAS 9.1, 2009). Results showed that egg production and daily egg mass were tended to be influenced in hens fed on 4.5% Ca and supplemented with vitamin D_3 1000IU plus 50µg 25-(OH)D_3 in per kg diet group among the treatments but egg weight and feed conversion ratio had not differed by the treatments. Egg production tended to be higher by about 1.86% due to the effect of increasing level of Ca 3.5 to 4.5% in diet while significantly higher egg weight and egg mass in hens fed on diet containing 4.5% Ca was observed. Also, significantly greater egg production was found due to reducing level of vitamin D_3 with increasing 25-(OH)D_3 up to 50 µgkg^{-1} diet group. Egg quality in terms of albumen height, Haugh unit, shell weight, shell with egg weight ratio were not affected by treatments or dietary Ca levels or vitamin D_3 concentration in diet except for egg shell thickness that was altered by the treatments, increased egg shell thickness when 4.5% Ca was fed but had no effect for the levels of vitamin D_3 or/with 25-(OH)D_3 concentration. Serum Ca concentration was statistically influenced by vitamin D_3 with level of 25-(OH)D_3 supplementation in the diet. However, increasing the concentration of 25-(OH)D_3 to 50 µg/kg diet significantly enhanced the concentration of Ca in the serum. On the other hand, the serum concentration of phosphorus, total protein and albumin, however, were not affected. Thus, the results suggest that supplementation of 25-(OH)D_3 can be substituted for vitamin D_3 in layer diets to improve egg production and increase Ca retention in plasma.

Key Words: calcium, cholecalciferol, 25-hydroxycholecalciferol, performance, laying hens

P349, **Effects of different sources and levels of copper and manganese on performance and carcass yield of broilers chickens** Priscila Groff-Urayama [1], Karolina Augusto [2], Yanming Han [2], Cássio Oura [1], Tatiane Santos [1], Jéssica Cruvinel [1], Fernanda Lima-Krenchinski [1], Julianna Batistioli [1], Andrey Assunção [1], Érica Mello [1], Giovana Alves [1], Gustavo Barbosa [1], José Sartori*[1] *[1] São Paulo State University (UNESP), School of Veterinary Medicine and Animal Science, Botucatu., [2] Trouw Nutrition R&D, 3800 AG Amersfoort, Utrecht, the Netherlands*

Hydroxychloride is the latest source of copper (Cu) and manganese (Mn) in broiler nutrition. It has emerged as an alternative source of mineral supplementation and is known for its great strength in chemical bonding. Thus, the objective of the present study was to evaluate the effect of different sources and levels of Cu (Cu Sulfate monohydrate - CSM and IntelliBond® C - IBC) and Mn (Mn sulfate monohydrate - MSM and IntelliBond® M - IBM) on performance (1 to 42 d-old), carcass and breast yield, as well as skin resistance and elasticity. A total of 1,920 1-d-old male Cobb chicks were distributed in a completely randomized design with a factorial arrangement of 2x3+2: IBC (15 and 150 ppm) × IBM (40, 80, 120) + CSM and MSM (15 and 80 ppm) and CSM and MSM (150 and 120 ppm) with 8 treatments and 8 replicates of 30 birds each. The diets were based with corn and soybean meal according to the recommendations of each phase: pre-starter (d1-d7), starter (d8-d21), grower (d22-d35) and finisher (d36-d42). At the end of the experiment, three birds per replicate were slaughtered for carcass and breast yield assessment, and the left drumstick skin was used to evaluate skin resistance and elasticity. Data were analyzed with Minitab® 18, and the means were compared by the Tukey test ($P<0.05$). No statistical difference was found for performance. Broilers fed diets containing 150 ppm IBC showed higher breast yield ($P=0.049$) compared with those fed diets containing 15 ppm. Breast yield was positively influenced ($P=0.030$) by the inclusion of 40 ppm IBM, comparing with 120 ppm. There was an interaction ($P=0.017$) between the IBC and the IBM for skin elasticity, and the highest elasticity found was when the supplementation levels were 150 ppm IBC and 40 ppm IBM. In conclusion, the results of this study indicate that supplementation of broiler diets with 150 ppm IBC and 40 or 80 ppm IBM levels enhance breast yield. Furthermore, by supplying 150 ppm IBC plus 40 ppm IBM it is possible to improve skin elasticity.

Acknowledgment: Thanks to Trouw Nutrition and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) finance Code 001 for finacing this study.

Key Words: feed conversion, hydroxychloride, mineral poultry nutrition, weight gain

P350, **Performance and relative organ weight of broilers supplemented with copper and zinc hydroxychlorides in substitution to sulfate sources and antibiotic growth promoters** Cassio Oura [1], Priscila Groff-Urayama [1], Karolina Augusto [2], Yanming Han [2], Jessica Cruvinel [1], Julianna Batistioli [1], Tatiane Santos [1], Robert Araujo [1], Érica Mello [1], José Sartori*[1]
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The objective of this study was to evaluate hydroxychloride copper (Cu) and zinc (Zn) sources (IntelliBond®C; IBC and IntelliBond®Z; IBZ) in comparison with the conventional inorganic minerals (copper sulfate monohydrate - CSM and zinc sulfate monohydrate - ZSM), and also compared them with the use of antibiotic growth promoters through growth performance. A total of 2,100 male Cobb 500 broilers (1-d-old) were distributed in a completely randomized design, consisting of 5 treatments with 14 replicates, totaling 70 experimental units (pens) of 30 birds each. Broiler chickens were given with a dose of Coccidiosis vaccine 10x the commercial dose on their first day of life and with a reused litter of wood-shavings. From 1-42 d-old, the broilers were submitted to the following treatments: T1: (positive control) 10 ppm Cu (CSM) and 80 ppm Zn (ZSM) plus 50 ppm Avilamycin; T2: 125 ppm Cu (CSM) and 80 ppm Zn (ZSM); T3: 125 ppm Cu (IBC) and 80 ppm Zn (IBZ); T4: 200, 150, 125, 125 ppm Cu (IBC) and 80 ppm Zn (IBZ) for the pre-starter (PS: 1-7 d-old), starter (S: 8-21 d-old), grower (G: 22-35 d-old) and finisher (F: 36-42 d-old); T5: 250, 200, 125, 125 ppm Cu (IBC) and 80 ppm Zn (IBZ), for PS, S, G and F phases, respectively. Data were submitted to analysis of variance using Minitab® 16, and the means were compared by the Tukey test (P<0.05). The performance presented statistical difference for body weight (BW), weight gain (WG), and feed conversion ratio (FCR). For BW, WG and FCR T1 and T3 presented the best results. The relative weight of organs was not affected by treatments. Based on this study, it is concluded that 125 ppm Cu IBC and 80 ppm Zn IBZ improve growth performance and can replace the use of antibiotic growth promoters. Acknowledgment: Thanks to Trouw Nutrition for finacing this study.

Key Words: inorganic minerals, broiler chickens, feed conversion ratio, weight gain

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