



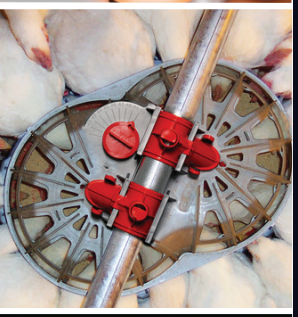

2019

INTERNATIONAL POULTRY SCIENTIFIC FORUM

FEBRUARY 11 – 12

ABSTRACTS
2019 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
February 11 - 12, 2019

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ABSTRACTS

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Milton Y Dendy Keynote Address

B313 Courage Over Comfort - How Can You Cultivate Food Safety? Lone Jespersen *Cultivate*

Impact of organizational culture on food safety is a much discussed topic in the global food industry and have directly been connected with consumer sickness and death. Companies who take this discussion serious have learned that it takes courage not only to understand and potentially assess their culture of food safety but also to ongoingly cultivate food safety to protect consumers, employees, and brands. In this session you will learn from both science and practical experiences how to breakdown your culture of food safety and what tactics you can apply tomorrow to create change and cultivate food safety in your company.

***Author presenting paper**

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Monday, February 11, 2019

Physiology, Endocrinology and Reproduction

M1 Broiler breeder hen zona pellucida B2 protein expression in the granulosa cell and perivitelline layer Josh Steed*[1], Andrew Benson[1], R Bramwell[2], Adam Davis[1] *[1]The University of Georgia; [2]University of Arkansas*

Prior to ovulation, the avian oocyte is surrounded by the theca, granulosa, and inner perivitelline layer (IPVL). The IPVL contains the zona pellucida (ZP) family of proteins which are required for sperm binding and fertilization. Previously we reported that ZPB2 mRNA expression was greatest in the granulosa cells of pre-hierarchical follicles and declined as the follicle matured to ovulation. In addition, mRNA expression of ZPB2 was greater in the granulosa cells from the germinal disc (GD) region of hierarchical follicles than in granulosa cells from nongerminal disc (NGD) regions. The aim of the present research was to determine if the mRNA and protein expression of ZPB2 correlated with one another. To determine ZPB2 protein expression in granulosa cells, the F1, F2, F3, and F4 hierarchical follicles were removed as were the small yellow (SYF) and large white (LWF) pre-hierarchical follicles from twelve 63 week old broiler breeder hens. The SYF and LWF were further subdivided into two groups based on diameter. The tissue from 3 individual birds were pooled to generate 4 replicates samples of granulosa tissue for each follicle size. Protein was isolated from GD and NGD sections of the IPVL of freshly laid eggs from twelve 44-week-old broiler breeder hens from 4 genetic lines of breeder hens. Individual GD and NGD sections were combined from 2 eggs from each line to create 6 replicate GD and NGD samples per genetic line. One dimensional gel electrophoresis was performed using stain free technology, and gels were transferred to PVDF membranes for Western blot analysis. A polyclonal antibody (rabbit anti-chicken) against ZPB2, and HRP conjugated secondary antibody (goat anti-rabbit) were used for the Western blot analyses. Relative ZPB2 expression was determined using Image Lab Software (BioRad). Granulosa cell ZPB2 protein expression was greater in the smallest LWF relative to any of the hierarchical follicles ($P < 0.05$). Protein expression of ZPB2 was greater in the GD region than NGD region of the perivitelline layer. Overall, ZPB2 protein expression reflected mRNA expression in broiler breeder hens. These findings suggest an undetermined role for ZPB2 in early follicular development and for the preferential binding of sperm at the GD region of the IPVL.

Key Words: Broilers, Fertility

M2 Gut health priming from breeders to chicks – Evidence of direct pathogen transfer and shared environments Terry Parrott, Elizabeth Kim, Jordon Gruber* *DuPont Animal Nutrition*

Foundational studies of vertical and horizontal transmission of pathogens have established the baseline understanding for diseases in poultry systems. However, as production practices change and new influences evolve, transmission events should be re-evaluated to better determine new critical control points to prevent the spread of disease. In the current context of antibiotic-free production practices, new interventions that reduce pathogen spread, limit disease progression, and/or fortify the health of the animal is crucial. Strategies that reduce disease and build health as upstream in the production process as possible can significantly aide metrics and bolster holistic health treatments. Breeders, as an upstream parameter, and their health is an established co-factor in production of hatchable eggs, but little is known about the influence of breeder gut health on downstream chick health. A direct line of transmission is predicted following the process of depositing fecal material onto egg shells and the association of chicks to eggs shells after hatch. To investigate relationships of pathogens in the breeder/hatch system, the enteric pathogen Avian Pathogenic E. coli (APEC) was isolated and measured for its possible transmission through the hatchery environment and ultimately to the gastrointestinal track (GIT) of the chick. Using RAPD fingerprint analysis of bacterial isolates, APEC from breeder GITs were matched to APEC found on the surface of egg shells hatchery environment, and within the 1 – 2 day-old chick GITs. This relationship was found to be statistically significant using non-parametric methods for comparison. Other relationships were identified, but to a lesser degree including hatchery air samples and dead eggs, and dead eggs and chick GITs. Together, this work helps to highlight the importance of pathogen transfer from breeder to chick, including the hatchery as a vehicle for moving pathogens through the air. Importantly, the direct influence of gut health from breeder to chick demonstrate how gut health and pathogens share a common vehicle within poultry production and should be targeted for interventions.

Key Words: breeder, pathogen, transmission, gut-health

M3 Bacillus subtilis 29784 prevents a pro-inflammatory response in an induced inflammation condition using the Caco-2 cells model Damien Preveraud*[1], Lamya Rhayat[2], Marc Maresca[3], Erik Eckhardt[2], Estelle Devillard[2] *[1]Adisseo France SAS; [2]Adisseo CERN; [3]Aix Marseille University CNRS*

The protection of the animals against exogenous challenges, including infectious diseases and digestive disorders, is mainly provided by the intestine. Maintaining intestinal barrier integrity and functions is therefore critical to avoid deterioration of animal health status. Among the different ways to achieve this, probiotic supplementation could turn out to be a good solution.

In a previous study, we have shown in a Caco-2 cells model that *Bacillus subtilis* 29784 is able to prevent the disruption of the intestinal barrier by improving TransEpithelial Electrical Resistance (TEER) by reducing IL8, a pro-inflammatory cytokine. The objective of the present study was to explore the mechanisms involved into the anti-inflammatory properties of *B. subtilis* 29784.

NFkB signaling pathway is known to be a major player in inflammation. Vegetative cells of *B. subtilis* 29784 were applied for 16 hours to a 14 day-differentiated Caco-2 cells monolayer established in a Transwell system. Caco-2 cells exposed or not to bacterial cells were then stimulated basolaterally with IL-1β, and nuclear and cytosolic fractions were extracted. Western blotting was finally carried out to measure IkB protein level in the cytosol and nuclear translocation of NFkB.

IL-1β treatment on Caco-2 cells induced a fivefold increase in NFkB amount in the nuclear fraction, demonstrating activation of the signal pathway by the pro-inflammatory stimulus. When cells were pre-incubat-

ed with *B. subtilis* 29784, there was no increase in nuclear level of NFkB. This indicates that the bacterial strain is able to prevent the translocation of NFkB from the cytoplasm to the nucleus. In order to activate the NFkB signaling pathway, IkB must be first degraded. As expected, cytosolic level of IkB decreased in the IL-1β treated cells. When the Caco2 cells were exposed to *B. subtilis* 29784, IkB degradation was very much reduced, explaining the decrease in NFkB translocation.

The results obtained from this study suggest that *B. subtilis* 29784 exerts its immunomodulatory properties by inhibiting IkB degradation, thus preventing NFkB translocation and, by doing this, the expression of pro-inflammatory cytokines, such as IL8.

Key Words: bacillus, Inflammation, NfkB, Caco-2

M4 Reproductive toxicity of RoundUp® in broiler breeder roosters Zachery Jarrell*[GS], Muslah Ahammad, Andrew Benson *University of Georgia*

RoundUp Ready® cultivars constitute a large portion of the crops grown in the United States for animal feed due to their relative ease of production contributed by their resistance to RoundUp® and other glyphosate-based herbicides (GBHs). As a result, these feedstuffs are at an increased risk exposure to glyphosate throughout production. Levels of these residues are maintained well below their lethal dose, but little work has gone into investigating the tissue-specific toxicity of GBHs. The purpose of this experiment was to evaluate the toxic effects of RoundUp® on broiler breeder rooster sperm quality. A total of 144 Cobb 500 broiler breeder roosters were assigned to individual cages at 25 weeks old and allowed 2 weeks to adapt to cages and a corn-soy basal diet. At 27 weeks old, roosters were randomly assigned to 1 of 4 treatment diets: a control basal diet, 0.30% humic acid (HA) added, 0.15% RoundUp® added and 0.30% RoundUp® added. An HA treatment was used parallel to the control diet as HA is a reported neutralizer of the glyphosate residues already present in the basal feed. The treatment period lasted 18 weeks and was followed by a 4-week recovery period on basal feed. During all 22 weeks, no significant differences were observed in bodyweight. Per treatment, 4 semen samples were collected weekly and analyzed for sperm count by hemocytometer counting, viability by eosin-nigrosin viability staining and mobility by Accudenz assay. Throughout the treatment period, sperm mobility displayed a dose-dependent response to RoundUp®, with mobility decreasing in response to higher levels of exposure to RoundUp® ($p < 0.001$). Similarly, HA neutralizer added to feed promoted sperm counts during the treatment period when compared to other treatment groups ($p < 0.001$). After 4 weeks of recovery, no significant differences were observed across parameters. These results indicate that presence of RoundUp® residues in the feed of broiler breeder stock may contribute to the gradual decline in broiler breeder fertility. Furthermore, either usage of a Roundup®-free feed formulation or inclusion of a neutralizing additive may provide a method for improving the fertility of broiler breeder stock.

Key Words: broiler breeder, Roundup, reproductive toxicology, sperm physiology, fertility

M5 Effect of amino acid levels during broiler breeder pullet rearing on: 2. Egg weight and characteristics from 26 to 34 wk of age Yilmar Matta*[1,2UG], Edgar Oviedo-Rondon[1], Andres Ortiz[1,2], Ivan Ospina[1], Hernan Cordova[1], Lina Penuela[1,2], Miguel Chico[1], Viviana San Martin[1], Justina Calda[3] *[1]North Carolina State University; [2]Universidad del Tolima; [3]Cobb Vantress Inc.*

Amino acid pullet nutrition affects growth and development and it is important to evaluate potential effects on egg traits. One experiment was conducted to determine the effects of four amino acid (AA) dietary levels fed to broiler breeder pullets during the rearing phase from 5 to 24wk of age on egg characteristics. A total of 1,360 Cobb-500 slow-feathering pullets were placed in 16 floor pens (85/pen). Up to 4wk all pullets were fed one starter diet in crumbles and after 29d of age fed with four mash grower diets containing 4 AA levels (0.40, 0.54, 0.60, and 0.66% of dLys with balanced protein) and 2,700 kcal/kg ME. From 16wk to 5% egg production, developer mash diets with 2,800 kcal/kg ME and 0.51, 0.57, 0.63, and 0.69% of dLys were offered. Feed amounts varied slightly (±3 g/d) among treatments in the developer phase to maintain BW close (±2%) to Cobb guideline. After 5% egg production all hens were fed one common layer diet and feed increments were made according to egg production by treatment. Onset of egg production occurred at 165d and reached 5% at 167d of age. Egg weights (30/pen) were evaluated after all treatments reached 50% egg production at 26, 28, 30, 32, and 34wk of age. Eggshell color, strength, and thickness were evaluated in 6 eggs/pen at 30wk. Egg components, albumen, yolk and eggshell percentages were calculated and Haugh units measured. Data was analyzed in a CRD with 4 AA treatments and 4 replicate pens each. One-way ANOVA and regression analyses were conducted. Results indicated that AA levels affected ($P < 0.05$) egg weight up to 28wk. Hens fed the highest AA level during rearing had eggs up to 2 grams heavier than hens fed the lowest AA level and the other treatments were intermediate. There was a linear effect ($P < 0.01$) of AA levels on egg weight up to 28wk. However, no effects ($P > 0.05$) of dietary treatments during rearing on egg weight were detected at 30, 32 and 34wk. No effects ($P > 0.05$) on eggshell color, thickness, yolk color or egg components were observed. Eggshell strength and elasticity ($P < 0.05$) of hens fed the highest AA level during rearing was lower (37.9N) than eggs from hens fed the two lowest AA levels (42.5N). In conclusion, AA dietary levels during rearing affected initial egg weight and eggshell strength.

Key Words: amino acids, pullet breeders, egg weight, eggshell strength

M6 Incubational and embryonic temperature relationships in Ross 708 broiler hatching eggs Lauren Lindsey*[1UG], Katie Elliott[1], Seyed Fatemi[1], Peter Ishola[1], Patrick Gerard[2], Edgar Peebles[1] *[1]Mississipp State University; [2]Clemson University*

The standard temperature to incubate broiler hatching eggs has traditionally been 37.5°C. However, incubating eggs containing highly selected modern strain broiler embryos at 37.5°C may be too high due to their increased growth rate and associated production of metabolic heat. The purpose of this experiment was to determine if lowering incubation temperature has a direct effect on the body temperature of fast growing modern strain (Ross 708) broiler embryos throughout incubation, and if it impacts hatching performance. This was accomplished by telemetric technology, using temperature transponders that were aseptically implanted in the air cells of Ross 708 broiler hatching eggs. From 0 to 12 days of incubation, all eggs were incubated under standard conditions in a common incubator. Between 12 and 18 days of incubation, 120 eggs received transponder implants, with 30 eggs incubated in each of four incubators: two at 37.5°C and two at 35.6°C. Transponder readings were recorded thrice daily with a wireless probe. Fertile egg hatchability was 93.3% and 100.0% in the 37.5°C and 35.6°C treatment groups, respectively. Mean hatch time was delayed 9 hours at the lower temperature, and embryo temperature was significantly ($P \leq 0.0001$) lower in the 35.6 than in the 37.5°C treatment group. A significant ($P \leq 0.0001$) positive correlation existed between incubation and embryo temperature in the 37.5°C treatment group, but did not exist ($P = 0.4553$) in the 35.6°C treatment group. Nevertheless, embryo temperature in both treatment groups increased similarly over time during the 12 to 18 day incubation period. Incubating Ross 708 eggs at 35.6°C may be economically advantageous in commercial hatcheries, but a short delay in hatch time may be realized along with a greater dependence on the energy reserves in the egg to maintain embryo body temperature.

Key Words: broiler, embryo, incubation, telemetry, temperature

M7 An immunostimulatory injection on day of hatch leads to altered cellular signaling later in the grow out period in modern broiler birds Bridget Aylward*[1GS], Casey Johnson[1], Iyana Admasu[1], Famatta Perry[1], Dr. Rose Whelan[2], Dr. Ryan Arsenault[1] *[1]Department of Animal and Food Sciences, University of Delaware; [2]Evonik Nutrition & Care GmbH*

The study was conducted to compare modern broiler breeds and heritage Athens Canadian Random Bred (ACRB) birds' response to an immune stimulant, in order to determine if modern strains exhibit comparatively inappropriate or insufficient immune responses to pathogenic threats. We focused on the gastrointestinal tract and associated lymphoid tissues, as there is an increasingly clear link between immunology and metabolism and the gut is the crossroads. We administered CpG (25 µg/bird), a synthetic oligonucleotide mimetic of bacterial DNA, intra-abdominally to modern broiler chicks and ACRB chicks. Five birds in each treatment group were weighed, sacrificed and tissue samples were snap frozen or stored in RNAlater. We analyzed immunometabolic signaling changes in cecal tonsil and jejunal tissue samples on days 3, 15, and 34. We performed qRT-PCR with RNA extracted from the same cecal tonsil and jejunum samples and primers for key cytokines: IL-1beta, IFN-gamma, IL-6, and IL-18. The modern birds treated with CpG had significantly lower body weights than control birds on days 18, 21, and 29 (p values = 0.02, 0.02, and 0.02 respectively), whereas this difference was not seen in ACRB birds. In the CpG treated modern birds on Day 15 there is a decline in activity in the cecal tonsil in an important immunometabolic pathway, PI3K-Akt, when compared to control birds, and this is not seen in the ACRB birds. In the ACRB birds' cecal tonsils on day 15 there is NF-kB activation and insulin signaling activation. In the modern birds, insulin signaling is deactivated and there are indicators of oxidative stress leading to apoptosis. The qRT-PCR results showed an initial elevation of expression of all four cytokines in the cecal tonsils and jejunums of both modern and ACRB birds at day 3 in response to the CpG treatment. These results show a decrease in expression of IL-6 and IFN-gamma in the treated modern birds' cecal tonsils on day 15. Targeting intermediates of the insulin sensitivity and mitigating oxidative stress pathways could be potential targets for therapeutic intervention in the modern birds to bolster their immune responses as they age. Ideally, this would maintain the performance observed in modern broiler chickens without sacrificing immune robustness.

Key Words: broilers, immunology, kinomics, metabolism

M8 Effect of dietary protein source and litter condition on macrophage and mitotically active cell densities in the duodenum of broiler chickens at 3 days of age A. Jake Keel*[UG], Allan Calderon, Oscar Tejeda, Jessica Starkey, Charles Starkey *Auburn University*

Efficient feed utilization and growth performance requires proper absorptive and protective functions of the gastrointestinal (GI) tract. These functions depend on the continuous self-renewal of intestinal epithelial cells and the presence and activity of immune cells, which aid in pathogen defense. Without proper GI tract function, feed efficiency will suffer. To explore the effect of dietary protein source and litter condition on macrophage and mitotically active (proliferative) cell density in the duodenum of broiler chickens, a randomized complete block design experiment with a 3 x 2 factorial treatment arrangement was conducted. The 3 different dietary protein sources fed were soybean meal, 50% poultry by-product meal and 50% feather meal, and porcine meat and bone meal. Water and pelleted and crumbled corn and soybean meal-based diets were offered *ad libitum*. Birds were reared on either new litter (NL; fresh pine shavings) or used litter (UL; litter 3 flocks were previously reared on). On d 0, Yield Plus x Ross 708, female broiler chicks (Aviagen, Huntsville, AL) were randomly allotted to 1 of 6 treatments, and placed in an environmentally-controlled, raised floor pen facility. On d 3, 6 birds per treatment from 6 blocks (total n = 36) were randomly selected and injected intraperitoneally with 5'-bromo-2'-deoxyuridine (BrdU) 1 h prior to duodenal sample collection to label mitotically active cells. The duodenal samples were frozen in liquid nitrogen and subsequently analyzed using cryohistology, immunofluorescence staining, and digital fluorescence microscopy procedures to determine the density of macrophages and mitotically active (BrdU+) cells. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) and means were declared significantly different when $P < 0.05$. Dietary protein source did not alter macrophage density ($P = 0.2336$) or mitotically active (BrdU+) cell density ($P = 0.9859$) in duodenal tissue. Litter condition did not impact duodenal macrophage density ($P = 0.8414$). However, broilers reared on UL had greater density of mitotically active (BrdU+) cells than those reared on NL ($P = 0.0126$) at d 3. These data indicate that litter conditions may impact GI physiology. The mechanisms behind this change will warrant further investigation.

Key Words: Broiler chicken, duodenum, macrophage, bromodeoxyuridine, intestinal cell proliferation

M9 Effect of different basal culture media and sera combinations on primary broiler chicken muscle satellite cell proliferation and heterogeneity Joshua Flees*[GS], Cierla McGuire Sams, Jessica Starkey *Auburn University*

Chicken satellite cells (SC) isolated from *pectoralis major* muscles provide a useful *in vitro* model to study muscle development and growth in avian species. There is little consistency in the current literature regarding optimal basal culture media (BCM) and sera combinations for promoting SC proliferation. The 3 BCM assessed were: 1) McCoy's 5A (MCCOY), 2) High Glucose Dulbecco's Modified Eagle's medium (HGDMEM), and 3) Low Glucose DMEM (LGDMEM). The sera were: 1) 15% chicken serum (CS) and 2) a combination of 5% horse serum (HS) + 10% CS (HSCS). For each of the 3 independent replicates of the experiment, SC isolated from 22-d-old, male broilers were plated at 1.66 x 10^6 cells per well on 24-well plates coated with 0.1% gelatin. Parallel plates were cultured at 40°C in a 5% CO_2 incubator and fixed at 48, 72, and 96 h post-plating. Fixed cultures were immunofluorescence (IF) stained to detect cells expressing 3 myogenic regulatory factors (MRF); myogenic differentiation factor 1 (MYOD), myogenic factor 5 (MYF5), and Paired Box 7 (PAX7)-expressing cells. Following IF staining, a DAPI nuclear counterstain was applied. NIS-Elements imaging software was used to capture and analyze 2 images per well (8 images per treatment). Densities of MYOD+, MYF5+, PAX7+, MYOD+:MYF5+, MYF5+:PAX7+, MYOD+:PAX7+, and MYOD+:MYF5+:PAX7+ cells are reported on a mm^2 basis. Total myogenic cell density is a sum of all cells expressing one or more MRF. Cells expressing none of the MRF (MYOD-:MYF5-:Pax7-) cells were considered non-myogenic. Doubling time (DT) of all cell populations was calculated using 48 and 96 h post-plating cell densities. Data were analyzed using the GLIMMIX procedure of SAS (V9.4) with well as the experimental unit. Means were considered different when $P < 0.05$. At 48, 72, and 96 h post-plating, SC cultured in MCCOY+HSCS had the highest non-myogenic cell density of all treatments ($P \leq 0.0001$). At 48 h post-plating, MCCOY cultures had fewer MYF5+:PAX7+ cells ($P = 0.0074$) and more MYF5+:MYOD+ ($P = 0.026$) and MYF5+:MYOD+:PAX7+ ($P = <0.0001$) cells. At 96 h post-plating, broiler SC grown in HGDMEM+HSCS tended to have the highest myogenic cell densities compared with all other treatments ($P = 0.06$). HGDMEM+HSCS appears to best support broiler chicken SC proliferation.

Key Words: muscle satellite cell, broiler chicken, cell culture, media, serum

M10 In ovo injection of probiotic bacteria and its influence on the broiler immune system during a coccidia challenge Chrysta Beck*[1GS], Christopher McDaniel[1], Kelley Wamsley[1], G. Pharr[2], Aaron Kiess[1] *[1]Department of Poultry Science, Mississippi State University; [2]Department of Basic Sciences, Mississippi State University College of Veterinary Medicine*

Feed applications of probiotics, such as *L. animalis* and *B. licheniformis*, have improved performance while stimulating the immune system of poultry. However, immunological attributes have not been confirmed when these probiotic bacteria are *in ovo* injected. Therefore, the current objective was to determine if the *in ovo* injection of *L. animalis*, *B. licheniformis* and their combination influence the broiler immune system. Treatments were randomly applied to 2,880 fertile broiler hatching eggs on d18 of incubation and included: T1=Marek's Disease (MD) vaccination, T2=MD + *L. animalis* (~10^6 cfu/50µl), T3=MD + *B. licheniformis* (~10^6 cfu/50µl), and T4=MD + *L. animalis* + *B. licheniformis* (~10^6 cfu + ~10^6 cfu/50µl). Hatch of transfer and hatch residue data were collected. Following hatch, a 49 d grow out with a d14 coccidiosis challenge was performed. Immunological parameters were collected on d0, 14, 21 and 28. There was no difference in hatch of transfer (P=0.3), but T2, T3 and T4 had fewer % late dead eggs when compared to T1 (P=0.002). T2 had higher ileum lesion scores on d21 as compared to T1 (P=0.04). An interaction was detected for avg bursa follicle area in which treatments were similar on d0 and d14, however on d28, T2 was larger than T3 and T4 but T1 was larger than T4 (P=0.05). Treatment interactions and effects were also observed for several interleukin cytokines (P<0.05). Total white blood cell counts indicated that T2 was greater than T4 (P=0.02), and T4 had greater % heterophil than T1 (P=0.01). However, interactions were detected in which T2 and T3 absolute heterophil counts (thou/µl) were more elevated than all other treatments on all other days (P=0.04), T2 absolute lymphocyte counts (thou/µl) on d28 were more elevated than all other treatments (P=0.03), and T2 and T3 % lymphocytes on d0 were greater than T1 (P=0.005). These results indicate that the *in ovo* injection of probiotic bacteria can alter physical and chemical immunological responses post-hatch when birds are subsequently challenged with coccidiosis, and the injected probiotic bacteria may be capable of reducing inflammatory responses. Future research should examine the *in ovo* injection of probiotic combinations with dietary probiotic supplementation under a pathogenic bacteria challenge.

Key Words: in ovo, Probiotics, Immunity, Broiler, Coccidiosis

M11 Role of the HPT axis in the regulation of the preovulatory surge in low and high egg producing turkey hens Kristen Brady*[1GS], Julie Long[2], Tom Porter[1] *[1]University of Maryland; [2]USDA ARS Beltsville Agricultural Research Center*

The hypothalamo-pituitary-thyroid (HPT) axis has been shown to influence plasma progesterone levels of the preovulatory surge (PS) that triggers follicle ovulation and ultimately egg production. Dysregulation of the PS leads to lowered egg production, leaving the poultry industry to compensate with larger breeding flocks. The presence of nuclear and cell membrane thyroid hormone receptors (THRs) in the tissues of the reproductive axis indicates possible genomic and non-genomic effects of thyroid hormone on the reproductive axis. Characterization of HPT axis plasma hormone concentrations and gene expression surrounding the PS was performed in average egg producing hens (AEPH), low egg producing hens (LEPH), and high egg producing hens (HEPH) (n=3). Data were analyzed using the mixed models procedure of SAS, with significance indicated at P<0.05. AEPH displayed lower levels of triiodothyronine (T_3) and higher levels of thyroxine (T_4) inside of the PS. HEPH showed $T_3$and T_4levels consistent with those seen in AEPH, while LEPH showed inverse $T_3$and T_4 levels relative to the PS. HPT axis expression of mRNA for hypothalamic thyrotropin-releasing hormone, pituitary thyrotropin, and thyroid hormone metabolism enzymes were downregulated during the PS in AEPH. LEPH displayed higher expression of mRNA for pituitary thyrotropin and thyroid hormone metabolism enzymes. Additionally, in AEPH, expression of THR mRNAs was upregulated during the PS in the hypothalamus but downregulated in the pituitary. HEPH showed decreased expression of THRs in both the hypothalamus and pituitary when compared to LEPH. In regards to ovarian follicle expression, THR mRNAs were more prevalent in the thecal layer of the follicle wall rather than in the granulosa layer and expression of mRNAs for THRs tended to decrease with follicle maturity. In AEPH, THR genes showed upregulation during the PS. In more mature follicles, LEPH displayed higher expression of THRs while in less mature follicles, HEPH displayed higher expression of THRs. HPT axis plasma hormone concentration and gene expression were not only affected by the PS but also differed between LEPH and HEPH. Further studies will be necessary to pinpoint the role of the HPT axis in the regulation of ovulation and egg production rates.

Key Words: turkey, egg production, HPT axis, HPG axis, preovulatory surge

Teaching, Pedagogy, Extension

M12 Use of shape index to determine differences of eggs from Heritage breeds vs. commercial white egg strain Lauren Nolan*[GS], Anthony Pescatore, Micheal Pescatore *University of Kentucky*

This study was conducted to determine the relationships between egg shape (shape index) and egg strength in heritage breeds and a commercial white egg strain (WL). The 3 heritage breeds used were Barred Plymouth Rock (BPR), Black Australorp (BA), and Rhode Island Red (RIR). Heritage hens were housed in floor pens and provided ad libitum access to feed and water. The commercial strain was housed in cages and provided feed and water ad libitum. Thirty eggs from each breed were collected at 50 wks of age (34 wks of production) and analyzed for breaking strength (kgf), and measured for width, length, and volume, using the Volscan Profiler. The Volscan Profiler is benchtop laser scanner that measures different parameters such as volume, density, length, and width; while creating a 3D image of the scanned object. Shape index was calculated using the following equation, x 100. Width was taken at the widest part of the egg. Lower shape index indicates a more rounded, spherical egg, while a higher shape index indicates a longer, narrower egg. Data were analyzed using ANOVA in SAS. The commercial white egg strainproduced significantly lighter eggs (53.78 g) compared to RIR, BA, and BPR (54.70 g, 55.12 g, and 55.00 g respectively). However, WL had significantly higher egg volume, 55.63 mL, compared to 54.12 mL, 53.95 mL, and 54.07 mL for RIR, BA, and BPR respectively. From this experiment, it was determined that RIR and BPR produced significantly stronger eggs, 3.812kgf and 3.369kgf respectively, compared to WL (2.921 kgf). Eggs from WL had a significantly higher shape index (75.25) compared to the RIR, BA, and BPR (74.47, 71.05, 71.19 respectively). Shape index can be used as an indicator of the likelihood of an egg to break. This data shows that eggs within a 'normal' egg shape range (RIR, BA, BPR) have higher breaking strengths (3.426kgf, 3.330kgf, 3.369kgf respectively) compared to 'ovid' eggs of the WL (2.921kgf). Changes to shape of the egg has occurred as commercial egg strain were selected over time, moving away from spherical shape, which has impacted the geometry and physical breaking strength of the egg.

Key Words: egg shape, egg quality, Heritage breeds

Processing and Products

M13 Enhanced statistical process control systems for monitoring and predicting live operations and plant performance and pathogens Isaac Duerr* *Smart Data Science Solutions*

Purpose: To combine and improve historically successful Statistical Process Control (SPC) algorithms with machine learning techniques to build advanced SPC "systems" capable of monitoring Key Performance Indicators (KPIs) and pathogen results in live operations and the processing plant. Based on a producer's historical data, SPC systems determine if observed variation in an outcome is due to natural fluctuation or is indicative of a production problem or outside factor. Machine learning algorithms generate predictive Outcome Scores that guide decision making under uncertainty.

Experimental Design: We combine historically successful SPC algorithms, producer-specific data from 3000+ commercial bird placements, and machine learning techniques to build an SPC system to monitor key poultry production parameters. We augment this system with a machine learning algorithm that predicts likely outcomes with sufficient lead-time to allow for changes in the production environment based on these predictions.

Results: The resulting SPC system was back-tested using historical data to confirm that it was capable of detecting short-term aberrations as well as incremental long-term changes to an underlying process. It was then implemented in a real production environment for >6 months and showed successful detection of more than 10 short-term events and 3 long-term shifts in underlying baselines across multiple production levels (breeder and grow-out) and regions. The predictive machine-learning algorithm was able to identify with 80% accuracy which flocks were most likely to have positive pathogen-related tests on the final (raw) outgoing product.

Conclusion: Historical SPC algorithms can be combined with modern machine-learning techniques and trained on historical producer-specific datasets to produce robust SPC systems that both monitor and predict KPIs and pathogens in live operations and the processing plant. Accurate (80%) prediction of outcomes is possible with lead times of up to two weeks.

Key Words: statistical process control, KPI, pathogen, monitoring, machine learning

M14 Impact of postmortem holding temperature on feather retention force and broiler carcass microbiology Dianna Bourassa*[1], Caitlin Harris[2,3], L Bartenfeld[2], Stephanie Richter[4], Wayne Daley[4], R Buhr[2] *[1]Auburn University; [2]US National Poultry Research Center, USDA-ARS; [3]University of Georgia; [4]Georgia Tech Research Institute*

Commercial on farm slaughter could eliminate many potential animal welfare issues associated with cooping, transport, dumping, and shackling of live broilers. However, concerns regarding carcass processing efficiency and microbiological status following the delay between slaughter on the farm and scalding at the processing plant following transport must be investigated. Live broilers obtained from a commercial processing plant were transported to Auburn University, stunned at 15 V for 10 s, and then bled for 2 min in batches of 5. Carcasses that were delayed prior to scalding were held suspended in shackles for 4 h at refrigerated (4°C), room (27°C) or body (40°C) temperatures. One control batch of 5 carcasses was processed without scalding delay. Prior to scalding, 6 wing secondary remiges and 6 tail rectrices were extracted and maximum feather retention force (FRF) measured. All carcasses were hard scalded at 60°C for 60 s, defeathered for 30 s, and sampled post-evisceration for microbiological analysis. Whole carcass rinses (WCR) were sampled for aerobic plate count (APC), Enterobacteriaceae (EB), *Salmonella*, and *Campylobacter*. Ilea and ceca were sampled for *Lactobacillus*, EB, total anaerobes (TA), *Salmonella*, and *Campylobacter.* Carcass holding temperature significantly impacted wing and tail FRF for all four treatment groups (P<0.0001). Carcasses held at 4°C had the highest peak FRF for both wing and tail feathers (4.26, 1.96 kgf) followed by the non-delayed control group (3.19, 1.66 kgf), followed by the group held at 27°C (2.49, 0.94 kgf), and the group held at 40°C (0.89, 0.52 kgf). No significant differences in bacterial counts were detected for WCR, APC, or EB or for ilea or ceca *Lactobacillus*, EB, or TA (P>0.1970). There were significantly fewer *Salmonella* WCR positive samples for the 40°C carcasses in comparison to 4°C or 27°C carcasses (20% vs. 100%). Significantly fewer 27°C held ilea were positive for *Campylobacter* when compared to 40°C held carcasses (20% vs. 100%). No other treatments were significantly different for *Salmonella* or *Campylobacter* prevalence. This study revealed that carcass holding temperature following slaughter significantly impacts the force required for feather removal while having only minimal impact on carcass microbiology.

Key Words: feather retention force, delayed scalding, Salmonella, Campylobacter, on-farm slaughter

M15 Whole Carcass Salt-Uptake Tendencies during Immersion Chilling Stephanie Richter*, Daniel Sabo, Comas Haynes *Georgia Tech Research Institute*

Poultry skin is commonly said to act as a barrier against water and salt uptake during the processing chilling phase. This experiment was developed to determine salt uptake tendencies of whole carcasses (WOGs) during immersion chilling. High salt uptake and presence in final meat product has labeling and consumer avoidance implications.

WOGs were collected post-evisceration and kept at temperature. WOGs were chilled for 60 minutes by either air chill (4°C), chilled water (5°C) with 50 ppm peracetic acid (PAA), or 4.5% salinity chilled water (5°C) with 50 ppm PAA. Pre- and post-chilling, all WOGs were weighed and inner breast meat temperatures were logged. Post-chill, three sample types were collected per carcass (breast skin, white meat, and dark meat) from different locations. Carcass samples were analyzed for salt concentration.

This initial investigation includes three replicates (n=45 carcasses; total 135 samples). Salt was recovered from the skin and meat samples using a standard organic dry-ash method. The total salt concentration for each sample was determined using an ion chromatograph (IC). Statistical analysis was performed from each sample's IC data, combining sample weight and dilution factor correction. Chloride concentration was calculated as ppm per gram of sample. One-way ANOVA test used to compare chloride concentrations [ppm/g] given groups defined by chilling treatment and carcass skin/meat sampling type, considering mean ± SD (p-value = ≤ 0.05). Initial results show, of all nine groups (3 chilling types * 3 sampling types), 4.5% salinity chilled water skin samples were the only statistically significant group (p = <0.0001) in chloride ppm/g. 4.5% salinity skin samples (38.1 ppm/g) averaged 14x and 22x higher than chilled water and air chilled skin samples respectively. Results show no statistical significance ($P > 0.05$) between white and dark meat samples between all three chilling forms.

Results indicate that salt concentrations increase in the skin and does not affect white or dark meat. The skin acts as a barrier that prevents salt penetration into the white and dark meat. Further testing are underway to examine higher salinity immersion chilling to see if salt can penetrate through skin.

Key Words: Salt-uptake, Immersion chilling, Whole carcasses, Sodium chloride

M16 Poultry parts cooling Aklilu G Giorges*, John Pierson *Georgia Institute of Technology*

Poultry (Chicken) parts dominate most of the poultry market, while whole chicken sold is shrinking. Thus, FSIS has established pathogen reduction performance standards for *Salmonella* and *Campylobacter* in raw

chicken parts. The new performance standard for chicken parts is one of the current challenges confronting the poultry industry. Complying with new standards for chicken parts has a significant impact on secondary processing. Dipping tanks are one of the new approaches used to address the chicken parts performance standard. Although this may be an easy and short-term fix, it is not completely clear that the approach is an efficient and cost-effective way of poultry processing. The goal of this study was to generate data that can be used to evaluate the feasibility of chilling process modifications as well as the impact of new cooling technologies and approaches. This study has generated air and water chilling data for whole chicken and chicken parts. The data can be used to establish the foundation for quantitative evaluation of chicken parts chilling instead of whole bird chilling. The water chilling data show significant cooling time difference between whole and chicken parts. The data of individual parts under forced convection cooling show that it took between 17-26 minutes to cool thighs, 6-11.5 minutes to cool wings, and 10-20 minutes to cool drumsticks, while it took over an hour to cool whole chicken. In addition, the air chilling data of individual parts under low relative humidity air chilling show that it took between 30-40 minutes to cool thighs, 20-29 minutes to cool wings, and 29-36 minutes to cool drumsticks, while it took over 1.5 to 2.5 hours to chill whole chicken to 40°F. As expected, the data show significant cooling time difference between whole and chicken parts. The chilling time is also found to be strongly affected by the weight and initial temperature. Indeed, one of the major drawbacks of an air chilling system is that it takes longer time to chill whole birds, consequently requiring a very large air chilling room. However, if the chicken parts are chilled with new air chilling systems, the footprint of the chilling system can be significantly reduced, re-hang can be eliminated, and water can be saved.

Key Words: chicken parts cooling, chicken parts processing, cooling time

M17 Differential effects of formaldehyde, formic acid, and mono-glycerides on carcass and meat quality traits of Cobb 700 broilers Joshua Jendza*[1], Kristina Feye[2], Juan Caldas-Cueva[2], Barbara Mallmann[2], Dana Dittoe[2], Casey Owens[2], Mike Kidd[2], Steve Ricke[2] *[1]BASF Corporation; [2]University of Arkansas*

Feed treatment with formaldehyde has been linked to lower availability of nutrients, particularly amino acids, and thus to lower performance. Therefore, a study was performed to ascertain the impacts of feed treatment with formaldehyde and two alternatives on performance, digestibility, and carcass and meat quality traits of broilers. Carcass and meat quality data are presented below. Eight-hundred sixty-four Cobb 700 broilers were randomly assigned to 48 pens at day of hatch. Treatments were assigned to pens according to a randomized complete block design with blocking based on location within the barn. Diets were based on a negative control (NC) formulated to meet the breeder specifications. Treatments consisted of the NC, the NC + 0.25% formalin, the NC + 0.25 and 0.50% Amasil NA (61% formic acid and 20.5% Na-formate), and the NC + a mixture of monoglycerides (SILO Health 104L; 0.5% from 0 to 14 d, 0.4% from 14 to 28d, and 0.2% from d 28 to 42). On day 43, a total of 288 birds were processed and carcass traits evaluated (n=48 birds for NC and n=60 birds for each of the remaining treatments). Data were analyzed as a randomized complete block design with means separation based on Dunnett's test and the NC + formalin treatment set as the control. Treatment of feed with formalin had only small effects on carcass yield. Feed treatment with formalin tended to reduce whole breast yield relative to treatment of feed with 0.5% Amasil NA (659 vs 691g; $P < 0.10$). It also reduced rack size as a percent of the total carcass relative to the untreated NC (21.6 vs 20.9%; $P < 0.05$). No differences were noted in other parts. Formalin had more obvious effects on quality attributes of breast fillets. In non-marinated fillets, formalin increased cook loss relative to Amasil NA (26.8% vs 22.5 and 23.9%; $P < 0.01$), but decreased cook loss relative to the mono-glyceride (26.8% vs 30.7%; $P < 0.01$). Formalin also increased the shear force in non-marinated breast fillets relative to 0.50% Amasil NA (12.6 vs 11.6; $P < 0.01$). In conclusion, treatment of broiler feed with 0.25% formalin resulted in small but significant negative changes in carcass quality when compared with treatment of broiler feed with 0.25 or 0.50% Amasil NA.

Key Words: broiler, carcass, formaldehyde, formic acid, monoglyceride

M18 Survey of egg quality in commercially available table eggs Cirenio Hisasaga*[UG], Sara Shinn, Katy Tarrant *Fresno State*

Increase demand in consumer choice has resulted in a wide variety of egg selection available in the retail market. Specialty designer eggs, including those that have been nutritionally supplemented, or are produced from hens with various levels of enrichment in their environment, represent a portion of the table egg market, which is increasing in size. Egg quality is known to be of great importance in all eggs as it relates to food safety, consumer preferences, and product value. In this study, egg quality characteristics were analyzed using a one-way analysis of variance and Tukey's test using JMP v.13.0.0. to evaluate two commercially available conventional egg brands (Brands A - white and B - brown) and four commercially available designer egg brands (Brand C-F). Eggs were of the same age and were sampled twice over a seven-month span. A total of 309 eggs, were evaluated for shell, content weight, dimensional measurements, and breakage force. Calculations were completed to determine percent yolk and albumen, yolk index, and Haugh units, followed by an accelerated lipid oxidation study. No significant variation exists in breakage force. Brands A-E meet AA grade standard at a score of 72 Haugh units or above, while Brand F, a pasture-raised brand, meets the A grade standard, falling between 60 to 71 Haugh units. Brand F has the highest yolk fan color value (10.41 ± 0.193, $P<0.001$), and the lowest yolk index (0.523 ± 0.013, $P< 0.05$). Additionally, Brand F has the smallest albumin height ($P<0.001$). As albumin height is an indication of freshness, and as all eggs were of equivalent age, it is possible that Brand F exhibits overall lower quality compared to other brands. The conventionally raised white eggs of Brand A experienced the greatest increase in % free fatty acids during oxidation study, which would likely result in off-flavors from hydrolytic rancidity to be more prevalent in this brand. The organic, cage free Brand D eggs have a significantly greater peroxide value (17.3 ± 2.9, $P< 0.001$), relative to all other brands, and is over the 10 mEg/kg threshold, which would be considered a unsuitable product for consumption. Ultimately, the measures of quality used in this study are essential for evaluating the sustainability of the specialty egg market.

Key Words: Egg

M19 Effect of red seaweed (Chondrus crispus) on laying hen eggshell thickness Cassie Stupart*[GS], Bruce Rathgeber, Janice MacIsaac *Dalhousie University*

There has been a directional movement in the poultry industry toward including feed ingredients that naturally modify bacteria in the digestive tract in an effort to reduce the use of antibiotics. Previous studies have indicated the seaweed *Chondrus crispus* (CC) provides prebiotic benefits in short term laying hen studies. The purpose of this study was to monitor egg quality of laying hens fed CC over 9-months to ensure eggshell quality was not compromised, as eggs with weaker shells are more susceptible to breakage. The experiment was a 2 x 4 x 2 factorial in a completely randomized design with processing method of the CC (ground and extruded), inclusion level (0, 0.5, 1.75, 3%) and strain of hen (Lohmann Lite-LSL White and Lohmann Lite Brown) as the main effects. A total of 400 birds were used for the study, with 4 replicate cages per treatment combination (5 birds per cage). The 9-month trial was divided into 9 periods, each consisting of 28 days. At the end of each period, 3 eggs per cage were collected and analyzed for shell thickness using a TA.XT Plus Texture Analyser (Texture Technologies Corp, New York, NY, USA). The results were analyzed as a factorial arrangement using the Proc Mixed Procedure of ANOVA. An interaction effect ($P<0.05$) was observed between

inclusion level and strain of hen whereby Lohmann Lite Brown birds had thinner shells when fed the 0.5% (422 µm) and 1.75% (424 µm) inclusion levels compared to the control birds (433 µm). Eggshell thickness for the Lohmann Lite Brown hens fed the 3% (428 µm) inclusion level was intermediate between the control and the lower CC inclusion levels. Dietary seaweed did not influence shell thickness for eggs from Lohmann Lite-LSL. Thus, further investigation as to the effect of CC on egg quality, specifically eggshell thickness, is needed before a conclusion can be made regarding its value as a feed additive.

Key Words: red seaweed, laying hen,, eggshell thickness

M20 Assessment of peroxyacetic acid as a post-defeathering antimicrobial intervention during broiler processing Ella Lawley*[UG], Dianna Bourassa *Auburn University*

During broiler processing, defeathering is known to lead to increases in microbial levels on broiler carcasses due to squeezing of the carcass and expulsion of fecal material. The immediate application of an antimicrobial processing aid following defeathering may help to minimize the impact of carcass contamination that occurs during defeathering. In a commercial processing plant on three consecutive weeks, broiler carcasses were dip treated with 250, 500, or 650 ppm peroxyacetic acid (PAA) for 5 seconds immediately following defeathering. Each week on three consecutive sampling days, 10 carcasses were sampled after defeathering prior to PAA treatment (controls) and 10 carcasses sampled following PAA treatment. After treatment carcasses were removed from the processing line and allowed to drip for 1 min prior to bagging and sampling by whole carcass rinse using 400 mL buffered peptone water with 1 g/L sodium thiosulfate added to neutralize potential PAA carryover. Rinsates were collected and sampled for Enterobacteriaceae (EB) and *Campylobacter* counts. Rinsates were then enriched for *Salmonella* prevalence. Post-defeathering PAA treatments of 500 and 650 ppm significantly decreased *Campylobacter* levels (2.00, 1.92 $\log_{10}$CFU/mL, P<0.0001) and PAA at 650 ppm significantly decreased EB levels (3.32 $\log_{10}$CFU/mL, P=0.0069) in comparison to non-treated controls (2.55 $\log_{10}$CFU/mL *Campylobacter*, 3.90 $\log_{10}$CFU/mL EB). PAA treatment at 650 ppm decreased enriched *Salmonella* prevalence from 73% to 50% (P=0.0245) and non-enriched *Campylobacter* prevalence from 98% to 83% (P=0.0105). These results indicate that PAA at 650 ppm as a dip treatment following defeathering could be used as an intervention to minimize the levels and prevalence of foodborne pathogens on broiler carcasses.

Key Words: peroxyacetic acid, post-pick, Salmonella, Campylobacter, Enterobacteriaceae

M21 A scald water detergent combined with an organic acid carcass dip reduces microbial loads on post-harvested broiler carcasses Grayson Walker*[1GS], Dianna Bourassa[2], Rasha Qudsieh[1], Brittany Wall[1], Matthew Livingston[1] *[1]North Carolina State University; [2]Auburn University*

Organic acid dips or sprays are commonly applied to poultry carcasses during meat processing to reduce or eliminate foodborne pathogens and spoilage microorganisms. Scald water detergent agents employed to improve feather removal may have similar effects in the early stages of processing. The purpose of this study was to investigate the effects of concurrent application of these agents on the microbial loads of carcasses harvested in a small-scale production model. Broilers were reared in litter floor pens to 47 d of age and harvested under standard conditions. Carcasses were subjected to either control or detergent scald water initially, and either 0.02% organic acid dip or water dip after feather removal in a 2 by 2 factorial design with n=15 carcasses per treatment group. The commercially available scald water additive increased water pH from 7.6 to 7.8, while the organic acid dip consisting of lactic and citric acids was maintained at pH of 2.5. Approximately 10 g of neck skin was collected 1 minute post-dipping and placed in buffered peptone water with an added neutralizing agent, sodium thiosulfate. Serial dilutions were performed to determine aerobic (AC), coliform (CC), and *E. coli* (EC) counts as CFU per gram of skin sample. A significant 1.6, 0.6, and 0.8 log reduction each of AC, CC, and EC, respectively, was attributed to use of the organic acid carcass dip ($P \leq 0.01$). There were no significant differences in carcass microbial reduction due to detergent scald water alone. The interaction of detergent scald water and organic acid dip resulted in significant reduction of AC ($P \leq 0.05$). Both control and detergent carcasses dipped in water had the greatest AC loads, the control carcasses dipped in acid were statistically intermediate, while the greatest reduction of 1.96 log was attributed to the detergent scalded, acid dipped carcasses. These data demonstrated that a synergistic effect of microbial reduction on broiler carcasses exists when detergent scald water and an organic acid dip are employed simultaneously during broiler processing.

Key Words: E. coli, organic acid, aerobic counts, coliform, detergent

M22 NIRS analysis to assess the nutritional value of two corn hybrids dried at different temperatures: a comparative analysis between laboratories Hernan Cordova-Noboa*[GS], Edgar Oviedo-Rondón, Ivan Ospina-Rojas, Viviana San Martin, Miguel Chico, Andres Ortiz-Diaz, Yilmar Matta-Lozano, Lina Peñuela-Sierra *Prestage Department of Poultry Science North Carolina State University*

Near infrared reflectance spectroscopy (NIRS) is nowadays a common tool to analyze the nutritional content of feedstuffs. Corn hybrids and drying temperatures could affect the nutritional value of the grain. It is important to determine if these factors are detected by NIR. Two corn hybrids (Dekalb 68-05 and Dekalb 65-20) varying in kernel hardness (average and hard respectively) were dried at three temperatures (35, 80, and 120°C) to investigate the effects on its nutritional content. Subsequently, 5 samples of whole and grounded corn per treatment were collected and read in a FOSS (DS2500) NIR. The spectrum of each sample was sent to 5 commercial companies (AB Vista, Adisseo, Cargill, DSM, and Trouw Nutrition) to use their respective calibration models. Results from each commercial company were codified as laboratories A to E independently of the alphabetical sequence presented above. Treatments resulted from a 2 x 3 factorial arrangement of two corn kernel hardness and three drying conditions. Data were analyzed in a completely randomized design and mean separation with Tukey's test. Whole grain samples of non-dried grain showed that the corn with hard kernel had greater ($P < 0.05$) moisture in all models, vitreousness (A: 89.17 *vs.* 83.44%), starch (A: 83.57 *vs.* 78.71%), and fat (B: 4.28 *vs.* 3.88%) compared with the corn with average hardness. In contrast, no differences ($P > 0.05$) were observed in protein with these samples. However, once the corn hybrids were dried, results indicated that both grain hybrids dried had the lowest ($P < 0.01$) protein at 80°C and higher ($P < 0.01$) fat content at 35°C in grounded samples. At 35°C higher ($P < 0.01$) content of Lys, Met+Cys, and Thr were observed in corn with average hardness. Fatty acid content was greater ($P < 0.001$) in corn with hard kernel compared with the corn of average hardness and effects of drying temperatures also were detected. Hard kernel corn had less starch ($P < 0.001$) than corn with average hardness when dried at 80 and 120°C in all models. These results could affect the predicted energy for feed formulation. In conclusion, NIRS was able to detect differences between corn hybrids dried at different temperatures independently of the calibration model, but there was variation in results among models.

Key Words: NIRS, corn hybrids, nutritional value, drying temperature

M23 Effect of alternative lighting program and low protein starter feed on growth and meat quality parameters. Brittany Wall*[UG], Matthew Livingston, Daniel Adams, Kimberly Livingston *North Carolina State University*

The current lighting program used in the United States relies on minimal dark and maximal lighting periods to improve performance parameters including feed intake, body weight, and feed conversion ratio. Alternative lighting programs are recommended by some third-party certification

programs for their perceived animal welfare benefits. However, there are limited studies that have examined their effect on meat quality. Furthermore, myoblast cell differentiation and ultimate density has been linked to initial dietary protein concentration, which may impact breast muscle myopathies including wooden breast and white striping. This experiment sought to investigate alternative lighting methods and low protein starter diets on growth performance and meat quality characteristics including wooden breast and white striping. One experimental house was placed with 26 Ross 708 male chicks into 32 floor pens and split into a 2x2 factorial design of diet x light. Dietary treatments of Control (24% CP) and Low Protein (15% CP) were given from 0–3 d, upon which all treatments were moved to a common 24% CP starter feed until moving to a common grower at 17 d and common finisher at 29 d. For the first three days, the lighting programs remained the same across the house, starting on day three one half of the house was changed to the alternative lighting program of eight hours dark and fourteen hours of light until 28 d. The control lighting program consisted of 23, 22, and 21 h light for week 1, 2, and 3; respectively. At day 22 both treatments were placed on a natural lighting program. Birds were processed at 42 and 49 days of age. After processing birds were chilled for 24 hours, and cut up for meat yield, cook loss, drip loss, meat color, and myopathy scores. Data were analyzed as a two-way ANOVA using JMP. To conclude the prestarter diet had no deleterious effects on HCW, CCW, dressing percentage, frame or breast meat yield. However, following a standard lighting program resulted in increased dressing percentages, and breast muscle yield with a reduced frame percentage when compared to broilers raised utilizing alternative lighting programs providing birds with less light in early life.

Key Words: Protein, Lighting, Meat quality

M24 Evaluating the effects of Functional Ice (FICE) on shelf life and quality of raw poultry meat Jasmine Kataria*[GS], Avery Smith, Laura Garner, Amit Morey *Auburn University*

Raw poultry meat is a highly perishable commodity due to its high moisture content and nutritional value. Therefore, there is a need to develop novel hurdle technologies to prevent microbial spoilage without affecting the meat quality. Thus, Functional Ice (FICE) is developed to facilitate "Sustained Antimicrobial Release Mechanisms"(SARM) besides cooling effect which can reduce the growth of spoilage microorganisms during transportation and storage (TS) and improve quality. The study aimed to investigate the effects of FICE on the shelf-life and quality parameters of fresh poultry thigh meat during TS. FICE was prepared by freezing aqueous solutions of sodium tripolyphosphate (STPP) 5%(w/v) and sodium lactate-sodium diacetate (L+D) 2.5%(v/v); tap water ice served as control treatment. Fresh thigh meat was procured from a local poultry processor and was stored in FICE treatments (STPP 5% and L+D 2.5%) and control ice at 4°C for 48 h (Meat: Ice =1:2). After 48 h, thighs were packed in Styrofoam trays (4/ tray), saran wrapped and stored at 4°C. For microbiological and quality analysis, five tray packs/ treatment were randomly chosen on day 0, 2, 4, 6 and 8. Two thighs/tray pack/treatment were sampled for Aerobic plate count (APC), Lactic Acid Bacteria (LAB) and Psychrotrophic pate counts (PSY) on every sampling day (3 Trt. x 5 tray packs/treatment x 2 thighs/ treatment x 5 sampling days x 3 trials; n= 450). One thigh/ tray pack was sampled for color, (L*, a* and b*), cook loss and texture analysis (3 Trt. x 5 tray packs/treatment x 1 thigh/ treatment x 5 sampling days x 3 trials; n= 225). Statistical differences among treatments were determined using ANOVA with Least Square Means at $p \leq 0.05$. Results indicated that FICE treatments significantly reduced the PSY and APC compared to control treatment which leads to an extension in shelf-life by 1-2 days, whereas LAB counts were relatively similar by the end of storage. Cook loss% of samples stored in 5% STPP was 7-12% less than the other two treatments and lower a*-values were observed for 5% STPP. No significant differences ($p \geq 0.05$) were observed in the average peak force, L* and b* values by the end of the study. Thus, FICE can potentially reduce spoilage and extend the shelf-life of raw poultry meat during TS.

Key Words: Functional Ice, poultry meat, shelf-life, quality, storage and transportation

M25 Woody breast incidence in commercial broiler strains at various ages Barbara A. Mallmann*[1GS], Ashunti Jackson[2], Andy Mauromoustakos[1], Casey Owens[1] *[1]University of Arkansas; [2]Cobb-Vantress*

Woody breast (WB) continues to be an issue in the poultry industry. The objective of this study was assess the impact of strain, sex, and age on WB and white striping (WS). A total of 13,024 broilers were processed on 5, 6, 7, 8, 9, and 10 weeks of age in multiple trials over a three-year period which included multiple strains from modern and heritage type broiler flocks. Due to a low overall incidence in some strains (e.g., heritage type or small bird market type), data from three of the top commercial crosses (n=5,550) including standard and high breast yielding lines was focused on for the main effects. Following processing, carcasses were deboned and breast fillets were weighed, and measured for length, width, and thickness of breast fillet. Fillets were hot-boned and scored for WS and WB at approximately 1 h postmortem. WB were scored 0 to 3 with 0.5 increment scale. For analysis, categories were combined and referred to as Normal (0+0.5), Mild (1+1.5), and Severe (2+2.5+3). Data were analyzed using generalized regression and SLmeans in JMP Pro 14 with strains, age, sex, breast fillet weight, and measurements fit as the effects. At 5 weeks of age, the overall incidence of mild was 10.4% and severe was 2.7% and this incidence increased to 27.9% and 22.3%, respectively at 10 weeks for the entire data set with males and females. For the three commercial strains, broiler size (body weight, fillet weight) and incidence of mild and severe WB increased as age increased from 5 to 10 wks ($P<0.01$). Males had greater WB severity than females in all the age periods ($P<0.01$) or when data was grouped together by weight classes. However, there were no differences in WS scores due to sex at a given age, but females had greater degree of WS than males at a given weight ($P<0.01$). The thickness of the breast fillet has a high impact on the WB, whenever the thickness increases the WB severity also increases. Generally, as the broilers got larger, there was an increase in SEV WB as fillet yield increased. The incidence of WB is still an issue in current commercial broiler flocks.

Key Words: Wooden Breast, incidence, ages, White Striping, measurements

M26 Influence of utilizing breast meat afflicted with woody breast myopathy on sausage textural properties Macc Rigdon*[1GS], Alexander Stelzleni[1], Brian Bowker[2], Hong Zhuang[2], Dean Pringle[1], Harshavardhan Thippareddi[1] *[1]University of Georgia; [2]USDA-ARS*

Woody breast (WB) myopathy reduces the utility and value of breast meat for the broiler industry. It is hypothesized that WB meat may be included in comminuted products to increase utility and ultimately add value to the broiler industry. Current literature is void of the effects that WB inclusion has on further processed products. The objective of this research was to evaluate the quality of sausage made with WB meat of varying degrees of severity. For each of three replications broiler breast meat (normal, moderate WB, and severe WB) and abdominal fat were obtained from a commercial poultry processor. Breast meat was coarse ground (19-mm) and combined with fat (targeting 15%) to produce 10-kg batches representing 25%, 50%, and 100% moderate WB meat, 25%, 50%, 100% severe WB meat, and a 100% normal control. The batches were then reground (4.8-mm), mixed for 1 min with 1.5% salt, and stuffed into 35-mm natural casings. Links were placed in individual bags, cooked to 70°C in a water-bath, and allowed to cool to room temperature before hardness, cohesiveness, springiness, gumminess, and chewiness were evaluated using texture profile analysis. Individual sausage links were weighed before and after cooking for the calculation of cook loss. Data were analyzed using SAS version 9.3 as a fixed effects design with replication as a random effect.

Sausage hardness tended to be softer (P=0.06) as WB inclusion rate and severity increased. Cohesiveness and springiness were not different between treatments (P=0.53, P=0.95, respectively). Gumminess decreased (P<0.05) as severity and inclusion of WB increased indicating a lack of bind, which was further supported by the decline in chewiness (P<0.05). Only 25% moderate WB and 50% severe WB were as light in color (L*) as the normal breast meat control. In cooked sausage, 25% and 50% inclusion of WB meat regardless of severity were as light in color (L*) as 100% normal formulations. With no difference in cook loss (P=0.08), the data presented indicates that moderate and severe WB meat can be included in the formulation of linked sausages to increase utility and value of broiler WB meat.

Key Words: Woody, Breast, Broiler, Meat, Texture

Pathology

M27 Tannins to control Salmonella in poultry Leandro Redondo[1], Natalia Casanova[1], Enzo Redondo[1], Patricia Joaquim[1], Johana Dominguez[1,2], Mariano Fernandez Miyakawa[1,2], Pablo Chacana*[1] *[1]Instituto Nacional de Tecnología Agropecuaria; [2]Consejo Nacional de Investigaciones Científicas y Técnicas*

Salmonella infection is a major cause of bacterial enteric illness in both humans and animals; and this food-borne microorganism is usually associated with poultry production. Also, in several countries fowl typhoid (FT) caused by *S*. Gallinarum (SG) still represents an important economic problem for the poultry industry. Among other measures, antimicrobials are traditionally used to control *Salmonella* which which results in the selection and spread of resistant microorganisms; therefore new alternatives are needed. The aim of this study was to determine the efficacy of chestnut and quebracho tannins against motile and non-motile *Salmonella*. The minimal inhibitory concentration (MIC) of the tannins was assessed for 4 serotypes of *Salmonella* isolated from poultry. Activity of tannins against cell invasion and adhesion and bacterial cell damage was evaluated for *S*. Enteritidis (SE) and SG. In addition, *in vivo* efficacy of tannins was evaluated. For SE, one-day-old broiler chickens were fed regular feed added with tannins, then challenged with SE and excretion of the pathogen was determined. For SG, groups of laying hens were fed the tannins, challenged with SG and mortality due to FT was recorded. MIC values for chestnut and quebracho ranged from 4 to 1 mg/mL; a fractional inhibitory concentration of 0.5 was determined for quebracho/chestnut suggesting that both tannins have synergistic effects. Alterations of cell morphology were observed on cell surface of the bacteria indicating interaction between outer membranes and tannins. Both tannins impaired adhesion and invasion ability towards epithelial cells, suggesting that tannins can inhibit colonization and invasion of *Salmonella* in the intestine. Significant reduction of the excretion of SE (P<0.01) and significant reduction of mortality due to FT (P= 0.0465) was observed when the birds were treated with a blend enriched in quebracho or in chestnut tannins respectively. The results show that both tannin blends have *in vitro* inhibitory activity against *Salmonella*, and also were effective to reduce the excretion of SE or to reduce mortality due to FT. Future studies should evaluate the efficacy of tannins additives in farms to determine practical applications of these natural compounds to control the pathogen.

Key Words: Salmonella, tannins, alternative to antimicrobials

M28 Survey of Mycoplasma synoviae prevalence and economic impact in the Middle East and North Africa area Husam Bakri*, Entisar Al-Hallaq *Vaxxinova International BV*

The bacteria *M. synoviae* (MS) is a member of the Mycoplasma genus. It causes disease in the joints, bones, respiratory tract and oviduct of birds. A MS infection can result in big economic losses due to a drop in egg production and eggshell quality. During last few years, it was noticed that some layer and breeder flocks faced a drop in egg production of ~10%, some after the peak, while other before reaching the peak of production, along with poor eggshell quality. Publications from A. Feberwee et al* describes the association of MS with egg quality/shell problems in layers and breeders. Therefore, it was believed that it was valuable to investigate the prevalence of MS in the regions.

Blood samples have been collected from the Middle East and North Africa area from flocks suffering from production drops, respiratory signs, and poor eggshell quality. Also, samples were taken from broilers showing respiratory problems.

Blood samples were analyzed using serology-ELISA technique to detect the disease presence. 7951 blood samples were collected from layers, breeders and broilers. At age < 16 weeks, 10% of the samples were found positive; While at age > 16 weeks, ~ 45% of the samples were found positive. Moreover, 4% of broiler samples were positive. In addition, effect of its impact on economy was estimated based on drop in egg quality/ production and medication cost.

Key Words: M.synoviae prevalence, Middle East, North Africa, Production drop, economic impact

M29 The effects of in ovo administration of 6/85 Mycoplasma gallisepticum vaccine on layer chicken embryo hatchability and early post-hatch performance Abdulmohsen Alqhtani*[GS], Scott Branton, Jeff Evans, Katie Elliott, David Peeples *Mississippi State University*

Mycoplasma gallisepticum (MG), the causative agent of chronic respiratory disease in chickens, has maintained its presence in commercial layer flocks. The 6/85 MG vaccine exhibits a low level of virulence, is licensed for use in the U.S., and is currently administered by either spray or eye-drop. *In ovo* administration as a possible alternative method of 6/85 vaccination was assessed. Each treatment group contained 240 eggs, which were randomly placed on each of 8 replicate tray levels of a single-stage incubator. On 18 d of incubation, the eggs were administered a particular dosage of a live attenuated 6/85 strain MG vaccine in either the air cell (A) or amnion (M). The treatments included control non-injected eggs, and eggs injected in the A or M with diluent alone, or diluent containing 1.73, 10^2, or a 10^4 CFU of 6/85 MG. Hatch success and residual embryonic mortality were determined at 22 d of incubation. One hatched chick per treatment replicate was swabbed for the detection of 6/85 MG in the choanal cleft. Six chicks were placed in each of 4 replicate cages per treatment and raised for 3 wk for initial post-hatch assessments. On d 21, 6 birds per treatment replicate were swabbed and bled. Swab samples were analyzed by real-time PCR for the detection of 6/85 MG, and blood samples were analyzed for MG antibody production by serum plate agglutination (SPA) and ELISA tests. Hatchability was decreased by the 10^4 CFU dose in the M group (P=0.004), and was associated with a significant (P<0.0001) increase in late dead mortality. Hatch of fertile eggs was 83.3% in the 10^4 CFU-M treatment group, whereas it was 90% or greater in all other treatment groups. There were no significant (P>0.05) differences for feed intake, BW, BW gain, and FCR among all treatment groups. Chick mortality during the first 3 wk was 42% (P=0.0001) in the 10^4 CFU-M treatment group, but was minimal (<3%) in all other treatment groups. The PCR, SPA, and ELISA tests confirmed that 6/85 MG was only successfully transmitted to embryos in the 10^2 and 10^4 CFU-M treatment groups. The highest dose (10^4 CFU) of 6/85 MG injected into the M influenced chick hatchability, post-hatch survival. Further research is needed to examine the influence of the 6/85 MG *in ovo* vaccine on layer immunocompetence.

Key Words: embryo, layer, in ovo, Mycoplasma gallisepticum, strain 6/85

M30 Examining intestinal microenvironments using immunohistochemistry to evaluate the chicken immune response to Eimeria infection Maria Arendt*[GS], Jonathan Elissa, Emily Michael, Laura Knoll, Mark Cook *University of Wisconsin - Madison*

Eimeria species are intestinal protozoan parasites that cause lack of production, malabsorption and mortality in floor raised chickens. *Eimeria* infection causes an increase in host intestinal and systemic interleukin 10 (IL-10) levels. Administering an oral antibody to IL-10 reduces the symptoms of coccidiosis in broilers, indicating IL-10 is key to *Eimeria* pathology. IL-10 is an anti-inflammatory cytokine and acts as a stand down signal to reduce inflammation and host pathology during disease. Related protozoan parasites exploit IL-10 to reduce pathogen-damaging host inflammatory responses.

In this study we aim to determine if IL-10 increase is a normal host immune response or acting as a traitor to benefit *Eimeria* pathogenesis. We hypothesize that *Eimeria* upregulate the production of IL-10 in microenvironments to allow for evasion of the host immune response and down regulation of a key pro-inflammatory cytokine, interferon gamma (IFNγ). Immunofluorescent histochemistry was used to measure IL-10 and IFNγ in intestinal mucosal microenvironments. 19-day old Cobb broilers were administered either a 10x dose of Advent® *Eimeria* vaccine or saline. Duodenum, jejunum and cecum samples were collected on days 3-7 post infection at 12 hour intervals, processed, stained and imaged. Images were taken of the villi tip, middle and crypt of chicken intestines assessing IL-10 and IFNγ presence in intestinal mucosa in regions with a visible *Eimeria* burden and regions with no *Eimeria* visible. IL-10 and IFNγ were quantified using image J software to assess percent area of fluorescence. The experimental design is a completely randomized design with subsampling. Data analysis was done by a linear mixed effects model on SAS 9.4.

IL-10 and IFNγ had significant changes around days 4-7 post infection. During *Eimeria* infection the duodenum had increased IL-10 presence and increased IFNγ presence, and the jejunum and cecum had decreased IL-10 presence and decreased IFNγ presence. Duodenal and cecal results were opposite of previously quantified luminal IL-10 protein presence, which may indicate that cytokines are excreted from the intestinal mucosa into the jejunal and cecal lumen during infection.

Key Words: Eimeria, broiler, interleukin-10, interferon gamma, intestine

M31 Heat stress causes inhibition of Eimeria replication in broiler chickens Gustavo Schneiders*[GS], James Foutz, Alberta Foutz, Romdhane Rekaya, Samuel Aggrey *University of Georgia*

Eimeria infection is one of the most important diseases affecting poultry production, and is characterized by bloody or watery diarrhea, weight loss, poor feed conversion and moderate to high mortality. Heat stress (HS) is among the major environmental stressors in poultry, predisposing broiler chickens to immunosuppression and rendering them susceptible to diseases. There are some suggestions that HS reduces *Eimeria* oocyst output in chickens, however, the relationship between HS and coccidiosis is not elucidated. The objective of this study was to investigate the effect of HS on the development of *E. tenella*. Fifty-four 21 day old Cobb500 broiler chickens were infected via gavage with $15x10^4$ *E. tenella* sporulated oocysts suspended in water and raised in either a thermoneutral (control: 25°C) or a heat-stressed (treatment: 35°C) environment. At 6 days post-infection (dpi), 9 birds in each group were euthanized humanely, and the caecal lesion scores, merozoite and oocyst counts were evaluated. The rectal temperatures were also taken. Data were analyzed using Mann-Whitney test at the significance level of 5%. Heat stress was confirmed in the group exposed to 35°C by visual evaluation of the chickens, including panting, opened wings. The HS group had significantly higher cloacal temperature (43.03 ± 0.45°C versus 40.72 ± 0.40; $P<0.001$) as compared to the control group. At 6 dpi, merozoites, caeca lesion scores and oocyst counts were evaluated in both groups. The HS chickens had lower caeca lesion scores (0.33 ± 0.16 versus 1.89 ± 0.45; $P=0.014$), merozoite (26.67 ± 24.26 versus 823.21± 262.31; $P=0.0002$) and oocyst counts (80.40 ± 24.36 versus 1802 ± 266.34; $P=0.008$) as compared to control chickens. Overall our results indicate an interruption of the cycle of *E. tenella* in chickens housed under heat stress conditions.

Key Words: Eimeria, Coccidiosis, Heat stress, Merozoites, Oocysts

M32 Characterization of growth performance changes and lesion development in commercial toms infected with intestinal parasite Tetratrichomonas gallinarum Kelly Grace Keen*[GS], Robert Beckstead *North Carolina State University*

Tetratrichomonas gallinarum is an intestinal parasite commonly found in commercial turkeys. While prevalent in the industry, its virulence has yet to be defined. Published literature has described clinical signs specifically attributed to *T. gallinarum*, which include lesion formation in the ceca and liver of birds. However, the validity of these reports has been disputed due to diseased birds also being positive for *Histomonas meleagridis*, a parasite which causes similar lesions. The purpose of this study was to investigate the effects of a *T. gallinarum* field isolate on growth performance and lesion formation in turkeys. We hypothesize that birds infected with *T. gallinarum* will not show differences in growth performance or clinical signs when compared to non-infected birds. 120 commercial toms were separated into 2 treatments, infected and control. There were 4 replicate pens per treatment and 15 birds per pen. On day 19, each infected bird was intracloacally inoculated with 200,000 *T. gallinarum* cells. Infection percentage was determined 5 days post-infection through *in vitro* culturing of cloacal swabs. Body weight (BW), body weight gain (BWG), feed intake (FI), and adjusted feed conversion ratio (FCR) were determined on a weekly basis up to 49 days. On day 49, all birds were euthanized and necropsied for ceca and liver scoring. Performance data was analyzed through JMP Pro 13 using the student t-test to determine differences between control and infected treatments ($p\leq0.05$). There was no statistical difference in BW, BWG, FI, or FCR between the two treatment groups. There were no lesions observed during necropsy in any control or infected bird. These results demonstrate that this field isolate of *T. gallinarum* does not cause disease signs in commercial toms under research conditions. It is possible that *T. gallinarum*, when combined with other pathogens or stressors, could cause disease in turkeys. Potentially, other, more virulent field strains of *T. gallinarum* could also cause disease signs. Future research will be conducted looking at the additive effect of other *T. gallinarum* isolates under various field conditions on disease development in turkeys.

Key Words: Tetratrichomonas gallinarum, turkey, growth performance

M33 Genetic resistance to blackhead disease in commercial turkeys Alessandro Ferrarini*[GS], Robert Beckstead *NORTH CAROLINA STATE UNIVERSITY*

The protozoa *Histomonas meleagridis* infects a wide range of gallinaceous birds causing histomoniasis (blackhead disease). Blackhead disease manifests with necrotic lesions in the liver and ulceration of the ceca. Variation in susceptibility of gallinaceous birds to blackhead disease have been observed; chickens are typically asymptomatic and turkey mortality is common in the 80-100% range. This variation suggests that there is a genetic basis of the immune response to *H. meleagridis* infection. Based on our observations of *H. meleagridis* infected turkeys, we hypothesize that a small percentage of turkeys will be resistant to blackhead disease. To test this hypothesis, two flocks of 2,000 turkey poults were infected with a virulent strain of *H. meleagridis*. The first flock had 2,000 males, while the second flock had 1,000 males and 1,000 females. Turkeys were placed at a density of 16 poults/m² with 1000 birds in each room having *ad libitum* access to feed and water. Birds were neck tagged and blood collected on day 16. Poults in the first flock were cloacally inoculated on days 17, 38 and 59 with 100,000 histomonads, while poults in the second flock were inoculated with the same dose on days 23, 40 and 57. Mortality

was monitored and recorded twice daily with each bird scored (0-4) for blackhead disease in the ceca and liver. The first and second trials were terminated on day 80 and 81 respectively. A second blood sample was taken from turkeys that were alive at the end of the trial. All surviving birds were euthanized and scored as above. In the first flock, 223 toms (11%) were alive at the end of the trial with 101 (5.1%) showing no lesions. From the second flock, 146 (7.3%) turkeys survived with 45 toms (4.5% of the males) and 38 hens (3.8% of the females) showing no signs of disease. A Student's t-test showed no statistical difference in susceptibility based on sex ($p \leq 0.05$). Both flocks averaged a cecal score of 3.86 and liver score of 3.88. The identification of blackhead resistant turkeys supports our hypothesis and suggests that turkeys can be bred for resistance. To identify the genetic basis of this resistance, genome wide association studies will be performed using SNPs genotyping on DNA isolated from the blood of sensitive and resistant turkeys.

Key Words: Histomonas Melagridis, Turkeys, Resistance

M34 Utilization of sub-lethal bacteriostatic tetracycline co-administration for development of a novel in ovo challenge model for virulent Escherichia coli strains Danielle Graham*[GS], Callie Selby, Kyle Teague, Lucas Graham, Christine Vuong, Juan Latorre, Guillermo Tellez, Billy Hargis *University of Arkansas*

During the hatching process, chicks are exposed to opportunistic and/or pathogenic organisms, such as virulent or avirulent *Escherichia coli.* Previously, virulent *E. coli* strains have not been a feasible option for induction of neonatal colibacillosis via *in ovo* challenge due to resulting high embryonic mortality. In the following experiments, the addition and co-administration of the bacteriostatic antibiotic tetracycline to a virulent *E. coli* challenge culture improved hatchability and livability of seeder chicks while allowing robust horizontal transmission in the hatching cabinet to contact chicks. Experiment 1 consisted of 3 trials. Experiment 1, trial 1 was conducted to determine an effective ratio of *E. coli* challenge and tetracycline doses to be utilized in the seeder model. Trial 2 and 3 were conducted to evaluate the transmission efficacy of *E. coli* from seeder to contact chicks. Experiment 2 consisted of 3 independent 7-day trials wherein body weight gain, mortality, and selected enteric bacterial recovery was evaluated. In trials 1-3, significantly ($P < 0.05$) more Gram-negative bacteria were recovered from whole gut samples (GIT) vs. negative controls on day-of-hatch, from both seeder and contact chicks. At d7 in trial 1, contact chicks had significantly ($P < 0.05$) more Gram-negative bacteria recovered from the GIT than the negative controls, but not in trials 2 and 3. Presumptive lactic acid bacterial recovery was significantly ($P < 0.05$) elevated in contact and seeder chicks compared to negative controls in all 3 trials. Contact-challenge caused a significant ($P < 0.05$) reduction in body weight gain in 2 out of 3 trials at d7 and there was a significant ($P < 0.001$) increase in mortality as compared to the negative controls in all trials. These data suggest that co-administration of a virulent *E. coli* strain with tetracycline allows for hatch of directly challenged chicks and effective horizontal transmission to contact chicks during the hatching process, as evidenced by reduced d7 performance and altered selected enteric bacterial recovery.

Key Words: Escherichia coli, in ovo, broiler, model, hatchers

M35 Supplementation of a probiotic alone or in combination with prebiotics and phytogenics alleviates the negative impact of subclinical necrotic enteritis in broiler chickens Nima Khodambashi Emami*[1], Emily Kimminau[2], Ali Calik[1], Mallory White[1], Aaron Oxendine[1], Rami Dalloul[1] *[1]Avian Immunobiology Laboratory, Department of Animal and Poultry Sciences, Virginia Tech; [2]Huvepharma, Inc., 525 Westpark Drive*

The withdrawal of antibiotic growth promoters from poultry feed has increased the risk of necrotic enteritis (NE) outbreaks in poultry production with clinical and subclinical forms accountable for annual loss of $6 billion worldwide. This study examined the effects of a probiotic (OptiBac-L; OBL) alone or in combination with natural feed additives on performance and body composition of broiler chickens under a naturally occurring NE model. In total, 960 d-old male Cobb 500 broilers were randomly allocated to 4 treatments (8 floor pens, 30 birds/pen) including 1) Negative control (NC): corn-soybean meal basal diet; 2) Positive control (PC): NC+20 mg Virginiamycin (VA)/kg diet; 3) NC+227g OBL/ton diet; and 4) NC+453 g OBL+prebiotic+phytogenic (Essential premix; EP)/ton diet. One day after placement, all birds were challenged by a live oocyst coccidia vaccine as a precursor to naturally occurring NE. Performance was measured at the onset of NE (d8) and end of starter, grower, and finisher periods. On d8, the small intestine of 3 birds/pen were examined for NE lesions. Body composition was measured on d42 (3 birds/pen) by dual energy X-ray absorptiometry. Data were analyzed in JMP with significance between treatments identified by LSD ($P<0.05$). Diets supplemented with VA, EP and OBL significantly reduced d1-14 mortality compared to NC (1.66, 0.83, 1.66 and 5% respectively), while overall mortality was only numerically lower. Pathology data showed significantly lower NE lesion scores for EP and OBL compared to PC. Despite higher average daily gain in EP and PC groups compared with NC, average daily feed intake was similar in all treatments. In addition, supplementation of OBL and EP significantly improved FCR (1.55 and 1.54 vs. 1.58 in NC) for the overall experimental period, and PC had the lowest FCR (1.53) among all the treatments. There was no significant difference in carcass fat and lean percentage in birds fed the different dietary treatments, but these were improved numerically in OBL and EP compared to PC group. Collectively, these data indicate that supplementation of a probiotic alone or in combination with a prebiotic and phytogenic could improve broiler performance, and lower mortality rates and lesion scores in a subclinical NE challenge model.

Key Words: Necrotic enteritis, broilers, mortality, performance, body composition

M36 Stimulation of endogenous butyrate producers in the intestinal tract of broilers by in ovo exposure inoculation with lactic acid bacteria Denise Russi-Rodrigues*, Kimberly Wilson, Whitney Briggs, Audrey Duff, Kaylin Chasser, Lisa Bielke *Ohio State University*

Butyrate-producing bacteria have recently gained attention because of their role in control of pathogens and inflammation, and enhancement of intestinal integrity. Butyrate is a major energy source for epithelial cells and is related to optimal gut health of poultry. Many previous studies have shown that butyrate producing bacteria consume substrates produced by lactic acid bacteria (LAB) via cross-feeding interactions. Therefore, it was hypothesized that *in ovo* inoculation by LAB may affect growth and metabolism of butyrate producers. The aim of this study was to evaluate the influence of two apathogenic Gram negative isolates or a LAB-probiotic as pioneer colonizers on the establishment of butyrate producing communities. At ED18, embryos were inoculated with either saline (S), ~ 10^2 CFU of *Citrobacter freundii* (C1), *Citrobacter* sp (C2) or LAB (L) in the amnion. DOH GIT, duodenum to ceca, plus ileum and ceca at 3d and 10d were collected for microbiome analysis. Once the DNA was isolated from mucosal and digesta contents, samples underwent 2 x 300 paired-end Illumina MiSeq library preparation, targeting the V4-V5 region of the 16S rRNA gene for microbiome analysis. Data were analyzed using ANOVA with means separated using Student's t-test. An increased abundance of *Erysipelotrichaceae, Ruminococcaceae,* and *Lachnospiraceae* were observed in the L group at DOH. At 10d, the abundance of *Faecalibacterium prausnitzii* in the ceca was significantly higher in C an L (C: 22.88% ± 3.05%; L: 22.12% ± 3.99%) compared to S and C2 (S: 7.90% ± 2.24%; C2: 6.41% ± 2.06%, $p < 0.001$). In the ileum, there was a significant increase of *Erysipelotrichaceae* population at 3d and 10d ($p < 0.05$). At genus level, an evident increase in relative abundance of *Blautia* at 3d and 10d, as well as *Dorea* at 10d were observed ($p < 0.05$). Minimal changes in relative abundance of *Faecalibacterium prausnitzii* occurred in ileum, the fraction was only 0 to 3.14%, however at 10 d, L group had a numerally increased population. These results suggest that LAB-probiotic as

pioneer colonizers may stimulate butyrate-producing bacteria in the GIT of young broilers.

Key Words: Butyrate, microbiome, pioneer colonizers, next-generation sequencing

M37 Effects of dietary supplementation of OPTIGUT on the performance and intestinal microbiota in a necrotic enteritis challenge model in broiler chicks Vasileios Tsiouris*[1], Ioanna Georgopoulou[1], Tilemachos Mantzios[1], Kostas Kiskinis[1], Angelo Lauwaerts[2], Giorgos Filiousis[1], Giorgos Papadopoulos[1], Paschalis Fortomaris[1] *[1]Aristotle University; [2]Proviron Industries nv*

Inflammation of intestinal mucosa can directly affect intestinal barrier function, impairing absorption of nutrients and slowing down growth of birds. The aim of the present study was to investigate the effect of OPTIGUT™ (a mixture of glycerol esters of fatty acids) on the intestinal ecosystem and on the pathogenesis of experimental necrotic enteritis in broiler chicks. One hundred and twenty (120) 1-day old broiler chicks were randomly allocated to four treatment groups according to the following experimental design: group A, which served as negative control, group B, to which dietary supplementation of OPTIGUT™ product was applied, group C, to which challenge of birds *C. perfringens* and *Eimeria* spp. was applied and group D, to which dietary supplementation of OPTIGUT™ and challenge of birds was applied. At day 36, from each bird, the intestine, gizzard and liver were collected and scored for gross lesions. The intestinal digesta was collected for pH and viscosity determination. One caecum from each bird was taken for microbiological analysis. The statistical analysis and evaluation of the experimental data revealed that the dietary supplementation of OPTIGUT™ reduced significantly ($P \leq 0.05$) the severity of necrotic enteritis lesions. In addition, the dietary supplementation of OPTIGUT™, the challenge as well as their combination increased significantly ($P \leq 0.05$) the *Bifidobacterium* spp. and *E. coli* counts in caecum. The study provides evidence that the dietary supplementation of OPTIGUT™ can significantly affect necrotic enteritis and intestinal microbiota.

Key Words: OPTIGUT, performance, intestinal microbiota, necrotic enteritis, broiler chicks

SCAD

M38 Comparison of the impact of monobutyrin, other glycerides, and glycerol (SiloHealth 104), sodium formate (Amasil NA) and bacitracin methylene disalicylate (BMD) on intestinal and processed parts bacteria count when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp). Mike Coelho*[1], Peter Ader[2] *[1]BASF Corporation; [2]BASF SE*

A total of 3,744 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 9 replications). Birds were blocked by weight and sex. The treatments were T1=PC, no additive, no Cp; T2=NC, no additive, Cp; T3=NC +4/4/4 kg/MT SiloHealth 104 (SH) in starter/grower/finisher; T4=NC+ 4.0/2.5/1.5 kg/ MT SH; T5=NC+2.5/1.50/0.75 kg/MT SH; T6=NC+8/8/8 kg/MT Amasil NA (NA); T7=NC+2.5 kg SH + 4.0 kg/MT AN/ 2.5 kg SH + 4.0 kg/MT AN/2.5 kg SH + 4.0 kg/MT AN; and T8=NC+ Coccidiostat+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1-8 had litter inoculated on D0 with 2,500 oocytes of E. acervulina and E. maxima/bird, and T2-8 had litter inoculated with $5*10^4$ CFU/bird of E. coli and $5*10^4$ CFU/bird of Cp. On D21 and 42, 3 birds from each pen (27 birds/treatment) were sacrificed and data collected on Small Intestine (SI) and caecal (C) bacteria. At 43-45 days of age, 10 birds (5M and 5F) were processed from each replicate (9-replicates), to determine lesion scores and bacteria count. D21 SI lesion scores (0-3) (0.204, 1.667, 1.019, 1.037, 1.333, 0.778, 0.667 and 0.796, P<0.05, respectively): D21 SI Clostridium (CL) (3.595, 4.519, 4.209, 4.136, 4.388, 3.954 and 3.683 CFU log 10, P=<0.05, respectively); D21 SI Campylobacter (CA) (3.343, 4.489, 4.243, 4.074, 4.414, 3.834, 3.576 and 3.956, P<0.05, respectively); D42 SI CL (3.526, 4.534, 4.200, 4.074, 4.168, 3.973, 3.733 and 4.035 CFU log 10, P<0.05, respectively; D42 SI CA (3.329, 4.276, 3.93, 3.957 4.088, 3.829, 3.531 and 4.002 CFU log 10, P<0.05, respectively; caecal CL (3.480, 4.485, 4.277, 4.059, 4.165, 3.975, 3.678 and 4.042 CFU log 10, P<0.05, respectively; caecal CA (3.330, 4.243, 3.940, 4.055, 3.778, 3.521 and 3.930 CFU log 10, P<0.05, respectively; D45 processing breast CA (1.482, 2.850, 2.496, 2.655, 2.482, 2.094, 1.920 and 2.753 CFU log 10, P<0.05, respectively; breast salmonella incidence (1.111, 25.556, 13.333, 18.889, 15.556, 10.000, 7.778 and 6.667 %, P<0.05, respectively. In conclusion, monobutyrin, sodium formate and BMD significantly reduced bacteria count in small intestine, caecal and processing breast versus NC.

Key Words: necrotic enteritis, clostridium perfringens, momobutyrin, calcium formate

M39 Field observations on Activo Liquid as an intervention for Clostridial Dermatitis in Turkeys Mark Blakley*, John Smalley, Rafael Cabrera *EW Nutrition, USA*

Clostridial Dermatitis of turkeys is an acute disease with signs of serosanguinous subcutaneous dependent edema marked by sudden increases in mortality and where carcasses decompose quickly. Its etiology is believed to be multifactorial with several theories as to the route of entry of the Clostridial organism and physiology behind the onset of disease.

Clostridial Dermatitis was first observed in the upper Midwest in the mid-1990s and has increased in its scope, duration, and reoccurrence since. Penicillin has been used as the treatment of choice and has been quite successful in halting mortality. As concerns have arisen about antibiotic resistance and market and consumer trends have moved away from antibiotic use, alternative therapies have entered the marketplace.

One such alternative product is Activo Liquid. It is a proprietary blend of specific plant compounds which act to improve gut health by modulating the immune system, helping to control gut bacterial pathogens, and by improving digestibility. This emulsified plant extract is blended with organic acids to improve efficacy. Activo Liquid is water miscible and may be used in the drinking water to help control gut associated bacterial diseases in poultry.

Field data will be presented on the use of Activo Liquid as an intervention for Clostridial Dermatitis control in meat turkeys from multiple integrators in different regions of the U.S.

Key Words: Clostridial Dermatitis, turkeys, alternative product, Activo Liquid

M40 Characterizing and assessing the safety and immunogenicity of Chitosan and Polyanhydride nanoparticle vaccines loaded with Clostridium perfringens antigens. Gabriel Akerele*[2GS], Renukaradhya Gourapura[1], Ramesh Selvaraj[2] *[2]University of Georgia; [1]Ohio State University*

This study was conducted to identify the immunogenicity of a synthetic nanoparticle (Poly (methyl vinyl ether-co-maleic anhydride)) and a natural nanoparticle (chitosan) vaccine loaded with Clostridium Perfringens antigens. Solubilized cell wall and the extracellular proteins from 24-h old broth cultures of a mildly virulent strain of Clostridium perfringens (CP6) was precipitated using salt displacement method. This crude extract was loaded onto chitosan (CS-NP) and polyanhydride (PLN-NP) nanoparti-

cles with 60% and 70% entrapment efficiency respectively. The synthesized nanoparticle vaccine was analyzed for pH stability by identifying the amount of protein released in different pH conditions. At 2 h of incubation between pH 2.5 to 8.0, CS-NP and PLN-NP released 1.7% to 8.0% and 0% to 20% respectively. To test for vaccine safety and immunogenicity, sixty-three Cobb 500 birds were randomly allocated into 9 treatments of 7 birds per treatment. The treatments were 3 levels of oral gavage of chitosan and polyanhydride (10µg, 50µg, 100µg), unloaded chitosan, unloaded polyanhydride and PBS. The birds were housed in individual wire cages for 28 days on standard, antibiotic-free starter diets. Feed and water were provided ad-libitum. Serum was analyzed on d 0, 7, 14, 18, 21, 25 and 28 for Clostridium Perfringens specific IgG. Bile was analyzed on day 28 to measure Clostridium Perfringens specific IgA. Bile IgA was significantly more in vaccinated vs control (p=0.04) at 5% level of alpha. Indirect ELISA assay did not detect differences in serum IgG at other time-points. Western immunoblot however, showed marked differences in the reaction between pooled control and vaccinated sera to denatured CP6 supernatant proteins at all-time points. Since birds were not specified pathogen free, CP6-specific antigens may be required to reliably check for Serum IgG through indirect ELISA. There was no visible pathology directly related to vaccine administration. This study demonstrates the safety and immunogenicity of CS-NP and PLN-NP vaccines and provides a foundation for studies on their protective efficacy.

Key Words: nanoparticle, vaccine, chitosan, polyanhydride, clostridium

M41 Novel subunit vaccine targeting Clostridium perfringens mucinases for control of necrotic enteritis in broilers Audrey Duff*[1GS], Kendal Searer[1], Whitney Briggs[1], Kimberly Wilson[1], Christine Vuong[2], Billy Hargis[2], Luc Berghman[3], Lisa Bielke[1] *[1]Ohio State University; [2]University of Arkansas; [3]Texas A&M University*

Necrotic enteritis (NE) is a pervasive enteric disease responsible for large scale economic losses within the global poultry industry. The etiologic agent of NE is *Clostridium perfringens* (CP), a pathogenic bacteria that possesses many toxins and glycoside hydrolases (GH) as key virulence factors. Importantly, some GH, mucinases, are used to degrade mucin in the gastrointestinal tract (GIT) as an energy source. Two experiments were completed that proposed and evaluated mucinase vaccine candidate antigens sourced from conserved peptide sequences of carbohydrate binding module 32 (CBM32) of specific mucinases. In experiment 1, 37 antigens were sequenced and evaluated based on ability to obstruct CP growth *in vitro*. Total CFU of CP were measured at 6h and 8h to determine growth in the presence of anti-mucinase hyper-immune serum. Peptides 4, 5, 22, 24, and 30 were selected for further *in vivo* proof of concept testing based on conservation or ability to inhibit growth by over 50% at 6h and 8h. In experiment 2, peptide candidates were conjugated to anti-CD40 antibody-guided vaccine complex (AGC) and evaluated *in vivo*. Broilers were given an *Eimeria maxima* (EM) and CP induced NE challenge to evaluate vaccine efficacy. Treatments included a non-vaccinated non-challenged control (NVNC), non-vaccinated challenged control (NVCC), vaccination with peptide (VP) 4, 5, 22, 24, or 30, and a combination of all 5 peptides (MC). No differences were observed in body weight gain (BWG), but VP22, VP30, and MC exhibited greater numerical values than NVCC. The MC group had the numerically highest BWG of all vaccinated groups of 428.80±37.99, 68.9g greater than NVCC 359.90±32.10. There was a significant increase ($p<0.05$) in the percent change in BWG (%ΔBWG) relative to NVCC for VP22 and MC of 18.54% and 17.43%, respectively. Lesion scores (LS) were low in all groups, but lowest in MC with a mean score of 0.63±0.18. These results suggest the MC combination was the most successful in alleviating overall performance losses associated with NE in broilers, and encourage the future testing of the MC combination in the development of an NE vaccine.

Key Words: Necrotic enteritis, Clostridium perfringens, Mucinase, Subunit vaccine

M42 Effects of glycinated zinc on host immune response to necrotic enteritis in broilers Theros Ng*[GS], Ramesh Selvaraj *Department of Poultry Science, University of Georgia*

This experiment studied the effects of glycinated zinc (B-TRAXIM®2C Zn, Pancosma, CH) on broilers infected with necrotic enteritis (NE). Broiler chicks were randomly distributed into four treatments with six replications each: uninfected group fed 40 mg/kg of glycinated zinc and infected groups fed 40, 80, or 120 mg/kg of glycinated zinc. Chickens were infected with 5,000 *Eimeria maxima* oocysts per bird on day 14 and 10^8 CFU *Clostridium perfringens* on d 19, 20, and 21 by oral gavage. Feed and water were given *ad libitum*. On day 21, three bird/pens were euthanized for lesion scoring and sample collection. The remaining birds were euthanized on day 35 to evaluate growth performance during the recovery phase. Average daily gain, average daily intake, and anti-*Clostridium perfringens* IgG in serum and IgA in bile were not different on day 21 ($P > 0.05$). NE infection increased feed conversion ratio by 18.0% on d 21 ($P < 0.05$) and by 21.6% on day 28 ($P < 0.05$). Uninfected birds had the lowest intestinal lesion scores ($P < 0.01$), infected birds fed 40 and 80 mg/kg of zinc had the highest lesion scores ($P < 0.01$), and infected birds fed 120 mg/kg of zinc had lower lesion scores than birds fed 40 and 80 mg/kg zinc. Pro-inflammatory and anti-inflammatory cytokines, tight junction proteins, and zinc transporter and anti-oxidant proteins mRNA gene expressions will be analyzed in jejunum, cecal tonsils, spleen, and liver tissues. Inclusion of zinc above 40 mg/kg from glycinated zinc improved production performance from NE infection; but doses of 120 mg/kg of zinc was needed to alleviate from intestinal damages.

Key Words: Necrotic enteritis, zinc, immunology, broiler chickens, Gut health

M43 Necrotic enteritis predisposed by coccidiosis: efficacy of challenge models in broilers Shelby Curry*, G. Raj Murugesan, Chasity Pender *BIOMIN America, Inc*

Necrotic enteritis and coccidiosis are considered two of the most common and economically afflictive challenges in broiler production. To understand these challenges as well as to evaluate potential mitigation strategies, an appropriate model is needed. The utilization of both *Clostridium perfringens* (CP) and *Eimeria* strains has become the most commonly accepted method to induce necrotic enteritis by predisposing the gut with coccidiosis. However diverse thoughts exist on the levels and effects of litter quality, amount of each pathogen, age of challenge, and targeted mortality. The purpose of this abstract was to compare different challenge models and their outcomes. In the first model, birds on battery cages were orally gavaged with 5,000 oocysts of *E. maxima* on d 14 and 10^8 CFU of CP once daily on d 19, 20, and 21, which significantly reduced performance on d 28 (-80 g/bird in BWG and +24 points in FCR in comparison to unchallenged control; $P<0.05$) but had mild mortality (3.1%). In the second model, oral gavaging birds raised in fresh litter with 60,000 oocysts of *E. maxima* on d 18, and 0.25 ml of $4x10^9$ CFU CP on d 22 resulted in significantly reduced performance on d 35 (-108 g/bird in BWG and +15 points in FCR compared to unchallenged control; $P<0.05$) but again with mild mortality ($P>0.05$). In the third model, when birds were raised on fresh litter, then exposed to *Eimeria* and CP contaminated litter on d 17, their performance was not affected in comparison to unchallenged control (-3 g/bird in BWG and + 1 points in FCR; $P>0.05$) but again with mild mortality (2.27%). However in the last model, when birds raised on *Eimeria* and CP contaminated litter were fed CP at $4.13x10^8$ CFU/bird on d 17, their overall performance on d 28 was significantly reduced (-53 g/bird in BWG and +4 points in FCR compared to unchallenged control; $P<0.05$) but with much higher mortality (22.92%; $P<0.05$). Thus it can be concluded that there is not a 'one' model fits all solution. A challenge

model that has higher sensitivity to produce the desired outcome has to be utilized, especially when evaluating intervention strategies.

Key Words: necrotic enteritis, coccidiosis, model, Eimeria, Clostridium perfringens

M44 Evaluating effectiveness of live Salmonella vaccination of broilers from day of age to the carcass rinse Charles Hofacre*[1], Roy Berghaus[2], Doug Cosby[3], Mark Berrang[3], Arthur Hinton, Jr.[3], Kalen Cookson[4], Manuel DaCosta[4] *[1]Southern Poultry Research Group; [2]University of Georgia; [3]USDA-ARS; [4]Zoetis Global Poultry*

USDA-FSIS and FDA NARMS 2002-2012 reported *Salmonella enteritidis*and *S. heidelberg*were the two most common Salmonella associated with human foodborne illness from poultry. A study determined the effectiveness of vaccinating broiler chickens with a live *S. typhimurium*vaccine (Poulvac ST) at 1 day (spray) and 14 day (drinking water) to reduce *S. heidelberg*colonization of broilers ceca, environment (bootsock swabs) and whole bird carcass rinse (hot rehang). The challenge was a nalidixic acid resistant S.H. at 4 days of age to one-half of broilers (direct) in each pen. The non-challenged were tagged and dyed to identify the horizontal challenged. A typical feed withdrawal was performed prior to catching and transport to the USDA, U.S. National Poultry Research Center Pilot Processing Plant at 42 days. Salmonella prevalence and enumeration were performed with tetrathionate, then XLT-4 (25 mg/ml nalidixic acid). Enumeration was performed by micro most probable number (MPN) method of Berghaus, et al., 2013. Ten horizontal birds/pen were processed for carcass rinse and ceca while five direct challenged had ceca removed. Bootsocks were tested at 14 and 42 days from each pen. The bootsocks at 14 days were 100% positive for non-vaccinated and 93% for S.T. vaccinated; while at 42 days, both were 100% positive. The 42 day MPN enumeration were a mean $\log_{10}$of 2.34^{a}(control) and 1.94^{a}(vaccinated). Prevalence of S.H. in 42 day ceca of both direct and horizontal were not significantly different, control (18.1%^{a}) and vaccinated (17.4%^{a}). However, there was a significant reduction in number of S.H./ceca $\log_{10}$(MPN/g) with the control , 0.76^{b}and vaccinated, 0.10^{b}. Prevalence of S.H. on carcasses were numerically reduced (P = 0.102) control, 31.5%^{a}and vaccinated, 11.3%^{a}with the MPN/carcass reduced by the vaccine (1.29^{a}) versus control (1.36^{a}). The challenge control had 3/16 pens with 100% of the carcass rinse positive while the vaccinated group had no pen (0/16) with 100% of the carcasses positive. In conclusion, use of the live Salmonella vaccine (Poulvac ST) reduced the S.H. colonization of the ceca, which resulted in lower environment S.H. and lower S.H. on the carcasses in the processing plant.

Key Words: live Salmonella vaccine, Salmonella Heidelberg, carcass rinse, processing

M45 Cross protection effects of live salmonella typhimurium vaccination against early and late Salmonella spp. challenges Manuel Da Costa*, Kalen Cookson, Jean Sander, Andy McRee, Tak Niino, Jon Schaeffer, John Dickson *Zoetis - U.S. Poultry*

Live *Salmonella typhimurium* (Poulvac®ST) vaccination is currently seen as an effective live side intervention that helps to reduce Salmonella load at the processing plant in broilers. Three broiler studies evaluated the effect of Poulvac®STvaccination against early and late Salmonella challenges from 3 different O serogroups. Poulvac®ST vaccine was given by spray at d 0 and boosted by water/oral gavage at 14 d. Birds in studies 1 and 2 were exposed to an early challenge (4 and 5 d of age) of Salmonella from groups D and C, respectively. For study 3, birds were challenged at 35 d of age with a group B Salmonella. Salmonella prevalence and enumeration were evaluated in cecae and liver/spleen for the all studies. In addition, bird rinses and boot swabs were taken for study 3 as an indication of environmental Salmonella contamination. Study 2 birds were progeny from *Salmonella enteritidis* vaccinated breeders whereas studies 1 and 3 were progeny from breeders not vaccinated for Salmonella.All statistical analysis were conducted at the 0.05 level of significance (0.10 considered a trend) using two-sided tests. Birds in study 1 were sampled at 21d (17d post-challenge [dpc]) of age and Poulvac®ST birds tended (P=0.077) to have lower loads (- 0.6 log) of Salmonella D group in the liver/spleen with a concomitant 0.9 log numerical reduction in the cecae (P=0.234). Study 2 birds were sampled at 14 d (9dpc) of age with Poulvac®ST vaccination resulting (P=0.072) in lower loads (-0.4 log) of Salmonella C group in the cecae. Study 3 terminated at 49 d of age (14 dpc) and prevalence of Salmonella B group was significantly reduced (P<0.05) by Poulvac®ST in both cecae (- 39% positive samples) and liver/spleen (- 75% positive samples). Salmonella load in Poulvac®ST vaccinated birds was also significantly lowered (P=0.05) in cecae, boot swabs and carcass rinses by - 1.5, - 4 and - 0.5 logs, respectively. In conclusion, Poulvac®ST was shown to effectively cross-protect against Salmonella from groups B, C and D by lowering the loads of early and late Salmonella challenges. It is noteworthy that even when the challenge was faced a few days post vaccination, Poulvac®ST allowed birds to mount an effective immune response against the Salmonella challenges evaluated herein.

Key Words: salmonella, vaccination, broilers, cross protection

M46 Efficacy of chitosan based nanoparticle vaccine administration in broiler birds challenged with Salmonella enterica serovar enteritidis Keila Acevedo-Villanueva*[1GS], Renukaradhya Gourapura[2], Ramesh Selvaraj[1] *[1]University of Georgia; [2]The Ohio State University*

This study analyzed the protective effects of an oral chitosan-based *Salmonella* nanoparticle vaccine loaded with *Salmonella* outer membrane proteins (OMPs) and flagellin proteins (OMPs-F-CNPs). One-day-old Cobb-500 broilers (n=18) were orally gavaged with PBS (mock) or 500, 1000 or 2000 µg of OMPs-F-CNPs. A booster was given at 1wk-of-age. At 2wk-of-age, birds were challenged with 5.4 x 10^5 CFU of live *S. enteritidis* orally. All birds were euthanized at 11d post-challenge. At 8h-post-challenge, birds vaccinated with 1000µg OMPs-F-CNPs had 39 % higher (P<0.05) OMPs-specific serum IgY titers compared to that from the control group. Serum-OMP IgA was higher (P<0.05) in the 1000µg vaccinated group compared to that from the mock, 2000µg, and 500µg treatment groups by 185%, 117% and 375%, respectively. At 25d-of-age, birds that were vaccinated with 1000µg OMPs-F-CNPs had higher(P<0.05) bile OMPs-specific IgA titers compared to that from the mock, 2000µg, and 500µg treatment groups by 73%, 73% and 63%, respectively. Bile-flagellin IgA was higher (P<0.05) in the 1000µg vaccinated group than the control and 500µg group by 12%, and 13%, respectively. Cloacal -OMP IgA titers in the 1000µg treatment group was 208% higher (P<0.05) at 8h-post-challenge compared to that from the 2000µg group. Cloacal flagellin-OMP IgA was higher (P<0.05) in the 500µg vaccinated group compared to that of the mock treatment by 142% and 218%, respectively. Macrophage nitric oxide production at 25d-post-challenge was higher (P<0.05) in the 500µg vaccinated group compared to that from the mock, 2000µg, and 1000µg treatment groups. Cecal tonsil IL1 mRNA amount was 8-fold lower (P<0.05) in the 1000µg vaccinated group compared to that of the mock treatment. Cecal tonsil IL10 was 8-fold higher (P<0.05) in the 1000µg vaccinated group compared to that of the 500µg treatment. FCR (d0-25) was not significantly (P>0.05) different between any of the treatment groups. It can be concluded that vaccinating birds with 1000 µg of nanoparticle vaccine can provide optimal protection from *S.* enteritidis infection.

Key Words: Salmonella, Enteritidis, Nanoparticle, Vaccine, Chitosan

M47 Mucosal immunity of the broiler chicken vaccinated with nanoparticle vaccine and challenged with Salmonella enterica Enteritidis Bailey Lester*[1GS], Ramesh Selvaraj[1], Renukaradhya Gourapura[2] *[1]Department of Poultry Science, University of Georgia; [2]Food Animal Research Program, OARDC, The Ohio State University*

The objectives of this study were to create and evaluate a novel killed subunit nanoparticle vaccine against *Salmonella enterica* that can be de-

livered orally to the intestinal cell wall in broiler birds that can induce a protective immune response against *Salmonella*. A killed subunit polyanhydride nanoparticle based vaccine was designed with *S.enterica* serovar Enteritidis antigen. The aim of this study was to evaluate the anti-Salmonella immunoglobulin titers post vaccine administration. 15 birds were randomly distributed into four treatment groups with 3 replications. Birds were inoculated orally with either 0, 250, 500, and 1000 µg of nanoparticle vaccine on d0 and d7. Serum was collected on day 0, 7, 14, 17, 20, 23, and 25. Birds were challenged with 1 X 10^6 CFU of a *Salmonella enterica* Enteritidis wild type strain on day 14 to identify protective antibody response and pro and anti-inflammatory cytokine production. Anti-Salmonella specific serum IgY and bile IgA was measured at several time points post vaccine administration and post-Salmonella challenge. Serum and bile immunoglobulin data were analyzed using one-way ANOVA. Birds inoculated with 250 µg of nanoparticle vaccine and challenged with *Salmonella* had 73% decrease (P =0.0542) in bile IgA compared to the unvaccinated challenged control. Bird inoculated with 250 µg and 500 µg of nanoparticle vaccine had 82% and 47% decrease(P=0.0047) in IgY serum antibodies respectively. The relative expression of pro-inflammatory cytokines IL1 and IFN-g and anti-inflammatory cytokines IL4 and IL10 were not significantly different in the cecal tonsils of birds from different treatment groups. Further tests will evaluate the shedding of *Salmonella* in birds vaccinated with nanoparticle vaccine and challenged with Salmonella. It can be concluded that birds vaccinated with a killed subunit nanoparticle vaccine decrease the level of *Salmonella* specific antibodies, shortening the immune response compared to *Salmonella* challenged and unvaccinated birds.

Key Words: Salmonella enterica, Enteritidis, vaccine, mucosal immunity

M48 Evaluation of a Subunit Salmonella Vaccine to Control and Reduce the Contamination of Salmonella Heidelberg in Cecae of Broiller Chickens Igor Praxedes-Campagnoni*[1,2GS], Fabrizio Matte[2], Fábio Gazoni[2], Thalia Malta[2], Emanuel Gumina[3], Sherry Layton[4] *[1]UDESC - Universidade do Estado de Santa Catarina; [2]Vetanco do Brasil; [3]Vetanco SA; [4]BV Science*

Salmonella spp. is a major cause of bacterial enteric illness in both humans and animals. Vaccination is one of the most prevalent control strategies for Salmonella in the commercial poultry industry. We have previously reported the efficacy of a commercially available subunit vaccine, Biotech Vac Salmonella (BTVS), to protect against and control *Salmonella Infantis* (SI) and*Enteritidis* (SE). The aim of this study was to evaluate the performance of BTVS to reduce *Salmonella Heidelberg* (SH) contaminationin cecas of broiler chickens. The experiment was conducted using 100 day of hatch broiler chickens (Cobb) split into 4 treatment groups (n=25/group). Chicks received either 0.2ml of Saline or BTVS by oral gavage on d3 and 17 of life and were challenged as follows on d5 with 0.5ml of SH containing 10^9CFUs: Group 1 (saline) and Group 2 (BTVS) 5 seeder chicks (20%) were challenged to determine horizontal transmission and Group 3 (Control) and Group 4 (BTVS) 100% of the birds were challenged. On d28 birds were humanly sacrificed, ceca collected, contents diluted, enriched, plated on SH selective media and incubated overnight, the following morning the plates were read for the presence or absence of SH. SH positive plates were further analyzed, and SH quantified. Data was analyzed for significance using Kruskal-wallis (<0.05). Results showed that horizontal transmission was significantly decreased in the birds receiving BTVS compared to the Saline treated chicks: 15% and 55% SH positive. In Groups 3-4 (100% birds challenged), vaccinated birds again showed significant reduction in colonization when compared to the saline treated birds (30% and 85% respectively). Quantitative analysis also confirmed significant reductions in bacterial load of the ceca of SH positive chickens: average log10 CFU in the vaccinated group was 1.000 while the average was 2.301 saline treated group. These results proved similar to those obtained previously when chickens were challenged with SE and SI, demonstrating the cross protection efficacy of the vaccine to control several serotypes of salmonella. In conclusion, the vaccine combined with biosecurity may be a useful tool to control *Salmonella spp.*by reducing the contamination in the birds and in the environment.

Key Words: Salmonella, Mucosal Health, vaccine, public health, subunit

M49 In vitro and in vivo assessment of probiotics as an on-farm control strategy against Campylobacter jejuni in broilers Mohamad Mortada*[1GS], Revathi Shanmugasundaram[1], G Raj Murugesan[2], Ramesh Selvaraj[1] *[1]The University of Georgia; [2]2BIOMIN America Inc.*

Campylobacter is one of the major foodborne pathogens associated with the consumption of poultry. *In vitro* and *in vivo* studies were conducted to evaluate the effectiveness of a synbiotic containing *L. reuteri*, *B. animalis*, *E. faecium*, *P. acidilactici* and fructooligosaccharide for the reduction of *C. jejuni* in broilers. The objective of the *in vitro* study was to identify the minimum inhibitory concentration(MIC) of cell-free supernatant (CFS) of the 4 probiotic strains. *C. jejuni* was co-cultured with the 4 different CFSs at supernatant:*Campylobacter* dilutions of 0:1, 1:1, 5:1, or 10:1, in triplicates (n=3) in 96-well plates. Plates were incubated overnight at 37°C. The MIC of *E. faecium* and *P. acidilactici* CFSs against *C. jejuni* was at 1:1. While the MIC of *L. reuteri* and *B. animalis* CFSs against *C. jejuni* was at 5:1. An *in vivo* 2(0 and 0.1% synbiotic) x 2 (unchallenged and challenged) factorial study was conducted to evaluate the effect of *C. jejuni* challenge and synbiotic supplementation on performance and immune parameters in broilers. Birds were randomly assigned to 4 treatments with 6 replicates/treatment (n=6) and 8 birds/replicate. Treatments 1 and 3 received synbiotic supplementation daily in feed (0.1% wt/wt) while treatments 2 and 4 received a basal diet. Birds in treatments 1 and 4 were orally challenged with 1 X 10^8 CFU/bird of *C. jejuni* on d 14. *C. jejuni* challenge decreased BWG by 82g on d 35 (p=0.22). In addition, challenged birds had a significantly higher (p<0.01) $CD4^+CD25^+$ % in cecal tonsils when compared to unchallenged birds at 7 d post challenge (5.56% vs. 0.76%). Treatment 1 had decreased $CD4^+CD25^+$ % by 38.7%, 39.7% and 12.3% when compared to treatment 4 on 7, 14 and 21 d post challenge, respectively. Treatment 1 had a lower CD8+/CD4+ ratio when compared to the other treatments on d 21. In conclusion, the 4 probiotic strains showed an inhibitory effect against *C. jejuni in vitro* and were able to decrease $CD4^+CD25^+$ % and Th1/Th2 ratio in *C. jejuni*-challenged birds *in vivo*. The increase of Tregs after *C. jejuni* challenge might explain the commensal nature of *Campylobacter* in broilers.

Key Words: Probiotics, Campylobacter, jejuni, Tregs, Immune response

M50 Validation of ATP bioluminescence swabs to determine microbial loads in a controlled laboratory setting Julia McElreath*[1GS], Brian Jordan[1], Miguel Barrios[2] *[1]University of Georgia; [2]Synexis Biodefense Systems*

Swabs utilizing ATP bioluminescence technology for measuring microbial load offer a fast and inexpensive alternative to traditional plating methods, reporting values as relative light units (RLU). These assays function through a chemical reaction involving the ATP present in living cells to generate luminescence detectable by a luminometer. However, swabs measuring different targets (total bacteria, specific class of bacteria, etc.) can produce inconsistencies in a field setting when compared to traditional agar plate inoculation. For this trial, three types of ATP bioluminescence swabs were obtained from a commercial company: Hygiena UltraSnap, Hygiena MicroSnap Total Viable Count (TVC), and Hygiena MicroSnap Enterobacteriaceae (EB). UltraSnap swabs detect total surface ATP regardless of source; MicroSnap TVC swabs detect ATP from all bacterial cells and are AOAC performance tested, and MicroSnap EB swabs select ATP deriving only from Enterobacteriaceae cells. The objective of this study was to compare sampling methods and identify the most accurate method for measuring microbial load in a controlled laboratory setting using a known bacterial concentration. An *Escherichia coli* ATCC-

25922 strain was obtained from Microbiologics, and prepared in a tryptic soy broth (TSB) stock culture. Ten-fold serial dilutions were made in phosphate buffered saline (PBS) in quadruplicate, and direct plating was performed by inoculating tryptic soy agar (TSA) plates with 0.1mL of each dilution. Additionally 0.1mL of each dilution was used to inoculate 100cm^2 stainless steel coupons. Coupons were swabbed with one of four swab types: Hygiena UltraSnap, Hygiena MicroSnap TVC, Hygiena MicroSnap EB, or a cotton swab saturated with PBS. UltraSnap swabs were analyzed immediately using a Hygiena Ensure luminometer while both MicroSnap swab types were incubated for 7 hours. Cotton swabs were suspended in 1.0mL PBS and vortexed before 0.1mL was pipetted on TSA plates to measure microbial load recovery using indirect plating methods. By comparing colony forming units (CFU) obtained from traditional plating methods to Hygiena swab RLU and indirect plating CFU, the most accurate and sensitive bacterial detection method available can be selected for field application in poultry settings.

Key Words: Microbiology, ATP bioluminescence, Hatchery

M51 Impact of dietary chitosan and coccidia challenge in broiler chickens Saheed Osho*[GS], Olayiwola Adeola *PURDUE UNIVERSITY*

Two experiments were conducted to investigate the effect of graded concentrations of dietary chitosan and coccidia challenge (CC) on growth performance, nutrient digestibility, jejunal morphology, plasma cytokine and gene expression in broilers chickens (Cobb 500). Experiment 1 was conducted to validate the optimum dietary concentration of chitosan in the diets of broiler chickens. A total of 384 one-day-old male broiler chicks were randomly assigned to 6 dietary treatments in a randomized complete block design. Corn-soybean meal-based diets were formulated to contain 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 g of chitosan/kg of diet. There were linear effects ($P < 0.05$) of graded chitosan concentration on body weight (BW), BW gain, feed intake, jejunal villus height and villus height to crypt depth ratio at d 22 post-hatching as well as linear increases ($P < 0.05$) in ileal dry matter and energy digestibility. Experiment 2 was conducted to investigate the immune-protective properties of chitosan on broiler chickens during a coccidia challenge. A total of 256 male broiler chicks were randomly assigned to a 2 × 2 factorial arrangement of treatments with two chitosan concentrations (0 or 1 g of chitosan/kg of diet) and with or without CC. Birds in the challenged group received 68x, which is 2.7 times the recommended coccidia vaccine dose of 25 doses/kg BW, while the nonchallenged group received physiological saline. Coccidia challenge reduced ($P < 0.05$) and dietary chitosan increased ($P < 0.05$) BW gain and gain;feed. Chitosan supplementation increased ($P < 0.05$) the gain: feed in birds challenged with coccidia as indicated by the interaction ($P < 0.05$) between chitosan supplementation and CC. Coccidia challenge reduced ($P < 0.05$) jejunal villi height whereas dietary chitosan supplementation increased ($P < 0.05$) jejunal villi height. Dietary chitosan supplementation attenuated the CC-induced effects ($P < 0.05$) on the expression of plasma interleukin-6 and 10, claudin-1 and occludin genes. In conclusion, dietary chitosan improved growth performance, nutrient digestibility, and jejunal morphology. In addition, dietary chitosan reduced the impact of coccidia challenge and may provide immune-protection to avian coccidiosis.

Key Words: Broiler chickens, chitosan, coccidia challenge, concentration, gene expression

M52 Effects of glutamine supplementation on broiler performance and intestinal immune parameters during an experimental coccidiosis 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 coccidiosis infection. Ninety-one-day old Cobb 500 male broilers were randomly distributed to three experimental groups with 0, 0.5, and 1.0% of supplemental Gln to a basal diet. 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 five replicates (5 battery cages) of 6 birds per replicate with water and feed being provided *ad libitum*. On d 14 of the study, each bird was given an oral gavage of 20x dose of Coccivac B52® suspended in distilled water. Bw, feed intake, and mortality were recorded throughout the study. Six d post challenge *Eimeria* lesion scores, cytokine gene expression, intestinal histomorphology, and cecal tonsil T cell population percentages were analyzed. Data were analyzed using the PROC GLM procedure in SAS. Orthogonal contrasts were used to compare Gln supplementation levels (0.0 vs. 0.5% and 0.0 vs. 1.0%) with differences between levels being considered significant at $P < 0.05$. Jejunum INF-g and IL-10 gene expression was 11.8 and 25.3 fold lower in birds fed 0.5% Gln versus 0.0% ($P = 0.02$ and $P = 0.02$; respectively). Cecal tonsil INF-g gene expression was significantly ($P < 0.01$) lower in the 0.5 and 1.0% Gln supplemented groups compared to 0.0% (8.1 and 7.9 fold; respectively). Gln supplemented at 1.0% significantly reduced IL-10 cecal tonsil gene expression by 6.8 fold ($P < 0.01$). Both 0.5 and 1.0% Gln supplemented groups Claudin-1, Claudin-2, and Zonula occuloden-1 gene expression were significantly higher ($P < 0.01$, $P < 0.01$, and $P < 0.01$; respectively). The two groups that were supplemented with Gln had improved crypt depths and villus height to crypt depth ratios compared to that of the unsupplemented group ($P < 0.01$). No Significant effects ($P > 0.05$) were observed on any performance parameter or T cell population percentages. These results show that glutamine supplementation upregulated tight junction cytokine gene expression, improved intestinal morphology, and down-regulated pro and anti-inflammatory cytokine gene expression.

Key Words: Glutamine, Eimeria, Coccidiosis, Broiler

M53 Next-generation Illumina sequencing for improved speciation of common Eimeria species infecting chickens Grace Albanese*[1], Dong-Hun Lee[2], Brian Jordan[1] *[1]University of Georgia; [2]University of Connecticut*

Coccidiosis is a costly gastrointestinal disease of commercial chickens caused by the protozoan parasite *Eimeria*. There are 7 species of *Eimeria* known to infect chickens, and 2 additional strains which have yet to be confirmed. The classical method for identification and differentiation of *Eimeria* species is with the use of morphological measures, such as oocyst and sporozoite size and shape, and infection dynamics, including zone parasitized within the chicken intestine, number of asexual replication cycles, region of the epithelial cell infected, and occurrence of intestinal lesions. Classical methodology for speciation of *Eimeria* is quite useful, however, it can also be subjective, especially when speciating based on morphology alone. Because of potential issues using classical methods alone, molecular methods of species identification are being employed more frequently. Typically, molecular speciation has involved a PCR reaction to detect a specific gene region, which may be followed by another PCR or by Sanger sequencing. Sanger sequencing is only able to determine sequence for the predominant species in the sample, and minor populations may be missed. New technology using next-generation Illumina sequencing can be used instead of the two-step PCR, and all genomic populations present within a sample will be detected. In this study, 3 regions of the *Eimeria* genome were compared for the ability to differentiate between species. Two regions were from the ribosomal DNA, the 18S and internal transcribed spacer region 1 (ITS-1) genes, and one region was from mitochondrial DNA, the cytochrome c oxidase subunit I (COI). Generic PCR amplification for each genome region was performed on an *Eimeria* monoculture and on a commercial vaccine containing known species of *Eimeria*. For each region, the generic PCR product was sequenced using Illumina sequencing, and bioinformatic analysis was performed to show all *Eimeria* genomic populations present and the proportion of each within the samples. Results showed that the sequence identities between species for both the 18S and ITS-1 regions were very high, making speciation dif-

ficult. The COI region was found to be the most suitable for metagenomic analysis, and appeared to be the best target for species identification.

Key Words: Eimeria, PCR, Illumina

Metabolism and Nutrition

M54 Black Soldier Fly larvae oil and meal as dietary ingredients for laying hens Luke Trimble*[UG], Arianna Ferguson, Heather Sciubba, Paul Patterson, Nuket Acar *The Pennsylvania State University*

Methionine supplementation is essential for healthy, productive laying hens. Synthetic methionine is added to diets to meet this requirement, however synthetic methionine supplementation is limited in organic diets. Recently, the Food and Drug Administration and Association of American Feed Control Officials approved Black Soldier Fly Larvae (BSFL) as a feed ingredient for poultry. In two studies, BSFL oil and BSFL meal were fed to White Leghorn hens from 43 to 47 and 51 to 55 wks of age. The 216 hens were divided into 4 groups of 54 hens with one control group and 3 treatment groups. The hens were fed iso-caloric, iso-nitrogenous diets that had 3 levels of BSFL oil (1.5, 3 and 4.5%) in study 1; and 3 levels of BSFL meal (8, 16 and 24%) in study 2. Results indicated that increasing levels of dietary BSFL oil yielded greater albumen height (P=0.0649) and yolk color (P < 0.0001) in the first study. Control yolks had a color score of 7.37 and significantly less than those of BSFL oil treatments averaging 7.87. This indicates that with the inclusion of BSFL oil a more desirable and higher quality egg can be obtained. It is also worth noting that there were no significant changes in hen body weight, feed intake, feed conversion, percent production or egg size as the amount of BSFL oil increased in each of the diets (P > 0.05). Feeding hens BSFL meal at levels greater than 16% was found to have a negative effect in the second study. Hens fed a diet devoid of soybean meal and completely replaced with BSFL meal reduced hen body weight (P=0.0509), feed consumption (P < 0.05), total egg production (P <0.05) and increased feed conversion (P=0.0584). The percent egg productions was reduced to 77.01% and was less than the average for the control, 8 and 16% BSFL meal treatments which was 85.14%. However, intermediate dietary levels of BSFL meal (8 and 16%) had no negative impact on these performance parameters and were not significantly different than hens fed the control diet. Both studies yielded eggs that had enhanced yolk color from feeding the BSFL ingredients. It is concluded from these studies that both the oil and meal can be used as sources of energy and protein, while BSF meal could be used as a natural source of methionine.

Key Words: Black Soldier Fly, Laying Hens, Oil, Meal, Dietary Ingredients

M55 Evaluation of differential scanning calorimetry to assess starch gelatinization of multi-component feed processed with different conditioner temperatures and Hygieniser retention times Lindsey Spencer*[GS], Luis Avila, Allan Calderon, Danny Patino, Wilmer Pacheco, Emefa Monu, Oladiran Fasina, Charles Starkey *Auburn University*

Starch can gelatinize in the presence of heat and water during thermal processing, the opening of the starch granule may enhance energy digestibility in broilers. An experiment was performed to investigate the effect of a Hygieniser® (HYG, CPM, Crawfordsville, IN) in addition to a typical conditioner on starch gelatinization of a typical broiler breeder feed. Nine treatment combinations were analyzed with 3 conditioning temperatures (75, 85, and 95 °C) and 3 retention times (RT) in the HYG (80, 160, and 240 s). Samples (n = 198) for differential scanning calorimetry (DSC) analysis of starch gelatinization were collected on 3 separate d. Feed samples (10 mg each) were weighed into Al hermetic pans with 20 µL of deionized water and left to equilibrate overnight. DSC thermograms were analyzed using TA Universal Analysis 2000 software to assess the enthalpy (J per g) for each sample. Data were analyzed using the GLIMMIX procedure of SAS (V9.4). Means were considered significantly different when $P \leq 0.05$. Post-conditioner samples conditioned at 75 °C had higher enthalpy ($P \leq 0.0182$) than those conditioned at 85 and 95 °C. Enthalpy of samples collected from the HYG conditioned at 75 °C was higher ($P = 0.0486$) than those conditioned at 85 °C. An interaction ($P = 0.02$) was observed for the enthalpy of samples collected after pelleting with feed conditioned at 75 °C and retained in the HYG for 80 s having greater enthalpy than all other time and temperature combinations. Starch gelatinization is calculated using: $(H_0\text{-}H_1/H_0)*100$, where H_0 represents the enthalpy of uncooked samples and H_1 represents the enthalpy of cooked samples. For this study, the analyzed enthalpy values from the uncooked meal samples were lower than the enthalpy values from the cooked samples. Therefore, the equation cannot be used to properly determine degree of starch gelatinization. In order to accurately determine starch gelatinization in animal feed samples, the validated AOAC 996.11 method must be used. The combination of proteins, fats, and other carbohydrate fractions do not appear to allow for the use of DSC as an alternative to AOAC 996.11 in complex animal feed. Based on these results, DSC is not a suitable method to assess starch gelatinization in multi-component animal feed.

Key Words: starch gelatinization, Feed manufacturing, differential scanning calorimetry, Hygieniser

M56 Determining the influence of diet formulation, particle size, and analytical method on the crude protein predictability of Near Infrared Reflectance Spectroscopy Caitlin Evans*[1GS], Nana Frempong[1], Chad Paulk[1], Thomas Nortey[2], Charles Stark[1] *[1]Kansas State University; [2]University of Ghana*

The accuracy of prediction using near infrared reflectance spectroscopy (NIRS) may be influenced by sample particle size, shape and arrangement. The purpose of this study was to determine the accuracy of the NIRS in predicting the crude protein (CP) content of varying broiler diet formulations containing different corn particle sizes when using the factory calibrations provided with the instrument. Treatments were arranged in a 4×3×3 factorial with diet formulation, corn particle size, and analytical method serving as the main effects. Varying inclusion levels of SBM, DDGS, Fish meal and/or Wheat bran were used to create 4 individual diets each formulated to contain 20% CP. Each of these diets was manufactured using 3 different corn particle sizes (400, 600 and 800 µm) and then analyzed for crude protein via 3 methods (laboratory, NIRS-ground and NIRS-unground). Mash samples undergoing NIRS analysis were scanned as-is or were ground through a 0.5 mm sieve prior to analysis. Both unground and ground mash samples were scanned on a Foss NIRS D2500 with a wavelength range of 400 to 2,500 nm at a reflectance of log (1/R) at 2 nm intervals for each sample. Laboratory values for CP were determined using ground mash samples based on the combustion method as outlined by AOAC official method 990.03. Values obtained from the standard method were compared to results from the NIRS. No diet × particle size × analytical method interaction was observed. There were interactions ($P \leq 0.05$) between diet × method and particle size × method. When analyzing CP using the laboratory or NIRS-ground methods there were no differences between diets within method. However, CP differed when using the NIRS-unground method. When analyzing CP using the NIRS-ground or NIRS-unground methods there were no CP differences between particle size within method. The CP concentration differed between particle sizes when using the laboratory. Overall, analyzed CP content of laboratory and NIRS-ground samples (20.3 and 19.6%) were greater ($P \leq 0.05$) than

that of NIRS-unground (17.8%). Thus, analyzing unground, mash feed samples using factory calibrations yielded less accurate results compared to ground samples analyzed with either NIRS or the standard laboratory method.

Key Words: Near Infrared Spectroscopy, Prediction, Chemical Composition, Analytical Method

M57 Effects of pellet diameter and crumble size on the growth performance and relative gizzard weight of broilers Frank Idan*[1GS], Thomas Nortey[2], Chad Paulk[1], Scott Beyer[1], Chance Fiehler[1], Courtney Truelock[1], Christopher Delfelder[1], Charles Stark[1] *[1]Kansas State University; [2]University of Ghana*

Feeding broiler chicks with either pelleted or crumbled diets stimulates feed intake and improves growth performance and feed conversion ratio (FCR). A 21-d experiment was conducted to compare the growth performance, FCR, and relative gizzard weight (RGW) of broiler chicks fed diets, in the form of a mash, micro pellets, or coarse and fine crumbles either with or without the fines removed. A total of 300 male Cobb 500 broiler chicks (initial BW of 44 ± 0.4 g) were randomly distributed into 60 Petersime battery cages with 5 birds per cage. Cages were randomly assigned to one of six dietary treatments to provide 10 replicates per treatment in a CRD. Treatments were: 1) mash (M); 2) micro pellets (MP); 3) coarse crumbles with fines (CF); 4) coarse crumbles with fines removed (CN); 5) fine crumbles with fines (FF); and 6) fine crumbles with fines removed (FN). The BW and feed intake were measured and used to calculate ADG and ADFI at 7, 14 and 21 d. Data were analyzed using the GLIMMIX procedure of SAS. Overall, BW and ADFI were increased ($P < 0.001$), FCR improved ($P < 0.001$) and RGW decreased ($P < 0.001$) for birds fed pellets or crumbles vs. the mash diet. The BW and ADFI at 21 d were 891, 1049, 1008, 1043, 994 and 1030 g and 55, 61, 58, 58, 58 and 59 g for M, MP, CF, CN, FF and FN respectively. The BW of birds fed MP, CN and FN was greater ($P < 0.001$) than those fed CF and FF but collectively were higher ($P < 0.001$) than birds fed M. The FCR was 1.37, 1.29, 1.29, 1.24, 1.30 and 1.29 for birds fed M, MP, CF, CN, FF and FN respectively at 21 d. Birds fed the CN and mash diets had the best and worst FCR respectively. The RGW was 1.96, 1.61, 1.58, 1.60, 1.82 and 1.72 % for birds fed M, MP, CF, CN, FF and FN respectively. Birds fed M had the highest ($P < 0.001$) RGW while those fed CN had the lowest. The results of this study indicated that feeding MP or crumbles improved broiler BW, ADFI and FCR but decreased RGW compared to mash diet. Removing fines resulted in further improvement in BW for birds fed crumbles. The study also indicated that feeding a good quality crumb will yield similar growth performance in broiler chicks as a micro pellet and does not require a pellet die change.

Key Words: Micro pellets, crumbles, body weight, feed conversion ratio, relative gizzard weight

M58 Determining the average particle size (APS) consumed between two broiler strains (S) receiving diets varying in feed form (FF) and feed quality (FQ) and the subsequent impact on starter performance (0-6 and 0-18 d) Andrew Brown*[1GS], Mark Lemons[1], Christopher McDaniel[1], Joseph Moritz[2], Kelley Wamsley[1] *[1]Mississippi State University; [2]West Virginia University*

A previous, companion study utilized a 2 S (Fast Growing (FG) or High Yielding (HY)) x 2 FF (Crumbles (C) or Intact Pellets (IP)) x 3 FQ factorial arrangement to determine the impact of these treatments on broiler performance. The two FF, C or IP, were fed only in the starter diet (d 0-18) as one of three FQ: Low-2210 µm (C) or 40% IP, Medium-3010 µm (C) or 60% IP, and High-3388 µm (C) or 80% IP. Data from 0-18 d showed that feeding increased FQ for IP diets enhanced d 18 chick BW and BW gain. Carryover effects (18-63 d and processing) exhibited that FF/FQ fed from 0-18 d may need to vary upon grow-out length and S utilized. However, the current study was warranted to determine the average particle size actually consumed from d 0-6 and d 0-18 among these S, FF, and FQ. This study utilized a sub-sample of pens from the companion study, where d old chicks (480/S; 0.093 m^2/bird) were randomly allocated by S to 48 pens. On each day of the starter phase for each treatment, the APS placed was determined by hand sieving 2x the breeder's recommendations for FI/bird/day, using a stack of eight sieves (over a collection pan) for 2 min. Remaining feed on each sieve was recorded for particle size classification, then recombined before being fed. Each day, the previous days' feed was sieved in the same manner to determine feed remaining on each sieve. Strain was significant for d 0-6 (P=0.02) and 0-18 (P=0.04) with HY having a higher APS consumed. A SxFF interaction from d 0-18 (P=0.008) revealed that APS consumed only differed between S when pellets were fed, with HY consuming a larger pellet size. Significance for FFxFQ on 0-6 and 0-18 d (P<.01) demonstrated in general, when broilers are fed High FQ, they consume a larger APS regardless of FF. For birds fed Low and Medium FQ, they consume a large APS when fed as C, but a smaller APS when fed as IP. Significant correlations of APS consumed with d 0-6 BW, BW gain, and FCR showed that broilers consuming a higher APS had improved performance (P=0.01; R=0.35, 0.37, -0.38, respectively); this was also true for d 0-18 FCR (P=0.04; R=-0.30). These data suggest that APS consumed is dependent upon S and FF presented; further research is needed to verify S preference of APS when provided ad libitum amounts of each particle size.

Key Words: Pellet, Crumble, Feed Form, Feed Quality, Bird Performance

M59 Effects of corn particle size and formic acid on productive and processing performance of broilers Andrea Rubio*[1GS], JT Pope[1], Joshua Jendza[2], Adam Fahrenholz[1] *[1]North Carolina State University; [2]BASF*

Previous research has reported that inclusion of coarse corn particle size in broiler diets can improve gastro-intestinal tract (GIT) function. Formic acid decreases the feed pH which could create a less favorable environment for bacteria and alter buffering capacity which may improve the efficacy of digestive enzymes. However, research evaluating the interactive effects between coarse corn particle size and dietary levels of formic acid throughout the production period of broilers is sparse. The objective of this study was to evaluate the effects of corn particle size and formic acid on broiler growth performance and carcass characteristics. A total of 2,034 male Ross YPM × 708 broiler chicks were randomly distributed among 6 dietary treatments with 12 replicate pens/treatment and 32 birds/pen, which constituted a 3 × 2 factorial arrangement of 3 corn particle sizes (395 µm fine corn (FC), 850 µm coarse corn (CC) obtained with greater inclusions of 1642 µm corn (CC-Hi) or lesser inclusions of 2290 µm corn (CC-Lo) when blended with FC and 2 dietary levels of formic acid (0.0% and 0.4%) fed from 11-49 d of age. Feed intake and BW were determined at 10, 21, 35, 42 and 48 d of age and feed conversion (FCR) was calculated and adjusted for mortality. At 43 d, 2 birds/pen were processed for the determination of carcass characteristics and hot carcasses were deboned to determine total breast meat yield (pectoralis major and minor muscles). Data were statistically evaluated as a 3 × 2 (corn particle size × formic acid) factorial arrangement in a randomized complete block design. Although there were no significant differences in FI attributed to the inclusion of formic acid ($P>0.05$), birds fed CC-Hi diets had a lower FI compared with those fed CC-Lo diets at ($P<0.05$) at 48 d. At 35 d, birds fed FC diets with 0% formic acid had a lower BW compared with those fed FC diets with 0.4% formic acid. Birds fed CC and 0.4% formic acid exhibited an improved FCR compared with those fed diets with FC and 0% formic acid at 48 d. Dietary inclusion of formic acid increased pectoralis minor yield at 43 d ($P<0.05$). These data indicated that up to 0.4% of formic acid can be fed with a coarse corn particle size without negatively affecting broiler performance or carcass characteristics.

Key Words: carcass, coarse, corn, formic acid, performance

M60 Using individual ingredients with Ark NE system in broiler diets Katie Hilton*[GS], Antonio Beitia, Pramir Maharjan, Jordan Weil, Naiwin Suesuttajit, Craig Coon *University of Arkansas*

The classical method of net energy (NE) only assesses the value of heat increment (HI). Analyzing classic NE can be misleading as more calorie efficiency (NE/ME) is given to fat deposition than lean mass deposition. It is therefore desirable to provide an improved process of formulating energy requirements of feed ingredients that overcomes the shortcomings of the classical equation. Experiments were conducted to create the methodology of a new NE system that includes both HI and body composition. Two test diets were developed for each feeding phase with two different samples of SBM evaluated for starter (0-10d, 3,008 kcals/kg, 21%CP), grower (10-22d42, 3,100 kcals/kg, 19%CP) and finisher (22-42d, 3,200 kcals/kg, 18%CP) feeding periods. The highest concentration of lysine/CP/kcal for SBM was utilized as control SBM when evaluating the SBM samples. The control SBM was formulated in a corn soybean diet to provide 80%, 100% and 120% AA requirements for starter, grower and finisher diets with both AA levels and ME set according to the breeder recommendation. The control SBM dictated the formulation inclusion levels utilized for all of the test SBM. Each of the SBM samples were fed equally on a percentage basis as determined for the control SBM. Birds were moved to the respiratory chambers 1d before evaluation for a period of adaptation. Heat production (HP) Kcal=3.872*VO_2 (L/d) + 1.195 VCO_2 (L/d) (Farrell, 1974) was measured for 1d. After HP was measured, fasting heat production (FHP) was measured for 24h. HI was determined HI=HP – FHP (Farrell, 1974). Body composition was measured on throughout the experiments by dual energy X-ray absorptiometry (DEXA) to determine net energy gain (NEg). Simple linear regression indicated a high level of predictability (R^2, P=0.029) for Ark NE with total digestible amino acids as the predictor. The regression model gives the equation Y=13.881x+2707.4, showing an Ark NE value of 2,976 kcals and 3,457 kcals for corn and SBM, respectively. This ongoing study of testing the best regression predictor shows a good relative accuracy to the development of the precision of the Ark NE regression with chosen ingredients. Additionally, utilizing the Ark NE system gives more calories to protein ingredients than traditional energy ingredients.

Key Words: Net Energy, Metabolizable Energy, Ark NE

M61 Influence of coccidiosis vaccination on nutrient digestibility of feed ingredients in broilers Alyson Gautier*[GS], Samuel Rochell *University of Arkansas*

Subclinical infections elicited by coccidiosis vaccination can impair nutrient digestibility in broilers, but the impact on individual feed ingredients is unknown. Two experiments were conducted to determine the impact of coccidiosis vaccination on the apparent ileal digestibility (AID) of nutrients and energy of commonly used feed ingredients in broilers. Eight experimental treatments based on a factorial arrangement of 2 coccidiosis control methods [control group with in-feed diclazuril (CTL) or vaccine (VAC)] and 4 different diets were administered to male Cobb 500 broilers in floor pens (8 pens per treatment) with 12 birds per pen (0.09 m^2/bird). For the vaccinated group, a 3x dose of a live coccidiosis vaccine was given via oral gavage on day of hatch. Experimental diets consisted of a basal diet and 3 test diets in which 30% of the basal diet was replaced with either corn, soybean meal (SBM), or distillers dried grains with solubles (DDGS) to allow for calculation of nutrient digestibility of individual ingredients by difference. Broilers were fed a common diet from 0 to 7 d and experimental diets from 7 to 12 d. On d 12, blood and ileal digesta were collected to measure plasma carotenoids and determine AID of nitrogen, ether extract (EE), energy (experiments 1 and 2), and amino acids (AA) (experiment 2). Vaccination increased ($P < 0.05$) excreta oocyst counts and decreased ($P < 0.05$) plasma carotenoids when compared with CTL birds. No differences ($P > 0.05$) in AID of nitrogen or energy were observed between VAC and CTL birds in either experiment, whereas vaccination decreased ($P < 0.05$) AID of EE for all diets. In experiment 2, the vaccine-induced reductions in AID of EE were greatest for the corn diet and lowest for the DDGS diet. Interactive effects ($P < 0.05$) were observed for AA digestibility, whereby VAC decreased digestibility of all AA in SBM diets but generally increased AA digestibility of DDGS diets, with no impact on corn diets. In conclusion, these data demonstrated that the impact of coccidiosis vaccination on nutrient and energy digestibility varies among ingredients; however, fat digestibility was most consistently impacted, with the greatest reduction observed for corn.

Key Words: coccidiosis, digestibility, vaccination, amino acid, broiler

M62 Supplementation of betaine in diets adequate in choline and methionine improves breast meat yield of broilers under varying experimental conditions Kenia Mitre Herrera*[1GS], Brooke Bodle[1], Franco Mussini[2], Chance Williams[3], Samuel Rochell[1] *[1]University of Arkansas; [2]DuPont; [3]Wayne Farms LLC*

Betaine (BET) is a methyl-donor that has been shown to spare choline and improve live performance and meat yield of broilers. Outcomes in poultry nutrition research are influenced by the number of birds in an experimental unit, number of replications per treatment, and method of bird selection when all birds in an experimental unit can't be feasibly sampled. Two experiments (EXP) were conducted to evaluate dietary BET supplementation on broiler performance and meat yield under varying experimental conditions. In EXP 1, 480 Ross 708 broilers were allocated to 4 treatments based on a factorial arrangement of sex and BET supplementation [0 or 0.2% anhydrous BET (97%)] with 10 replicate pens of 12 birds reared to 41 d and processed at 42 d post-hatch. Methods for selecting processed birds were evaluated post hoc to compare results when 12 birds/pen were selected with those based on 6 or 4 birds selected randomly or with individual weights closest to the mean pen weight. In EXP 2, 384 Cobb 500 broilers were allocated to a factorial arrangement of BET (0 or 0.2%) and pen size [large (2.22 m^2; 24 birds; 4 pens/diet) or small (1.11 m^2; 12 birds; 8 pens/diet)], reared to 46 d, and processed on d 47. Treatments were maintained across starter, grower, and finisher diets containing adequate methionine and choline in both EXP. In EXP 1, BET increased ($P < 0.01$) weight gain and lowered ($P < 0.05$) feed conversion ratio during the starter phase, regardless of bird sex, but did not influence ($P > 0.05$) overall (0 to 42 d) bird performance. Dietary BET increased breast weight in males but not in females, leading to a BET × sex interaction ($P < 0.01$). Bird selection method had no impact ($P > 0.05$) on means of breast meat data, but SEM were decreased by almost half when means were based on 12 birds/pen versus 6 randomly-selected birds/pen. In EXP 2, no effects ($P > 0.05$) of pen size, BET supplementation, or interactions were observed on overall live performance, but CV were lower for large pens than for small pens. Dietary BET increased ($P < 0.05$) breast yield in EXP 2. In conclusion, bird selection method and pen size influenced the variability of selected outcomes, yet dietary BET improved breast muscle accretion of broilers under varying conditions in both experiments.

Key Words: Broiler, betaine, breast yield, pen size, meat yield

M63 Effects of hatch basket feed and water access and hatch window on broiler performance and processing yield Joshua Deines*[1GS], R Bramwell[2], Doug Yoho[1], Samuel Rochell[1] *[1]University of Arkansas; [2]Jamesway Incubator Company*

The objectives of these 2 experiments (EXP) were to investigate the effects of hatch window and feed and water availability in hatch baskets on subsequent broiler performance and processing yield. In both EXP, hatching eggs (Cobb 500) were assigned to control (no nutrient provisions; CTL) or treatment (feed and water provided; FAW) groups and placed in a lighted hatch cabinet at transfer. Chick hatch window (HW) was divided into periods based on hours until pull, translating to hours of access chicks had to feed and water in the baskets (24, 18, 12, and 6 h, EXP 1 or 18, 12, and 6 h, EXP 2). At the end of each HW period, hatched chicks were weighed and placed into corresponding CTL or FAW baskets until time of pull. After pull, 1,352 (EXP 1) or 1,500 (EXP 2) chicks were distributed

in floor pens (1.524 x 3.048 m) in the same house. In EXP 1, there were 13 replicate pens of CTL and FAW groups, with 13 chicks from each HW period in each pen (52 total/pen). In EXP 2, there were 5 replicate pens of 50 chicks for each combination of HW and basket (CTL or FAW). Feed used in FAW baskets was the same crumbled starter diet subsequently fed during rearing. Common starter, grower and finisher feeds and water were provided *ad libitum* from placement to 42 d. Bird weights and feed consumption were recorded weekly by pen. At 43 d, 16 (EXP 1) and 14 (EXP 2) males from each pen were processed. Period of HW influenced ($P < 0.05$) early feed intake, live bird weight, and carcass weights. In EXP 2, weight loss of chicks between hatch and placement was greater for chicks in the CTL than those in FAW group from the early HW period, whereas there was no difference in weight loss between CTL and FAW chicks that hatched during late HW period (interaction: $P < 0.01$). In both EXP, chicks in the FAW group were 1 g heavier ($P < 0.01$) than CTL chicks at placement and remained heavier through 28 d in EXP 1 and 7 d in EXP 2; afterwards, they were similar ($P > 0.05$) in weight. No differences ($P > 0.05$) in feed conversion, mortality, or processing data were observed between CTL and FAW groups in either EXP. These trials indicate that feed and water access in hatch baskets increases the weight of broilers up to 4 wk, but has no influence on processing yield, feed conversion, or livability.

Key Words: broiler, nutrition, early, feeding, hatch

M64 Role of Biocholine on growth performance, hepatic gene expression, and adiponectin in broilers Dima White*[1GS], Woo Kim[1], Shivi Maini[2] [1]*University of Georgia;* [2]*Indian Herbs Specialities Ltd.*

Choline, a water-soluble vitamin and essential for physiological functions, optimum performance and lowering of fat content of liver and body in broiler birds. If choline is inadequate in the diet, the liver fat content and abdominal fat content increases, causing fatty liver syndrome. Choline chloride supports the digestion and utilization of dietary energy in poultry birds. Biocholine, a natural alternative for choline chloride, contains glycerol, phosphatidyl inositol and phosphatidylserine that play significant role in metabolism, enzymic modulation and biosynthesis of phosphatidylcholine that can produce significant growth response. The objective of this study was to evaluate the effects of choline chloride and Biocholine on BWG, FI, FCR, and liver metabolic gene expression in broilers over a 42-day period. A total of 240 1-day old Cobb 500 broilers were randomly allocated into two treatment groups (2 trt x 6 rep x 20 birds/pen). The two treatments were (T1= Synthetic choline chloride 60%, and T2= Biocholine with 35% of synthetic choline chloride). Quantitative real time reverse transcription polymerase chain reaction (qRT-PCR) and Adiponectin ELISA was performed at five weeks of age for assessment of Peroxisome proliferator-activated receptors (PPAR) gene expression in the liver. Results were subjected to a one-way ANOVA using the GLM procedure, with means deemed significant at $P<0.05$. Results indicated no significant difference in FI or FCR, however six-week average body weight in Biocholine group was numerically higher than the choline chloride group. qRT-PCR and ELISA results indicated that Biocholine supplementation leads to hepatic gene expression of PPAR receptors with a 39.03% increase compared to the choline chloride group. The chicken Adiponectin mRNA displayed high expression levels in liver tissues with Biocholine supplementation at 14.61% higher versus what was observed in choline chloride supplemented group. Bioactive of Biocholine and phosphatidyl choline conjugates are agonists of PPARs, therefore Biocholine activates PPAR receptors leading to signal transduction and release of Adiponectin hormone. In conclusion, results indicate that Biocholine can replace synthetic choline chloride in poultry dietary feed.

Key Words: Biocholine, choline chloride, metabolism, lipolysis, broiler

M65 Immunometabolic influences of Salmonella enteritidis and Salmonella heidelberg in chicken macrophages Famatta Perry*[GS], Ryan Arsenault, Casey Johnson *University of Delaware*

It is difficult to identify chickens that harbor *Salmonella* because they are often asymptomatic. The prevention of S*almonella* contaminated chicken products is of significant concern to the poultry industry, as is developing an antibiotic free treatment for *Salmonella* in chickens. To develop an antibiotic free treatment for *Salmonella* infection, it would be beneficial to better understand the immune and metabolic effects of *Salmonella* on host cells. In this study, HD11 chicken macrophages were infected with either *Salmonella* enteritidis (SE) or *Salmonella* heidelberg (SH). A gentamicin treatment was administered to cells for 30minutes, 2hours and 6hours to eliminate extracellular bacteria. To study the intracellular changes in host during *Salmonella* infections, a kinome peptide array analysis was performed to show the phosphorylation states of peptides in the infected cells at the 3 time points. Seahorse Xfp metabolic flux assays were performed to understand the metabolic effect of the two strains of *Salmonella* on macrophages by measuring the oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). Based on the results of kinome analysis, there were significant changes ($p<0.05$) in energy production pathways that indicate an inflammatory state in infected cells. In contrast to SE, SH at 30minutes post-infection induces a glycolytic pathway that is void of PfK-1 but uses enzymes of the pentose phosphate pathway that lead to the production of iNOS, an agent of inflammation. Data analysis also revealed different changes in the phosphorylation of at least 3 sites on the energy sensor protein AMPK during each infection. Analysis of Seahorse metabolic flux results with ANOVA ($P<0.01$) revealed that: (i) SE at 30minutes is more inflammatory than SH; indicated by the increase in ECAR at 30minutes SE infection and the phosphorylation of an apoptosis inducing protein JNK at 30minutes post infection. (ii) SH delays the classic inflammatory response to bacterial infection as indicated by delayed ECAR spike until 2hours post infection. From this data, we conclude that SH is better at manipulating the host immunometabolic responses to increase its chance of survival and SE induces the classic inflammatory response of host macrophages to bacterial infections.

Key Words: Immunometabolism, AMP-activated protein kinase, Kinome peptide array, Salmonella, antibiotic free treatment

M66 Effect of broiler breeder age and different dietary oil sources on gene expression and egg fatty acid profile Antonio Beitia*[1GS], Justina Caldas[2], Nirun Boonsinchai[3], Katie Hilton[1], Pramir Maharjan[1], Nawin Suesuttajit[1], Garrett Mullenix[1], Michael Schlumbohm[1], Judith England[1], Craig Coon[1] [1]*University of Arkansas;* [2]*Cobb-Vantress;* [3]*Charoen Pokphan Group*

As breeders age, they undergo reproductive changes that cause an increase in egg and yolk size, ultimately affecting progeny, hatchability, and growth. Two experiments were conducted. Exp. 1 was utilized to evaluate the effect of breeder age on gene expression. Exp. 2 was utilized to evaluate the effects of three different oil sources fed to aging parent stock hens. Previous research suggests that young breeders may be limiting in providing adequate palmitic or oleic acid to the progeny. Exp. 1: A PCR array gene expression analyses were performed. Yolk sac (YS) and liver were taken from 3 embryos on embryonic d17 from hens at 26 and 35wk of age. Liver and abdominal fat were also taken from 3 hens from same ages. 48 selected genes relating to FA, glucose, and AA metabolism were profiled from each sample. Exp. 2: 1008 pullets were reared in floor pens and later transferred to individual cages. At 24wk, 162 pullets were randomly assigned to 3 dietary treatments (54 hens/trt). All diets were isocaloric at 2925 kcal/kg and contained either a 2.10% fat or oil addition, with T1 (control) containing poultry fat, T2 containing corn oil, and T3 containing canola oil. Egg FA was determined at 29 and 39wk. Exp. 1 the fold regulation comparison was performed between treatment and control groups with a cut off at 2.0 and P-value cut off at 0.05. For Exp. 2 an ANOVA analysis and a chi-square test were performed using JMP Pro 13. In Exp. 1

the top over-expressed genes in YS of the embryo from young hens were PC, GPT2, and LPL with fold regulation of 75.5, 28.0, and 23.9. The top over-expressed genes in the liver of young hens over the control were FA metabolism genes SCD, ELOVL6, and ACACA, with fold regulation of 7.3, 4.5, and 4.4, respectively. In Exp. 2 the C16:1, Δ9; C18:2, ω6, 9; and PUFA's concentrations were higher in eggs from old hens fed T1 ($p<0.05$). C18:2 concentration was higher in young hens fed T2 ($p<0.05$). C18:1 and PUFA's were higher in young hens fed T3 ($p<0.05$). In conclusion, young hens have a lower concentration of C16:1, Δ9; C18:2, ω6, 9; and PUFA's in the eggs in comparison to old hens. Over-expressed genes involved in FA metabolism such as SCD in liver shows that young hens lacked the ability to provide essential FA for optimum embryonic development.

Key Words: Fatty acids, Gene expression, Dietary oils, Broiler breeders, Hen age

M67 Effects of inositol and gradient phytase supplementation on growth performance and meat yield of broilers from 1 to 41 days of age Ruben Kriseldi*[1GS], Carrie Walk[2], Mike Bedford[2], William Dozier, III[1] *[1]Auburn University; [2]AB Vista*

An experiment was conducted to evaluate effects of inositol and gradient phytase supplementation on growth performance and meat yield of broilers from 1 to 41 d of age. A total of 1,920 Yield Plus × Ross 708 male chicks was placed in 64 floor pens (30 birds/pen). Each pen received 1 of 8 dietary treatments (8 replicate pens) from 1 to 15, 16 to 29, and 30 to 40 d of age. A negative control (NC) diet (Diet 1) was formulated to contain 0.16 and 0.15% lower Ca and P concentrations, respectively, than the positive control (PC) diet (Diet 7). Diets 2 to 6 were formulated similar to Diet 1 with phytase addition at 500, 1,500, 4,500, 13,500, and 40,500 phytase unit (FTU)/kg, respectively. Diet 8 was formulated similarly to Diet 7 with inositol addition, which was calculated based on the expected inositol liberation in Diet 6. Feed and birds were weighed at 1, 15, 29, and 40 d of age to determine BW gain, feed intake, and feed conversion. Birds were processed at 41 d of age to determine carcass characteristics. From 1 to 40 d of age, log-quadratic effects of phytase (Diets 1 to 6) were observed for BW gain ($P = 0.002$) and feed conversion of broilers ($P = 0.018$), whereas feed intake increased log-linearly ($P = 0.045$) as phytase addition increased. From 1 to 40 d of age, BW gain of broilers was highly correlated ($r = 0.73$, $P < 0.001$) with feed intake. The addition of 40,500 FTU/kg phytase increased cumulative BW gain ($P = 0.001$) and decreased cumulative feed conversion ($P = 0.005$) of broilers by 4.4 and 3.1%, respectively, compared with birds fed PC diet. Log-quadratic effects of phytase additions were observed for carcass ($P < 0.001$) and breast meat weight ($P = 0.004$). Cumulative growth performance and carcass characteristics of broilers fed PC diet were similar ($P > 0.05$) to birds fed Diet 8, which indicated that inositol addition did not alter growth rate and meat yield. These data demonstrate that the benefits of phytase addition in increasing BW gain of broilers may be associated with increased feed intake in addition to phytase effects of increasing phosphorus liberation, amino acid digestibility, and apparent metabolizable energy. Inositol supplementation did not provide additional benefits to growth performance or meat yield of broilers in this study.

Key Words: Broiler, Phytase, Inositol

M68 Impact of calcium to available phosphorus ratio and phytase calcium matrix values on broiler live performance Shuja Majeed*[1GS], Rasha Qudsieh[1], Jon Broomhead[2], Mike Lanahan[2], John Brake(†)[1] *[1]North Carolina State University; [2]Agrivida*

Determining the correct calcium to available phosphorus ratio (Ca:AvP), with or without phytase addition, is important for optimal broiler growth. A 35 d broiler trial was conducted to determine the live performance effects of either decreasing Ca:AvP ratio as bird ages, without phytase, or increasing Ca matrix credit when feeding phytase. A total of 1152 d-old male birds were assigned to six dietary treatments (Diets A-F) with 16 birds per pen and 12 replicate pens per treatment. Diet A and B containing no phytase and 0.5% or 0.45% AvP during the starter (0 – 15 d) or grower (16 – 35 d), respectively. Diet A was 2:1 Ca:AvP, continuously, where diet B changed from 2:1 (starter) to 1.85:1 (grower) Ca:AvP. The remaining diets contained 3000 FTU/kg phytase, with diet C being the same as diet A but with phytase added on top. Diets D, E, and F are based off diet A with AvP reduced by 0.12% and Ca reduced by 0.11, 0.15 or 0.17%, respectively. Body weights (BW) were measured at 15, 28, and 35 d and feed intake (FI) measured within each of the periods. Body weight gain (BWG) and feed conversion (FCR) was calculated for each period and overall. Data was analyzed as one way ANOVA using GLM of SAS. No live performance differences ($P > 0.05$) were observed between birds fed diets A or B. With the exception of 28-35 d BWG being similar ($P > 0.05$) between birds fed diet A and F, birds fed phytase had higher ($P < 0.05$) early (0-15 and 16-28 d) and overall BW, BWG and FI compared to those fed diet A or B. Assigning different Ca matrix values, within phytase treatments, had no effect ($P > 0.05$) on live performance measurements. No significant differences ($P > 0.05$) in FCR was observed during 0-15 d and 16-28 d. During 29-35 d, using 0.15% Ca and 0.12% AvP matrix credit or no Ca and AvP credit with phytase improved ($P < 0.05$) FCR compared to Diet A and B. Overall (0-35 d) FCR was improved for all phytase supplemented diets compared to Diet A. Results demonstrate that adding phytase improved live performance and reducing Ca:AvP ratio as the bird ages did not affect performance in diets without phytase. In addition, with a 0.12% AvP matrix value, adjusting Ca matrix values between 0.11 and 0.17% did not have a large effect on animal performance when 3000 FTU/kg phytase was fed.

Key Words: Broilers, Calcium, Phosphorus, phytase matrix value

M69 Effect of a combination of phytase and multi-carbohydrase on broiler growth performance and bone mineralization Jinquan Wang*[1GS], Robert Patterson[2], Woo Kim[1] *[1]University of Georgia; [2]Canadian Bio-system*

An experiment was conducted to evaluate the effect of phytase and multi-carbohydrase (MC) on growth performance and bone ash in broilers. A total of 480 Cobb 500 male broilers were randomly allocated to eight experimental diets with six replicates as 10 birds per replicate from 0 to 18 d of age with a completely randomized design. Treatments consisted of 1) a positive control diet (PC) to meet the Cobb nutrition recommendations based on corn and soybean meal; 2) a negative control diet (NC) with a reduction of 75 kcal/kg ME, 1.5% of crude protein, and 0.15% of Ca and avP; 3) NC plus 500 FTU/kg of phytase; 4) NC plus 1,000 FTU/kg of phytase; 5) NC plus 1,500 FTU/kg of phytase; 6) NC plus a combination of MC at 500g/ton with 500 FTU/kg of phytase; 7) NC plus a combination of MC at 500g/ton with 1,000 FTU/kg of phytase; and 8) NC plus a combination of MC at 500g/ton with 1,500 FTU/kg of phytase. Body weight, feed intake, feed conversion ratio was recorded at d7, d14 and d18. Data was subjected to SAS using one-way ANOVA following GLM procedure. Significance level was set at $P<0.05$. Means were separated using Duncan's Multiple Range Test. For growth performance, adding a combination of phytase at 500, 1,000 and 1,500 FTU/kg and MC increased ($P<0.05$) BW and BWG compared to the NC at d 18. A reduction of 75 kcal/kg ME, 1.5% of crude protein, and 0.15% of Ca and avP (NC) increased ($P<0.05$) FCR compared to PC during d 0-18 of age. Supplementation of phytase at 1,500 FTU/kg or a combination of phytase at 500 FTU/kg and 1,500 FTU/kg decreased ($P<0.05$) FCR compared to the NC, and it reached the same level with the PC during d 0-18 of age. For bone ash, a reduction of nutrients (NC) decreased ($P<0.05$) the ash weight and ash percentage. Supplementation of phytase at 1,000 and 1,500 FTU/kg with MC increased ($P<0.05$) the ash weight compared to the NC and it reached to the same level with the PC. Supplementation of phytase or a combination of phytase and MC increased ($P<0.05$) ash percentage compared to the NC and it reached to the same level with the PC. In conclusion, supplementation of a

combination of phytase and MC could compensate the decrease on growth performance and bone ash caused by the nutrient reduction.

Key Words: Broiler, Phytase, Carbohydrase, Growth performance, Bone mineralization

M70 Effects of limestone particle size, limestone source, and phytase on Ca and P digestibility in broilers Stuart Taylor*[1,2GS], Micaela Sinclair Black[1], Roselina Angel[3], Christine Jansen van Rensburg[2], Peter Plumstead[1] *[1]Chemunique PTY LTD; [3]University of Maryland; [2]University of Pretoria*

Standard industry practice is to formulate broiler diets to a total Ca and available or digestible P. The limitation with this is that inherent characteristics of the limestone source (LS) and particle size (PS) of limestone added to feed may affect phytate P utilization and dietary Ca and P digestibility. The objective of this study was to determine how LS and PS affected Ca and P digestibility with and without the use of phytase from *Buttiauxella* spp. Two groups of 600 Ross 308 male broilers were fed experimental diets in two blocks from days 19-21 and 23-25, respectively, in order to determine the effects of LS, PS, and phytase on apparent ileal digestibility (AID) of Ca and P. The experiment was a 3x2x2 randomized block design with 3 LS (A, B, C), 2 PS (0.8mm and Commercial) and 2 phytase levels (0 and 1000 FTU/kg feed). The particle size and solubility of limestone was determined as per method of Kim *et al*, 2019. A basal corn/soy-based diet with no added inorganic P was mixed to which limestone and phytase was added to make 12 treatments (Trt). The experiment was repeated twice (block) with 5 cages per Trt per block. Each block consisted of 100 cages with 6 birds/cage. Digesta from the distal half of ileum was collected, freeze dried, and analyzed for Ca, P, and Cr marker to determine the AID of Ca and P. Data was analysed as a full factorial using Proc Mixed (SAS Institute 2012) with block included as a random effect. Results showed AID of Ca was altered by LS and phytase (P<0.01), with no interaction of LS, PS and phytase. A significant three-way interaction of LS, PS, and phytase was observed for AID of P. In the absence of phytase, a larger limestone PS consistently increased AID of P. With phytase added, the effect of PS on AID of P differed between LS. The increment in AID of P from added phytase was also dependent on the LS (P<0.01). Effects of LS and PS on P digestibility were significantly correlated with *in-vitro* solubility of limestone. These data suggest that LS and phytase can alter the utilization of total dietary Ca by broilers. Further, since LS and limestone PS altered the increment in AID P from added phytase, the characteristics of limestone should be considered when assigning matrix values to phytase in practical broiler diets.

Key Words: Limestone, Calcium, Phosphorus, Phytase, Broiler

M71 Effects of superdosing phytase and use of carbohydrase enzymes in low energy diets on 56 day male broiler performance and processing Courtney Ennis*[1GS], Mark Jackson[2], Omar Gutierrez[2], Staci Cantley[2], Kelley Wamsley[1] *[1]Mississippi State University; [2]Huvepharma, Inc.*

Combined enzyme strategies are being investigated to further the efficacy of commonly used phytase and carbohydrase enzymes for optimal broiler performance. The objective of this study was to determine the effects of phytase dose (PD; *E. Coli* derived, 6-phytase) and carbohydrase enzyme (CE) on d 0-56 broiler performance and processing. This study utilized a 2 PD (250 or 1500 FTU/kg (superdose (SD)) x 3 CE (xylanase (X), mannanase (M), or their combination (XM)) factorial arrangement. All vegetable basal diets were formulated to 95% of breeder recommendations, with Ca and aP reduced an additional 0.145%, as well as AME reduced 100 kcal/kg beyond breeder recommendations. Male Ross × Ross 708 chicks were equally dispersed as a RCB to 60 pens (23 birds/pen; 0.265 m^2/bird) with 10 replications for each diet. On d 14, 28, 44, and 55, variables of BW, BW gain (BWG), feed conversion ratio (FCR), feed intake (FI), and adjusted FCR (aFCR) were recorded. On d 45 and 56, 4 birds/pen were selected for processing. Birds fed SD demonstrated a 2-3 pt improvement in d 0-44 FCR and aFCR as compared to those fed 250 FTU/kg (P<0.03). Additionally, for the main effect CE, d 0-44 FCR and aFCR benefits were observed for birds fed CE-X (P<0.04). No PD effects were found for d 55 BW, 0-55 BWG, FCR, or aFCR (P>0.05); however, similar CE effects as those from d 0-44 were established from feeding CE-X for d 0-55 FCR and aFCR (P≤0.03). For d 0-44 and 0-55, the main effect of CE was significant for FI, BWG, and BW; generally, performance was enhanced with the inclusion of CE-X (P<0.05). While not significant, d 45 processing showed that SD reduced fat pad weight (P=0.09) and increased tender weight (P=0.06); however, when these data were based on yield relative to carcass weight, it became significant (P<0.05). Also at d 45, feeding CE-X increased drumstick weight (P=0.10). While not significant, d 56 processing data demonstrated that birds fed diets containing CE-X had numerically increased wing, tender, thigh and drumstick weights (P<0.15). Day 56 yield data was not significant (P<0.05) or trending (P>0.15). These data demonstrate clear live performance and some processing benefits of feeding diets with CE-X and SD; however, more research is needed using different formulation strategies.

Key Words: exogenous enzymes, phytase, carbohydrase, broiler performance, processing yield

M72 Effects of dietary lysine and exogenous protease inclusion on turkey growth performance. Courtney Truelock*[1GS], Christopher Delfelder[1], Miguel Barrios[2], Charles Stark[1], R Beyer[1], John Gonzalez[1], Chad Paulk[1] *[1]Kansas State University; [2]Jefo*

A 42-d experiment evaluated the effects of dietary lysine level and exogenous protease inclusion on turkey growth performance. Female poults (Hybrid Genetics, Ag Forte LLC, Aurora, MO; n = 780) were weighed and randomly assigned to 1 of 32 floor pens with 24 or 25 poults per pen. Poults were maintained on a 24-hr lighting schedule in an environmentally controlled room with *ad libitum* access to feed and water. Poults were randomly assigned to 1 of 4 treatments within location block for a total of 8 replications per treatment. Treatments were arranged in a 2 × 2 factorial of standardized ileal digestible (SID) lysine and commercial protease. Poults were fed a starter diet from 0 to 28 days and a grower diet from 29 to 42 days. Starter diets were formulated to provide 1.52% and 1.62% SID lysine for the low and adequate SID lysine treatments, respectively; grower diets were formulated to provide 1.39% and 1.49% SID lysine, respectively. Exogenous protease was added to the protease diets at 125 g/tonne, and the same inclusion of sand was added to the diets without protease. Diets were balanced by energy and had equal amino acid ratios. Pen weights and feed consumption were recorded on d 14, 28, and 42 for calculation of BW, ADFI, and FCR. Data were analyzed using the GLIMMIX procedure in SAS 9.4 with pen as the experimental unit and pen location as the blocking factor. There was no evidence of a lysine level × protease interaction (P > 0.12) for BW, ADFI, or FCR. From d 0 to 42, poults fed adequate Lys had greater (P < 0.01) BW and ADFI and improved (P = 0.01) FCR compared to poults fed decreased Lys concentrations. From d 0 to 28, there was no evidence of difference (P > 0.43) in poult performance due to protease inclusion. From d 29 to 42, poults consuming protease tended to have greater (P = 0.06) final BW and greater (P = 0.02) ADFI than those not consuming protease. There was no evidence of difference (P = 0.53) in FCR due to protease inclusion in the grower phase. Overall, greater SID lysine increased growth performance in poults up to 42 d of age and there was no benefit of protease inclusion. However, poults fed protease had improved BW and ADFI during the grower phase.

Key Words: exogenous protease, growth performance, lysine, turkey

M73 Effects of xylanase and xylo-oligosaccharide application in corn-soy diets on performance of male broilers fed diets with two different corn sources Kyle Brown*[1GS], Austin Jasek[1], Gemma González-Ortiz[2], Craig Wyatt[3], Tri Duong[1], Jason Lee[1] *[1]Texas A&M University; [2]AB Vista; [3]AB Vista NAM*

The objective of the current experiment was to evaluate the inclusion of xylanase and a fermentable xylo-oligosaccharide (XOS), separately and in combination, in corn-soy based broiler diets with two different corn sources. Cobb 500 male broiler chicks (3,240) were placed in twelve experimental treatments, with a 2 (corn source) x 2 (xylanase) x 3 (XOS) factorial design. Each treatment had ten replicate pens with 27 chicks/pen. Starter (d 0-17), grower (d 17-32) and finisher (d 32-42) diets, and water were available *ad libitum*. Statistical comparisons were performed using a 2 x 2 x 3 factorial ANOVA. If significant interactions were present, a one-way ANOVA was used to determine differences (P≤0.05) between individual treatment means. Main effect and treatment means were separated by Duncan's Multiple Range Test. Multiple interactions were observed between factors for body weight (BW) and mortality corrected feed conversion ratio (FCR), mainly present between xylanase inclusion and corn source. No three-way interactions were observed between corn source, xylanase, and XOS; as well as no interactions between corn source and XOS. On d 18, the inclusion of xylanase increased (P<0.05) BW and decreased (P<0.05) FCR regardless of corn source. Increasing levels of XOS increased (P<0.05) grower feed consumption (FC) which led to increases in d 33 BW regardless of xylanase inclusion or corn source. During the grower phase, impact of enzyme inclusion was dependent on corn source as FCR was improved with corn 2, with no effect observed for corn 1. Differences in finisher FC were observed with corn source, however this had no impact on BW at 42 d. From d 0-33 the inclusion of xylanase improved (P<0.05) cumulative FCR in corn 1, however, had no effect on corn source 2. At the conclusion of the experiment, the inclusion of xylanase reduced (P<0.05) FCR as compared to non-enzyme supplemented treatments, as well as, cumulatively through d 42 (P=0.006). These data continue to highlight the ability of xylanase to positively impact early BW and improve FCR in corn/soy diets.

Key Words: xylanase, xylo-oligosaccharide, corn, broiler, performance

M74 Administration of refined functional carbohydrate with yeast culture improves growth performance and reduces pathogen colonization in broiler chickens Lindy Froebel*[1GS], Sangita Jakular[2], Theresia Lavergne[2], Jason Lee[1], Tri Duong[1] *[1]Department of Poultry Science, Texas A&M University; [2]Arm and Hammer Animal and Food Production*

Prebiotics are potentially important alternatives to antibiotic growth promoters in poultry production because of their beneficial health effects. The administration of prebiotic Refined Functional Carbohydrates (RFC) derived from *Saccharomyces cerevisiae* has been demonstrated to improve animal health, growth performance, and microbial food safety in poultry. In this study, we investigated the administration of a prebiotic product composed of RFCs with yeast culture in broiler chickens. Broilers were fed rations supplemented with a low (50 g t^{-1}) or high (100 g t^{-1}) dose of prebiotic and administered untreated or prebiotic-treated water in the final 72 h of production, while untreated or BMD-treated feed and untreated water served as controls. Growth performance was evaluated through 42 d of production; gastrointestinal microbiota were evaluated at d 42 and 8-h after feed withdrawal, and litter microbiota were evaluated at d 0 and 8 h after feed withdrawal. Growth performance and bacterial counts were analyzed using ANOVA, and pathogen prevalence was analyzed using the χ^2 test. Administration of the high prebiotic dose increased final BW (P = 0.002) by up to 376 g, and administration of either prebiotic dose improved ADG when compared to the control diets (P = 0.033). A significant main effect of prebiotic dose on final BW (P = 0.002), finisher phase ADG (P = 0.004), and finisher phase FI (P = 0.012) was observed, with the high dose improving each performance measure. Administration of the prebiotic in water over the final 72 hours of production was not observed to have a significant main effect. Prebiotic administration decreased counts of *Campylobacter jejuni* at d 42 (P = 0.012) in comparison to the control groups. Although the effects were not significant, *Campylobacter* prevalence in the cecum after feed withdrawal was 17 % lower when broilers were administered the high prebiotic dose as compared to the other treatments and up to 50 % lower in the litter when broilers were administered prebiotic as compared to the control treatments. These data suggest that administration of RFCs with yeast culture as a dietary prebiotic can be used to improve growth performance and reduce human foodborne pathogens in poultry.

Key Words: refined functional carbohydrates, prebiotics, Campylobacter

M75 Effects of the combination of mannan-oligosaccharides and β-glucan on growth performance, intestinal morphology and immune gene expression in broilers Po-Yun Teng*[1GS], Roshan Adhikari[2], Woo Kim[1] *[1]University of Georgia; [2]Kerry Inc.*

The study was conducted to evaluate the effects of the combination of mannan-oligosaccharides (MOS) and β-glucan on growth performance, intestinal morphology and immune gene expression in broilers. A total of 640, 1-day-old male Cobb 500 broilers were randomly allocated into 32 pens with 20 birds per pen. Thirty-two pens were divided into four treatments, including control, 0.04% MOS, 0.002% β-glucan, and a combination of MOS and β-glucan with eight replicates per treatment. Performance of birds (FI, BWG, FCR) was measured on d 14, 28, and 35. The ileum, and cecal tonsils were collected from one bird per pen at 21 and 35 day of age for further analyses of immune gene expression. The duodenum, jejunum, and ileum were collected for intestinal morphology at d 35. Data were analyzed as the PROC GLM in SAS (9.4) and the Duncan test was used to separate means with significance levels at $P \leq$ 0.05. The β-glucan and the combination of MOS and β-glucan treatments both significantly increased BWG compared to the control group at d 14 (P< 0.05), but only the β-glucan group had higher BWG than the control group at d 28 (P< 0.05). The results of ileum gene expression showed that Interleukin-10, an anti-inflammatory cytokine, was significantly higher in birds fed the combination of MOS and β-glucan than the control group at d 21 (P< 0.05). Moreover, dietary supplementation of MOS and β-glucan significantly up-regulated gene expression of Interferon-γ in the cecal tonsils at d 21 (P < 0.05). However, there is no significant difference in growth performance, intestinal morphology, and immune gene expression among the treatments at d 35. In conclusion, the diets supplemented with β-glucan or combination of MOS and β-glucan increased body weight gain in starter and grower periods as well as up-regulated immune gene expression in the ileum or ceca tonsils of 21-d old broilers.

Key Words: Mannan-oligosaccharides, β-glucan, prebiotic, broiler

M76 Pellet mill die feed retention and enzyme thermostability. J. T. Pope*[1GS], Joel McAtee[2], Paul Steen[2], Adam Fahrenholz[1] *[1]North Carolina State University; [2]AB Vista*

An experiment was conducted to investigate the impact of pellet mill die feed retention on enzyme thermostability. The experiment was a 4 x 2 factorial arrangement with 4 pellet mill throughputs (PMT; 227, 454, 908, and 1816 kg/hr) and 2 mash conditioning temperatures (CT; 75 and 86°C). A 1910 kg batch of a broiler starter diet was blended, conditioned for 30 s, and then pelleted under the interaction of the aforementioned factors and the process was replicated three times. Unconditioned mash, conditioned mash, and pellet samples were obtained over a three-minute period of pelleting for each interaction and were then blended into composite samples for determination of phytase and xylanase activity. Each pellet sample was subjected to pellet durability testing after cooling utilizing a Holmen pellet durability tester for a duration of 30 s to determine pellet durability index (PDI). None of the applied factors affected xylanase recovery, which indicated that the xylanase utilized was thermostable. There were no significant differences in the level of phytase recovered in conditioned

mash relative to unconditioned mash, which indicated that neither PMT nor CT affected phytase denaturation in the conditioner. However, a significant interaction between PMT and mash CT was observed with regards to phytase recovery in pellets relative to unconditioned mash (P:UCM; $P \leq 0.01$). The lowest phytase recoveries in P:UCM were observed when diets were conditioned at 86°C at the slowest PMT (227 kg/hr). However, when diets were conditioned at 86°C at the fastest PMT (1816 kg/hr), the phytase recoveries in P:UCM were statistically similar to all the diets pelleted at 75°C. A significant inverse association between pellet durability index and phytase recovery in P:UCM was observed when the mash CT was 86°C (R^2=0.96; $P \leq 0.01$). These data indicated that some enzymes may be sensitive to the residence time within the pellet mill die and that poor recoveries may be alleviated by increased PMTs.

Key Words: phytase, xylanase, thermostability, pellet durability index

M77 Replacement of antibiotic growth promoters by a combination of eubiotics and an enzyme blend in broilers diets under commercial conditions. Oscar Sanabria-Bernal*[1GS], Carlos Lozano-Poveda[2], Javier Bohórquez-Quevedo[3], Douglas Korver[1] *[1]University Of Alberta; [2]DSM Nutritional Products Colombia S.A; [3]Avícola San Isidro S.A. Avisid*

Antibiotic growth promoters (AGP) have been used in poultry diets for many years; however, the poultry industry needs new alternatives due to changing demands. Our objective was to evaluate the effect of a combination of eubiotics (additives that promote a healthy balance of the microbiota in the gastrointestinal tract) and a mixture of enzymes as an alternative to AGP under commercial conditions. A total of 2,066,000 broiler chickens (51% Cobb 500 - 49% Ross AP) were housed in a completely randomized design in 56 environmentally-controlled houses (experimental unit), distributed across 6 farms in the province of Guayas, Ecuador. A four-phase feeding program (pre-starter, 0-7d; starter, 8-20d; grower, 21-35d; and finisher, 36d-end of the cycle) in mash form was used. Diets were based on corn, soybean meal, oats, and rice bran. The treatments were: Control (T1): AGP + butyrate + prebiotic (enzymatically hydrolyzed yeast walls) + phytase (1,000 FYT/Kg); Treatment 2 (T2): T1 without AGP, but with phytase increased to 2,500 FYT/Kg + probiotic B (*Bacillus subtilis* and *Bacillus licheniformis)*; Treatment 3 (T3): T2 without butyrate or prebiotic. The basal diet was the same in each treatment and included probiotic A (*Enterococcus faecium*NCIMB 10415), essential oils (thymol, eugenol, piperine), enzymes (xylanase (2,000 FXU/g), amylase (600 KNU/g), and protease (75,000 PROT/g)). Feed conversion ratio, body weight, feed intake, European efficiency factor, viability, carcass yield, footpad lesion score, intestinal integrity index, litter moisture, and total litter nitrogen were measured. Data were analyzed using one-way ANOVA with a P<0.05 level of significance. There were no effects of treatment on productive performance(P> 0.1). Viability tended (P = 0.1052) to be greater in T3 (97.24%, SEM 0.160) compared to the control (T1: 96.74%, SEM 0.165). There were no differences between the treatments on carcass yield, intestinal integrity index, footpad lesion score, nor litter humidity and total nitrogen (P> 0.1). Because there was no detrimental effect of the T2 or T3 treatments, it is feasible to replace AGP with one of the proposed strategies (T2 or T3) under commercial conditions without causing harmful effects on bird health and productive performance.

Key Words: Broilers, Production, Antibiotic growth promotor, Eubiotics, Field trial

M78 The effect of dietary inclusions of guanidinoacetic acid on broiler performance and carcass yield Nathaniel Barrett*[1GS], Paul Patterson[1], Fausto Solís[2], John Boney[1] *[1]The Pennsylvania State University; [2]Wenger Feeds*

Creatine is a central constituent in energy metabolism, especially in those cells with variable energy demand, such as muscle cells. Animal proteins such as fish, poultry, and meat meals may provide adequate amounts of creatine when included in diet formulation. The objectives of this experiment were to describe the broiler performance and carcass yield effects when guanidinoacetic acid (GAA) was added as a top dress to broiler diets containing either animal protein or non-animal protein ingredients. Treatment structure consisted of a 2x2 factorial arrangement varying in basal ingredients (conventional (CON) or non-animal protein (NAP)) and GAA inclusion (0 g/ton or 600 g/ton) in a randomized complete block design. Treatments were applied to 12 replicate pens of 36 Hubbard x Cobb 500 straight-run broilers from hatch through d42. One pen of broilers represented the experimental unit. Broilers were provided crumbles during the starter (d1-21) and pellets throughout the grower (d22-35) and finisher (d36-42) periods. Body weight was measured on days 0, 21, 35, and 42. Feed intake, live weight gain and mortality corrected feed conversion ratio (FCR) were calculated over the feed phase periods. On d42 broilers were weighed by pen and the pen nearest the treatment average pen weight was selected for yield determination. Overall period (d1-42) results indicate 0.019 FCR improvement when GAA was included to broiler diets (P=0.0024). Similar results were apparent in each feed phase period (P<0.05). A spike in d22-35 mortality likely contributed to overall mortality differences where mortality increased when broilers were provided the NAP diet (P=0.0105) compared to those provided the CON diet. Carcass yield results indicate a 45-g increase in breast weight when GAA was included in broiler diets (P=0.0354). Basal ingredients and GAA inclusion effects interacted affecting d42 breast yield (P=0.0443). The inclusion of GAA in NAP diets did not affect breast yield; however, breast yield was reduced by 1.78 percentage points when broilers were provided the CON diet devoid of GAA. In conclusion, GAA has the potential to improve broiler performance metrics per se, as well as improving breast yield when included to broiler diets with conventional animal protein sources.

Key Words: Broiler, Performance, Yield, Creatine, Guanidinoacetic acid

M79 Dietary methylsulfonylmethane supplementation and oxidative stress in broiler Muhammed Shameer Abdul Rasheed*[1GS], Maci Oelschlager[1], Brooke Smith[1], Laura Bauer[1], Rose Whelan[2], Ryan Dilger[1] *[1]University of Illinois; [2]Evonik Nutrition & Care GmbH*

An experiment was conducted to study the effects of feeding methylsulfonylmethane (MSM) to broilers exposed to diet-induced oxidative stress on tissue MSM distribution, growth performance, oxidative stress biomarkers, and immune responsivity. A total of 528 birds were allocated 12 birds per brooder battery cages, to 11 replicate cages per treatment with four dietary treatments. A two by two factorial design was used to test the effects of MSM (0.00 or 0.05% inclusion) in diets with 5% inclusion of either fresh or oxidized oil. Growth performance was calculated, and blood and tissue samples were collected at d 7, 14, and 21 to analyze MSM concentrations, and oxidative stress biomarkers including TBARS, total antioxidant capacity (TAC), total glutathione (TGSH), glutathione peroxidase (G-Px) and glutathione reductase (GR) activities. Blood samples were collected at d 25 (end of the study) to quantify T-cell (TC) populations using flow cytometry. Overall, MSM was quantified in all tissues and plasma samples of MSM-treated groups at all time-points. Oxidized oil reduced ($P = 0.006$) feed intake over the –21-d feeding period, but MSM did not affect growth at any time-point. No effects ($P > 0.2$) of MSM or oil type were observed on TC populations. In the presence of oxidized oil, MSM reduced ($P = 0.013$) plasma TBARS and increased ($P = 0.02$) liver GPx at d 21, and increased ($P = 0.06$) liver GR at d 7. Irrespective of dietary oil type, groups supplemented with MSM had higher ($P < 0.05$) TAC in plasma at d 7, and liver GPx (d 21), and liver GR (d 7) compared with groups not receiving MSM. In conclusion, 0.05% dietary MSM supplemented may partially protects birds from oxidative stress, but did not affect TC populations.

Key Words: methylsulfonylmethane, oxidative stress, immunity, feed additive, broiler

M80 The effect of exogenous enzyme supplementation on performance and AME in broiler chickens challenged with coccidia vaccine, 7- and 14-days post challenge Andrew Dunaway*[GS], Sunday Adedokun *University of Kentucky*

The purpose of this study was to examine the effect of exogenous enzyme supplementation (xylanase, β-glucanase, and pectinase) to a corn-SBM (CS) and a wheat-CS-based (WCS) diets on birds that were challenged with coccidia vaccine (Coccivac B-52™). A total of 448 Cobb500 male broilers were used in this study. A CS-based starter diet that met or exceeded nutrient and energy requirements of the birds was fed from d 0 to 14. On day 14, birds were weighed individually and allotted in a completely randomized design with a 2x2x2 factorial arrangement of treatments. The treatments were 2 diets (CS or WCS), 2 levels of enzyme (0 vs. 10%), and 2 levels of coccidian vaccine challenge (CVC, 0 vs 20x). The CVC birds were orally gavaged with 0.6 mL of either distilled water or distilled water containing 20x of coccidia vaccine. On days 21 (4 birds/cage) and 28 (4 birds/cage), ileal digesta from the distal two-third of the ileum while excreta was quantitatively collected on day 19, 20, and 21, and on days 26, 27, and 28. Ileal digestibility and total tract retention of N, energy, as well as apparent metabolizable energy (AME) of the diets were determined. Individual birds and feed were weighed on days 14, 21, and 28 for determination of performance. Jejunal digesta from the heaviest bird within each cage was collected for digesta viscosity determination. Statistical analysis was performed using Proc GLM of SAS, with alpha set at ≤ 0.05. Feed intake (FI) of birds fed the WCS-based diet was significantly higher from day 14-21. Coccidia vaccine challenge reduced ($P < 0.05$) BW gain, FI, and FE (day 14-21). However, FE of the CVC birds was higher ($P < 0.05$) than the control unchallenged on day 21-28. On day 21, significant interaction between diet, CVC, and enzyme supplementation was observed for N and energy digestibility, as well as AME and AMEn. The effect of viscosity was significant for diet, CVC, and enzyme, and was decreased ($P < 0.05$) in birds fed WCS. By day 28, viscosity was again significant for the main effect of diet, challenge, and enzyme, and was again lowered in birds fed WCS. This study confirms that birds challenged with coccidia have decreased ileal viscosity and decreased performance, but are able to compensate for the losses in performance.

Key Words: Broiler Chicken, Coccidiosis, Enzyme, Wheat, Corn

M81 Effect of feeding pronutrient (Alquernat Nebsui L) on hen day egg production, egg quality and small intestine histomorphology of laying hens. Milan Sharma*[1GS], Carlos Domenech[2], Pratima Adhikari[1] *[1]Mississippi State University; [2]Biovet S. A*

An experiment was conducted to determine the effect of a phytogenic compound (Alquernat Nebsui L) applied through drinking water on hen day egg production (HDEP), egg quality, and small intestine histomorphology of 65 weeks-old Hy-Line W36 laying hens. The hens were allowed to adapt for two weeks before the start of the research after which the trial lasted for 9 weeks. A total of 90 hens were randomly allocated into two treatment groups with 15 replicate cages and each cage containing 3 hens. Hens were housed in the conventional cage with *ad libitum* commercial layer feed and water, with 16 hours light period. The two treatment consisted of either with or without a water additive, Alquernat Nebsui L provided via drinking water (0.5 mL/ liter). The HDEP, egg quality, and small intestine histomorphology data were analyzed using PROC GLM procedures of SAS 9.4 and *P* value less than 0.05 was considered as statistically significant. Average HDEP (%) was found significantly higher in the group supplemented with Alquernat Nebsui L for weeks 1, 2, 3, 4, 6 and 9 (all $P < 0.05$). Haugh unit was found significantly higher for Alquernat group at weeks 1, 5, 6 and 8 ($P < 0.05$). There were no significant differences between other internal egg quality parameter. No significant difference was found on villi height (VH), crypt depth (CD), and ratio of villi height to crypt depth (VLCD) of duodenum, jejunum or ileum. However, villi width (VW) in duodenum was observed significantly higher (56.35 vs. 46.95 µm, $P < 0.05$) in treatment compared to the control group. There was no significant difference in relative intestinal length (as a fraction of body weight) in either the Alquernat Nebsui L or control group. The total weight of small intestine was higher in control compared to treatment group (55.70 vs. 54. 65 gm, $P < 0.05$). The result of the experiment suggests that Alquernat Nebsui L can be used in laying hens to increase the HDEP % and HU without any negative effect. In comparison to control group, VW of duodenum was found higher thus increasing surface area for nutrient absorption, and total intestinal weight was lower compared to control, which indicates that the additive might increase intestinal efficiency.

Key Words: laying hens, small intestinal histomorphology

M82 Effects of FortiShell® on performance and egg shell quality of Hy-Line W-36 laying hens under late egg production (46 to 70 weeks of age) Kolby Foltz*[1GS], Nicholas Evans[2], Michael Persia[1] *[1]Virginia Tech; [2]PMI Nutritional Additives*

An experiment was conducted to determine the effects of supplementing laying hen diets with FortiShell® (FS) on post-peak egg production and shell quality. The FS feed additive is a multi-component supplement comprised of bioavailable vitamins, minerals, and patented medium chain fatty acids. Diets were corn, soybean meal, and corn DDGS-based and treatments included no feed supplement (control) or FS supplementation (2 lb/ton) beginning at 46, 54, or 58 wk of age for a total of 4 treatments. In total, 432 Hy-Line W-36 laying hens were randomly placed in high rise 3-tier cages with 3 birds per cage (0.05 m^2/bird) and two adjacent cages served as one experimental unit (EU) with 18 EU/treatment. Hens were control fed 97 g/hen/day and egg production was recorded daily, with performance data summarized over 2-wk periods. Every 4 wk, eggs (9/EU) were collected over two consecutive days to determine egg breaking strength, shell weight, and shell thickness. Data were analyzed by repeated measures ANOVA using the MIXED procedure in SAS 9.4 and LSMEANS were separated using Tukey's HSD with alpha level set at 0.05. Additionally, pre-planned contrasts were used to compare hens fed the control diet vs those fed the diet supplemented with FS for all phases (wk 46-70). Hens fed FS for all phases had higher ($P \leq 0.02$) hen-day and hen-housed egg production than hens fed the control diet (77.8 vs 75.2% and 77.8 vs 74.6%, respectively). There were no differences ($P > 0.05$) in body weight or feed intake between treatments, but egg weights (g/egg) of hens fed the control or FS diet beginning at wk 54 were 1.0-1.2 g larger ($P \leq 0.01$) than those fed FS at all phases or FS beginning at week 58. Egg mass and feed efficiency were similar ($P > 0.05$) among all treatments. Egg shell breaking strength was 5.6% higher (4.12 vs 3.90 kgf; $P = 0.02$) for hens fed FS for all phases compared to those fed the control diet, while hens fed FS beginning at 54 or 58 weeks were intermediate (4.07 and 4.02 kgf, respectively). No differences ($P > 0.05$) between treatments were observed for shell thickness or shell weight. In the current experiment, long term supplementation of FS in laying hen diets improved egg production and egg shell breaking strength compared to hens that were not supplemented with FS.

Key Words: post-peak egg production, egg breaking strength, shell quality

M83 Dietary supplementation of an antioxidant (EconomasE™) to ameliorate the effect of stressors on physiological parameters, intestinal morphology, intestinal integrity, and cellular immune response in broilers Opeyemi Olojede*[1GS], Sunday Adedokun[1], Anthony Pescatore[1], Tuoying Ao[2] *[1]University of Kentucky; [2]Alltech Inc.*

There are multiple physiological responses to stress, often quantified by an aggregate of parameters whose biological importance is crucial to understanding the role of stressors. In the present study, the effect of dietary supplementation of a commercial algae-based antioxidant, containing Se yeast EconomasE™ (EcoE) to alleviate effect of different stressors on intestinal damage, cellular immunity, and other physiological responses was

observed in broilers. Chicks were raised for 21 d and arranged in a 2 x 2 x 2 factorial in a completely randomized design with 7 replicate cages and 6 birds/cage. The effect of the combination or standalone coccidia challenge (with or without), dexamethasone (DEX) (with or without), and supplementation of EcoE (0 or 0.2g/kg diet) was observed in 336 birds. The birds were orally gavaged with 20x commercial coccidia vaccine, while DEX was supplemented in the diet at 1.5ppm. Exposure to both DEX and cocci oocysts lasted for 7 successive days. On day 21, birds were killed to obtain blood, jejunal tissue, and mucosal samples for antioxidant, histological, gene expression and mucosal immunity analyses. Dexamethasone in the feed decreased body weight (21 d), gain, feed intake, and feed efficiency ($P < 0.0001$); increased liver weight, decreased spleen weight, bone breaking strength and % ash in the tibia and femur ($P<0.0001$); decreased villus height, crypt depth, and villus width ($P<0.0001$); decreased activity of superoxide dismutase, and increased catalase activity ($P<0.0001$). Furthermore, DEX challenge decreased ($P < 0.05$) expression of IL-6, IFN-γ, and claudin 1 but upregulated levels of IL-10, and occludin. The coccidia challenge was not effective in inducing any stress but a combination of both coccidia and DEX challenge reduced crypt depth ($P<0.0001$); BW (d 21), gain, and feed intake ($P < 0.05$). While the feed additive did not ameliorate the effect of the stressors, it upregulated ($P < 0.001$) the mRNA expression of IL-6, IL-10, IFN-γ, CXCLi2, IL-8, ZO-1, and claudin 1 in the gut. This study provides evidence that glucocorticoids inhibit gastrointestinal functioning when delivered orally and can be used as a model to understand the intricacies of the gut and decipher the immune response associated with a bacteria challenge.

Key Words: gut, immune response, dexamethasone, tight junction, broiler

M84 Effects of riboflavin on growth performance, processing yield, and internal organ development of Ross 708 male broilers Bo Zhang*[GS], Wei Zhai *Mississippi State University*

Effects of various dosages of riboflavin (vitamin B_2) on growth performance, processing yield, and internal organ development of Ross 708 male broilers were studied. A total of 840 broilers were randomly assigned to 10 blocks, 7 pens per block with 12 birds per pen. The 7 experimental diets were supplemented with 0 (riboflavin deficient negative control, NC), 6.61 (normal control, at recommended level), 10, 20, 40, 80 or 160 ppm riboflavin, with a corn-soybean meal as the basal diet. Data were analyzed using one way ANOVA of PROC GLM in SAS 9.4. The results indicated that body weight (BW) was improved by supplementation of riboflavin on d 14 ($P < 0.0001$), 28 ($P < 0.0001$), 36 ($P = 0.0002$) and 42 ($P = 0.0004$) as compared to the NC. However, BW were not different among all riboflavin-supplemented groups. The difference in BW among the 7 treatments diminished on d 56 ($P = 0.146$), which may due to broilers' riboflavin requirement decreasing with aging. The feed conversion ratio (FCR) of NC was higher than that of the other 6 treatments ($P < 0.0001$) during d 0-14, then remained higher than that of 20, 40 and 160 ppm groups ($P = 0.013$) during d 0-28, but was not different during d 0-42 ($P = 0.407$) and d 0-56 ($P = 0.714$). Weights of carcasses, breasts, drumsticks, and thighs were not different among treatments on d 56 ($P > 0.05$). However, the 160 ppm riboflavin supplementation increased the weights of fat pad ($P = 0.058$), wings ($P = 0.026$), and tenders ($P = 0.056$) as compared to NC. On d 35, birds from NC, 6.61, 20, and 160 ppm groups were necropsied, internal organs (including small intestines, heart, spleen, liver, pancreas, and bursa) were weighed, and relative organ weights to BW were calculated. NC and 160 ppm groups exhibited heavier absolute ($P = 0.034$) and relative ($P = 0.054$) bursal weights than the normal group. Other organs were not different among treatments. In conclusion, riboflavin deficiency decreased broiler growth performance before d 42. Supplementation of riboflavin alleviated the depression on growth performance. Supplementation of a dose higher than 6.61ppm could not improve BW further. However, a higher level, 160ppm, may alternate tissue deposition, as indicated by the changes in abdominal fat pads, wings, tenders, and bursa.

Key Words: broiler, growth performance, organ, processing, riboflavin

M85 Effects of the in ovo injection of vitamin D3 and 25-hydroxyvitamin D3 on immunity and small intestine morphology in broilers fed commercial or calcium and phosphorous-restricted diets Saman Fatemi*[1GS], Katie Elliot[1], Abiodun Bello[2], Bradley Turner[3], Haijun Zhang[1], Edgar Peebles[1] *[1]Mississippi State University; [2]University of Alberta; [3]DSM*

Effects of the *in ovo* injection of vitamin D_3 (D_3) and 25-hydroxyvitamin D_3 ($25OHD_3$) on broiler immunity and small intestine morphology were investigated. At 18 d of incubation (doi), live embryonated Ross 708 broiler hatching eggs were *in ovo*-injected with a 50 µL solution of one of the following treatments using an Inovoject multi-egg injector: 1) diluent (control); diluent containing either 2) 2.4 µg D_3; 3) 2.4 µg $25OHD_3$; or 4) 2.4 µg D_3 + 2.4 µg $25OHD_3$. At hatch, male broilers were randomly placed in floor pens and were fed either a commercial diet or a diet restricted in calcium and phosphorus (ReCaP) content by 20% for the starter, grower and finisher dietary phases. Eighteen birds were placed in each of 6 replicate pens in each *in ovo* injection and dietary treatment group combination. Blood samples were collected from 1 bird per pen for determination of serum IgG at 14 doa, and α-1-acid glycoprotein (AGP) at 40 doa. At 14 and 40 doa, duodenum, jejunum, and ileum villus length (VL), crypt depth (CD), villus length to crypt depth ratio (RVC), and villus surface area (VSA) were determined in 1 bird per pen. There was no significant diet x *in ovo* injection treatment interaction for any variable. However, serum IgG levels were higher ($P=0.013$) in $25OHD_3$ than in diluent or D_3 *in ovo*-injected birds. Also, AGP tended ($P=0.079$) to decrease in broilers that received *in ovo* $25OHD_3$ in comparison to those that were *in ovo*-injected with diluent or D_3 alone. At 14 doa, a higher jejunal RVC ($P=0.001$) was observed in birds that were *in ovo*-injected with $25OHD_3$ alone as compared to all other *in ovo* injection treatments, and a $25OHD_3$ *in ovo* injection resulted in a shallower CD ($P=0.001$) in comparison to the diluent or D_3 treatments. At 40 doa, VL increased ($P=0.017$) in the ileum and CD ($P=0.001$) decreased in the jejunum in commercial diet-fed birds compared to ReCaP-fed birds. Higher VL or RVC is associated with improved nutrient absorption and a higher CD is associated with a higher energy maintenance by the gut. In conclusion, *in ovo* injection of $25OHD_3$ increased antibody secretion, tended to decrease inflammation, and improved small intestine morphology; however, a ReCaP diet is detrimental to broiler small intestine morphology.

Key Words: In ovo injection, 25-hydroxyvitamin D3, small intestine morphology, immunity, broilers

M86 Feed ingredient handling characteristic evaluation of lipid encapsulated versus non-encapsulated poultry vitamin and trace mineral premixes Olivia Wedegaertner*[1GS], Adam Fahrenholz[1], Jean-Christophe Bodin[2], Jean Fontaine[2], Peter Ferket[1] *[1]North Carolina State University; [2]JEFO Nutrition, Inc*

Handling characteristics of feed ingredients are important to the efficiency of all stages of animal feed manufacturing. The ability of feed ingredients to change physically or chemically when exposed to moisture, heat or pressure determines how well they flow through a bin and disperse throughout the feed. Vitamin and trace mineral premixes are known to be dusty, electrostatic, hygroscopic, and chemically reactive. Encapsulation is used to reduce reactivity and improve storage and handling characteristics of nutrients. We hypothesize that lipid encapsulation of vitamin and trace mineral premixes significantly improves their handling characteristics making them more desirable products for feed mills. Handling characteristics evaluated were solubility, hygroscopicity, lumping, compressibility, particle size (Dgw), particle size variability (Sgw), density, and flowability (measured by angle of repose). Triplicate samples of un-encapsulated

(free) vitamin and mineral premixes and lipid-encapsulated (protected) vitamin and mineral premixes were evaluated for their handling characteristics. Results were analyzed with JMP Pro 14 to determine differences in free and protected premixes. Encapsulation increased Dgw of vitamin and mineral premixes from 200 to 611 and 79 to 723 microns, respectively ($p<.0001$), increased density of the vitamin premix by 139.22 kg/m^3($p<.0001$), lumping score of the mineral premix by 3 points ($p<.0001$) and angle of repose of vitamin and mineral premixes by 7.45°and 18.81°, respectively ($p<.0001$). Encapsulation decreased density of the mineral premix by 713.85 kg/m^3($p<.0001$), lumping score of the vitamin premix by 2.3 points ($p<.0001$) and Sgw of the vitamin premix by 0.18 ($p=.0002$). Encapsulation decreased moisture uptake by 0.77% and 0.66% ($p\leq.0001$) and moisture retention by 0.45% and 0.41% ($p<.0001$) of vitamin and mineral premixes, respectively. Free premixes were both soluble in an aqueous solution, while protected premixes were insoluble. Lipid encapsulation of vitamin and trace mineral premixes decreases solubility and protects the nutritional constituents from chemical degradation, decreases hygroscopicity allowing for longer premix shelf life and increases particle size and flowability for more efficient feed milling operations.

Key Words: encapsulation, vitamin premix, mineral premix, handling characteristics, feed milling

M87 Zinc requirements of broiler breeder hens Elisa Francois*[GS], Sergio Vieira, Liris Kindlein, Andre Mayer, Eveline Berwanger, Thiago Noetzold *UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL*

Zinc (Zn) is an essential mineral with important functions in animal metabolism. The objective of this study was to assess the different level of Zinc supplementation of broiler breeder hens using Zn sulfate as the source of the added Zn. One hundred and twenty Cobb 500 broiler breeder hens, 20 wks old, were individually placed in cages (0.33 m length x 0.46 m deep x 0.40 m height) whereas 30 Cobb breeder males were placed in 3 collective floor pens (2.0 x 1.5 m) for semen collection. The study was composed of 3 phases, each one having different diets as follow: adaptation to cages (basal diet) for 6 wks; depletion (deficient diet) 26 to 32 wks in order to deplete the hens body Zn storage and experimental phases (treatment diets) at 33 wks. The experimental diets were composed of 6 different Zn concentrations. Each treatment had 20 replicates and one hen was the experimental unit. Supplementation was done using laboratory grade Zn sulfate heptahydrate (ZnSO 4 7H 2 O). The supplemented levels were 0, 30, 60, 90, 120, and 150 mg Zn/kg of feed, and Zn analyzed levels were 18.7 + 0.47; 50.3 + 10.6; 77.3.0 + 11.0; 110.2 + 12.8; 140 + 12.2 and 170.6 + 13.2 mg Zn/kg, respectively. The experimental phase was divided into 3 periods of 28d from 33 to 44 wks of age. Eggs were classified daily as hatchable or not (broken and deformed eggs). Requirements of Zn were done using quadratic polynomial (QP), broken line quadratic (BLQ), and exponential asymptotic models (EA). Requirements obtained for hen day egg production and settable egg production were 114.1, 83.3, 78.6 ppm and 112.6, 61.4, 65.4 ppm for period of 33 to 36 wks, and 116.4, 63.3, 53.1 and 113.2, 60.4, 46.1 ppm for period of 37 to 40 wks, and 116.3, 62.8, 52.8, and 120.0, 67.7, 62.1 ppm for period of 41 to 44 wks, respectively, using QP, BLQ and EA models. Total eggs and total settable eggs produced per hen had Zn requirements estimated as 116.4, 75.7, 64.7 ppm, and 115.2, 56.5, 41.5 ppm, respectively for QP, BLQ and EA models, whereas for ALP were 161.8 and 124.9 ppm using QP and BLQ models. EA and BLQ models provided better fits for most measurements and estimated lower requirements than the QP model. Reported requirements varied from 41.5 ppm (6.3 mg/hen/day) to 161.6 ppm (24.7 mg/hen/day) Zn.

Key Words: breeders, egg production, mineral, zinc, supplementation

M88 Effect of dietary supplementation of layer hens with a hydroxy-analogue of selenomethionine on performance and egg quality Luis Avila*[GS], Diego Puron, Mario Sierra, L. Gabriela Aragon, Jessica Starkey, Charles Starkey *Auburn University*

Selenium (Se) is metabolically important for immunity, reproduction, and health of layer hens. Selenoproteins (SeP) have antioxidant, enzymatic, storage and transport functions. The purpose of this experiment was to evaluate the effect of 2 dietary Se sources and on layer hen performance, Haugh units (HU) as a freshness parameter, and Se content of eggs (µg Se per 100 g). A 12-wk randomized complete block design experiment with 3 dietary treatments was conducted. Dietary treatments were: 1) negative control without added Se (Se0; 0.07 mg per kg of feed), 2) sodium selenite (SeSod; 0.37 mg per kg), and 3) hydroxy-analogue of selenomethionine (SeHDXM; 0.37 mg per kg). Added Se sources provided Se at a rate of 0.30 mg per kg of feed. Forty-two wk-old White Dekalb layer hens (n = 420; 1.6 kg BW) were deprived of added dietary Se for 4 wk (42 to 46 wk of age) before the 8 wk (47 to 54 wk of age) Se feeding period. There were 6 complete blocks with 12 replicate cages with 7 hens per cage (420 cm² per hen). All hens were fed equal amounts of feed per week (104 g per hen per d). Measured variables were: laying rate (%), egg weight (g), egg mass (g), feed conversion ratio (FCR), and wk mortality (%). HU determination was done at 0, 1, 2, 3, 4 wk post-collection at wk 8 with unwashed eggs (n = 250) stored at room temperature of 21 ˚C. Se contents in whole egg were determined from samples (n = 80) collected at wk 4 and 8. Data were analyzed using the GLIMMIX procedure of SAS (V9.4). Means were declared different when $P < 0.05$. Hens fed SeHDXM had a higher laying rate (96.36%) than hens fed SeSod (94.70%; $P = 0.023$). Hens fed Se0 had the highest egg weight (63.28 g; $P = 0.004$). Se source did not impact egg mass *(*$P = 0.166$), wk mortality *(*$P = 1.000$), or FCR ($P = 0.106$). At wk 4, eggs from hens fed SeHDXM had the lowest Se content ($P = 0.031$). At wk 8, Se content of eggs was similar among all treatments ($P = 0.724$). Se source did not alter HU at wk 0, 1, 2, 4 post-collection. Eggs from hens fed Se0 had the lowest HU (47.05) 3 wk post-collection ($P = 0.015$). Overall, neither added Se source improved hen performance compared to Se0; however, egg quality (HU) was improved by added Se.

Key Words: selenium, layer hens, egg quality, selenomethionine

M89 Amino acid digestibility of broilers fed diets varying in amino acid density and supplemental copper concentration from 1 to 14 days of age Stephanie Philpot*[1GS], Kurt Perryman[2], William Dozier[1] *[1]Auburn University; [2]Micronutrients USA, LLC*

Preliminary research indicates that feeding high concentrations of Cu may enhance amino acid (AA) digestibility, which could support improved growth performance in broilers fed low AA density diets. An experiment was conducted to determine the interactive effects of dietary AA density and supplemental Cu concentration on AA digestibility of Yield Plus × Ross 708 male broilers. A total of 672 chicks was randomly distributed to 48 floor pens (14 birds per pen; 0.16 m²/bird). Six dietary treatments consisting of a 2 × 2 factorial arrangement of 2 dietary AA densities and 2 Cu concentrations with positive (PC) and negative (NC) controls were used, with 8 replicate pens per treatment. Moderate and low AA density diets were formulated to 95 and 88% of the recommended AA specifications of the 2014 Ross 708 Broiler Nutrition Specifications, respectively. Supplemental Cu was provided at 135 or 270 mg/kg in the form of tribasic Cu chloride (Micronutrients USA, LLC). Positive control and NC birds were fed moderate AA diets with approximately 13 mg/kg Cu. In addition, PC birds were unvaccinated and received diets with diclazuril, whereas all other birds received a single dose of Coccivac® B52 at hatch. Broilers were fed starter diets (1.22 or 1.13% digestible Lys and 3,000 kcal ME/kg) in crumble form and included 0.5% TiO_2. Diets were formulated to contain digestible Met + Cys, Thr, Val, and Ile ratios relative to Lys of 0.74, 0.67, 0.75, and 0.69, respectively. At 14 d of age, 8 birds per pen were euthanized and ileal digesta was collected. Digestibility of essential AA was not significantly affected ($P > 0.05$) by the main effect of AA density or by

the contrast assessing the effect of vaccination against coccidiosis vs. non-vaccinated birds. Cysteine digestibility decreased ($P < 0.004$) in broilers fed diets containing 135 or 270 mg/kg of Cu compared with NC-fed birds. Digestibility of Thr, Ile, Leu, and Phe was increased ($P < 0.001$) with birds fed 270 mg/kg Cu compared with those fed 135 mg/kg Cu. Significant interactions ($P < 0.05$) between AA density and Cu concentration were observed for Lys, Val, Trp, His, and Arg. This research indicates that Cu supplementation influences AA digestibility, with the direction and magnitude of the effect depending on the specific AA.

Key Words: Amino acid, antibiotic free, broiler, copper, digestibility

M90 Determination of the digestible valine to lysine ratio in broilers from 1 to 14 days of age Tanner Wise*[1GS], Paul Tillman[2], Kevin Touchette[3], William Dozier III[1] *[1]Auburn University; [2]Poultry Technical Nutrition Services LLC.; [3]Ajinomoto Animal Nutrition North America Inc.*

Valine is the 4th limiting amino acid for broilers fed corn-soybean meal based diets. Two experiments were conducted to determine the optimal digestible (dig) Val to Lys ratio for the growth performance of broiler chickens from 1 to 14 d of age. In each experiment, 1,600 Yield Plus × Ross 708 male chicks were placed in 64 floor pens (25 birds/pen) at 1 d of age. Each pen received 1 of 8 dietary treatments (8 replicate pens) from 1 to 14 d of age. In both experiments, diets were formulated with corn, soybean meal, and peanut meal as primary ingredients. L-Valine was added to create various concentrations of dig Val among dietary treatments. All diets were formulated to be adequate in all essential amino acids except for Val and Lys. Diet 1 was the positive control formulated to contain 1.25% dig Lys with a dig Val to Lys ratio of 0.79. Diet 2 was the negative control with a dig Val to Lys ratio of 0.55. Diets 2 to 8 were formulated to contain 1.15% dig Lys with dig Val to Lys ratios ranging from 0.55 to 0.91 in increasing increments of 0.06. Feed and birds were weighed at 1, 7, and 14 d of age to determine body weight gain (BWG), feed intake, and feed conversion ratio (FCR). Male broilers fed gradient concentrations of dig Val displayed significant linear ($P \leq 0.022$) and quadratic ($P \leq 0.023$) responses for BWG, feed intake, and FCR in both experiments. In experiment 1, quadratic broken-line analysis indicated that optimal dig Val to Lys ratios of 0.77 and 0.789 for BWG ($P < 0.001$) and FCR ($P < 0.001$) of broilers, respectively. In experiment 2, linear broken-line analysis determined optimum BWG ($P < 0.001$) and FCR ($P < 0.001$) with dig Val to Lys ratios of 0.753 and 0.775, respectively. Based on these data, the estimated optimal dig Val to Lys ratio for BWG and FCR ranges from 0.75 to 0.79 for Yield Plus × Ross 708 male broilers from 1 to 14 d of age.

Key Words: valine, broiler, amino acid

M91 Effect of amino acid levels during broiler breeder pullet rearing on: 1. Growth and development Andres Ortiz*[1,2UG], Edgar Oviedo-Rondon[1], Ivan Ospina[1], Hernan Cordova[1], Viviana San Martin[1], Camila Alfaro[1,2], Gustavo Quintana[1,2], Ivan Cardenas[1,2], Miguel Chico[1], Yilmar Matta[1,2], Lina Penuela[1,2], Justina Calda[3] *[2]Universidad del Tolima; [1]North Carolina State University; [3]Cobb Vantress Inc.*

Adequate pullet nutrition is essential to obtain the BW and development suitable for reproduction. An experiment was conducted to determine the effects of four amino acid (AA) dietary levels fed to broiler breeder pullets during the rearing phase from 5 to 24wk on BW, abdominal fat deposition and development. A total of 1,360 Cobb-500 slow-feathering (SF) pullets were placed in 16 floor pens (85/pen). Up to 4wk all pullets were fed one starter diet in crumbles and after 29d of age fed with four mash grower diets containing 4 AA levels (0.40, 0.54, 0.60, and 0.66% of dLys with balanced protein) and 2,700 kcal/kg ME. From 16wk to 5% egg production, developer mash diets with 2,800 kcal/kg ME and 0.51, 0.57, 0.63, and 0.69% of dLys were offered. Feed amounts varied slightly (±3 g/d) among treatments in the developer phase to maintain BW close (±2%) to Cobb guideline. Individual BW was obtained at 4, 10, 15, 20 and 25wk of age. At these days 3 pullets per pen were selected, sacrificed and breast muscle with bones, liver, abdominal fat and intestines were collected and shanks measured. Fleshing scores were assessed at 11, 16, 21 and 25wk of age and shank length measured. Data was analyzed in a CRD with 4 AA treatments and 4 replicate pens each. One-way ANOVA and regression analyses were conducted. Results indicated no differences in BW ($P > 0.05$) at 4 wk. The two higher AA levels resulted in heavier ($P < 0.01$) pullets throughout the experimental phase being 2.4 and 3.6% above Cobb guideline at 20wk, while the other two levels were 0.6 and 0.4% below. At 16, 20 and 21wk of age pullets fed diets with the highest AA level resulted in greater ($P < 0.05$) relative breast muscle weight or fleshing score and shorter shanks as compared with pullets fed diets with the lowest AA levels. Pullets fed the lowest dietary AA level had the longest ($P < 0.05$) intestines at 10wk of age, the largest deposits of abdominal fat at 15 and 25wk, and the smallest liver at 20wk. Fleshing increased linearly ($P < 0.01$), while abdominal fat and shank length decreased ($P < 0.05$) as AA increased. In conclusion, AA dietary levels have important effects on pullet BW, fleshing, abdominal fat and organ development during rearing, but the best egg production was observed with the two highest AA levels.

Key Words: amino acids, pullet breeders, growth, development, breast muscle

M92 The effect of nutrient density on performance and processing yield in Cobb 700 mixed-sex broilers Corey Johnson*[1GS], Rocky Latham[2], Rob Shirley[3], Tri Duong[1], Jason Lee[1] *[1]Poultry Science Department, Texas A&M Agrilife Research; [2]Tyson Foods; [3]Adisseo USA, Inc.*

The objective of this study was to define the effect of digestible amino acid (dAA) density on the performance and processing yield of Cobb 700 × Cobb MV, mixed-sexed broilers. In a four phase feeding program (starter: d 0-12, grower: d 12-26, finisher: d 26-36, and withdrawal: d 36-49), five levels of AA density were evaluated (Trt 1: Control, Trt 2: Con +5%, Trt 3: Con +10%, Trt 4: Con +15% and Trt 5: Con +20%). These treatments were allotted in a randomized, complete block design, with 12 replicate pens/trt and 28 birds/replicate. From the starter to the withdrawal, the dLys content of control diets was 1.18, 1.07, 0.96 and 0.89% dLys, respectively. Treatments were formulated to maintain equivalent dEAA: dLys ratios across all diets within a given phase. Body weight (BW), feed intake (FI), and mortality-adjusted feed conversion ratio (FCR) were evaluated on d 12, 26, 36 and 49. On d 50, 4 males and 4 females from each pen were processed to determine boneless-skinless breast, tenderloin and fat pad yield. Data were analyzed via ANOVA and means were separated using Duncan's Multiple Range test ($p < 0.05$). Increasing the dAA density reduced feed intake during the starter ($p = 0.06$) and grower phases ($p = 0.03$). Compared to the control, BW increased with +5% dAA at 12 and 26 days-of-age ($p < 0.01$). Improvements in BW were also observed at 26 d ($p < 0.01$) and 36 d ($p = 0.06$) when broilers were fed > +5% dAA. Compared to the control, FCR were incrementally reduced throughout the starter, grower and finisher phases when +5%, +10%, +15% and +20% dAA were fed ($p < 0.05$). Increasing the dAA density by +10% and +15% reduced cumulative 49 d FCR by 6 points and by 8 points, respectively. There was an increase in boneless, skinless breast yield (as a % of live weight; males and females combined[SR1]) at 20% dAA (+ 5.75%). Tenderloin yield was increased at 10% dAA (+ 4.6%), with no further response at 15 or 20% dAA. Fat pad yield followed a classical decrease as dAA increased, ranging from 1.56% in the Control to 1.11% when +20% dAA were fed ($p < 0.01$). Improvements in performance and yield support the need for higher dAA supplementation in the Cobb 700 x MV cross.

Key Words: Heavy Broiler, digestible amino acids, breast yield, Cobb 700, amino acid density

M93 Impact of feeding varying grower digestible lysine and energy levels to female Cobb MV × Cobb 500 broilers on 42-day growth performance and processing yield Rosana Hirai*[1GS], Leonel Mejia[2], Cesar Coto[2], Justina Caldas[2], Christopher McDaniel[1], Kelley Wamsley[1] *[1]Mississippi State University; [2]Cobb-Vantress*

Previous research in our lab has revealed that feeding various levels of starter digestible lysine (dLys) and energy (AME) affects early bird performance and processing yield of 42d old Cobb MV × Cobb 500 male broilers, a relatively new commercial cross. Therefore, the objective of the current study was to evaluate a 3 dLys (1.00; 1.08; and 1.18%) x 4 AME (2,937; 3,028; 3,116; and 3,206 kcal/kg) factorial arrangement of Grower diet treatments (Gdiets) and their impact on d14-28 performance and processing yield of 42d old Cobb MV × Cobb 500 female broilers. On d0, chicks from a commercial hatchery were vent sexed and placed at 15 females/pen; they were provided a common starter diet (d0-14; 1.22% dLys+2,977 kcal/kg). On d14, 13 females were placed/pen (0.086 m^2/bird) and pen weights were equalized by block; 12 treatments were assigned as a randomized complete block design (8 replicate pens/treatment). A common finisher diet (d28-42; 0.97% dLys+3,151 kcal/kg) was fed. Measured variables were BW, BW gain (BWG), feed intake/bird (FI), FCR, and d42 processing data. In general, d14-28 and 14-35 FCR ($P<0.05$) interactions demonstrated that as birds were fed Gdiets increasing in dLys for each AME, FCR decreased. However, birds fed Gdiets at 1.08% dLys and 3,028 kcal/kg AME demonstrated a slight plateau in FCR. Feeding Gdiets of 1.18% dLys maximized d28 BW and d14-28 BWG ($P<0.001$), as well as d35 BW and d14-35 BWG ($P=0.05$). Feeding Gdiets of 1.18% dLys or 3,206 kcal/kg AME decreased d14-28 and 14-35 FI ($P<0.05$). Overall, d14-41 data exhibited improved BW and BWG when birds were fed Gdiets at 1.08 or 1.18% dLys ($P=0.03$). Also, feeding Gdiets formulated to 1.18% dLys or ≥3,028 kcal/kg AME optimized FCR by 4-5 points ($P<0.001$). Processing data demonstrated improved breast yield when birds were fed Gdiets formulated to ≥1.08% dLys or Gdiets formulated to 2,937 or 3,028 kcal/kg AME ($P<0.05$). Birds fed Gdiets at 3,116 kcal/kg AME had similar breast yield to those fed 3,206 kcal/kg AME, as well as similar yield to those fed 2,937 or 3,028 kcal/kg AME. In order to determine the best feeding regime for this new commercial broiler, future research should evaluate the effects of varying dLys and AME levels during the finisher phase on broiler performance and processing yield.

Key Words: new commercial cross, digestible lysine, energy, growth performance, processing yield

M94 Responses of Cobb 700 broiler chickens to eight levels of digestible lysine. Leasea Butler*[1,2], Andy Mauromoustakos[1], Cody Keen[2], Colin Scanes[1], Justina Caldas[2], Michael Kidd[1], Sam Rochell[1] *[2]Cobb-Vantress, Inc.; [1]University of Arkansas*

There is limited information on the nutritional requirements of Cobb 700 broiler chickens. This Cobb MVM x Cobb 700 broiler trial was a continuation to the digestible Lysine (dLys) titration trial for the first growing phase (GP) (GP1 = 0d to 14d) where 96 pens (48 male pens and 48 female pens) in a completely randomized block design were placed on eight different diets with increasing dLys levels [Treatment (T)1=0.90%, T2=0.98%, T3=1.06%, T4=1.14%, T5=1.22%, T6 1.30%, T7=1.38% and T8=1.46%] to determine the dLys requirements for body weight (BW) growth and feed conversion (FCR). It was considered that live performance or processing performance in the other GPs (GP2 = 14d to 28d, GP3 = 28d to 41d and GP4 = 41d to 53d) would not be well represented by placing them on isocaloric and/or isoprotein diets. Instead, birds were raised on the eight different planes of dLys adjusted lower for all treatments by the GPs, but remaining 0.08% different between the treatments. For instance, GP2 diets dLys levels were set at (T1 0.76%, T2 0.84%, T3 0.92%, T4 1.00%, T5 1.08%, T6 1.16%, T7 1.24% and T8 1.32%). Male and female Cobb 700 broilers were able to compensate for early BW deficits from GP1 and GP2 with increased feed intake ($P<0.05$) in GP3 and GP4. Carcass yield, breast fillet, and tenders as percentages of live BW were lower ($P<0.05$) for dLys planes below T4, however dLys planes above T4 may be sufficient and may be in excess of requirements at the levels of T5 or T6 ($P<0.05$). Woody breast was evaluated on the scale of 0= no incidence, 1 = slight incidence, 2 = moderate incidence and 3 = severe incidence. All male treatment WB scores were lower than 1.03 ($P<0.0001$), and lower than 1.45 ($P<0.0002$) for the females. However, higher ($P<0.0001$) pen average woody breast scores were observed in dLys planes above T5 for males and T4 for females ($P<0.0002$). These results may suggest that if the crude protein or dLys requirements of the Cobb 700 are not met in GP1 and/or GP2 the bird may be able to compensate with increased feed intake. However, BW gain with increased dLys in the later GP's is an expensive approach, but in scenarios of early health challenges may be an effective one.

Key Words: Cobb 700, Amino Acid, Lysine, Broiler

M95 Responses of varying levels of DL-methionine and TSAA and their subsequent effects on [1-13C] methionine and [1-13C] cysteine oxidation Jordan Weil*[1GS], 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 & Care*

As the industry moves towards ABF practices, it is important to understand the degradation of TSAA to the end-product of glutathione, which plays an important role in gut health. In two experiments, 2400 Cobb 500 chicks were reared to determine the rate of CO_2produced (VCO_2) through stable isotopes and indirect calorimetry as well as the oxidation of [1-^{13}C] methionine (Met) and [1-^{13}C]cysteine (Cys). In Exp 1, 1800 broilers were fed a starter diet (0-10d) and one of 5 grower diets (10-21d), each containing different TSAA ratios (Evonik AMINOChick®2.0). Similarly, 600 birds in Exp 2 were fed a corn-soy diet from 0-10d, followed by one of 3 grower diets at d10. At 21d old, birds were selected for either control or 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. Additionally, birds were selected for metabolic chambers or IG [^{13}C] $NaHCO_3$infusion. Blood was collected and $^{13}CO_2$enrichment was determined by isotope ratio mass spectrometry (IRMS). Results from IRMS were converted to atom percent excess (APE) and analyzed with pharmacokinetic modeling (JMP Pro 13.1). Comparison of infusion methods was analyzed using ANOVA, with means separated using Tukey HSD. Results revealed birds fed diets containing deficient TSAA with adequate Met levels had the highest FCR, suggesting birds consumed more feed to reach cysteine requirements (Exp 1). However, no differences exist between BW of those fed sufficient TSAA ($p>0.05$). The repercussions of deficiencies of TSAA was further supported in Exp 2, where birds consumed additional feed, resulting in higher FCR when fed deficient TSAA levels. Comparison of Met and Cys $^{13}CO_2$revealed differences in IG and IV infusions ($p<0.05$). Loss of [1-^{13}C] Met through $^{13}CO_2$in birds fed sufficient TSAA levels increased (18 vs 11%; $p<0.05$) due to the conversion to cysteine. Ideal, considering ^{13}C Met recovery signifies opportunity for glutathione synthesis. Ultimately, the loss of the Met and Cys labels to $^{13}CO_2$proves worthy to understanding TSAA, considering greater $^{12}CO_2$production from ^{13}C Met may lead to opportunity of producing additional glutathione, and consequently, more protection to broilers in ABF environments.

Key Words: broilers, metabolism, amino acids, stable isotope

M96 Evaluating the effects of isoleucine supplementation in low crude protein amino acid fortified diets on nitrogen retention, body composition and egg production in White Shaver hens 20-48 weeks of age Ilona Parenteau*[1GS], Marvin Stevenson[2], Elijah Kiarie[1] *[1]University of Guelph; [2]Halchemix Canada Inc.*

A study was conducted to investigate the effects of Ile supplementation in low crude protein (LCP) diets on N retention, body composition and egg production in White Shaver laying hens between 20 to 48 wks of age

(woa). Nineteen-week-old pullets were allocated to 30 cages (6 hens/cage, n=6) based on body weight (CV<4%) and fed either a control diet with standard crude protein (18%, HCP) or a LCP (16%) diet fortified with synthetic AA plus graded levels of Ile at targeted ileal digestible Ile:Lys (dIle:dLys) ratios of 70, 80, 90 and 100%. Egg production (EP) was recorded daily whereas egg weight (EW) and feed intake were recorded biweekly to 46 woa. At 28 woa, CP was reduced by 11% for phase 2. At 29 and 48 woa, excreta samples were collected, and two hens/cage were bled for plasma AA and serum uric acid (UA). At the end of the trial, one bird/cage was killed for whole body composition using Dual-energy X-ray absorptiometry (DXA). Breast muscle, liver and abdominal fat pad were dissected, weighed and further analyzed. Data were subjected to 2-way ANOVA in SAS Studio with diet and 4-wk period as fixed effects. Orthogonal contrasts were used to evaluate HCP *vs* LCP and effects of Ile supplementation in LCP diets. Analyzed dIle:dLys of LCP diets were 76.0, 84.7, 87.9, 97.7% and 68.1, 72.4, 82.2, 94.3% for phase 1 and 2 diets, respectively. Nitrogen retention improved (P<0.01) with a reduction in CP, however whole body, breast and liver fat increased (P<0.05) by 2.5, 2.4 and 2.6%, respectively, and egg mass was negatively (P=0.09) affected due to a lower EP (P<0.01), indicating that Ile was deficient. Isoleucine linearly reduced breast muscle fat:lean composition (P=0.07), increased EP (P<0.01), and was correlated (r^2≥0.49, P0.02) with N retention. Fat pad weight was lowest in hens fed the highest level of Ile and was positively correlated (r^2=0.41, P=0.02) with EW, suggesting that Ile may indirectly influence EW by altering body composition. Lowest serum UA and peak performance was observed in birds fed 90% dIle:dLys, suggesting that dietary CP levels for laying hens can be reduced by 2 percentage units if adjusted for Ile to this level.

Key Words: isoleucine, low protein, layers, egg production, body composition

M97 Application of a high density distiller's protein in diets for turkey poults 0-42 days of age; impact on performance and nitrogen retention Peter Williams*[1], Emily Burton[2], Dawn Scholey[2] *[1]Fluid Quip Process Technologies, LLC; [2]Nottingham Trent University*

The corn distilling industry is a major processor of grain but the high fiber co-product (DDGS), has limited application in monogastric and neonate nutrition. Simple mechanical processing downstream of fermentation produces a high protein (52.4 % DM), highly digestible, low crude fiber (4.4% DM) product containing approximately 24% of DM as spent brewer's yeast, suitable for monogastric and neonate nutrition (high density distiller's protein: HDDP). The protein is valuable and is not in competition with human food. 250-day-old male BUT6 turkey poults were weighed before randomly allocated to pens and bedded on wood shavings. Feed and water were available *ad libitum*. A standard commercial-practice rearing system was used. Starter diets were provided as crumbs and grower diets as pellets. 5 dietary treatments were formulated based on a commercial formulation A) control containing standard hi pro soya; B) 4% HDDP; C) 8% HDDP; D) low quality soy plus 4% HDDP; E) Soy protein isolate. The diets were formulated to be iso proteic in terms of ileal digestible amino acids and isoenergetic. HDDP was included at either the 4 or 8% level partially replacing soya bean meal in the ratio 1.55:1 respectively. Soya bean meal was reduced from 47.3 to 41.9 in the starter and from 40.5 to 35.1 in the grower diets in the controls compared with 8% HDDP diet respectively. The starter formulation was offered from day 0 to 21 and a grower from day 22 to 42. Birds were weighed by pen and weekly for the duration of the trial. Statistical analysis was carried out using SPSS v.24. After KS testing to confirm normality, data were analysed using one way ANOVA to investigate the effects of dietary treatment. Where appropriate, Bonferroni post hoc testing was used to test differences between treatments. There were no significant differences between individual treatments in feed intake, live weight gain or feed conversion efficiency however when data was pooled to compare diets with or without HDDP the addition of HDDP to the diets at 42 days of age significantly increase weight gain (+143g: +6%; P<0.03) and tended to increase feed intake (+156g: +4%; P<0.11). Inclusion of HDDP in the diet significantly increased N retention/kg diet (3gN/kg diet; +16% p<0001).

Key Words: protein, turkey, poults

M98 Beneficial effects of different extracts of edible mushroom species on production performance of healthy and Eimeria infected commercial broiler birds Muhammad Ullah*[1], Masood Akhtar[1], Mian Awais[1], Muhammad Anwar[1], Kashfa Khaliq[2] *[1]Bahauddin Zakariya University; [2]Government College University*

In this experiment, three edible mushroom species including *Pleurotus (P.) ostreatus*, *P. sajor-caju* and *Lentinus (L.) edodes* were processed for aqueous, methanolic and polysaccharide extracts. These extracts were administered to commercial broiler birds. Production performance in terms of weight gains and feed conversion ratios (FCR) were determined in healthy birds on weekly basis. Further, post Eimeria infection, weight gains were monitored on alternate days and different organs including bursa of fabricious, thymys, spleen and caecal tonsils were weighed and evaluated for organ to body weight ratios.

Results revealed significant higher (P<0.05) weight gains and FCR in mushroom administered groups as compared to control. Weights of experimental birds of all groups were monitored daily from days 4 to 12 after Eimeria infection. As compared to control, all mushroom extracts administered experimental groups revealed significantly higher (P<0.05) weight gains from days 4 to 12 post Eimeria infection. Organs to body weight ratios of bursa of fabricious, thymys, spleen and caecal tonsils were monitored and found higher in mushroom extracts administered groups as compared to control; however, results found were statistically non significant (P>0.05).

Results from this experiment demonstrate that mushrooms species including *P. ostreatus*, *P. sajor-caju* and *L. edodes* have ability to enhance the body weight in healthy and diseased commercial broiler birds.

Key Words: Poultry, Broiler, Mushrooms, production, immunity

M99 Effect of dried moringa oleifera leaves on growth and immunity of broilers Anjum Khalique*, Kaleem Ul-Hassan, Talat Naseer *University of Veterinary and Animal Sciences*

In developing counties, average daily protein intake in human diets is well below recommended standards. Poultry production is playing a major role to fill this gap. However, rising prices for oilseed meals and expected feed crises due to rapid increase in human and livestock population motivate nutritionists to look for good quality alternative protein sources. A study was conducted with the aim to evaluate the effects of *Moringa oleifera* leaf meal (MOLM) on growth performance, mortality percent, immunity, carcass characteristics, meat quality and bone strength in broilers. For this purpose, total 300-day-old broiler chicks were allotted 5 treatments (Control, 5% MOLM, 10% MOLM, 15% MOLM, 20% MOLM) divided into 30 replicates having 10 chicks each. The obtained data were subjected to one-way analysis of variance (ANOVA) and means were compared through Duncan's Multiple Range test. Results revealed that body weight of birds on diet 0%, 10% and 15% MOLM were significantly higher (P≤0.05) than others while, control group consumed significantly higher feed intake. On the other hand, 15% MOLM showed significantly improved mortality (%) and antibody titer against NDV while feed efficiency was not affected by any treatment. Birds fed 15% MOLM in feed also improved (P≤0.05) carcass weight and dressing% and carcass cut-up parts including breast, thigh and drumstick percentage. Similar group having 15% MOLM significantly (P≤0.05) improved ultimate pH and more appealing color of meat along with better bone strength. In conclusion, MOLM inclusion at 15% in broiler diet improves the overall growth performance and immunity in broilers.

Key Words: broilers, Moringa oleifera, growth, immunity, meat quality

M100 Effect of feeding spray-dried plasma in the starter diet at different doses and duration in broilers undergoing a severe health challenge Ricardo Gonzalez-Esquerra*[1], Joy Campbell[1], Javier Polo[1], Vieira Sergio[2], Liris Kindlein[2], Andre Favero[2] *[1]APC, Inc.; [2]Universidade Federal de Rio Grande do Sul*

The literature reports better performance, gut integrity, development and functionality and reduced susceptibility to disease and stress when feeding spray-dried plasma (SDP) to companion and farm animals including chickens. The effect of SDP relative to dose and duration fed to broilers was studied. Ninety floor pens with reused litter were distributed in a commercial barn of 25,000 chickens in production. Each 2.1 m2 pen had 25 male Cobb[JC1] 500 1-d old chicks. SDP was fed for 0, 4, 7 or 10 days at levels aiming to attain SDP intakes of 0, 1.5, 3 or 6 g/bird. Treatments were: 1) No SDP [Ctrl]; 2) 2% SDP for 4d aiming 1.5 g/bird [4d-1.5g]; 3) 4% SDP for 4d aiming 3 g/bird [4d-3g]; 4) 1.8% SDP for 7d aiming 3 g/bird [7d-3g]; 5) 3.6% SDP for 7 d aiming 6 g/bird [7d-6g]; 6) 1% SDP for 10 d aiming 3 g/bird [10d-3g] and 7) 3.6% SDP for 10 d aiming 6 g/bird [10d-6g]. All Corn-SBM diets had similar nutrient content across groups and from 10 to 42 d all birds were fed the same grower and finisher diets. An unintended health condition developed and low performance was observed from the start. Thus, SDP intakes were 29% lower vs target on average. Still, from 0 to 10 d, significantly better BWG (170 vs 153 g) and FI (227 vs 205 g) were observed in SDP vs Ctrl birds, respectively (P<.0001).. By 18 d, daily mortality peaked and norfloxacin (20 mg/kg live BW) was added in the water for 5 d. *E. coli* and *Staphylococcus spp* were isolated from necropsied birds and a thick layer of white exudate was found in their inflamed ileal and jejunal mucosa. By 42 d, Ctrl birds had 83.9% mortality vs. 54.4 and 39.1% in groups 4d-1.5 g and 4d-3 g; 35.7 and 35.9% in groups 7d-3 g and 10d-3 g, and 16.7 and 14.1% in groups 7d-6 g and 10d-6 g, respectively (P<.0001). Similar effects were observed in BWG (CTRL=1.26 vs 10d-6g=1.97 kg), FI (CTRL=2.23 vs 10d-6 g=3.28 kg) and FCR (Ctrl=1.77 vs 10d-6 g=1.66) with the best results seen at the highest SDP intakes when fed for 7 or 10 d (P<.001). Adding SDP in starter diets significantly reduced mortality and improved performance in birds under a severe health challenge. Benefits were observed feeding as little as 2% SDP for 4 d and were significantly greater when used for 7 or 10 d.

Key Words: Broilers, E coli, Staphylococcus, Spray Dried Plasma, Challenge

M101 Growth performance of commercial breed broilers raised on imported versus commercial feed in Nigeria – A preliminary study Akinniyi Dare*[1], Adelumola Oladeinde[2] *[1]Obafemi Awolowo University, Ile-Ife; [2]USDA-ARS*

In this study, we assessed the growth performance of Arbor Acre (AA) broilers raised on imported versus commercially available feed in Nigeria. A total of 60 AA chicks were used for the study in a completely randomized design. Chicks were individually weighed at a day-old and randomly divided into 2 equal groups (A and B) of 30 chicks/pen group in 8 x 6 feet per floor pen. Birds were allowed ad libitum access to feed and water. Group A were fed an Imported Commercial Single diet feed (ICs-USA) while Group B were fed a Local Commercial phases feed (LCp-Nigeria) for 25 days. After which Group A and Group B broilers were switched to a National Research Council (NRC) formulated phases feed (NRCp-Nigeria) for 31 days. Live Lentogenic (LaSota) Strain BP (Vet) was administered intraocular to the birds at age 7 and 21. AMPRol® 25% Feed Premix at a dose of 0.0125% was added to the LCp-Nigeria feed to match the ICs-USA amprolium dose. No other medication was used throughout the experiment. Pen live weight (LW), average daily feed intake (ADFI), average daily gain (ADG) and feed conversion ratio (FCR) were determined. Additionally, 10 birds per pen group were slaughtered at 8 weeks of age for carcass evaluation. Results revealed significant differences ($P < 0.01$) in LW from week 1 to week 8, with Group A having higher LW (range - 0.3 - 5.4 lbs) than Group B (range - 0.2 - 4.8 lbs). Importantly, Group A broilers required shorter grow-out (45 days) to reach a LW of 4.6 lbs compared to 52 days for Group B broilers. However, drumstick yield (DSY), wing yield (WGY) and gizzard yield (GZY) was significantly higher ($P < 0.05$) for Group B (DSY = 15.1 ± 1.37 %, WGY = 16.3 ± 5.36 %, GZY =3.1 ± 0.56 %) broilers compared to Group A (DSY = 14.0 ± 0.80 %, WGY = 11.2 ± 0.89 %, GZY= 2.4 ± 0.26 %). Our study suggests that the higher neutral detergent fiber (29.7 %) and lower energy composition (1146 KC/lb) of ICs-USA feed compared to LCp-Nigeria may have contributed to the observed growth performance difference between group A and group B AA broilers.

Key Words: Growth, Arbor Acre broilers, feed utilization, Antibiotic-free, Nigeria

M102 Evaluation of flowability characteristics of feed ingredients for poultry Ahmet Pekel* *Istanbul University-Cerrahpasa*

The objective of the current study was to determine the flowability characteristics of feed ingredients and develop prediction equations for flowability based on physical and chemical parameters. Thirty five different feed ingredients with a total of 66 samples were used including protein feeds, energy feeds, by-products, amino acids, mineral and vitamin sources. The angle of repose for each ingredient was determined in triplicate by funnel test and used as an indicator for the flowability characteristic. The ingredients were evaluated for compressibility, tapped density, aerated bulk density, mean bulk density, and nutrient content in triplicate. To evaluate the effect of feed particle size on flowability, samples were ground to pass through a 1−mm or 0.5−mm screen. Proc corr and reg procedures were used to analyze the data. The compressibility values (%) for samples ground through a 1−mm or 0.5−mm screen ranged from 7 to 69% and from 7 to 57%, respectively. The angle of repose values for the samples ground with 1−mm or 0.5−mm screen ranged between 22 and 47 and between 24 and 42, respectively. Cotton seed meal had the highest compressibility and angle of repose values, while soybean meal, sunflower meal, and corn gluten meal had the lowest compressibility and angle of repose values. Feed ingredients ground with 0.5−mm screen had significantly higher angle of repose and compressibility values than ingredients ground with 1−mm screen ($P<0.05$). As mean bulk density increased, the compressibility and angle of repose decreased ($P<0.05$). The multiple linear regression analysis showed that the angle of repose of feed ingredients were positively correlated to compressibility and negatively correlated to mean bulk density ($r^2=0.64$). Compressibility and angle of repose were positively correlated with ether extract content and negatively correlated with CP content of the feed ingredients ($P<0.05$). A significant ($P<0.001$) multiple linear relationship showed that angle of repose can be estimated from mean bulk density, compressibility and crude protein content. In conclusion, there is a wide variation in angle of repose value among different ingredients tested and mean bulk density, compressibility, fat and protein content can be good indicators for feed flowability.

Key Words: angle of repose, compressibility, feed, flowability, mean bulk density

M103 Field observation: Urease activity (Delta pH Method) versus urease activity (European Method) determined on 45 commercial soybean meal samples and correlations with trypsin inhibitor contents. Nelson Ruiz*[1], Fabiola de Belalcázar[2], Jorge Castillo[3] *[1]Nelson Ruiz Nutrition, LLC; [2]Nutrianálisis Ltda.; [3]Castillo Consulting, Inc.*

A total of 45 commercial soybean meal (SBM) samples were analyzed for urease activity (UA) by both the AOCS Method (Official Method Ba 9-58, 2011) and the European Method (Journal officiel des Communautés européennes, n° L 155, 1971). Also, each sample was analyzed for trypsin inhibitor (TI) contents by the ISO Method (Animal feeding stuffs: determination of trypsin inhibitor activity of soya products. ISO 14902:2001). The 45 SBM samples were selected from a database of previously analyzed samples by the AOCS Method (Delta pH) and ranged from 0.000 to

2.051 pH units. Re-analysis yielded a range from 0.000 to 1.822 pH units with an r-square of 0.72 (P<0.001) for the correlation between previous analysis and the re-analysis. Analysis of the 45 samples by the European Method (EM) yielded a range from 0.000 to 1.440 mg N/g/min. The correlation between the Delta pH Method and the EM was 0.80 (P<0.001). TI contents ranged from 1.34 to 20.68 mg/g SBM. The correlation between Delta pH and TI contents was 0.81 (P<0.001). However, the correlation between the EM and TI contents was only 0.52 (P<0.001). Nevertheless, the regression equation for each method vs. TI contents provide similar estimates of TI values. These data indicate that there is a statistical correlation between the trypsin inhibitor concentration in SBM and the urease activity as determined by the Delta pH Method and the EM and both methods are highly correlated.

Key Words: urease activity, trypsin inhibitor, soybean meal, delta pH, European Method

M104 Evaluation of the combined effect of probiotics and coccidia vaccine in coccidia-challenged broilers Luis Valenzuela[1], Eduardo Vicuna*[1], Marc Pages[2], Martina Dardi[2] *[1]BIOMIN; [2]HIPRA*

Growth promoters have been banned in many countries, and as a result scientific and academic institutions are conducting research and investing significant resources to create alternatives and to reduce the incidence of other microorganisms such as *Eimeria*. The objective of this trial was to evaluate the protective effect of a synbiotic (PoultryStar®, BIOMIN GmbH, Austria) in broilers vaccinated against coccidiosis with a live attenuated vaccine, HIPRACOX® (marketed by HIPRA and containing precocious sporulated oocysts of *E. acervulina*, *E. maxima*, *E. mitis*, *E. praecox* and *E. tenella*) immediately after hatch and challenged with a coccidia species mixture at d15. Therefore, 456 day old Ross 308 chicks were housed for 35d and randomly assigned to one of the three groups, each consisting of 8 replicates. Treatment groups consisted of a non-challenged negative control (NEG), a challenged positive control (POS), and a challenged group supplemented with a synbiotic (PoultryStar sol®; 20 mg/bird/day; HCPS). One day old chickens of the HCPS group received a dose of HIPRACOX® vaccine. Birds at d15 in the POS and HCPS groups received 1ml/bird of coccidia challenge solution. PoultryStar® sol was applied via drinking water for the first 3 days of age and PoultryStar® me was applied via feed at 1 kg/ton during the starter phase (1-14) and 0.5 kg/ton during the grower phase (15-35). Enteric lesion scores were assessed on days 21 and 22 and fecal samples were collected from each group on days 6 and 7 post-vaccination, and 7 and 14 days post-challenge. Lesion scores in birds from the HCPS group were consistently lower compared to the IUC group. HCPS group showed less than half of the oocyst output level showed by the POS group on day 22. Daily weight gain was significantly better in NEG and HCPS groups. HCPS performed better when comparing BWG and FCR to birds from the POS group. In conclusion, the combination of the anticoccidial vaccine and the multi-species synbiotic had a positive impact on the zootechnical performance of the birds and on the coccidiosis lesion scoring after experimental induction of coccidiosis in this trial. Therefore, the combination of HIPRACOX® and PoultryStar® suggests a beneficial effect on broiler performance and gut health.

Key Words: Vaccines, synbiotic, coccidiosis, broilers

M105 The impact of mycotoxins (deoxynivalenol, fumonisin, and their combination) on performance in broiler chickens. 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 study was to evaluate the effects of the mycotoxins deoxynivalenol (DON), fumonisin (FUM), and their combination on growth performance in broiler chickens. A total of 480 Cobb-Cobb male broilers were obtained on the day of hatch and placed 10 birds per cage into 8 replicates battery cages. The experiment consisted of 6 treatments: (1) control; (2) DON 1.5 mg/kg; (3) DON 5.0 mg/kg; (4) FUM 20.0 mg/kg; (5) DON 1.5 mg/kg + FUM 20.0 mg/kg; (6) DON 5.0 mg/kg + FUM 20 mg/kg. All birds were fed *ad libitum* with a standard corn-soybean meal starter diet (in mash) from d 1 to 21. Total pen and feed weights were assessed on d 0, 8, 15 and 21 for weight gain and mortality-adjusted feed conversion ratio. Results showed no significant effect of mycotoxins on growth performance to d 8. From d 8 to 15, both DON and FUM combination treatments (DON 1.5 mg/kg + FUM 20 mg/kg; DON 5.0 mg/kg + FUM 20 mg/kg) showed impaired feed intake ($P \leq 0.05$) compared with the control treatment. Similar results continued from d 15 to 21 ($P \leq 0.05$). However, no significant effects were noted for BW gain or mortality-adjusted feed conversion ratio after adding single or combined mycotoxin in the diets on d 8 and 15. At d 21, cumulative BW gain was less ($P \leq 0.05$) in birds fed diets with DON 1.5 mg/kg + FUM 20 mg/kg and DON 5.0 mg/kg + FUM 20 mg/kg compared with the control. Mortality was unaffected by treatment. In conclusion, results from the current study showed that the DON (1.5 or 5.0 mg/kg) and FUM (20.0 mg/kg) combination contamination diets affected feed intake. The combination of DON and FUM (DON 1.5 mg/kg + FUM 20 mg/kg or DON 5.0 mg/kg + FUM 20 mg/kg) had negative effects on BW gain in broilers from d 0 to 21; whereas the individual additions of these mycotoxins did not significantly impact BW gain.

Key Words: mycotoxin, deoxynivalenol, fumonisin, broiler

M106 Trends in mycotoxin contamination in the United States corn 2015-2018 G. Raj Murugesan*[1], Erika Hendel[1], Paige Gott[1], Shelby Curry[1], Ursula Hofstetter[2] *[1]BIOMIN America, Inc.; [2]BIOMIN Holding GmbH*

Mycotoxins are harmful secondary fungal metabolites, and are of key concern to food and feed safety globally. In addition to compromised performance, mycotoxins negatively impact animal health. Although classic signs such as decreased feed intake and vomiting are known in the field as indicators for exposure, mycotoxins act as predisposing factors for diseases by immune suppression, causing inflammation, and modulating the gastrointestinal environment, even at low levels. This survey presents mycotoxin levels of corn samples from the 2018 harvest and compares these levels with those in previous years. Similar too previous years, new crop corn samples from various sources, were submitted for analysis starting from mid-August 2018 and consisted of corn (74%), corn silage (16%), and corn byproduct (10%). Samples were analyzed utilizing the liquid chromatography and tandem mass spectrometry (LC-MS/MS) method 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). Data are presented for major mycotoxin classes in Table 1. An average of 2 of the major mycotoxins were present in each sample tested positive, similar to 2016 but greater than 2017 ($P<0.05$). Prevalence of B-Trich has decreased compared with previous years, but average ppb is higher than 2017 ($P<0.05$). Prevalence, median, and average ppb of ZEN, FUM, and Afla are higher ($P<0.05$) than 2017. Contrary to previous years, average contamination level of Alfa has increased over 10X although not significantly different due to higher variation. The preliminary results from the 2018 corn harvest suggest a continued risk from mycotoxins produced by *Fusarium* fungal species (Deoxynivalenol, Zearaleone, and Fumonisins), and a potential increased risk of Afla compared to previous years. Because of the risk of multi-mycotoxin contamination in any given sample thus far, a comprehensive intervention strategy comprising of biotransformation of mycotoxins as well as support of the immune system and liver function beyond just adsorption.

Key Words: Deoxynivalenol, Aflatoxin, Fumonisin, Zearalenone, Corn

Environment, Management and Animal Well-Being

M107 Evaluation of the combined effects of moderate house relative humidity and increased air movement on bird health and welfare in a commercial broiler house Connie Mou*[GS], Michael Czarick, Brian Fairchild *University of Georgia*

High litter moisture (+35%) has been correlated with increasing risk factors relating to bird health and welfare. Litter moisture can be minimized with proper drinker management and maintaining a low house relative humidity (<40%) but doing so can come at a high cost. Depending on conditions, reducing Rh by just 20% could increase heating costs by 45% due to higher required air exchange rates. Alternatively, a grower could reduce litter moisture by maintaining a more moderate Rh level (<60%) and increasing air movement above the litter.

A field study was conducted on a commercial broiler farm to examine the effects of both maintaining a moderate Rh (<60%) and increasing air movement with the use of "high-volume" circulation fans. The study evaluated the effect on litter conditions, bird health and welfare. Two 12 m x 152 m houses were used for the study. One house had no circulation fans (CTL) and an adjacent house was equipped with eight-60 cm 1/3 hp circulation fans (TRT). These fans were capable of circulating 25% of house volume each minute, providing an air speed at floor level up to 1.25 m/s. Litter moisture, footpad scores, house temperature, and Rh were measured and recorded in both houses. The study was conducted from November to late March over three consecutive flocks.

Increasing air movement promoted litter drying and resulted in more uniform house/floor temperatures. Temperature sensors within TRT house differed by <1 C over 90% of the time compared to over 40% in CTL house. Uniform house/floor temperatures appeared to encourage young birds to spread themselves and their manure more evenly throughout TRT house enhancing litter drying. As a result of these effects along with moderate Rh, litter conditions were better. Litter moisture was below 25% on average versus CTL house that exceeded 35% on average. Drier litter conditions in TRT house resulted in improved paw quality. The TRT house had <20% occurrence of severe lesions by the end of each flock, compared to CTL that would exhibit >60% severe lesions. The TRT effect was less pronounced in Flock 3. Difference in litter moisture between CTL and TRT was <5%. Litter was drier in both houses during Flock 3 which may have been a result of higher air exchange rates due to warmer weather.

Key Words: litter management, air movement, bird welfare, footpad health

M108 Effects of dual warmth LED lights on broiler chicken performance Douglas Aldridge*[GS], Colin Scanes, Michael Kidd *University of Arkansas*

The development of new technology and its application through scientific based management practices have enabled continued advances in poultry production and improved well-being. Lighting sources and programs are an important factor in continuing these advancements. A choice approach was employed to determine the preference of 360, Cobb 700 broilers for two commonly used light emitting diodes (LED) of differing warmth: cool- 5000 K and warm- 2700 K, each at 20 lx. It was hypothesized that broilers would consume more feed under warm lighting while having better feed conversion under cool lighting. A choice system consisted of two pens (1.22 x 1.22 m) separated by a raised divider along a common sidewall allowing birds to pass between pens. Light treatments for the pens were the following: 1. cool-cool, 2. warm-warm and 3. cool-warm LED sources (n=6). Treatments began on day 14. Feed and water were available in each pen of the test systems. Feed consumption was measured on days 15, 23, 32 and 40 in trial one. During the second trial feed consumption was measured on day 15, 23, 32 and 43. Body weights were measured at day of age for both trials, day 40 in trial one and day 43 in trial two. In trial one feed consumption was 8% greater ($p<0.04$) for birds reared using cool-warm than those using cool-cool from day 32 till 40. In trial 2 birds reared using cool-warm had increased feed consumption between days 32 and 43 compared to both cool-cool ($p<0.02$) and warm-warm ($p<0.002$). However no difference were seen between treatments for feed conversion in trial one or two. Body weights were increased for the cool-warm treatment compared to warm-warm in both trials ($p<0.025$ trial 1, $p<0.022$ trial 2). This suggests that a uniform light warmth may not be optimal for broiler production

Key Words: Lighting, LED, Choice, Preference

M109 A new ventilation system to reduce temperature fluctuations in laying hen housing in continental climate Yang Wang*[GS], Weichao Zheng, Qin Tong, Baoming Li, Gang Chen *China Agricultural University*

Poultry suffers from large temperaturevariations with traditional tunnel ventilation and wet-pad evaporative cooling (TV) systemin continental climates, which negatively influences production performance, resulting in significant economic losses. An ideal ventilation system is desired for the retrofitted and new poultry houses. In this study, a new ventilation (NV) system based on the limited sticking jet principle was designed to mitigate the air temperature variations and improve the uniformity in poultry houses located in continental climate regions. The evaporative cooling pads are separately and uniformly installed on both sidewalls and exhaust fans are located on a gable wall in the NV system. The system can be operated in different modes for daytime and night time by automatic running or not running evaporative cooling according to indoor set-point temperature. The new ventilation is characterized by mixing fresh air with indoor air before it reaches the layer occupied zones, to provide air with uniform temperature in both vertical and horizontal directions. An analysis of the characteristics of the limited sticking jet is conducted and the design equations of the new tunnel ventilation cooling system are outlined. The performance of the NV system was evaluated in comparison with the TV system by measuring the thermal environment and layer performance of two poultry houses with different ventilation systems. The results showed that the NV system provided cooler air environment than the TV system, and the maximum fluctuation in average air temperature was reduced to 1 ºC (vs. 6.1 ºC in TV system,$P<0.05$). Compared to the TV system, the NV system provided more comfortable times than the TV system (83.3% vs 44.0%, $P<0.01$) evaluated on the basis of temperature humidity index, and reduced heat stress time by 29.9%, 42.2% and 69.3% along building length directions and by 54.2%, 24.0% and 16.7% along vertical directions based on temperature-humidity heat stress index (THI) values. The egg productions were higher in the NV system house than TV system house (20.3% vs 18.9%, $P<0.05$), the egg production increased by 1.4% and the mean bird's weights in NV system house were 21g higher than in TV system house.

Key Words: Poultry houses, Heat stress, Wet-pad evaporative cooling, Thermal environment, Layer performance

M110 Growth performance of four lines of chicken provided light during incubation Janessa Henry*[GS], Bruce Rathgeber, Xujie Li, Janice MacIsaac *Dalhousie University*

Getting chicks off to a good start in the rearing environment is key to health and productivity of the flock. One method of measuring how chicks are adjusting to their environment is weighing a select group of birds at placement and again 6 h later to determine early weight gain. In the current study two replicate trials were performed using 4 lines of chickens (Ross 308, Cobb 500, 1978 random bred male broiler line, Lohmann LSL-Lite) incubated in 4 different lighting treatments. Lighting treatments using LED bulbs were white (4100K), dim to blue and dim to red (ONCE Innovation) and a dark control. Lighting treatments were replicated twice in each of two trials by random assignment to 8 incubators. Photoperiod

was 12 h of light and 12 h of dark each day. A total of 768 birds were tagged at hatch and individually weighed at placement in the barn, and 6 h post placement. Birds of all strains were randomly assigned to one of two rooms and reared in free run environment with feed and water available ad libitum. Growth performance was monitored through the rearing period at d 7, d 14, d 25, and d 35. The experiment was a randomized complete block design using split plot with the main plot as a room of 4 incubators with each incubation treatment represented for each trial. The sub plot was individual tagged birds. An ANOVA using proc mixed procedure was used to differentiate the means. Body weight data were analyzed using repeating measures. Significant difference was determined when p-value was <0.05. The inclusion of light during incubation did not affect the hatch weights but had a significant impact on the body weight gain for all birds in the first 6 h. Birds incubated with blue or red light gained more during the first 6 hours after placement than those from dark incubators. Birds from incubators with white light were intermediate between the dark treatment and the other two lighting treatments. Analysis of growth data reported a light by strain by age interaction. The two commercial broiler strains exhibited a response to light at d 35. Cobb birds were heavier when incubated in red light compared to dark. Ross birds reacted differently to the light during incubation where birds hatched from white incubators were heavier than those from red lit incubators.

Key Words: incubation lighting, broilers, layers, body weight

M111 Impact of low protein starter feed and alternative lighting program on broiler performance parameters Daniel Adams*[GS], Matthew Livingston, Brittany Wall, Kimberly Livingston *North Carolina State University*

Consumers are becoming more concerned about the welfare of the animals raised for food. Consequently, some producers are obtaining various animal welfare certifications documenting birds are given proper nutrition that does not subject them to discomfort and "natural" light stimulus with continuous stretches of light and dark starting at an early age. These programs may alter standard production expectations. Therefore, this study evaluated how lighting program and a prestarter diet with reduced protein alters growth, feed consumption, and feed conversion ratio (FCR). A floor pen house with two distinguishable sides followed either standard commercial lighting program (0-7d, 23 h of light; 7-14d, 22 h of light, 14-21d, 21 h of light; 21-42, 12 h of light) or a welfare approved program (0-3d, 23 h of light; 3-21d, 16 h of light; 21-42, 12 h of light). Control diet contained 24% CP while low protein diet contained 15% CP. These two diets were given from 0-3d, after which all birds were placed on a 24% CP diet. A common grower diet was added at 17 d and a common finisher diet was added on 29 d. This resulted in a 2x2 factorial arrangement with light and diet as the independent variables. Eight hundred thirty-two Ross 708 chicks were allotted to 32 pens with 26 chicks per pen. Data were analyzed as a two-way ANOVA using JMP®. The low protein diet resulted in reduced body weight and greater FCR at 3 d of age (P<0.05). However, by 7 d of age the diet did not adversely affect performance (P>0.05). The lighting program affected the growth most consistently starting at 7 d of age where birds on the conventional lighting program had the greatest body weight (P>0.01). Interestingly the FCR was improved with the alternative lighting program at 14 and 35 d of age (P>0.05). However, no differences in body weight or FCR were observed at 42 d of age. In conclusion, having an initial low protein prestarter diet did not adversely affect performance after 3 days of age. Interesting, the alternative lighting program reduced body weight, but had a better FCR up to 35 d of age, and all differences disappeared by 42 d of age indicating there may be some compensatory gain.

Key Words: Lighting, Protein, Welfare, Broiler

M112 Evaluation of timing of hatching egg sanitization using the combination of hydrogen peroxide and ultraviolet light Karely Cantu*[GS], Craig Coufal *Texas A&M University*

Commercial chicken hatching eggs are often not washed or sanitized. Previous research with chicken hatching eggs has indicated that sanitization with the combination of hydrogen peroxide and ultraviolet light (H_2O_2/UV) decreases eggshell surface microbial loads and can improve hatchability. The objective of this study was to determine if the application time of H_2O_2/UV egg sanitization following collection would influence hatchability and chick quality. Treatments consisted of eggs that were untreated control, sanitized immediately after collection (SF), or sanitized following overnight storage in a cooler (S1). Surface eggshell microbial loads, hatchability, chick quality at hatch, and 7-day grow-out mortality were assessed. Approximately 2,400 broiler hatching eggs were obtained from a commercial broiler breeder flock at 62 weeks of age. SF and S1 eggs were treated on the breeder farm and then transported along with the control eggs to the lab for incubation. Ten eggs per treatment were sampled prior to incubation to enumerate eggshell microbial counts. Four incubators were used per treatment with 198 eggs placed per incubator. After 18 days of incubation, eggs were transferred to paired hatchers. At hatch, cull chicks and chicks with navel tags were counted. Good quality chicks from hatchers were placed in a corresponding floor pen and fed a standard broiler starter feed for 7 days. Mortalities were collected daily and recorded. A one-way ANOVA was used to analyze all data, and significant differences were determined at P<0.05. Eggshell APC for the control, SF and S1 treatments were 4.54, 2.71 and 2.21 $\log_{10}$ cfu/egg, respectively, and the SF and S1 treatments were significantly lower than the control. Hatch of fertile for the control, SF and S1 treatments were 84.2, 84.7 and 89.6%, respectively. Chick mortality at day 7 was 2.3, 1.3 and 1.1% for control, SF and S1 treatments, respectively; however, there were no statistical differences in hatchability, chick quality, or 7-day mortality. Data in this study demonstrated that using the H_2O_2/UV egg sanitization method on fresh or stored eggs could be an effective broiler hatching egg sanitization method to reduce eggshell microbial counts while maintaining hatchability and chick quality.

Key Words: hatching eggs, sanitization, hatchability, hydrogen peroxide, UV light

M113 Evaluation of dry hydrogen peroxide as a commercial hatchery sanitizer Laura Oxford*[1GS], Julia McElreath[1], Miguel Barrios[2], Brian Jordan[1], Jeanna Wilson[1], Nelson Cox[3] *[1]University of Georgia; [2]Synexis Biodefense Systems; [3]USDA-ARS*

Naturally occurring bacteria in a commercial broiler hatchery can be detrimental to hatchery performance as well as chick health even though sanitation and disinfection occur

routinely. Therefore, a method of constant sanitation could be a valuable tool to commercial hatcheries, and a commercially available product, gaseous dry hydrogen peroxide (DHP), was evaluated for this purpose. Preliminary studies have shown that DHP is effective at reducing microbial load on hatching eggs and does not negatively impact hatchability or chick health. The purpose of this study was to evaluate the effects of DHP on bacteria levels in a commercial hatchery. For this trial, a hatchery with two identical sides was used and half of the hatchery was treated with DHP through the HVAC system as well as with stand-alone units while the other half remained non-treated. Treated areas included an egg cooler, setter hall, hatcher hall, chick processing areas, and vaccine laboratories. The non-treated locations included an egg cooler, setter hall, and hatcher hall. Bacterial loads were measured by total ATP bioluminescence swab samples and static air plates using tryptic soy agar (TSA). Samples were taken from similar locations on each side of the hatchery for comparison, and from machinery in the chick processing areas. Static air samples and ATP swabs were collected for two weeks prior to treatment to establish baseline microbial load. During treatment, samples were initially obtained

bi-monthly, then weekly, then bi-weekly for 27 weeks. Data were analyzed using Prism statistical software with two-way ANOVA and Sidak's multiple comparisons test. In the treated egg cooler, there was a significant reduction from baseline in microbial load throughout the course of the experiment. There was no significant reduction from baseline in microbial load over the course of the experiment in the treated setter hall. Data from the chick processing areas was highly variable from week to week, which suggests that samples were more representative of post-processing sanitation than the effectiveness of DHP. This study suggests that a DHP product could be beneficial as a method of constant sanitation to reduce microbial load in commercial hatcheries.

Key Words: Dry hydrogen peroxide, Sanitation, Bacterial reduction, Commercial hatchery

M114 Impact of Every-day versus Skip-a-day feeding of broiler breeder pullets during rearing to compare intestinal development and overall performance Kelly Sweeney*[GS], Laura Oxford, Carla Aranibar, Woo Kim, Susan Williams, Jeanna Wilson *University of Georgia*

One of the most common feed restriction methods for broiler breeder pullets uses a variation of skip-a-day (SAD) feeding to prevent excessive body weight gains and poor flock uniformity. These feed restriction methods in the past have had a positive impact on flock performance. However, due to genetic improvements in feed efficiency of modern broiler breeders the intensity of feed restriction has increased. The objective of this experiment was to compare intestinal development and weight gain of breeder pullets fed a high fiber diet on either an every-day (ED) or SAD basis from standard chain feeders. The same developer ration and fed amounts were fed to both treatments (isocaloric and isonitrogenous). Day old Ross 708 pullet chicks (n=912) were randomly divided into four pens (n=228/pen) with two pens per feeding program. At 8, 12, 16, and 20 weeks five birds from each replicate pen were randomly selected to analyze intestinal development. Samples measuring approximately 2.54 cm in length were taken from the duodenum, jejunum, and ileum and were immediately placed in a 10% formalin solution. Routine tissue processing, embedding, sectioning and staining of slides was performed for measuring villi height and crypt depth using Lecia Application Suite V 4.8 software program. During sampling body and organ weights (liver, crop, proventriculus, gizzard, duodenum, jejunum and ileum) were measured and relative percentages calculated. All pullets were weighed at 4, 8, 12, 16, and 20 weeks of age and a sample of pullets (20% of the pullets) were weighed to maintain body weights according to the primary breeder recommendations. The data were analyzed by SAS SLICE using a significance level of $P<0.05$. Body weight gains were similar through 16 weeks with the ED pullets weighing 73.8 g more at 20 weeks. The relative liver and ileum weights are most consistently different over the rearing period with SAD pullet liver and ileum being heavier than ED. Greater liver and ileum weights in SAD pullets reflects the metabolic inefficiency of a SAD feeding method. Histological comparisons found that pullets fed ED had longer villi in the jejunum and ileum at 16 wk which suggests gastrointestinal tract changes that encourage greater nutrient absorption.

Key Words: Broiler Breeder Pullet, Skip-a-day, Every-day, Intestinal Development, weight

M115 Comparison of sites colonized in broilers through various routes when challenged with Salmonella Enteritidis at day 14 Lasheda Brooks*[GS], James Krehling, Kaicie Chasteen, Brittany Singh, Kenneth Macklin *Auburn University*

Salmonella Enteritidis (SE) is one of the major bacterial foodborne pathogens associated with poultry. Broiler grow-out facilities may contain this organism and it can be introduced to the broiler through various pathways. Five studies, oral (OR), subcutaneous (SQ), intra-tracheal (IT), ocular (OC), and cloacal (CL), were conducted to determine the influence of the inoculation route on colonization sites within broilers when challenged at d14. For each of the studies, 125 chicks were obtained from a commercial hatchery and 25 chicks were randomly assigned into five pens. Birds used in all studies were challenged with $1x10^4$CFU dose of SE on d14. At termination on d 35-38, the following tissue samples were collected from 100 birds: breast, pooled- bursa and thymus, ceca, crop, kidney, pooled-liver and spleen, skin, spinal cord, thigh, and, trachea. Also, swab samples were collected from the abdominal cavity, bone marrow, cloaca, and lung. Samples were incubated overnight at 37C and transferred into tetrathionate broth tubes and incubated for 48-72h at 37C. All samples were plated onto xylose-lysine-tergitol 4agar containing naladixic acid 100 ppb and novobiocin 15 ppb and incubated for 48h at 37C to determine SE presence. Data was analyzed using a general linear model to compare incidences based on the inoculation route and among samples within each of the studies. Differences were reported at $P\leq0.05$ with means separated using Tukey's HSD. There was a difference ($P<0.001$) in the total percent positive of samples collected for SQ, OR, CL, IT, and, OC with 2.3, 10.7, 15.5, 17.8, and, 23.1% positive, respectively. Numerically, the samples with the greatest SE recovery from highest to lowest include: ceca, bursa and thymus, cloaca, crop, skin, trachea, and, liver and spleen. All samples with the exception of abdominal cavity and bone marrow were found to be different ($P<0.001$) based on the inoculation route. Differences in the studies can be attributed to the ability of the organism to locate a hospitable colonization site and invade epithelial tissues. Based on these studies, the inoculation route could influence the tissues colonized. Future studies will continue to evaluate other *Salmonella* to determine if a similar pattern can be observed.

Key Words: Salmonella Enteritidis, challenge, colonize, inoculation route, incidence

M116 Effects of Stocking Density and Antibiotic-free Diet on Feeding and Drinking Behaviors of Broilers Guoming Li*[1GS], Yang Zhao[1], Joseph Purswell[2], Daniel Chesser[1], John Lowe[1] *[1]Mississippi State University; [2]USDA ARS Poultry Research Unit*

Stocking density and diet are among the core emphases in broiler production. Understanding their effects on broiler behavioral responses may provide valuable information to farm management and help to improve animal welfare and productivity. The objective of this study was to investigate broiler feeding and drinking behaviors at four stocking densities (SD, 27, 29, 33, and 39 $kg\cdot m^{-2}$) and with two diets [conventional vs. antibiotic-free (ABF)]. The treatment combinations of densities and diets were randomly assigned to 16 identical pens, with two pens for each combination. The conventional diet contained ionophores (Salinomycin, Saccox) and antibiotics (Bacitracin, BMD50), while the ABF diet without these additives. Ultra-high frequency radio frequency identification systems were used to monitor behaviors of 15 randomly selected broilers in each pen. Feeding and drinking behaviors of individual broilers were summarized in terms of daily time spent at feeder (DTSF) and at drinker (DTSD), daily percentage of time spent at feeder (DPTSF) and at drinker (DPTSD), daily number of feeder visit (DNFV) and drinker visit (DNDV), duration per feeder visit (DFV) and drinker visit (DDV), and hourly time spent at feeder (HTSF) and at drinker (HTSD). The results showed that the DTSF and DTSD were 51.2-90.2 and 22.7-32.7 $min\cdot bird^{-1}\cdot day^{-1}$, the DPTSF and DPTSD were 3.6-6.3% and 0.95-1.36%, the DNFV and DNDV were 48-64 and 42-54 $times\cdot bird^{-1}\cdot day^{-1}$, and the DFV and DDV were 1.14-1.41 and 0.54-0.69 $min\cdot visit^{-1}$. Statistical analysis showed that increasing SD significantly reduced DTSF, DTSD, DPTSF, DPTSD, DNDV, and DFV ($P<0.05$), but not DNFV ($P>0.05$). Additionally, the DDV under the SD of 39 $kg\cdot m^{-2}$ was significantly higher than that under other three SDs ($P<0.05$). Broilers fed with ABF exhibited lower DTSF, DTSD, DNDV, DFV, and DDV than those fed with conventional diet ($P<0.05$), but the effect of diets on DTSD, DPTSD, and DNDV was insignificant ($P>0.05$). The HTSF and HTSD peaked 2-3 h after lights were turned on and 2-3 h before lights were turned off. It is concluded that broilers alter their feeding and drinking behaviors at different SDs and diets, thus, farm manage-

ment needs to be adjusted accordingly to accommodate these behavioral changes and secure animal welfare.

Key Words: Broiler, stocking density, antibiotics-free, behavior, UHF-RFID system

M117 EFFECT OF DIETARY CALCIUM TO NON-PHYTATE PHOSPHORUS RATIO ON GROWTH PERFORMANCE OF BROILERS RAiSED UNDER LOW AND HIGH HEAT STRESS TROPICAL CONDITIONS Lorena Ramos de Mejia*[1GS], Jorge Camacho[2], Humberto Ugalde[3], Marlon Menjivar[3], Emilio Cura[3], Peter Ferket[1] *[1]North Carolina State University; [2]Serv Stats Camacho & Fernandez; [3]Cargill Protein Latin America*

Dietary Calcium:non-phytate phosphorus ratio (Ca:aP) may influence resistance to heat stress common in tropical regions where temperature-humidity index (THI=F+Humidity %) range is 150-170. Two trials were conducted on mixed-sex Cobb broilers to 35d to evaluate the responses of different dietary Ca:aP under low heat stress (LHS; 135-155 THI) and high heat stress (HHS; 160-170 THI). Trial 1 was a split-plot factorial arrangement of LHS and HHS conditions and 2.0 and 2.3 dietary Ca:aP, with 11 replicate pens of 50 birds per treatment. Trial 2 was a design of LHS and HHS conditions and dietary Ca:aP ratios of 1.85, 2.15 and 2.45, with 5 replicate pens of 50 birds per treatment. Body weight (BW) and feed intake were determined weekly, and mortality was recorded as it occurred. Level of heat stress was confirmed by a significant increase of ~0.5 C in cloacal temperature of 25 sampled birds per group at 3 and 4 wk. Carcass yield was determined at 5 wk. Data were analyzed using a randomized complete block design with a split plot over time, using the Mixed procedure of SAS® software. Dietary Ca:aP ratio or diet X environmental treatment effects on growth performance were not significant in either trial 1 or 2, although highly significant heat stress level effects were observed. There was no significant treatment effect on mortality rate, nor the incidences of locomotor problems and breast myopathies in either trial. In trial 1, LHS resulted in significantly higher 35d BW than HHS (1,882 vs 1,698g, P<0.0001) and lower 0-35d FCR (1.54 vs 1.61, P<.005), regardless of dietary treatment. Similar heat stress level effects were observed in Trial 2, favoring the LHS birds for 35d BW (2,075 vs 1,900g, P<0.002) and 0-35 d FCR (1.49 vs 1.54, P<0.07). The only significant treatment effect observed on carcass yield was on % backbone yield in trial 1, where Ca:aP of 2.0 was greater than 2.3 (11.5% vs 10.9%, P<0.01). As expected, growth performance of mixed-sex broilers decreased as the level of heat stress increased, but dietary Ca:aP ratio level did not influence this response to heat stress significantly. Therefore, we reject our hypothesis that dietary Ca:aP ratios between 1.85 and 2.45 influences resistance to low or high heat stress conditions in a tropical environment.

Key Words: broiler, heat stress, Calcium:phosphorus ratio, growth performance, carcass yield

M118 Amino acid chelated trace minerals reduces the severity of muscle myopathy in heat-stressed broilers Mikayla Baxter*, Sami Dridi, Elizabeth Greene, Juan David Latorre, Guillermo Tellez-Isaias, Casey Owens, Barabara Mallmann *University of Arkansas*

Heat stress (HS) causes a financial and physiological burden in poultry production and mitigating the negative effects of HS is essential. The purpose of this experiment was to determine if trace minerals supplementation would reduce the negative effects of HS. On day of hatch, 480 chicks were allocated into twelve environmental chambers in 2x2 factorial design. Each chamber was divided into two pens containing separate feeders and water jugs. Amino acid chelated trace mineral was supplemented in the water (½ oz per gallon) in one pen from each chamber. On d 28, temperature was increased to 35°C in 6 chambers and maintained until d42, with the remaining 6 chambers were maintained at 25°C. Feed and water intake were recorded daily, body weight and breast muscle palpation for woody breast was measured weekly. Blood and tissues including the gut were collected 2 hr post HS and at the end of the experiment to measure electrolyte levels and gene expression of stress and inflammatory markers. At the end of the experiment, the chickens were processed to determine carcass weight and composition. Data were analyzed by 2-way ANOVA with temperature and mineral supplementation as the main effects. Means were compared by Tukey's multiple comparison test and considered significant at $P<0.05$. Chronic HS caused an increase in core body temperature and water intake which decreased feed intake, body weight, and feed efficiency. HS reduced carcass yield, causing a decrease in the weight of all parts. HS also increased plasma corticosterone, blood partial oxygen and decrease partial CO_2, likely due to the panting occurring during HS. HS increased the expression of stress markers (HSP70), inflammasome (NLRP3) and proinflammatory cytokines (IL-6, IL-1β) in both circulation and gut. Although AA-chelated trace minerals had no statistical effect on growth performance, it reduced the expression of stress- and inflammation-related genes under HS but not under thermoneutral conditions. Interestingly, chickens treated with the mineral supplementation had a significant reduction in the incidence and severity of Woody breast syndrome by at least 20% regardless of environmental temperature.

Key Words: heat stress, performance, chickens, woody breast, amino acid-chelated minerals

M119 Identification of stress related to feed restriction and the use of a probiotic to modulate breeder pullet stress. Kristin Moncada*[1GS], Reagan Leger[1], Tara Price[1], Sohini Bhatia[1], Sean Davies[2], Suresh Pillai[1], Rosemary Walzem[1] *[1]Texas A&M University; [2]Vanderbilt University*

N-acyl-phosphatidylethanolamines (NAPEs) are the immediate precursors of N-acylethanolamines (NAEs) and can be produced by probiotic E. coli Nissle 1917 (EcN) carrying expression plasmid for the A. thaliana NAPE acyltransferase (At1g78690p, pNAPE-EcN). NAEs comprise a neuroactive lipid family; we've shown tissue specific expression of key NAE metabolizing proteins respond to feed intake in breeder hens. Breeder pullet management uses significant feed restrictions to maintain body weights suitable for reproduction. Increasingly viewed as a welfare issue, this study aimed to develop objective behavioral and physiological indexes of hunger related stress.

One hundred, day old, Cobb 500 broiler breeder females formed four groups of 25 per pen and reared according to breeder recommendations for 10 weeks with skip-a-day feeding started on week seven. Groups were naïve, and daily gavage with either vehicle, EcN or pNAPE-EcN. Recordings of behaviors on the same sequences of days ((skip day (S), a first on feed day (F1), and the second on feed day (F2)) were analyzed by trained students using Behavioral Observation Research Interactive Software (BORIS). State and point events involving drinking, feeding, and displacement behaviors were used in the development of an ethogram for chronic stress related to hunger. Birds processed on a skip day showed similar loss in body, liver and adipose weights ($p<0.05$) among groups. However, mean adipose weights were lowest in the pNAPE-EcN group on both fed and skip days ($p < 0.05$). Skip day blood samples had similar mean corticosterone (CORT) values that averaged 7.78 ng CORT /mL plasma; well below the 27.3 ng CORT /mL plasma average we observed in chicks of the same strain when housed singly. Adrenal gland weights were used to assess chronic stress. Adrenal weights of Naïve and pNAPE-EcN groups were not different while adrenal weights of vehicle and EcN gavaged groups were 24-25% heavier than pNAPE-EcN ($p < 0.03$). These outcomes support use of a chronic rather than single time point assessors of hunger related stress and the potential that pNAPE-EcN has in modulating hunger induced stress. AgriLife Research project #8738

Key Words: Probiotic, Breeder, Pullet, Feed Restriction, Stress

M120 Antemortem detection of wooden breast in commercial broilers and association with mortality, "turtle birds", and pulmonary disease Sesny Gall*[1], Hannah Sather[1], Marion Suyemoto[1], A. Sharpton[2], Luke Borst[1], H. Barnes[1], Rocio Crespo[1] *[1]North Carolina State University; [2]Perdue Farms*

Wooden breast is a production muscle disease of broiler chickens causing significant economic losses due to carcass rejection. The purpose of this study was to determine the associations between wooden breast, mortality, and pulmonary disease.

The total mortality of a 4,000 broiler chickens flock was 174 birds (4.35%). Between 40 to 56 days of age (the last 16 days of production), 14 chickens (0.35%) were culled and 49 (1.23%) were found dead. This means that 36% of the dead or culled were found in the last 16 days of production. Forty-six (73%) were males and significantly heavier than the targeted weight at the same age. Forty-four (70%) had wooden breast and 45 (71%) had pulmonary congestion and edema. Wooden breast and pulmonary disease occurred in 42 (68%), including 21 of 22 dead birds found on their back or "turtle birds." These turtle birds are birds that accidentally fall on their backs and cannot right themselves. They do not show any clinical signs except sometime respiratory distress that abated when the birds were manually turned over. These findings indicate wooden breast is associated with mortality prior to processing due to pulmonary disease in heavy male broilers.

In a follow-up study, we hypothesized that "turtle birds" were a pre-mortem presentation of wooden breast. The hardened or wooden breast muscle (*pectoralis major*) in "turtle birds" incapacitated them from raising their wings. An action they need to perform turn onto their legs after falling on their back. Inability to turn would cause respiratory distress and death after several hours on their back. In our experiment, the *pectoralis major* of 56-day-old Cobb 500 broilers were grossly scored for wooden breast by palpation, the ability of birds to touch their wings over their back, and ability right themselves when placed on their backs. Wing-touch ability was highly correlated with lower gross wooden breast scores ($P<0.05$). Antemortem wing-touch testing may serve as a tool to identify wooden breast in live birds.

In summary, although wooden breast has previously been considered primarily a meat quality issue only, it may also adversely affect live birds by increasing the prevalence of turtle bird syndrome and contribute to increased mortality.

Key Words: Wooden breast, Animal welfare, Meat quality, Turtle Bird

M121 Evaluation of electrocution, Koechner euthanasia device, Turkey euthanasia device, and carbon dioxide gas euthanasia methods on breeder Beltsville Small White turkeys Richard Buhr*[1], Caitlin Harris[1,2], Dianna Bourassa[3], Leonie Jacobs[4] *[1]USDA-ARS, USNPRC; [2]The University of Georgia; [3]Auburn Universtiy; [4]Virginia Tech*

On-farm euthanasia of individual broiler and turkey breeders using cervical dislocation is difficult due to the physical strength required to handle these older, large birds. Alternative euthanasia systems utilizing electrocution (120 V AC for 15 s), mechanical cervical dislocation (Koechner Euthanizing Device KED), captive bolt (Turkey Euthanasia Device TED), and carbon dioxide (30 to 70% in 4 min, CO_2) were developed and applied to Beltsville Small White breeder turkeys at flock termination. Euthanasia methods were evaluated for their ability to be administrated by a sole operator, relative safety, and physiological bird responses. On a single day, individual turkeys (40 hens 3.51 kg and 16 toms 8.26 kg) were placed head-first into a plastic cone positioned at approximately 45º that allowed access to the head for each euthanasia method and restricted the subsequent clonic/tonic musculoskeletal convulsions. Loss of induced nictitating eye membrane reflex, loss of pain reflex (toe pinch), cessation of musculoskeletal movement, skin damage, success of the initial application, and declaration of death were evaluated at 4 min post-application. All 14 turkeys euthanized using electrocution were determined dead with no observed induced reflexes or movements present at 4 min post application. For KED, 7/14 turkeys retained muscle movement and eye reflex, and 1/14 retained a pain reflex at 4 min. TED application resulted in 2/14 turkeys continuing to have muscle movement at 4 min post application. For CO_2, 2/14 turkeys retained muscle movement and eye reflex at 4 min. All turkeys euthanized with KED, TED, or CO_2 methods displayed clonic/tonic musculoskeletal convulsions during the 4 min. Broken skin/bleeding was present in all TED turkeys and in 12/14 KED turkeys. The rank order for ease of application by a sole operator was Electrocution : TED : KED : CO_2. Attaining a concentration of CO_2 at 30% within 1 min and then 70% within 3 min was variable. Presence of persistent musculoskeletal movements at 4 min (although brainstem reflexes were absent indicating unconsciousness) required a second euthanasia application by electrocution for 2/14 KED-treated and 1/14 CO_2-treated turkeys. Future research to refine euthanasia methods and apply for broilers breeders is planned.

Key Words: euthanasia, turkey breeders, electrocution, KED, TED

M122 Broiler chicken euthanasia by cervical dislocation: manual versus mechanical methods Leonie Jacobs*[1], Dianna Bourassa[2], Caitlin Harris[3,4], R. Jeff Buhr[4] *[1]Virginia Polytechnic Institute and State University; [2]Auburn University; [3]The University of Georgia; [4]USDA-ARS, US National Poultry Research Center*

Manual cervical dislocation (MCD) is a common method to euthanize broiler chickens, but can be challenging due to fatigue or a lack of strength or training. Mechanical cervical dislocation is a potential alternative. The aim was to assess the onset of brain stem death based on the birds' loss of induced reflex responses and skeletal muscle movements for two euthanasia methods. On three experimental days broilers were euthanized at 36 (n=60), 42 (n=80), and 43 days old (n=60), by MCD or the Koechner Euthanizing Device (KED). On the first day euthanasia was applied prior to bird placement in a cone. On days 2 and 3, euthanasia was applied to birds in cones and a modified KED treatment was added with the application of the KED, plus a head extension ~90˚ (KED+). On those 2 days, gap size was estimated between the atlas cervical vertebra and the skull (cm). The onset of brain death was assessed by recording the cessation of the nictitating eye membrane reflex, gasping reflex and skeletal muscle movements (sec). Additionally, neck skin damage was recorded (y/n).

In each batch ($P<0.05$ for all main effects, with exception of cessation of movement in batch 2, with $P=0.073$), MCD resulted in quicker loss of reflexes and movements (batch means of eye reflex duration: 2, 17, 19 sec; gasp duration: 0, 3, 20 sec; movement duration: 73, 88, 154 sec) compared to KED (batch means of eye reflex duration: 44, 58, 101 sec; gasp duration: 46, 64, 119 sec; movement duration: 89, 106, 193 sec), or KED+ (batch means of eye reflex duration: 46, 50 sec; gasp duration: 46, 56 sec; movement duration: 92, 110 sec). The eye reflex was absent and returned in 0, 10, and 15% of MCD birds, in contrast to 50 and 55% of KED birds (no data from batch 1), and 40 and 60% of KED+ birds. Skin damage occurred in 0% of MCD birds, 68, 75, and 95% of KED birds, and 85 and 95% of KED+ birds. In batch 2 and 3 KED birds had or tended to have a narrower skull-to-atlas gap compared to MCD and KED+ birds ($P=0.008$, $P=0.065$).

In all three batches, MCD resulted in quicker death in broilers of 36, 42 and 43 days old. Although the additional head extension in KED+ resulted in similar skull-to-atlas gap as MCD, reflexes and movements persisted. Therefore, MCD would be the recommended euthanasia method for broilers.

Key Words: Euthanasia, Broiler chicken, Brain death, Animal welfare

M123 Succession of broiler gastrointestinal microbiota from in ovo to harvest. Josh Rehberger, Sam Anderson, Rebecca Wujek, Eric Vang, Evan Hutchison, Jodi Delago*, Alexandra Smith, Tom Rehberger *Arm and Hammer Animal and Food Production*

Colonization of the gastrointestinal tract by beneficial bacteria is essential for broiler health, with early establishment of microbiota playing a vital role in stabilizing intestinal homeostasis and nutrient absorption, while promoting immunological protection. In general, microbiota is vertically transmitted from mother to offspring with some diversity gained through horizontal transmission from the environment. However, in commercial poultry production eggs are removed from hens, and many factors influence the establishment of bacteria such as use of antibiotics, sanitizers and egg handling methods. While these practices are credited with reducing pathogen loads and disease transmission, they may also be interfering with the colonization of beneficial bacteria. Our previous data indicated that microbial levels and compositions in day-old chicks is irregular and often colonized with pathogens such as avian pathogenic *Escherichia coli* and *Clostridium perfringens*. To understand microbiome succession and establishment, the microbes of breeder hens and their corresponding egg clutches within a large broiler integrator was analyzed. Gastrointestinal tracts from breeder flocks were sampled at lay of the targeted clutches. Eggs were sampled at early incubation, before transfer to the hatching room, 24 h post-transfer, at hatch and throughout production. Microbial analyses included plate counts as well as Illumina sequencing of microbial marker genes analysed with multivariate statistics using ordination methods. Counts of *E. coli*, clostridia and lactic acid bacteria were significantly lower *in ovo* and in day-old chicks compared to breeder hens and broilers. Bacterial populations differed between the shell and the interior of eggs. The greatest successional change in the microbiota occurred *in ovo* after eggs were transferred to the hatcher. The microbiota of the day-old chicks was similar to those *in ovo* post-transfer, but succession occurred rapidly over the first three weeks with stable communities at weeks 4 and 5. The results of this study provides insights into the means by which pathogenic organisms establish themselves *in ovo*, and will help implement interventions to improve chick quality.

Key Words: APEC, Hatchery, Clostridium perfringens, Microbiota

M124 The effects of downtime on key chicken production parameters Timothy Buisker*[1], Charles Corsiglia[2] [1]*Smart Data Science Solutions;* [2]*Foster Farms*

Purpose: To determine if a difference in downtime between bird placements is related to key production parameters of the subsequently placed flocks.

Experimental Design: This is a retrospective cohort analysis utilizing live operations and accounting data from 2009 commercial broiler flocks placed between January 2016 and October 2018. We analyze the effect of downtime on the following outcomes: average daily weight gain (ADWG), average final weight, week 1 mortality, livability, 6lb. adjusted feed conversion, total condemnations, and percent positive *Salmonella* boot sock tests. We then use machine-learning algorithms to model downstream effects and related costs that are associated with different proposed changes to downtime.

Results: Flocks with >= 18 days downtime prior to placement have statistically significantly higher average weight (6.12 vs. 5.98 lbs), ADWG (0.13 vs. 0.12 lbs), and livability (95.0% vs. 93.6%), and statistically significantly lower week 1 mortality (1.44% vs. 1.62%) and *Salmonella* boot sock prevalence (90% vs. 80%) compared to flocks with <=10 days downtime. The machine-learning-based model indicates inflection points at different days depending on the outcome of interest.

Conclusion: The number of days of downtime between bird placements has statistically significant effects on multiple key production parameters. Combined with standardized industry data or producer-specific expenditures, a machine-learning-based model can be used to optimize production costs relative to production-based constraints.

Key Words: downtime, production outcomes, weight, livability, Salmonella

M125 Effect of strain and stocking density on broiler conformation, performance, and welfare Shawna Weimer*, Darrin Karcher, Marisa Erasmus *Purdue University*

Broiler performance and welfare can be directly affected by stocking density. Consumer concern for broiler welfare has increased interest in raising broilers from slower growing strains at lower stocking densities; however, this will lower chicken production. Moreover, the impact of stocking density on the welfare of different broiler strains remains unknown. This study evaluated the effects of 2 stocking densities and 2 strains on broiler conformation, performance and welfare. Broilers from strains selected to reach market weight at 42 days (CONV) and at 63 days (SG) were exclusively placed into 16 pens at a stocking density of either 29 kg/m^2 (31 birds/pen) or 37 kg/m^2 (40 birds/pen) (n = 4 pens per replicate). Birds were provided the same starter, grower and finisher diets with phase changes occurring when SG birds matched CONV birds' body weight. Live body conformation measures of dorsal body length, pelvic width, shank length, shank width, keel length, breast width and breast depth were collected on 8 focal birds from each pen at Phase 1- start of the trial, Phase 2- starter (CONV days 1-13 and SG days 1-14), Phase 3- grower (CONV days 14-27and SG days 15-37) and Phase 4- finisher (CONV days 28-42 and SG days 38-63). At Phase 4, focal birds were scored for hock burn (HB), foot pad dermatitis (FPD) and toe damage (TD). Feed conversion ratio (FCR) and mortality for each pen were measured throughout the trial. CONV broilers had shorter dorsal body and shank length than SG at Phases 3 and 4 ($P < 0.001$). Phase 4 pelvic, shank and breast width of CONV broilers were wider than SG ($P < 0.05$). Phase 4 keel length was the longest for SG broilers stocked at 37 kg/m^2 compared to all other strain-stocking density combinations ($P < 0.01$). Final BW was similar (2.68 kg) amongst all strains and stocking densities ($P > 0.05$). CONV broiler FCR was 34% more efficient than SG (1.66 vs. 2.52; $P < 0.0001$). Broilers stocked at 29 kg/m^2 tended to have higher mortality ($P = 0.09$) than at 37 kg/m^2. SG broilers stocked at 29 kg/m^2 had the least HB but the most TD than other strain-stocking density combinations ($P < 0.01$). These results indicate differences in the effect of strain and stocking density on broiler conformation, performance and welfare.

Key Words: broiler, strain, stocking density, performance, welfare

M126 Performance evaluation of fan shades in reduction of light leakage and spatial variation of illuminance in commercial broiler houses John Linhoss*[1], Joseph Purswell[2], John Lowe[1], Gary Chesser[1] [1]*Mississippi State University Agricultural & Biological Engineering;* [2]*Joseph Purswell*

Lighting programs are an integral management tool for commercial poultry operations. Providing the optimum lighting environment is critical to physiological processes, welfare, and production efficiency. Recent research has shown that light intensity levels are dependent upon ambient sunlight conditions and control of light leakage can improve live performance. The objective of this study was to evaluate the performance of fan shades in reduction of light leakage and spatial variation of illuminance in commercial broiler houses. Data acquisition systems were constructed to measure both high-density static spatial illuminance and dynamic illuminance. Static illuminance was acquired at 605 or 810 locations for six commercial broiler houses with and without fan shades during a two-hour window around solar noon. Dynamic illuminance was acquired at nine locations in two commercial broiler houses with and without fan shades over a 24 hr period. Mean static illuminance at the fan end was significantly lower in houses with fan shades than in houses without fan shades (9.4 lx vs 80.6 lx, respectively, $P < 0.0001$). Mean dynamic illuminance at the fan

end was also significantly lower in the fan shade treatment than in the no fan shade treatment (18.5 lx vs 83.6 lx, respectively, P < 0.0001). Fan end static and dynamic illuminance values were significantly higher than pad and mid-house locations for both treatments (P < 0.0001). Results show that the fan shades were effective at reducing overall light intensity and improving uniformity of illuminance within a commercial broiler house.

Key Words: light intensity, illuminance, broilers, fan shade, lighting environment

M127 Air velocity under heat stress affects heavy broiler live performance and breast meat yield without changing meat quality or welfare parameters Edgar Oviedo-Rondon, Hernan Cordova, Viviana San Martin, Gustavo Quintana, Camila Alfaro, Ivan Cardenas, Ivan Ospina, Miguel Chico*, Bing Cheng, Yijia Zhao, Derek West, Lingjuan Wang-Li *North Carolina State University*

Heavy broilers with high breast meat yield are more susceptible to heat stress. Under high relative humidity, indoor air velocity (AV) becomes critical to offer optimal environmental conditions for growth and health. Four experiments were conducted under southeastern summer conditions to evaluate the effects of AV treatments (High *vs*. Low) on live performance, cut-up yields, meat quality and welfare parameters such as footpad dermatitis, hock burns, and leg angular deviations incidence of broilers raised from 28 to 61 d of age. In all experiments, a total of 264 Ross 708 28 d-old male broilers were randomly placed into 6 chambers for 3 replicates per AV treatment of 44 birds each. Broilers uniform in BW at 28d were fed grower, finisher and withdrawal diets in pellets. The AV of each chamber during each day were automatically adjusted to target broiler comfort zone according to environmental conditions and age, with average difference between treatments of 0.5 m/sec. Individual BW and incidence of leg problems were evaluated at 28, 42 and 61 d. Feed intake per chamber was recorded. Flock uniformity and FCR were calculated for each period. At 62 d of age, 18 broilers per chamber were processed, cut-up yields and breast meat quality evaluated. Data from all experiments were analyzed using the MIXED procedure of the SAS and arranged as a RCBD. Experiment was the blocking factor with chambers as the experimental units. Treatments were the fixed effect and the chamber effect was nested within treatment and considered as a random effect. The High AV improved ($P < 0.05$) BW gain (331 g) and FCR (18 points) in all periods and flock uniformity only at 61 d of age. The High AV also enhanced ($P < 0.05$) breast meat yield (0.79%) by increasing ($P < 0.05$) *Pectoralis major* muscle development. No effects ($P > 0.05$) of AV were detected on carcass, cut-up parts yields, meat quality (pH, cook and drip loss and shear force), pectoral myopathies (white striping, wooden breast, and spaghetti muscle), footpad dermatitis, hock burns, and bone relative asymmetry. Meat color was affected ($P < 0.05$) in 3 experiments. In conclusion, higher AV improved heavy broiler live performance, flock uniformity and breast meat yield without major impacts on meat quality or welfare parameters.

Key Words: Heat stress, broiler performance, ventilation air speed, meat yield, animal welfare

M128 Effects of butyrate and botanical feed additives on the performance of broilers raised on used pine shaving litter Mike Persia*[1], Nisana Siman-Tov[1], Haitham Yakout[2] *[1]Virginia Tech; [2]Adisseo North America*

An experiment was conducted to determine the effects butyrate, botanicals or the combination on the performance of 0 to 42 day old broiler chickens. In total, 2,380 male Hubbard x Ross 708 chicks were selected and allotted to 10 replicate pens of 34 chicks for the 7 dietary treatments. Treatments included a negative control (NC; birds raised on used litter without dietary supplements, 14But (500g/MT of a coated butyrate for the first 14 d followed by the NC diet), 42Bo (150g/MT of a blended botanical product for 42 d); 14But/42Bo (500g/MT of a coated butyrate for the first 14 d followed by 150g/MT of a blended botanical product until 42 d), 42But (500g/MT of a coated butyrate for 14 d followed by 250g/MT of a coated butyrate until 42 d), 42Tri 500g/MT of tributyrin for 14 d followed by 250g/MT of tributyrin until 42 d, and 42Co (500g/MT of Coban for 42 d). All diets were formulated using a basal diet and fed as a starter (0-14 d crumbled), grower (15-28 d pelleted) and finisher (29-42 d pelleted). Used litter was generated by placing 20 birds per pen on clean pine shavings for a 21 day period. These birds were provided 0.446 ml of CocciVac®B52 per pen in 1kg of feed. If significant ANOVA was detected, Fisher's LSD test was used to separate means ($P \leq 0.05$). Performance of the NC birds was poor (2.590 kg at 42 d) in comparison to commercial standards and was improved ($P \leq 0.05$) by 42Co, 14But, 42 But, 14But/42Bo treatments resulting in 2.718, 2.708, 2.707 and 2.693 kg at 42 d, respectively. Both 42Bo and 42Tri resulted in intermediate responses ($P > 0.05$) of 2.652 and 2.624 kg at 42 d. Over the 0-28 d period, mortality corrected FCR was improved with 14But, 14But/42Bo and 42Co treatments in comparison to the NC fed birds ($P \leq 0.05$), with 42Bo, 42But and 42 Tri resulting in intermediate responses. The response was lost over the 0 to 42 d period, but a trend ($P = 0.11$) was still evident. Although performance was reduced, lesion scores and oocyst shedding were low and not different among treatments ($P > 0.05$). Overall, supplementation of broiler chickens with butyrate for 14 or 42 d, a combination of butyrate and botanicals over 42 d or Coban for 42 days resulted in increased performance when raised on used liter.

Key Words: butyrate, botanicals, broiler, perfomrance

M129 Using machine learning based approaches to explore predictions in poultry production Tristan Bond[1], Todd Kelman[2], Roberto Carrasco[1], Joseph Gendreau[1], Nick Barbulesco[1], Maurice Pitesky*[1,2] *[1]AgriNerds; [2]UC Davis School of Veterinary Medicine-Cooperative Extension*

Optimizing poultry production requires a deep understanding of the data that is currently collected. Understanding specific on farm and processing data that positively and negatively affect production and food safety is essential toward optimizing food safety, production and profitability. Machine learning based techniques are capable of "searching" and "learning" for non-linear and interactive effects in order to create more reliable and accurate predictive models. This paper describes the utilization of various machine learning based models designed to better predict egg weight in commercial layers. Specifically, a training model and test model were created and a modification of the machine learning ensemble technique Random Forest was used to explore the ability to predict egg weights in various commercial flocks. In order to account for temporal variability, the utilization of 'lag data' to predict variables in a sequential sequence was employed. Results identified multiple variables including Age, Farm Location, Body Weight, Hen, Total Eggs, and House Style which were predictive of egg weight. For egg weight, the mean average error and root mean squared error were calculated to be 1.54 and 3.17 grams respectively. Random Forest and other machine learning based approaches offer a novel approach toward prediction and risk analysis in poultry production. The task of developing a robust model that accounts for intricate interdependence between several production parameters and outputs while predicting multiple sequential outputs is complex, machine learning techniques offer a novel approach towards overcoming this difficulty. The use of these models could offer a more comprehensive approach toward evaluating productivity and profitability in poultry production.

Key Words: Machine Learning, Poultry Production, Predictions

M130 ChickenBoy: a farmer assistance system for animal welfare and farm productivity Heiner Lehr*[1], Jörg Hartung[2], Johan van den Bossche[1], Maurice Mergeay[1], Daniel Rosés[1] *[1]Faromatics; [2]University of Veterinary Medicine Hannover*

The ChickenBoy is a robotic farmer assistance system. It is the worldwide first ceiling suspended robot that aims to assess aspects of animal welfare and help farmers increase farm productivity. At the current stage,

the ChickenBoy measures climatic conditions (temperature, relative humidity, CO2 and air speed), examines excrements using artificial intelligence, finds dead birds and defective nipple drinkers and identifies wet spots in the litter. Farmers access the robot via a cloud-based user interface or subscribe to one of the 15 available alerts that sends messages directly onto the farmers phones. We have created a full-scale economic model of farm performance to assess the impact of the ChickenBoy on farm productivity and to convince farmers that higher welfare can mean higher profits. In this contribution we will present the current state of the development with results from European farms.

Key Words: Broiler, Precision Livestock Farming, Welfare, Productivity, ChickenBoy

M131 Examination of the impact of range, cage-free, colony systems, and conventional cage environments on the labor inputs committed to bird care for three brown egg layer strains Kelly Brannan[1], Teresa Herman[1], Herbert Ledford[1], Anthony Thomas[1], Lisa Wilson[1], Kenneth Anderson*[2] [1]*NC Dept of Agriculture and Consumer Services, Piedmont Research Station;* [2]*North Carolina State University, Prestage Dept of Poultry Science*

The use of alternative production systems has increased in the commercial egg industry due to pressure arising from consumers concerns about hen welfare related to intensive production systems. Despite there being a paucity of research concerning how these changes will affect hen welfare, regulations are being established to promote alternative systems. One concern for egg producers is the extensive time commitment and labor costs required to manage alternative systems. Genetic selection for modern layers has improved hen performance but has been based upon intensive management systems. The objective of this study was to examine the labor-hours needed to manage 3 modern commercial brown egg layer strains in different traditional and alternative housing systems. All birds were managed under similar lighting and nutritional programs in either conventional cages (CC), enriched and enrichable colony cages (EC), cage free housing (CF), or free-range environment (R) from 33-81wk. Time data was collected daily using GLP compliant methods as employees completed their duties in each system. Wireless access within the houses ensured data was collected immediately upon completion of tasks using Google Drive. Animal Caretakers rotated between systems biweekly to prevent personnel bias and tasks consisted of routine animal care, egg collection, and collection of production data. Tasks were relatively uniform across all systems, with the exceptions of litter and pasture management required for CF and R, respectively. Data was transformed to reflect labor-hour per hen housed and hen surviving by strain and system for the production cycle. No significant differences for labor-hours were found between the strains, however highly significant differences were observed between systems. CC and EC required similar time commitments at 0.85 and 0.78h/hen housed respectively while R required significantly more time at 2.23 h/hen housed; CF provided a significant intermediate at 1.35h/ hen housed. Labor-hours for hen surviving were also significant and followed a similar trend. Although the time required had decreased compared to a previous time study at the same farm, extensive production systems were still observed to be highly labor intensive when compared to traditional housing.

Key Words: Alternative production, Labor, Range, Cage-free, Colony cages

M132 Behavior of laying hens reared in cage versus aviary pullet systems Maja Makagon*[1], Allison Pullin[1], Mieko Temple[2], Darin Bennett[2], Richard Blatchford[1] [1]*University of California, Davis;* [2]*California Polytechnic State University*

Pullet rearing affects keel bone development and the prevalence of keel bone fractures sustained by laying hens housed in enriched colony (EC) cages later in life. Collisions with objects and birds within the EC housing system are a main cause of keel bone fractures. We hypothesized that chicks raised in a multi-tier aviary rearing system would be better prepared to navigate (have fewer collisions with) their adult housing as compared to cage-reared chicks. At 19 woa, Lohmann LSL-Lite pullets were moved into a barn of 128 EC cages (approx. 25 hens/cage). Six of the 12 cages included in this study contained aviary-reared pullets, while the other six contained cage-reared pullets. Keel-mounted tri-axial accelerometers were used to detect the number of times that each of three focal hens per cage sustained impacts (accelerations >20G) at the keel over the course of two 10-day data collection periods (at 21 and 35 woa). Video recordings taken at the time the impacts took place were used to determine the number of times that each hen collided with an object or individual, and the action that led to the collision. The number of impacts and number of collisions were averaged by cage for each time period. Statistical analyses were conducted in SAS v9.4 using PROC GLIMMIX with pullet treatment (fixed effect), time (random effect), and their interaction. As predicted, cage-reared hens sustained more keel impacts (mean ± SE at 21 woa cage-reared: 20.3 ± 3.0, aviary-reared: 10.4 ± 3.0; at 35 woa cage-reared: 14.4 ± 3.0, aviary-reared: 4.7 ± 3.0; P=0.004) and collisions (mean ± SE at 21 woa cage-reared: 8.4 ± 1.4, aviary-reared: 4.8 ± 1.4; at 35 woa cage-reared: 3.9 ± 1.4, aviary-reared: 0.8 ± 1.4; P=0.03) over each 10 day period than aviary-reared hens. The number of collisions (P=0.008), but not total impacts (P=0.07), decreased over time. None of the time x treatment interactions were statistically significant (all P>0.05). The results indicate that pullet housing can have long term effects on the way in which hens use the EC system. These findings lend support to the assertion that the prevalence of keel bone fractures seen in adult hens might be reduced by helping hens develop better spatial abilities as pullets.

Key Words: pullet rearing, laying hen, keel, enriched cage, behavior

Tuesday, February 12, 2019

Metabolism and Nutrition

T133 Effect of in-feed supplementation of iron amino acid complex and iron sulfate on ascites mortality and blood indicators in broilers reared at high altitude. Miguel Forat[1], Manuel Gual[2], Marco Rebollo*[2], Duarte Neves[2], Leonardo Linares[2] [1]*Instituto Internacional de Investigacion Animal;* [2]*Zinpro Corporation*

Ascites is a leading cause of mortality and performance loss of broilers reared at high altitudes. Birds develop hypertension and compensatory polycythemia in response to this condition. Supplemental iron has the potential to improve oxygen delivery to alleviate the negative impact of ascites. Four dietary treatments were evaluated: 1) a standard non-supplemented diet (Control); 2) 40 ppm supplemental iron from $FeSO_4$; 3) 40 ppm iron from iron amino acid complex (Availa®Fe); and 4) 80 ppm iron from Availa-Fe. A total of 2640 Ross male broiler were randomly assigned to the treatments and housed in floor pens with 15 replicates each in a completely randomized design. Birds were raised to 49 days of age, at an altitude of 1800 m above sea level (Queretaro, Mexico), and were allowed *ad libitum* access to a high-density commercial diet. Performance, ascites mortality, and whole blood indicators (hematocrit, hemoglobin,

erythrocyte counts, total Fe content) were measured at 35 and 45 days of age. No statistical differences were found in performance to day 49. Ascites mortality was lower for all Fe supplemented treatments, compared to the Control ($P < 0.01$). Hematocrit and hemoglobin were higher for Fe supplemented treatments at 35 and 45 days of age ($P < 0.001$). Erythrocyte counts were higher for Fe amino acid complex treatments, compared to Control and $FeSO_4$, with a significant difference for 80 ppm Availa-Fe on d 35 ($P < 0.05$). Total Fe levels in blood were numerically higher for Fe amino acid complex treatments at 35 and 45 days of age. Birds consuming Fe sulfate had lower feed intake than all other treatments from week 2 to 5 ($P < 0.05$) and lower, though not significantly, performance. The use of supplemental Fe from metal amino acid complexes could help to reduce the negative impacts of ascites.

Key Words: ascites, broiler, Fe, high altitude, iron

T134 Dietary iron requirements for broilers fed different mineral sources Helvio Ferreira Júnior*[1], Bruno de Carvalho[1], Pedro Arnaut[1], Carolaine Ferreira[1], Filipe Monteiro[1], Raully Silva[1], James Pettigrew[2], Horacio Rostagno[1], Melissa Hannas[1] *[1]Universidade Federal de Viçosa; [2]Alltech*

A study was conducted to evaluate the iron (Fe) requirements for 20 d-old broilers fed iron from two different mineral sources. A total of 500 birds were randomly distributed into a 2 x 5 factorial arrangement (2 Fe sources x 5 Fe levels), totalizing 10 treatments, with 10 replicates of 5 birds per pen (considering as experimental unit). Iron sulphate ($FeSO_4.H_2O$) and iron proteinate (ProFe, Bioplex®Fe), at five supplementation Fe levels (0, 25, 50, 75 and 100 mg Fe/kg) were used to estimate the dietary Fe requirements for broilers. A semi-purified diet was used to obtain low levels of Fe, with 30% of corn, phytic acid and phytase to simulate a practical diet. At 20 d-old, birds and feeders were weighed to record body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE). At 21 d-old, one bird was selected for blood collection and slaughtered for Fe analysis in tissues. Data were subjected to ANOVA, with the model including source, Fe level, and their interaction. The requirements were estimated by quadratic polynomial model based on performance responses and iron concentration in tissues by orthogonal contrasts (at P<0.05). Increasing levels of Fe showed quadratic responses (P<0.05) for BW and ADG. Broilers fed ProFe showed quadratic responses (P<0.05) for final BW and ADG, while $FeSO_4.H_2O$ showed no quadratic response (P>0.05) for performance. No differences (P>0.05) were observed between the ProFe and $FeSO_4.H_2O$ for performance, Fe concentration in tissues and serum Fe (P>0.05). Considering the 29 mg Fe/kg of basal diet, Fe requirements to maximize final BW and ADG were 73.00 and 72.50 mg of Fe/kg, respectively, for increasing levels of Fe. Maximum BW and ADG were obtained from Fe levels of 72.30 and 72.20 mg Fe/kg for ProFe. Moreover, for the serum and Fe deposition in chest, liver and tibia, the Fe requirements were 95.80, 97.0, 120.3 and 106.0 mg Fe/kg, respectively for increasing mineral levels. Considering the above parameters, maximum responses were obtained with 94.6, 97.9, 116.1 and 99.5 mg Fe/kg for ProFe; while for $FeSO_4.H_2O$ were obtained with 96.9, 95.9, 124 and 116.9 mg Fe/kg. The values of Fe requirements in liver and tibia tissues for ProFe were lower compared to the inorganic mineral source.

Key Words: Iron, Requirements, Broilers

T135 The broiler breeder antioxidant vitamin status and impact on subsequent broiler performance Frank Parks*, mike coelho, Robert Jones *BASF Corporation*

As broiler breeders increase in size, the neutrophil function is depressed, requiring more antioxidant/electron transport vitamins (Brea, 2012). A total of 3000 female and 100 male Cobb 500 broiler breeders were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (60 females and 2 males/pen x 5 treatments x 10 replications). Treatments consisted of five broiler breeder vitamin supplementation levels (supp) (10 TIU A/3 TIU D3/ 30 mg E/9 mg Riboflavin/kg (10/3/30/9) (Low), 12/4/95/13 (AVG), 15/5/150/20 (high 25%), 19/6/227/25 (high 10%) and 22/8/295/30 (high 5%), while maintaining remaining vitamins at AVG survey supp (Coelho, 2018) and measured bacteria phagocytosis by neutrophils using a phagocytosis assay against clostridium perfringens and E.coli, Phagotest[R]. Breeders were fed mash corn/soy diets formulated to meet or exceed current cobb 500 breeder recommendations. Neutrophil bacteria phagocytosis by neutrophils increased up to high 10% vitamin supplementation (28, 52, 75, 91 and 92%, P=0.03, respectively. 33 weeks (wks) rate of lay (77.65, 82.21, 84.45, 91.21 and 92.54 %, P=0.04, respectively); Hatch (90.23, 92.41, 95.31, 96.57 and 97.12%, P=0.03, respectively); Fertility (90.91, 93.37, 96.51, 97.99 and 98.07 %, P=0.03, respectively); Hatching weight (40.71, 41.62, 43.87, 46.89 and 47.21, P=0.03, respectively. Hatching chick neutrophil bacteria phagocytosis by neutrophils increased up to high 10% vitamin supp (30, 55, 78, 93 and 93%, P=0.03, respectively. Broiler vitamin supp 9/4/30/8 (Low), 12/5/75/9 (AVG), 14/6/150/10 (high 25%, 19/6/187/15 (high 10%) and 22/6/265/20 (high 5%), while maintaining remaining vitamins at AVG industry supp (Coelho, 2018). Day 7 body weight (174, 178, 181, 183 and 184 g, P=0.02, respectively); FCR (0.87, 0.87, 0.85, 0.84, 0.82, P=0.02, respectively); mortality (3.2, 1.8, 1.3, 0.9 and 0.7%, P=0.02, respectively). In conclusion, broiler breeder and broiler performance parameters closely followed the neutrophil phagocytosis response. 33 wks broiler breeder performance significantly increased up to high 10% vitamin supplementation. D7 broiler performance significantly increased up to high 5% vitamin supplementation.

Key Words: broiler breeder, vitamin, antioxidant

T136 Improved growth performance response to hydroxychloride Zn and Cu partially driven by reduction in oxidative stress during growth Oluyinka Olukosi*[1], Sandra van Kuijk[2], Yanming Han[2] *[1]University of Georgia; [2]Trouw Nutrtion R & D*

A total of 1,080 Ross 308 male broiler chicks at zero-day old were allocated to two treatments in a randomized complete block design. Each treatment had 30 replicates with 18 birds each. The wheat-soybean meal diets had either of two sources of Cu (15 ppm added) and Zn (80 ppm added) (sulphate or IntelliBond® hydroxychloride). Birds and feed were weighed on days 0, 9, 21, 28 and 35. On day 28, blood was collected from two birds each from 15 randomly selected pens per treatment for analysis of plasma level of markers for oxidative stress. In addition, samples of pectoralis major muscle were collected from the same birds for analysis for expression of genes for protein synthesis and hydrolysis. On day 35, seven birds per pen were used for carcass evaluation and for visual scoring for white striping or wooden breast conditions. The litter in the pens and feet of 5 randomly selected birds per pen were assessed for litter or footpad dermatitis scores, respectively. There was no significant growth performance response to the dietary treatments until day 21, with exception of day 9 G:F which tended to be greater (P = 0.078) for birds receiving hydroxychloride Zn and Cu. Overall (day 0 to 35) weight gain was greater (P < 0.05) and feed intake tended to be greater (P = 0.071) for birds receiving hydroxychloride Zn and Cu. There were no treatment effects on the expression of genes for protein synthesis or degradation, except for 20S proteasome which had lower expression (P < 0.05) in birds receiving hydroxychloride Zn and Cu diet. The plasma levels for both uric acid and methyl malonic acid tended to be lower (P < 0.10) on day 28 in broilers receiving hydroxychloride Zn and Cu. There were no treatment effects on carcass yield measures, litter score, footpad scores or the scores for white striping or wooden breast. It was concluded from the current experiment that the superior growth performance observed in broilers receiving hydroxychloride Zn and Cu was partially driven by an attenuation of oxidative stress in birds during the period of the more rapid weight gain. The

implication of these, in combination with effect of protein synthesis and degradation, will need to be further investigated.

Key Words: Zinc, Copper, Oxidative stress, Broiler, Growth performance

T137 Physicochemical properties of ZnO correlated to their bioavailability in broilers Denise Cardoso[1], Denise Cardoso[2,3], Ian Cockshott*[3], Nathalie Meme[2], Yves Chevalier[1], Agnes Narcy[2] *[1]University of Lyon; [2]INRA; [3]Animine*

The bioavailability of a trace mineral source is related to its in vivo solubility, which in turn is determined by its physicochemical properties. It is still not clear which characteristics are more relevant in affecting solubility and bioavailability of feed compounds. Zinc oxide (ZnO) is a common feed additive used to supplement zinc in the diet of monogastric animals. However, different sources have shown different responses on animal bioavailability. This study hypothesized that different sources of feed grade ZnO have various physicochemical features that lead to distinct bioavailability values. Over 40 samples of ZnO have been collected from the feed industry worldwide. Samples were analyzed for density, tapped density, particle size, shape, specific surface area and dissolution kinetics. A principal component analysis (PCA) was performed to define the most relevant physicochemical characteristics and categorize the samples into groups. Representative products from each family were selected for in vivo trial to measure the effect of their characteristics on the zinc bioavailability in broilers. 135 animals were fed a standard starter diet from day 1 after hatching up to day 7. At day 8, animals were allocated in individual cages and fed one of each treatment. Treatments consisted of a basal diet with 23ppm of Zinc from the feed and 7 diets with supplement zinc oxide or sulfate at 6 or 12 ppm. Animals were slaughtered at day 22 and 23. Results indicated a significant interaction on source and the level of Zn ($p < 0.001$) on bone concentration. The bioavailability of the different sources varied from 37 to 132% in relation to zinc sulfate. In conclusion, ZnO sources have variable physicochemical properties, which affects its dissolution kinetics and bioavailability.

Key Words: Zinc oxide, Bioavailability, Characteristics, Dissolution

T138 Is the nutritional requirement of zinc different when supplied by organic source? Bruno Carvalho*[1], Helvio Ferreira Júnior[1], Diego Silva[1], Pedro Arnaut[1], James Pettigrew[2], Luiz Fernando Albino[1], Melissa Hannas[1], Horacio Rostagno[1] *[1]Universidade Federal de Viçosa; [2]Alltech*

The objective of this study was to update the nutritional requirement of Zn for broilers from organic and inorganic sources. To this, 500 male Cobb broilers from 1 to 17 d-old were used. The animals were housed (1-7d) following Cobb guidelines receiving a corn-soybean meal diet with low trace mineral content to avoid high body stores. At 8d, birds were weighed and randomly allocated in a 2x5 factorial design with 10 replicates of 5 animals each. The treatments consisted of 5 levels of supplemental zinc (0; 19; 38; 57 and 76 mg/kg) from 2 sources (ZnSO4, with Fe, Cu and Mn supplemented as sulphates and Se as selenite, or Zn proteinate, with Fe, Cu, Mn also supplemented as proteinates and Se-Yeast). The animals were housed in plastic cages with plastic feeders and received demineralized water and feed *ad libitum*. The experimental diets were semipurified (12mg/kg of basal Zn), with 30% of corn and cellulose, phytic acid and phytase to approach a practical diet. At 13 d, trays covered with plastic sheeting were installed to allow total excreta collection twice daily for 4 days to evaluate trace mineral balance. At 16d, the birds and feeds were weighed to determine BW, ADG, ADFI and feed conversion (FC). At 17 d, one bird per cage was slaughtered to collect tissues to determine mineral concentration. Data were subjected to ANOVA, with the model including source, Zn level, and their interactions, and the requirement was estimate using the quadratic response of each source by orthogonal contrats (if $P<0.05$). Considering 12 mg/kg of Zn in basal diet, the results suggest that the requirement to improve BW is 61.7 mg/kg of inorganic Zn or 57.9 mg/kg organic Zn. Alternatively, 64.7 mg/kg of Zn independent of source was determined to achieve the lowest FC. To maximize Zn retention, this requirement is 77.3 (inorganic) or 89.0mg/kg (organic) of Zn. The highest Zn deposition in liver and tibia were obtained with 81.5 and 74.0 mg/kg inorganic Zn and 78.3 and 89.9 mg/kg of organic Zn. However, with those requirements the highest liver and tibia deposition were 80.6 and 175.1 ppm for inorganic and 86.7 and 189.6 ppm of organic sources, respectively. In conclusion, there are significant differences in Zn requirement for broiler depending of source and the parameters evaluated.

Key Words: Zinc, Requirement, Trace mineral, Mineral balance, Broiler

T139 Performance and necrotic enteritis lesion scores of broilers fed diets supplemented with sodium bisulfate, by lowering salt, in two floor pen trials with live coccidia vaccination, used litter, and Clostridium perfringens challenge John Pitts[1], Michael Sims[2], Danny Hooge*[3] *[1]Jones-Hamiton Company; [2]Virginia Diversified Research Corp.; [3]Consulting Poultry Nutritionist*

Two feeding trials were conducted with straight-run Cobb 500 broiler

chicks to evaluate performance and necrotic enteritis (NE) lesion scores

when exposed to live coccidia vaccine (d 0), used litter (d 4), and *Clostidium*

perfringens (*Cp*; toxin producing, but BMD® resistant) challenges (d 17-19

by oral gavage and via water trough). There were 30 and 34 chicks started

per pen (4x5 ft, 1.22x1.52 m) in Trials 1 (42 d) and 2 (40 d), respectively,

and 10 replicate pens/treatment. In each trial, an unsupplemented,

unifected control (UUC) and unsupplemented, infected control (UIC) were

used. In Trial 1, the 3rd treatment was got dietary sodium bisulfate (SBS;

19.1% Na, 26% S) at 0.40% inclusion by lowering salt (NaCl) and challenge.

Trial 2 was a dose-response study with 0, 0.1, 0.2, 0.3, or 0.4% SBS in diets

by lowering salt or BMD® at 55 ppm 0-28 d than Stafac® 22 ppm 29-40 d

(AGP). Trial 1 had crumble/pellets and Trial 2 mash feeds. In each trial, NE

lesion scores (0=normal to 3=severe) were taken using 3 random birds/pen.

In Trial 2, ileum-jejunum and ceca contents were analyzed separately at 25 d

and end (pH, VFAs, minerals, microbes); that data is available but will not

be presented herein. Both trials used Completely Randomized Design ($P\leq0.05$)

with means separated in Trial 1 by Tukey's test ($P=0.05$) and in Trial 2 by LSD

($P=0.10$). In Trial 1, UIC had significantly ($P<0.001$) higher 21-d NE lesion score,

lower 42-d BW, and higher mortality-adjusted feed conversion ratio (MAFCR)

than UUC. The SBS (0.4%) FCR and MAFCR were improved ($P<0.001$; $P=0.016$)

vs. UIC, and BW (1.709 kg) was intermediate between UUC (1.874 kg) and UIC

(1.571 kg) ($P<0.001$). In Trial 2, 21-d NE lesion scores were higher ($P<0.001$)

for UIC than UUC, with SBS (0.1-0.2%) or AGP lower than UIC. The 40-d BW

was greater (*P*=0.01) for SBS 0.2% or AGP than UIC, and FCR was approaching

significance (P=0.106) with SBS 0.3% lower than UIC or AGP (LSD 0.1). The

0-40 d mortality % was lower (P=0.055; LSD 0.1) for SBS 0.3% than for UIC or

AGP and statistically equivalent to UUC. In conclusion, the SBS diets showed

significantly better performance than basal diets for broilers under *Cp*

challenge by improving NE lesion scores, BW, FCR, and mortality, with SBS

0.3% diets supporting best performance overall.

Key Words: antibiotic, broiler, Clostridium perfringens, necrotic enteritis, sodium bisulfate

T140 In vivo collagen synthesis in Pectoralis major over different ages of bird grow-out cycle in two strains of commercial broilers
Pramir Maharjan*[1], Jordan Weil[1], Antonio Guerra[1], Katie Hilton[1], Nawin Suesuttajit[1], Barbara Mallmann[1], Justina Caldas[2], Casey Hanning[1], Craig Coon[1] *[1]University of Arkansas; [2]Cobb-Vantress, Inc.*

A study was conducted to understand *in-vivo* soluble (S-) and insoluble (I-) collagen fractional synthesis rate (FSR) in breast muscle *(Pectoralis major*) using stable isotope flooding method in two commercial strains of broilers: strain A and strain B during grow out cycle (d 21, d 28, d 35, d 42, and d 57). Birds (n=900 each strain) were fed standard diets throughout the study as per recommended industry guidelines for the strain. FSR (% h^{-1}) was determined using precursor–product method: ΔEp/A* 1/t, where ΔEp enrichment change between baseline tissue sampling and tissue sampling taken at time 't' post infusion of tracer, and A is the area under the curve derived from the decay in plasma enrichment over time 't'. Birds (n=10) were infused with 1-^{13}C proline as tracer amino acid at each sampling age, and isotopic enrichment ^{13}C-to-^{12}C ratio for muscle and plasma samples were measured using the ratio of fragments 287 to 286 derived from GC MS. Breast fillet from sampled birds were also subjectively scored for manifestation of woody breast (WB) myopathy conditions. FSR on per hour basis were not detected for strain A for S- and I-collagen for earlier ages sampled whereas it was 0.06 % h^{-1} at d 56. FSR values for I-collagen tended to be lower for birds with higher WB scoring at d 56 when individual bird variation was taken into account. Higher FSR values were detected at earlier ages (d 21 and d 28) than later ages ($P < 0.01$) for strain B. FSR (% h^{-1}) at d 21 were 0.19 (± 0.01) and at d 28 were 0.052 (± 0.019) for I- collagen fractions. FSR values for S- and I- collagen tended to decrease at later ages of sampling. FSR values at d 56 were not detectable (0 % h^{-1}) on hourly basis for strain B. FSR values were not observed to be correlated with subjective WB scoring for the analyzed samples (R^2=0.01) in strain B. The findings suggest that there is marked age related changes in collagen metabolism for both S- and I- collagen protein in poultry breast muscle tissue for both strains. Since strain B had the highest WB scoring during the study, a slower synthesis rate for I- collagen in *P. major* for strain B at a later age may be an adaptive physiological mechanism to decrease synthesis and deposition rate of I-collagen in extracellular matrix.

Key Words: collagen, Pectoralis major, synthesis

T141 Improving laying hens' performance by using dietary probiotic
Mohamed EL-MANYLAWI*, Ahmed ABBAS *Animal Production Department, Faculty of Agriculture, Cairo University*

The present study was conducted to evaluate the lying hens performance by using dietary *Lactobacillus acidophilus* as a probiotic supplementation. One hundred-89 40-wk-old laying hens (Hy-line) were randomly assigned into 3 groups with 3 replicates of 21 birds each (63 laying hens per group) Lactobacillus Acidophilus were added to the hen's diet by 0.00 as control, 0.10 and 0.20% as supplementation for 6 wk.. All data were subjected to one-way ANOVA, and the means were compared for significance by Duncan's multiple-range tests (1955). Results showed that egg yolk and liver cholesterol, plasma lipid profile and hen layer performance were evaluated. The plasma HDL-cholesterol increased by 9.6 and 10.6%, respectively, while, plasma LDL-cholesterol significantly decreased by 10.9 and 16%, respectively, at the levels of 0.10 and 0.20% probiotic in diets compared by control. Moreover, the results showed significantly (P?0.05) reduction in the liver and egg yolk cholesterol concentrations compared with control group, for hens fed diets supplemented with 0.10 and 0.20% probiotic. Feed conversion ratio significantly improved, egg weight and feed intake significantly ($P < 0.05$) increased at the levels of at the level of 0.20%. Also,. However, Egg production was not affected during the 6-wk of feeding probiotic 0.10 and 0.20%. In conclusion, using *Lactobacillus acidophilus* as probiotic supplementation reduced plasma, liver, and egg yolk cholesterol. Therefore, it may be a recommended to commercial production of low cholesterol eggs along with positive on effects on performance of laying hens

Key Words: laying hen, probiotic, Lactobacillus acidophilus, cholesterol, performance

T142 Supplementation of Bacillus subtilis DSM 32315 in laying hen diets positively influences their production performance and intestinal microbial composition.
Bayo Sokale*, Anita Menconi, James Wen *Evonik Corporation*

Two independent field trials were conducted to evaluate the effects of *Bacillus subtilis* DSM 32315 (*B. subtilis*) on production performance and intestinal microflora of commercial laying hens. In trial 1, a total of 174,151, 18-week old Hy-Line W-36 strain pullets were allocated to 2 adjacent rooms within the same barn and fed diets supplemented with (test group) or without (control group) *B. subtilis* up to 72 weeks-of-age (woa). In trial 2, a total of 60,832, 18-week old, cage free Hy-Line Brown pullets were equally allocated to 2 separate barns of test and control groups up to 62woa. In both trials, the diets provided to birds in the test groups were supplemented with spores of *B. subtilis* at an in-feed concentration of 1×10^6 cfu/g of feed. In trial 1, average egg production from 23 to 72woa, and 45 to 72woa (post-peak laying phase) were 1.6 % and 3.0 % higher in the test group compared to the control group. The cumulative number of eggs per hen-housed (E/HH) from 23 to 72woa was 324 vs. 312 in the test and control group, respectively. During the post-peak laying phase, egg mass was 55.2 g/d and 54.0 g/d in the test and control group, respectively. Furthermore, feed conversion ratio (FCR) in the test and control group were 1.80 g/g and 1.79 g/g, respectively. At 72woa, the microbial composition of the small intestine (population of anaerobic and aerobic bacteria, C. *perfringens*, gram negative enteric bacteria, and Lactic acid bacteria) were evaluated. Overall, there were total higher bacteria counts in birds belonging to the test group compared to those in the control group (Log_{10} 5.68 vs. 4.62). In trial 2, average egg production from 23 to 62woa was 1.3 % higher in the test group compared to the control group. Cumulative E/HH and egg mass in test vs. control group from 23 to 62woa were 260 vs. 256, and 52.2 g/d vs. 51.2 g/d, respectively. In addition, from 45 to 62woa, egg mass was 50.0 g/d and 50.3 g/d, while FCR was 2.08 g/g and 2.10 g/g in the test and control group, respectively. In conclusion, these results indicate that dietary supplementation with *B. subtilis DSM 32315* could improve production performance of laying hens in the first laying cycle, by modulating the composition of the intestinal microflora.

Key Words: Bacillus subtilis, egg production, intestinal microflora, laying hens

T143 Effects of a multi-strain direct fed microbial (Bacillus subtilis DSM 32324, DSM 32325 and Bacillus amyloliquefaciens DSM 25840), on Salmonella Enteritidis colonization in laying hens Roland Koedijk*[1], Dorthe Sandvang[1], John Schleifer[2], Charles Hofacre[3], Anée Kehlet[1] *[1]Chr. Hansen A/S; [2]Chr. Hansen Inc.; [3]Southern Poultry Research Group*

The use of *Bacillus* strains as probiotic supplements in animal diets is expanding rapidly and is demonstrating immune stimulation, digestive enzyme production and competitive exclusion as the most prevalent modes of action. The objective of the current study was to investigate the effect of a combination of three Bacillus strains; *Bacillus subtilis* DSM32324, *Bacillus subtilis* DSM32325 and *Bacillus amyloliquefaciens* DSM 25840*,* on *Salmonella* colonization in laying hens. A total of 198 ten-weeks-old Hyline W-36 pullets were divided into two treatment groups and housed in individual cages. T1: Control, T2: Bacillus probiotic 1.6 x 10^6 CFU/g feed. At week 17 all birds were orally challenged with *Salmonella* Enteritidis (1.0 x 10^9 CFU/bird). Ceca samples were collected at day 7 (48 birds per treatment group) and at day 14 post-challenge (51 birds per treatment group). From each sample *Salmonella* was analyzed. In total samples from week 18 and 19 showed a significant ($P < 0.015$) reduction in *S.* Enteritidis positive samples in the probiotic group (52.5%) compared to the control group (66.7%). In conclusion, this study indicates that a *Bacillus*-based probiotic can reduce the prevalence of *Salmonella* and hence can be used as a valuable contribution to improve flock health and safety of poultry products.

Key Words: Salmonella, Food safety, Bacillus, Probiotic, Laying hens

T144 Effects of fumonisins and deoxynivalenol on layer hens and the efficacy of a mycotoxin deactivating enzyme and a bacterial strain to counteract Verena Starkl*[1], Barbara Doupovec[2], Sabine Masching[1], Dian Schatzmayr[2], Ursula Hofstetter[1] *[1]Biomin Holding GmbH; [2]Biomin Research Center*

The BIOMIN Mycotoxin Survey analysis since 2004 global mycotoxin contamination. More than 10 000 samples of different feed and raw materials were analyzed in 2018 and the global dominance of deoxynivalenol (69%) and fumonisins (68%) in all samples was shown.

Two components were tested to counteract effects of low level contamination of fumonisins and deoxynivalenol on layer. FUM*zyme*® (DSM 32159), a fumonisin esterase, biotransforms the toxic fumonisin B1 (FB1) cleaving off two tricarballylic acid side chains into the much less toxic hydrolyzed fumonisin B1 (HFB1). The levels of FB1 and HFB1 in feces are recognized biomarkers. BBSH797® (DSM 11798), a life microorganism of the *Coriobacteriaceae* family, that uses trichothecenes as a substrate and reduces their toxic 12,13-epoxide group into non-toxic metabolites.

Layers were fed a diet that contained 7.8 mg/kg fumonisin B1 (FB1), 1.7 mg/kg deoxynivalenol (DON) and 0.290 mg/kg zearalenone (ZEN). All levels are under the level of guidance for respective mycotoxins recommended by the FDA. 3 groups were formulated: a negative control without mycotoxins and without FUM*zyme*® and BBSH797®, a positive control with the amount of mycotoxins mentioned before, and a group with the same amount of mycotoxins treated with 15 Units of FUM*zyme*® and 1.7^10^8 cfu BBSH797® per kg finished feed. 150 layer hens (Lohmann Brown, 20 weeks of age) were randomly distributed to 30 pens. The trial lasted for 8 weeks and mashed feed was used.

The laying rate was numerically affected by mycotoxins in the diet. After 56 days the laying rate was 96.53% in the negative control, 94.75% in the group with mycotoxins and 97.62% in the group where the mycotoxins were counteracted with FUM*zyme*® and BBSH797®. When biomarkers in the feces of layers in the different groups were compared, a statistically significant difference ($p<0.05$) was observed. In the feces of the group with mycotoxins 11.2 nmol/g FB1 and 1.4 nmol/g HFB1 were found in the feces and in the group with mycotoxins and treated with FUM*zyme*® 4.8 nmol/g FB1 and 10.9 nmol/g of feces of HFB1 were found, demonstrating the degradation capacity of fumonisin esterase. Low levels of mycotoxins lead to numerical differences in performance and health of layers.

Key Words: fumonisin, deoxynivalenol, fumonisin esterase, layer

T145 The effects of refined functional carbohydrates (RFCs) supplemented to laying hens on egg production and mortality under commercial conditions Jill Nezworski*[1], Sangita Jalukar[2], Dan Karunakaran[2] *[1]Blue House Veterinary LLC; [2]Arm and Hammer Animal and Food Production*

Enzymatic hydrolysis of yeast produces refined functional carbohydrates (RFC) which have been shown to improve broiler performance and reduce *Salmonella* prevalence. This study was conducted to evaluate the effect of RFC supplemented to laying hens on egg production, mortality and *Salmonella* prevalence. A total of four commercial flocks were selected and assigned to two treatments. 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 till 45 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. 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 two control and two RFC houses were combined and means calculated for each parameter. RFC supplementation reduced cumulative mortality at 45 weeks of age from 2.86% in control to 1.82% in hens fed RFC. Average eggs per hen housed (EHH) were 159 for control and 169 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, egg production, Salmonella, Food Safety

T146 The impact of feeding Black Soldier Fly Larvae on laying hen performance and egg quality Paul Patterson*, Luke Trimble, Arianna Ferguson, Heather Sciubba, Nuket Acar *Mississippi State University, The Pennsylvania State University*

The Food and Drug Administration and Association of American Feed Control Officials recently approved Black Soldier Fly Larvae (BSFL) as a feed ingredient for poultry. Based on a favorable nutritional profile (3682 kcal/kg, 34% CP, 34% EE and 3% linoleic acid) and previous experience feeding BSFL to broiler chickens and turkeys, a study feeding BSFL to mature laying hens (60-64 wks of age) was undertaken. Two hundred and sixteen commercial Single Comb White Leghorn hens were divided into 4 groups of 54 hens with one control group and 3 treatment groups. The hens were fed nutritionally complete, iso-caloric, iso-nitrogenous diets based on corn and soybean meal with 3 treatment levels of BSFL (6, 12 and 18%) and a control.Hen body weight ($\bar{x}$=1737g), egg production ($\bar{x}$=85.02%) and egg size ($\bar{x}$=64.17g) were not significantly impacted by the BSFL treatment diets compared to the control ($P > 0.05$). However, feed intake was significantly reduced and feed conversion (kg egg/kg feed) was significantly improved by greater dietary levels of BSFL ($P < 0.05$). Measures of egg quality including blood spots, meat spots and yolk color were not impacted by the BSFL treatment diets ($P > 0.05$), although egg specific gravity was higher ($\bar{x}$=1.075 vs. 1.073) and albumen height ($\bar{x}$=7.99 vs. 8.55) and Haugh units ($\bar{x}$=87.54 vs. 91.28) were lower among the treatment eggs compared to the controls ($P < 0.05$). Egg yolk fatty acid profile was also significantly impacted by the BSFL dietary treatments. Greater amounts of yolk fatty acids (C12:0, C14:0, C14:1n5 and C21:0) were realized with increasing dietary levels of BSFL ($P < 0.05$), however,

there was no impact of the treatments on C18:2, linoleic acid or C18:3n3, alpha-linolenic acid (P > 0.05). Based on these results, BSFL appears to be a nutrient dense ingredient that is highly palatable up to 12% of the diet with no negative impact on egg production or size. Furthermore, there are were significant improvements in egg specific gravity and egg yolk medium chain fatty acid profile that could enhance their utilization for laying hens.

Key Words: Black Soldier Fly, larvae, laying hens, feed ingredient

SCAD

T147 An investigation of variables associated with mortality in a broiler complex in Mississippi Leslie Johnson*[1GS], Robert Wills[1], Brandon Carter[2], Phil Stayer[3], Alejandro Banda[1], Natalie Armour[1] *[1]Mississippi State University; [2]Elanco Animal Health; [3]Sanderson Farms, Inc.*

A southern Mississippi broiler complex in an area of high poultry density has experienced persistent lower livability and growth performance compared with company averages for the state. A retrospective analysis of data from a three-year period (March 2014 through March 2017) was designed to investigate the role of disease, settlement, geographic, and seasonal variables in broiler mortality. A database comprising diagnostic variables (processing-age ELISA titers for Infectious Bursal Disease (IBD), Infectious Bronchitis (IBV), Newcastle Disease (NDV), and Reovirus (REO)), settlement variables (downtime, age at processing, average weight at processing, week 1 mortality, breed, year, and broiler vaccination programs), geographic variables (number of commercial chicken farms and houses within 1 km, 5 km, 10 km, and 15 km radii), and weather variables (average temperature, average heat index, and average humidity for the first 7 days and last 14 days of grow-out) was created and analyzed using univariable and multivariable statistical analyses. First-week mortality, processing age, average processing weight, breed, NDV/IBV vaccination program, and heat index in the last 14 days of the grow-out period were found to be significantly associated with flock mortality in this broiler complex ($P \leq 0.05$). The results of this study should guide future management and disease control strategies aimed at reducing broiler mortality. Future studies with more diagnostic data are needed to further investigate the relative contribution of diseases to broiler flock mortality.

Key Words: Broiler mortality, Multivariable statistical analyses

T148 Comparison of the serological responses induced by a multivalent inactivated oil emulsion virus vaccine applied in two sites (breast intramuscular and inguinal subcutaneous), and gross evaluation of the local vaccine reaction. Eric Orozco*[1GS], Phil Stayer[2], Erin Riley[2], Rob Lawhon[2], Alejandro Banda`[1] *[1]Mississippi State University; [2]Sanderson Farms Company*

Thirty 15-week-old meat-type birds were assigned into two groups of fifteen birds each. One group was vaccinated with a commercially available multivalent inactivated oil emulsion virus vaccine including antigens of infectious bronchitis virus (IBV), Newcastle disease virus (NDV), infectious bursal disease virus (IBDV), and avian reovirus (ARV), applied intramuscularly in the breast muscle using a single needle continuous injection syringe. The second group received the same vaccine subcutaneously in the inguinal area using a double needle continuous injection syringe. The serological titers were evaluated by a commercial ELISA kit. The gross local vaccine reactions were evaluated weekly. There was no statistical difference in the ELISA titers between the two groups for IBV, NDV, and ARV. Titers vs IBDV were significantly higher in the birds that received the vaccine in the breast muscle. The local reaction was evident during the first two weeks post-vaccination and regression was observed from the third week after vaccination.

Key Words: vaccine, birds, serology, breast, inguinal

T149 Effects of Zn and Mn amino acid complexes in the chicken responses to IBV infections Ana Paula Da Silva*[1GS], Marco Rebollo[2], Rodrigo Gallardo[1] *[1]University of California, Davis; [2]Zinpro Corporation*

An IBV resistant (MHC-B2 haplotype congenic) line of chicken were fed one of three dietary treatments: 1) 60 ppm each Zn and Mn from sulfates (control); 2) 60 ppm Zn from zinc amino acid complex (ZnAA); or 3) 40 ppm each Zn and Mn from amino acid complexes (Zn/MnAA). Diets were fed for 3 weeks, after which birds were evaluated for IBV specific IgG antibodies (Ab) to determine maternal antibody presence. After confirmation of the absence of IBV Ab, chickens were challenged with IBV M41 at a titer of $10^{4.5}$EID 50%/mL. Non-challenged control birds were kept for comparison. Body weight, respiratory signs, viral load, mineral excretion, histomorphometry, airsacculitis, and level of IgG and IgA in tears were measured. The ZnAA and Zn/MnAA treatments induced a faster onset and recovery of clinical signs at 2 and 6 days post-infection (dpi) ($P<0.05$), and reduced airsacculitis at 14 dpi ($P<0.05$). A trend for lower viral excretion ($p>0.05$) was observed in the ZnAA treatment group. We observed an increase in body weight gain ($p<0.05$) of chickens fed with the amino acid complexed supplements, compared to the inorganic control before and after the IBV challenge. Viral load, mineral excretion in feces, and tear IgA levels inconclusive. Overall, the data shows that the zinc amino acid complex alone induced better performance after the IBV challenge.

Key Words: Infectious Bronchitis Virus, minerals, immune responses

T150 Comparison studies of a live GA08 vaccine on protection against GA13 and DMV/1639 IB viruses in SPF leghorns Jon Schaeffer, John Dickson, Manuel Da Costa, Kalen Cookson* *Zoetis*

Live infectious bronchitis (IB) vaccines are essential to helping limit IBV field infections and protect broilers from the consequences of IBV-induced respiratory disease. Because new IBV serotypes continue to evolve and emerge, it is important to determine how well IB vaccine serotypes cross protect against them. The purpose of this study was to evaluate protection of a commercial GA08 vaccine against two of the most prevalent new serotypes today—GA13 and DMV/1639. Study Design: In Study 1 (GA13 challenge), 135 SPF leghorns were placed into 9 different isolators (15 per) at day of age; eye drop was compared to spray administration. In Study 2 (DMV/1639 challenge) 150 SPF leghorns were placed in 10 isolators at day of age and the GA08 vaccine was given by cabinet sprayer. Vaccine "takes" were measured using real time PCR of choana swabs at 7 days of age. Birds were challenged with 3.5 EID50 of IB viruses at 21 or 25 days, respectively, and protection was assessed 5 days later based on real time PCR and histopathology of tracheas. Results: All birds were strongly positive for vaccine at 7 days with average Ct values of 23.9 and 24.5 in eye drop and spray vaccinated birds, respectively, in Study 1 and 27.5 in Study 2. After challenge, clinical signs were very mild in challenge controls in both studies. In Study 1, 54% of GA13 controls had moderate to severe histological lesions compared to 22% and 20% in eye drop and spray vaccinates, respectively. In Study 2, 74% of DMV/1639 controls had moderate to severe tracheal lesions compared to 9% in spray vaccinated birds—this difference was significant. Using a cut-off value of $Ct \geq 35$ (little to no live virus), 73% of eye drop birds and 80% of spray birds met this threshold compared to none of the GA13 controls. In Study 2, 95% of spray birds had little to no virus present in tracheas compared to only 9% of DMV/1639 controls; vaccinated birds were also significantly heavier

(350g vs. 313g). Discussion: GA08 vaccinated birds had lower rates of tracheal lesions compared to challenge controls (significantly reduced in DMV/1639 study), no weight suppression and significant levels of protection from viral infection. Spray vaccination was also similar to eye drop administration based on "take" levels and IBV protection.

Key Words: IBV, GA08, GA13, DMV/1639, crossprotection

T151 Use of sentinel broilers to isolate and identify existing populations of infectious bronchitis viruses in Northwest Arkansas Abigail Reith*[1], Lloyd Keck[2], Kalen Cookson[2], Brian Jordan[1] *[1]University of Georgia MAM; [2]Zoetis ; [2]Zoetis*

Infectious bronchitis (IB) is an acute, highly contagious viral disease of chickens. The disease causes an upper respiratory infection and subsequent airsacculitis results in broiler condemnation in the processing plant. IB can also affect laying hens causing a decrease in egg production and egg shell quality. Controlling IB and mitigating its negative economic impact is pivotal to the success of poultry companies. IB is caused by infectious bronchitis virus (IBV), a coronavirus known for its tendency to undergo genetic change which creates new serologically variant viruses. Serotype specific modified live vaccines are utilized to prevent infection from IBV, and therefore protect against IB. In order to have the most effective IBV vaccination protocol it is pertinent to know what serotypes are present within your poultry houses.To identify circulating IBV serotypes in Northwest Arkansas (NWA), sentinel birds were placed on broiler breeder farms. The sentinel birds were by-product day old Cobb 500 chicks that received only coccidiosis vaccine at the hatchery. The sentinels were reared to 28 days old in an isolated facility with no poultry contact. The sentinels were then placed on 10 different broiler breeder farms, with 10 birds placed on each farm. The ages of the individual broiler breeder farms ranged from 33-57 weeks. The birds were placed in cages inside the chicken houses in contact with the scratch area and provided feed and water. Two sampling sessions took place at 7- and 11-days post placement on the farms. 5 birds from each farm were necropsied at both sampling sessions and tracheas, cecal tonsils, and kidneys were harvested. The tracheas were sent to the Poultry Diagnostic Research Center in Athens, GA for quantitative real time RT-PCR. Initial results showed 10/10 farms were positive for IBV. Subsequent serotype specific PCR testing was then performed on the tracheas revealing 4/10 locations positive for DMV/1639, 3/10 positive for Ark, 8/10 positive for GA98, and 7/10 positive for GA08. Most locations were positive for multiple viruses. These results demonstrate the numerous IBV serotypes circulating in NWA and can assist in guiding the control mechanisms and vaccination protocols of the integrators in the area.

Key Words: Bronchitis, DMV/1639, Sentinels

T152 Alterations in H5 highly pathogenic and low pathogenic recombinant hemagglutinin tissue tropism associated with increasing age in selected Anseriformes and Galliformes bird species Carmen Jerry*[1,2GS], Christina Leyson[3], David Stallknecht[4], Monique Franca[1,2] *[1]Poultry Diagnostic and Research Center; [3]Southeast Poultry Research Laboratory; [4]Southeastern Cooperative Wildlife Disease Study; [2]Department of Pathology, College of Veterinary Medicine*

Intercontinental spread of Influenza A viruses (IAV) has been linked to introduction of novel H5 viruses to the United States, posing severe risks to poultry species. Outbreaks of highly pathogenic avian influenza (HPAIV) often causes infection and death in older birds (laying chickens and turkeys) compared to younger birds (broiler chicken). Conversely, experimental trials have demonstrated younger birds being more susceptible to clinical disease caused by HPAIV, compared to older counterparts.

To evaluate differences in H5 HA protein binding and tissue tropism, recombinant HA proteins were generated using gene sequences from low pathogenic (LPAIV) and HPAIV H5 IAVs. Broiler chickens, egg layers, turkeys, Mallard, Pekin, Muscovy, and Muscovy mule ducks were selected at the age groups: 1-2-weeks, 5-6-weeks, and 8-weeks-old. Samples of respiratory, and intestinal tract, cloacal bursa, kidney, brain and liver were selected for HA protein binding, using protein histochemistry.

Among *Anseriformes*, 5 and 8-week old birds of the order *Anseriformes*had stronger HPAIV HA binding to the trachea, lung and cloacal bursa compared to younger birds. Significant differences were not observed in other tissues. The LPAIV HA had no significant differences in binding across age groups. Among *Galliformes*, stronger HPAIV HA binding was observed in the trachea and bronchi of 1-day, and 6-week-old pullets, with no alterations in HA binding in other tissues. Turkeys had no significant differences among age groups with both HAs.

This studyis expected to provide insight into age-related differences in tissue tropism of IAV that may aid in explaining host-related differences in susceptibility to infection during outbreaks.

Key Words:

T153 Infection and transmission of recent LPAIV H9N2 virus isolates in SPF chickens. Elizabeth Pusch*, David Suarez *Southeast Poultry Research Laboratory, U.S. National Poultry Research Center, U.S. Dept. of Agriculture, Agricultural Research Service*

Poultry adapted H9N2 low pathogenic avian influenza (LPAI) isolates are responsible for disease in poultry in many countries in Asia, the Middle East, and Africa. Large economic losses are occurring in many countries where H9N2 has become endemic. Understanding differences in infectivity and transmission of this subtype is important for future management of this subtype. Using multiple strains of recent H9N2 LPAIV isolates from different countries, we conducted a study in which we inoculated 3-week old specific pathogen free (SPF) laying hens intranasally to evaluate the infectivity and transmissibility of these viruses. Oropharyngeal swabs were collected from inoculated birds 2, 4, and 7 days post-inoculation (dpi) to determine virus shedding. Naïve birds were added to each isolator 2 dpi and swabs were collected from naïve birds 2 and 5 days post contact to determine virus shedding. Whole blood was collected from all birds 14 days post challenge for serological analysis. The majority of SPF chickens inoculated with a 10^2 or10^4EID50 of foreign H9N2 LPAIV isolates resulted in no clinical signs, but a few birds challenged with the low (10^2) challenge dose showed mild conjunctivitis. One bird that was euthanized prior to experiment termination showed cecal tonsil hemorrhages. Tissues were collected for histopathalogical analysis. Recent foreign H9N2 isolates show greater infectivity and transmission to contact birds at the medium (10^4) challenge dose. We will discuss differences in seroconversion or transmission seen between the isolates and infectious doses used in this experiment.

Key Words: LPAI, H9N2, avian influenza

T154 Cross-protection efficacy of inactivated and recombinant vaccine against different lineages of H5 high pathogenic avian influenza viruses Miria Criado*, Kateri Bertran, Donghun Lee, Lindsay Killmaster, David Swayne *USDA-ARS, Southeast Poultry Research Laboratory*

Highly pathogenic avian influenza A H5Nx viruses have been causing outbreaks and affecting a wide range of avian species around the world. The emergence of novel reassortant viruses and their circulation among bird populations are of great concerns that require development of intervention strategies as vaccines. In the present study, three-weeks-old specific pathogen free (SPF) leghorn chickens received a single dose of either an inactivated vaccine (rgH5N1 contained the HA gene from clade 2.3.4.4 A/Gyrfalcon/Washington/40188-6/2014 (H5N8) HPAIV in the backbone of A/Puerto Rico/8/1934 (H1N1) common vaccine strain) or rHVT-H5 vaccine [containing the HA from clade 2.3.4.4 A/Gyrfalcon/Washington/40188-6/2014 (H5N8)]. Three-weeks post-vaccination birds were challenge against different lineages of H5 HPAI viruses to evaluate protection. Results showed all challenge groups vaccinated with the rHVT-

H5 had 70-100% protection. In contrast, the inactivated vaccine provided protection ranging 20-100%. All the sham-control birds after challenge developed acute severe clinical disease and died between 2 and 4 dpc. Therefore, our study shows that existing inactivated vaccines may not provide adequate protection in poultry challenge against specific H5 HPAIV lineages suggesting that updated H5 hemagglutinin should be genetically closer to the outbreak virus. However, live vectored vaccines can provide broader protection against antigenically diverse field viruses. Prime-boost strategy may be necessary for optimal protection in the field due to antigenic diversity of field viruses.

Key Words: HPAIV, H5 avian influenza, inactivated vaccine, recombinant vaccine, poultry

T155 Changes in the genome of an H5N8 highly pathogenic avian influenza virus after experimental infec-tion in chickens and mallards Christina Leyson*, Sungsu Youk, Dong-Hun Lee, Kiril Dimitrov, Diane Smith, Mary Pantin-Jackwood *Southeast Poultry Research Laboratory, National Poultry Research Center, US Department of Agriculture*

We previously reported differences between chickens and mallards in response to experimental infection with an H5N8 highly pathogenic avian influenza (HPAI) clade 2.3.4.4B virus, which is a virus similar to those that caused the HPAI outbreaks in Europe in 2016-2017 (A/Tufted duck/Denmark/11740/LWPL/2016). This virus was better adapted to mallards than chickens, with a lower dose of the virus needed to infect the mallards and better transmission to contact mallards. Since the same inoculum was used to infect both species, we sought to determine if viruses obtained from the infected chickens and mallards would reveal genetic changes that may be associated with host species adaptation. To this end, whole genome deep sequencing of viruses from swab and tissue samples from infected birds was performed. Relative to the viral genomic sequence of the inoculum, viruses obtained from the chicken samples (mean=6) had more changes across the genome compared to those from the mallard samples(mean=4). Furthermore, viruses sequenced from tissues in infected chickens had more changes in the viral genome then the oral and cloacal swabs. When we examined the viruses from the mallard samples, we observed that viruses from mallards that received a medium dose of the virus (10^4EID_{50}, 50% egg infectious dose units) also had more changes in the genome compared to mallards that received a high dose (10^6EID_{50}). A total of 89 nucleotide changes, relative to the inoculum, were identified from all samples sequenced. Thirty-eight of these changes result in amino acid change. A subset of these changes was well-represented in samples from chickens but not from mallards, and another subset was observed to be well-represented from contact-exposed mallards but not in directly-infected mallards. In conclusion, whole genome sequencing of swab and tissue samples from experimentally infected birds reveal that changes in an avian influenza virus genome can be affected by the virus dose given, the host-species, and the form of exposure (inoculated vs. contact). The observation that more changes occur in the viral genome upon infection of chickens compared to mallards provide further evidence that the H5N8 virus used in this study is more adapted to waterfowl species (mallards) than gallinaceous species (chickens).

Key Words: Avian influenza, clade 2.3.4.4, whole genome sequencing

T156 Avian paramyxovirus 13 replicates but does not induce cross-protective immunity in chickens against challenge with virulent Newcastle disease virus Iryna Goraichuk*[1,2], Kiril Dimitrov[1], Poonam Sharma[1,3], Timothy Olivier[1], Patti Miller[1], David Swayne[1], Claudio Afonso[1], David Suarez[1] *[1]Southeast Poultry Research Laboratory, US National Poultry Research Center, ARS, USDA; [2]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine; [3]Richard B. Russell Agricultural Research Center, US National Poultry Research Center, ARS, USDA*

A virus isolated from a white-fronted Goose in Ukraine in 2011was previously identified as a novel avian paramyxovirus serotype 13 (APMV-13). Whole genome random next-generation sequencing was performed, and phylogenetic analysis revealed that the isolate was most closely related to isolates from Japan and Kazakhstan, showing 98 and 97% nucleotide identity, respectively. Phylogenetic evaluation of the predicted amino acid sequences of the viral proteins revealed that among the rest of the serotypes, the new isolate was most closely related to APMV-12. Intracerebral pathogenicity index test resulted in a value of 0.34, which is indicative of a virus of low virulence for chickens. APMV13/White-fronted goose/Ukraine/Askania-Nova/48-15-02/2011, was further characterized *in vivo* to investigate its replication and transmission in chickens, and to define its protection against challenge with virulent NDV. Three groups (I, II and III, $n = 5$ each) of 3-week-old SPF chickens were inoculated oculonasally with 10^2, 10^4, 10^6 EID_{50}/ml of the virus per bird, respectively. At 2 days post inoculation (dpi) three additional SPF chickens were placed into each group to evaluate virus transmission. Serum samples were collected at 14 dpi. No chickens from groups I and II seroconverted after inoculation with APMV-13, only two out of five directly inoculated chickens from group III developed anti-APMV-13 antibodies as demonstrated by HI. No chickens showed any apparent clinical signs after inoculation with APMV-13. At 14 dpi chickens from all three groups were challenged oculonasally with 10^6 EID_{50} per bird of virulent NDV-California-2002. Cloacal and oropharyngeal swabs were collected at 2 and 4 days post challenge (dpc). Clinical signs with 100% mortality were observed in all chickens after challenge with the virulent NDV strain. The APMV-13 virus was able to replicate in chickens at higher challenge doses, but without causing any clinical signs. Also, no cross protection against virulent NDV challenge was observed.

Key Words: Newcastle disease virus, APMV-1, APMV-13, vaccine

T157 Avian reoviruses molecular characterization, looking for better classification methods Rodrigo Gallardo*[1], Sofia Egana[1], Simone Stoute[1], HL Shivaprasad[1], Beate Crossley[1], Ha-Jung Roh[2] *[1]University of California, Davis; [2]CEVA animal Health*

Reovirus variants induce high economic losses in the affected flocks due to lack of uniformity, poor feed conversion, increased condemnations and reduced animal welfare in meat-type poultry. In California, a reovirus tenosynovitis outbreak started in August 2015 affecting broilers from 14 to 47 days of age. We have focused in virus isolation, molecular characterization and pathogenicity of some of these isolates. Most of the recovered isolates are from tendons, followed by heart and joints. Six different genotypic clusters have been recognized with changes in their trend from 2015 to 2018. Pathogenicity studies show microscopic lesions in heart and tendons. Full genome sequencing techniques are helping us to determine associations between pathogenicity, antigenicity and certain genes.

Key Words: Reovirus, genetic variants, genetic classification

T158 Chicken macrophages' susceptibility and innate immune response to infectious bursal disease virus with the supplementation of 1,25(OH)2D3 Juan Rodriguez-Lecompte*[1], Jairo Jaime-Correa[2], Diana Vargas-Bermudez[2], Alexander Yitbarek[3], Julian Reyes[1] *[1]Atlantic Veterinary College -University of Prince Edward Island; [2]Universidad Nacional de Colombia; [3]University of Guelph*

The target cells for infectious bursa disease virus (IBDV) are actively dividing immunoglobulin M-bearing mature B-lymphocytes. Infected and activated monocyte-macrophage lineages produce abundant amount of pro-inflammatory cytokines that promote cellular damage and destruction. Vitamin D (vit D) is known to have an immunomodulatory property with the capacity to enhance immune competency. Therefore, the aim of this study was to evaluate the innate response modulatory effect of vit D ($1,25(OH)_2D_3$)on chicken (ch)-macrophages infected with IBDV. Ch-macrophages (HD-11) were cultured in triplicates at a concentration of $5x10^5$cells/well in 24-wells plates. After 12h, cells were supplemented with 500nM of $1,25(OH)_2D_3$. At 16h post vit D supplementation, cells were inoculated with 0.1 or 1 multiplicity of infections (MOIs) of a mild strain (ST-12)-live vaccine IBDV. Gene expressions of antiviral markers and vitamin D receptor (VDR) were measured at 0, 3, 6, 12, 24, and 36 h post infection (p.i.). Results: The earliest viral RNA was detected at 3 h p.i. with a peak at 12 h p.i. at MOI 1 and vit D supplementation ($p<0.05$). VDR expression was significantly upregulated ($p<0.05$) in vit D supplemented cells at 0.1 MOI during the first 6 hours p.i. The intracellular endosomal membrane toll-like receptor (TLR-3) and the signal adapter TRIF were significantly upregulated ($p<0.05$) at both MOIs without vit D during the first 3 h p.i. Conversely interferon regulatory factor 7 (IRF 7) was upregulated ($p<0.05$) just in vit D supplemented macrophages. Expression of interferon alpha (IFN-α) was significantly upregulated at 6 h p.i. at MOI of 0.1 and vit D suppmentation. IFN-inducible virus inhibitory proteins OAS, PKR and viperin were significantly upregulated ($p<0.05$) only at 24 and 36 h p.i. with vit D supplementation. There was a significant upregulation ($p<0.05$) of the pro-inflammatory cytokines IL-1β and IL-6 following the same patterns of IFN-inducible antiviral proteins. In conclusion: the results support that vit D supplementation has an important role in the expression of innate immune response genes on ch-macrophages during an IBDV infection. However, vit D supplementation did not decrease the infectious capacity of the virus or protect the cells from infection with IBDV.

Key Words: Vitamin D, Innate, IBDV, Macrophages, Antiviral

Metabolism and Nutrition

T159 Effect of a feed additive (EndoBan) on broiler performance Jesse Stoops, Hilke Willemsen, Kurt Van de Mierop* *Nutrex NV*

Endotoxins (lipopolysaccharides or LPS) are part of the cell wall of Gram-negative bacteria. During growth or bacterial lysis, LPS are released and can transfer to blood and tissues. In poultry, the intestinal microflora is the most important source of endotoxins, and the GI tract the main risk site for transfer from the lumen. Several factors (e.g. heat stress, mycotoxins, pathogens, feed transition) are known to increase gut permeability, promoting leakage of endotoxins into the blood. Once absorbed, endotoxins induce an inflammatory response, thus wasting energy and nutrients meant for growth and production.

EndoBan is a feed additive developed to minimize the negative effects of endotoxins. The objective was to study the effect of EndoBan on male and female broiler performance when combined with a commercial feed additive providing protection against mycotoxins. Two trials were conducted in which Ross 308 broiler chicks were reared in an environmentally controlled poultry house (Zootechnical Centre, Belgium), using a two phase dietary program (starter d0-14 and grower/finisher d14-d42) in which all diets were fed *ad libitum*. In trial 1, 352 male broilers were randomly allocated to 2 treatments with 8 replicates per treatment (22 birds/pen). Treatment 1 was a corn-soy-based control diet and treatment 2 an EndoBan supplemented (0.5 kg/T feed) control diet. In trial 2, 216 female broilers were randomly allocated to 6 replicates per treatment (18 birds/pen). Treatments included a corn-soy based control diet and an EndoBan supplemented (0.5 kg/T feed) control diet. In both studies, the control diet contained a feed additive against mycotoxins at 1 kg/T of feed. Body weight, feed intake and mortality were recorded on d14 and d42. In trial 1, the male broilers supplemented with EndoBan reached a higher body weight (3125 g) and lower feed conversion (1.58) compared to the control diet (3060 g and 1.60). In trial 2, supplementation of EndoBan improved ($p < 0.05$) final body weight of female broilers to 2558 g vs 2470 g for the control group. Feed conversion was similar for both treatments (1.53). These results indicate that EndoBan can improve growth performance of broilers, even when an additive protecting against mycotoxins is already present in the feed.

Key Words: Endotoxins, broilers, performance, feed additive

T160 Evaluation of broilers supplemented with Bastion® feed additive and challenged with Clostridium perfringens Nicholas Evans*[1], T Karnezos[1], Ronald Dvorak[1], Curtis Novak[2], Mahmoud Masadeh[2], Michael Sims[3] *[1]PMI Nutritional Additives; [2]Purina Animal Nutrition; [3]Virginia Diversified Research Corp.*

A 40-day (30 birds/pen @ 0.625 ft^2 and 15 replicates/treatment) broiler study was conducted to compare the lesion scores, performance, and mortality of straight-run Cobb 500 broilers. Birds were vaccinated with Coccivac®-B at day-of-hatch and challenged with *Clostridium perfringens* at day 19, 20, and 21. Experimental treatments were: 1) negative control (no additives); 2) positive control (challenge + no additives); 3) BMD®/Stafac® (challenge + BMD® 50 g/US ton in starter and grower, and Stafac® 20 g/US ton in finisher); and 4) Bastion® (challenge + Bastion® 1.25 lb./US ton in starter, 1 lb./US ton in grower, 0.75 lb./US ton in finisher). As expected, all three challenge treatments (positive control, BMD®/Stafac® and Bastion®) had significantly increased ($p\leq0.05$) lesion scores (day 23) compared to the negative control; however, lesion scores for the BMD®/Stafac® and Bastion® treatments were significantly decreased ($p\leq0.05$) compared to the positive control. Overall, performance (body weights and mortality adjusted feed conversion) was similar for the BMD®/Stafac® and Bastion® birds when compared to the negative control and these three treatments were all significantly better ($p\leq0.05$) than the positive control. Mortality was not significantly different among any of the treatments. In this study, Bastion® was comparable to the BMD®/Stafac® antibiotic growth promoter program in reducing necrotic enteritis lesion scores and supporting performance of broilers challenged with *Clostridium perfringens*.

Key Words: Clostridium perfringens, necrotic enteritis, broiler, feed conversion

T161 Bastion® feed additive improves performance of broilers in a commercial antibiotic-free production program Mahmoud Masadeh*[1], Nicholas Evans[2], Theodore Karnezos[2], Curtis Novak[1] *[1]Purina Animal Nutrition; [2]PMI Nutritional Additives*

A 42-day performance study was conducted in a commercial antibiotic-free production program to evaluate Bastion® feed additive, a proprietary blend of plant extracts, prebiotics and probiotics. Ross 708 male birds were provided a standard vaccination program including Coccivac® at day-of-hatch. Birds were then randomly assigned to floor pens (50 birds/

pen @ 1.0 ft^2) with 15 replicates per experimental treatment: negative control (no additives) and Bastion® (Bastion® 1.25 lb./US ton in starter, 1 lb./US ton in grower, 0.75 lb./US ton in finisher). Performance and mortality was evaluated at 14, 28, and 42 days. At 14 and 28 days there were no significant difference in performance; however, at 28 days, a trend towards a significant ($0.05<p\leq 0.10$) improvement in performance (body weight, weight and mortality adjusted feed conversion, and gain/day) was observed for the Bastion® treatment. By 42 days, body weight (0.15 lbs. heavier), weight and mortality adjusted feed conversion (3 points lower), and gain/day (0.004 lbs./day increase) were significantly ($p\leq 0.05$) better in birds fed Bastion®. In this study, Bastion® optimized performance in a commercial antibiotic-free production program.

Key Words: Performance, Antibiotic-Free, Body Weight, Feed Conversion, Gain/Day

T162 Effects of a commercial Macleaya cordata extract on broiler growth performance under a coccidiosis challenge Meghan Schwartz*[1], Nickki Tillman[2], Marcos Rostagno[1] [1]*Phytobiotics North America, LLC;* [2]*Nutritional Statistics, LLC*

A study was conducted to evaluate the optimum inclusion level of a standardized blend of isoquinoline alkaloids extracted from *Macleaya cordata* (Sangrovit®, Phytobiotics North America, LLC) in broilers, under a coccidiosis challenge. A total of 498 day-old Cobb 500 males were assigned to five dietary treatments with 8 replicate battery cages per treatment (10 or 17 birds/cage), in a randomized complete block design. The feeding program consisted of 3 phases using corn-soybean based diets. Five dietary treatments were evaluated, including 0 (control), 30, 60, 90, and 120 ppm of Sangrovit®. 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 14, 21, 28, and 35. Broken line quadratic ascending (BLQ) and quadratic polynomial (QP) models were used to determine the optimal Sangrovit® dosage which maximizes growth performance (body weight (BW) and feed conversion ratio (FCR)). The sum of squared residuals (SSE) and R-square (Rsq) were used to compare the models. Results show that BLQ provided lower estimation of the optimal Sangrovit® dosage and Rsq value while higher SSE compared with QP model. Thus, QP was used to determine Sangrovit® dosage for this dataset. The maximum BW and minimum FCR responses were observed at 76 ppm of Sangrovit® during 0-14 d of age. From 0-21d and 0-28d, optimal Sangrovit® levels went down to 72 and 67 ppm, respectively. From 0-35d, the maximum BW response was observed at 126 ppm while the minimum FCR response was observed at 86 ppm of Sangrovit®. These results suggest that the *Macleaya cordata* extract supports the intestinal health of the birds under a coccidiosis challenge, and warrants further investigation for larger bird size as suggested by 35 d result.

Key Words: feed additive, isoquinoline alkaloids, broilers, coccidiosis

T163 Use of Alquermold Natural to treat and prevent Clostridium perfringens induced necrotic enteritis in broilers David Díez[1], Carlos Domenech[1], Júlia Pié*[1], Manuel Rosemberg[2] [1]*Biovet, S.A.;* [2]*Universidad Científica del Sur*

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

Objective: An *in vivo* experiment was conducted to determine the effectivity of AMN as a prevention and treatment for necrotic enteritis (NE) in broilers. Productive performance, mortality, uniformity, carcass yield and *Clostridium perfringens* (CP) intestinal colonies were evaluated.

Methods: 504 broilers were divided into four treatment groups: non-infected control (T1); infected control (T2); infected group receiving AMN as a prevention at 0.5 kg/mT through feed, continuously during all the trial (T3); infected group receiving AMN as a treatment at 1 ml/L through water for one week, starting its administration the day after the infection (T4). Birds in T2, T3 and T4 were challenged with CP in drinking water on days 19, 20 and 21 of age with 10^8 UFC/bird. Data were analyzed using PROC GLM procedures of SAS 9.1 and P value less than 0.05 was set as statistically significant.

Results: Results showed that AMN as a preventive (T3) was significantly effective in controlling NE (91.51 more grams/bird, 1.17% feed conversion reduction; compared to T2, $P<0.05$). AMN as a treatment (T4) also resulted effective in counteracting the negative effects of NE (65.34 more grams/bird, 0.58% feed conversion reduction; compared to T2), although less efficient in maintaining productive performance, carcass yield and controlling the proliferation of CP, compared to T3.

Conclusions: In conclusion, AMN in feed can be used as a preventive for NE in poultry farms. Additionally, farms experiencing clinical NE can be treated with AMN in the water. These results were corroborated in a similar trial (same product, same experimental treatments) in the US in 2018.

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

T164 Metallo-phytobiotics; novel weapons against Clostridium perfringens Viviana Molnar-Nagy*[1], Zsofia Bata[1], Jozsef Gyenis[2], Valeria Stechova[2] [1]*BV Science Inc.;* [2]*Dr. Bata Ltd.*

Phytobiotics can be viewed as novel growth promoters due to their efficacy in combating intestinal pathogens, such as *Clostridium perfringens (Cp.)*. The largest limitation of phytobiotic use is that microbial susceptibility varies from isolates to isolates. The aim of the study was to develop a unique metallo-phytobiotic feed additive that controls the growth of *Cp.* in an effective and economic way both *in vitro* and *in vivo* as well.

The first step of the research was the development of a quick and efficient method for evaluating the susceptibility of *Cp.* field isolates. After isolating numerous *Cp*. strains from geographically different sources, known antibiotics (Doxycyclin, Spectinomycin, Enrocin, Tylosin and Zinc-bacitracin) were tested as positive controls to measure the susceptibility of *Cp.* growth. The thus validated kit was used for the screening of various phytobiotic extracts, combinations of phytobiotic extracts and metal complexes; metallo-phytobiotics. The efficacy of the developed metallo-phytobiotic feed additive was tested in three different *in vivo* trials with broilers, with modelling oral challenge in the form of litter filtrate addition. The bacterial composition of the litter filtrate was standardized and contained 2 x 10^8 coliform and 4 x 10^6 *Cp.* CFUs. Three factors modelling field conditions were varied between the trials; day old broiler health, oral challenge with litter filtrate, use of fresh or old litter. The three setups were the following: 1. healthy day old broilers, fresh litter and no oral challenge; 2. healthy day old broilers, oral challenge and used litter; 3. prone to disease day old broilers, oral challenge and used litter. In each trial birds were divided into a control and a metallo-phytobiotic treated (trial) group.

Results revealed that in ideal conditions (Trial 1.) there is no significant difference between the production parameters. As soon as the circumstances differ from the ideal - either the health of the day old chicks or cleanness of the houses are reduced– the difference in the production parameters becomes significant ($>p=0.001$) in favour of the trial group. Results suggest, that in non-ideal conditions, the addition of optimal metallo-phytobiotic products can avoid losses in the production parameters.

Key Words: metallo-phytobiotics, antibiotics, Clostridium perfringens

T165 Effect of a feed incorporated metallo-phytobiotic on the performance of broiler chickens given a non-defined challenge Bruno Vecchi*[1], Emanuel Gumina[1], Ariel Sugezky[1], Sherry Layton[2] [1]*Vetanco SA;* [2]*BV Science*

Currently there is growing concern with antibiotic growth promoters (AGP) and their effect on the bacterial resistance in poultry production;

necessitating a need for the development of natural products that increase production parameters with the same efficiency as AGPs. In this current study we tested a commercially available *Humulus lupulus* based metallo-phytobiotic, with specific action against *Clostridium perfringens*, to determine productivity parameters in broiler chickens. 400-day-old chicks (Cobb 500) were individually tagged, weighed and randomly assigned to one of 20 pens (n=20 birds/pen). Pens were randomly assigned per treatment group: 4 treatments with 5 replicates each (n=100 birds/treatment). All birds were fed a 4-phase standard commercial diet with or without the following treatments: T1 no modification, T2 AGP (BMD 11% at 0.5 kg/tn); T3 metallo-phytobiotic 1 kg/tn and T4 metallo-phytobiotic at 0.5 kg/tn. On d 7, 14, 15 of life, all birds in all treatment groups were challenged by drinking water (5 liters/pen/day) with a non-defined challenge derived from fermentation and subsequent crude filtration of used poultry litter from a flock that broke with necrotic enteritis. General microbial counts were determined using Petri-film: $6.4x10^7$ (aerobics), $1.5x10^5$ (fungi and yeast), $7.5x10^9$ (anaerobics) and $1.1x10^5$ (E.Coli). At day 35 all birds were again individually weighed for determination of body weight gain (BWG); additionally, mortality and feed conversion (FCR) were recorded and calculated. Statistical analysis was performed using a one-way ANOVA ($p<0.05$) followed by a Tukey test. Different letters represent difference between means. Productivity parameters calculated were: BWG (gm) were: T1 1584.55a ± 53.92; T2 1831.5ab ± 21.73; T3 1953.35c ± 35; T4 1858.2ab ± 25, FCR (gm) was: T1 1958.21a ± 66.6; T2 1610.88ab ± 19; T3 1536.33c ± 27.1 and T4 1668.85ab ± 22.4 and mortality was 5% for T1 and 3% for T2, T3 and T4. Data show that all treatment groups numerically performed better than the non-treated control group; however only T3 (metallo-phytobiotic at 1kg/tn) showed statistically significant increases in BWG and FCR; which could be attributed to the specific antimicrobial activity of the product.

Key Words: Phytobiotic, Clostridium perfringens, Broilers, Feed Additive, Humulus lupulus

T166 Augmented fat soluble vitamin absorption due to phytogenic feed additive supplementation in broiler chicks Chasity Pender*[1], Kimberly Gardner[2], Christopher Bailey[2], Raj Murugesan[1] *[1]Biomin America, Inc.; [2]Texas A&M University*

In recent years, there has been increasing interest in the use of phytogenic feed additives as non-antimicrobial means to improve gut health and digestive efficiency in poultry. The objective of this trial was to evaluate the effects of a phytogenic feed additive on the efficiency of absorption of the fat-soluble vitamins A, D, and E. A total of 50 day-old Cobb 700 broiler chicks were allocated within 10 stainless steel battery brooder pens and randomly assigned to one of two dietary treatment groups; a non-supplemented control and a supplemented group receiving a phytogenic feed additive (Digestarom® P.E.P. 125 Poultry; 125g/MT), each consisting of 5 replicate pens (5 birds/pen). After placement, birds were provided a diet consisting of 18% CP, 2850 kcal/kg, and lacking a vitamin premix for a one-week depletion period. Starting at 7 days of age, birds were supplemented daily with vitamins A, D, and E, via oral gavage at a ratio of 750:200:5 IU per kg of feed. On day 14, serum and liver samples were collected from 3 birds per pen. Data were analyzed using Welch's t-test procedure and the significance threshold was set at $P = 0.05$. Phytogenic feed additive supplementation numerically increased vitamin A concentrations in the serum and liver by 3.4% and 13.6%, respectively, when compared to the control. Vitamin D concentration in the serum was numerically increased ($P < 0.10$) by 86.2% due to phytogenic supplementation, while it was significantly increased by 100% in the liver. In the serum, vitamin E concentration was significantly increased by 55.9% in the birds provided the phytogenic feed additive, while, in the liver, concentrations were numerically ($P < 0.10$) increased by 31.6%. Taken together, these results suggest that phytogenic feed additives may provide a means to enhance digestive efficiency and utilization of vitamins in broilers.

Key Words: phytogenics, poultry, vitamins, antibiotic-free, nutrient utilization

T167 The optimum dose level of a phytogenic blend in diets for broilers challenged with a virulent coccidia live vaccine Francisco Dias*, Andreas Mueller, Jan Dirk van der Klis *Delacon Biotechnik GmbH*

This floor pen study was done to evaluate the efficacy of Biostrong® Protect supplemented at graded dose levels up to 2.0 lb/t in the starter diet, followed by 0.3 lb/t Biostrong® 510 till slaughter. The study was done at Southern Poultry Research Station in Athens, USA. 1500 one-day-old male broiler chickens (Cobb 500) were allotted to 5 treatments with 12 replicate pens of 25 birds each. All broilers were vaccinated with a live coccidiosis vaccine (Coccivac-B52, MSD Laboratory) at label recommended dose at the hatchery. After arrival at the farm, birds were assigned to the following experimental corn/soy diets with graded levels of Biostrong® Protect from 1-28 days of age: Group 1: 0 lb/t; Group 2: 0.5 lb/t; Group 3: 0.75 lb/t; Group 4: 1.0 lb/t; Group 5: 2.0 lb/t. At 28 days of age, Biostrong® Protect was replaced by 0.3 lb/t Biostrong® 510 until slaughter at 42 days of age. Basal diets were formulated to meet the bird's nutrient requirements. Feed and water were freely available. Body weight gain (BWG) and feed conversation ratio (FCR) were calculated from 1-14, 15-28 and 29-42 days of age, and total mortality rate was calculated from 1 to 42 days of age. In this trial, similar ($P>0.05$) results of BWG and mortality among treatment groups were observed. However, as of 28 days, dietary supplementation with Biostrong® Protect at levels of min 0.75 lb/t significantly ($P<0.05$) improved FCR, as compared to non-treated control. Based on the quadratic dose-response curve the minimum FCR was shown at an inclusion level of 1.2 lb/t. Results from this study showed that the Biostrong® program improved the resilience of broilers against a coccidiosis infection. A program to use of 0.8 to 1.2 lb/t of Biostrong® Protect up to 28 days of age followed by 0.3 lb/t Biostrong® 510 till slaughter is recommended.

Key Words: Biostrong® program, efficacy, dose-response

T168 Anticoccidial Efficacy of Amprolium 125 ppm Fed to Commercial Broiler Chickens Infected with a Mixture of Eimeria maxima Greg Mathis[1], Emily Kimminau*[2] *[1]Southern Poultry Research; [2]Huvepharma*

Amprolium is an anticoccidial drug that has been used in poultry production since 1962. Amprolium has historically associated with treatment of *Eimeria tenella* infections. The purpose of this study was to evaluate the anticoccidial activity of in feed Amprolium against a mixed *Eimeria maxima* infection. 224 chicks were placed in coccidia free battery cages and at 12 days of age (DOT 0), they were moved to experimental cages (24 birds/treatment). There were 7 total treatments: Nonmedicated-uninfected, non-medicated-1000 oocysts *E. maxima* (low), non-medicated-2500 oocysts *E. maxima* (medium), non-medicated-5000 oocysts *E maxima* (high), Amprolium 125 ppm- 1000 oocysts *E. maxima*, Amprolium 125 ppm- 2500 oocysts *E. maxima*, and Amprolium 125 ppm- 5000 oocysts *E. maxima*. On DOT 2, birds were inoculated with respective doses of E. maxima and the un-infected treatment was given 1 mL of distilled water. On DOT 8, all birds were weighed, sacrificed and lesion scored according to Johnson and Reid Method for *E. maxima* (1970). Microscopic *E. maxima* scores were also measured. Performance parameters were measured from D0-D8. Amprol 125 ppm significantly ($p<0.05$) reduced gross lesions compared to unmedicated controls (low, medium and high challenge). Amprol 125 ppm also significantly ($p<0.05$) reduced microscopic *E. maxima* scores compared to unmedicated controls (low, medium, and high challenge). Amprol 125 ppm significantly ($p<0.05$) improved FCR compared to unmedicated controls (low, medium and high challenge). There weren't significant differences ($p>0.05$) in body weight gain be-

tween medicated and unmedicated controls at any challenge level (low, medium or high). In conclusion, Amprolium 125 ppm in feed was efficacious in reducing lesions infection at various infection levels (high, medium and low) of *E. maxima*.

Key Words: Eimeria, anticoccidial

T169 Efficacy of different forms of benzoic acid and butyric acid in broilers subject to Eimeria challenge Frances Yan*, Juxing Chen, Vivek Kuttappan, Mercedes Vazquez Anon *Novus International Inc.*

Organic acids have been proven to be a valuable tool to maintain growth performance and gut health of broilers raised in AGP free production. However, their efficacy is both type and form dependent. A battery trial was conducted to evaluate the effect of benzoic acid and butyric acid on growth performance and gut health of male broilers subject to *Eimeria* challenge as affected by protection technology. The study consisted of 6 dietary treatments – negative control, free benzoic acid, benzoic acid protected by embedding in fat (AVIMATRIX®, Novus International Inc.), tributyrin, encapsulated calcium butyrate, and free sodium butyrate, each with 12 replicate pens of 8 birds. Rye (10%), canola meal (7.5%) and poultry byproduct meal (3%) were incorporated into a corn soybean meal based nutritionally complete diet in crumbles to provide some mild dietary challenge. All birds were orally gavaged with a coccidiosis vaccine (mixed species of *E. acervulina*, *E. tenella*, & *E. maxima*) at 10x the recommended dose on d 14. Growth performance was determined on d 7, 14, and 19. Ceca content bacteria were quantified by qRT-PCR on d 19, and intestinal morphometry was examined on d 20. Data were subject to one-way ANOVA and means were separated by Fisher's protected LSD test. On d 14, all acid treatments except encapsulated calcium butyrate had better FCR; on d 19, only protected benzoic acid, tributyrin, and free sodium butyrate improved FCR ($P \leq 0.05$). During the 14-19 d challenge phase, protected benzoic acid was more effective than free benzoic acid and encapsulated calcium butyrate in improving FCR. Protected benzoic acid and free sodium butyrate tended to reduce cecal *E. coli*, and protected benzoic acid, free benzoic acid, and tributyrin tended to lower cecal *C. perfringens* ($P \leq 0.10$). Only protected benzoic acid improved jejunal crypt depth to villus ratio suggesting better gut maintenance and growth efficiency ($P \leq 0.05$). In summary, protected benzoic acid was effective in improving growth performance, modifying gut microbiota, and improving gut morphometry of young broilers, and its efficacy was similar to tributyrin, but better than free benzoic acid and encapsulated calcium butyrate in improving morphometry and FCR during the *Eimeria* challenge phase.

Key Words: benzoic acid, butyric acid, Eimeria, broiler

T170 Evaluating the effects of different sources of butyric acid on growth performance in broilers Cinta Sol*[1], Mónica Puyalto[1], Juan Mallo[1], Justin Fowler[2] *[1]NOREL; [2]University of Georgia*

This study was performed in order to evaluate the effect of different butyric acid (BA) based additives on performance in broilers. A total of 1,000 broiler chicks at hatch day were distributed in 5 treatments among 45 floor pens (25 birds per pen, 8 replicates per pen). The treatments consisted of: T1 – Control feed without additives; T2 – GUSTOR N'RGY (1Kg/t of feed), based on 70% sodium butyrate protected with sodium salts of palm fatty acids distillates, BA=56%; T3 – GUSTOR COATED (2.19Kg/t) based on 32% sodium butyrate encapsulated with palm stearine, BA=25.6%; T4 – GUSTOR BCP5 (0.8Kg/t), based on 85% calcium butyrate protected with palm stearine, BA=69.9%; T5 – Tributyrin (0.65 Kg/t) based on 99% Glicerol tributyrate, BA=86.4%. The same amount of butyric acid was included in all treatments. The study lasted 35 days. The parameters evaluated were: body weight (BW), feed conversion ratio (FCR), feed consumption (FC) and European Production Efficiency Factor (EPEF). The results were analyzed by one-way ANOVA using the GLM procedure of SAS. Means separation was done by Duncan's Multiple Range Tests. There was a trend to have different Final BW (35 days), being T2 birds the heavier and T1 the lighter (2367^{y}, 2441^{x}, 2414^{xy}, 2374^{y}, and 2389^{xy} for T1, T2, T3, T4 and T5 respectively; P=0.088). FCR was significant different among treatments for the whole period, being the lowest for T5, which improved the T1 situation as well as T2 and T3 (1.51^{c}, 1.47^{ab}, 1.46^{ab}, 1.48^{bc}, 1.44^{a} for T1, T2, T3, T4 and T5 respectively; P=0.004). There were no significant differences in FC or EPEF. However, all treatments had numerically higher EPEF than the control (improvement of 3 – 5.5%). It can be concluded that the better results in performance were achieved with T2 and T5, GUSTOR N'RGY and Tributyrin, respectively, being only the GUSTOR N'RGY broilers heavier than the Control broilers. Moreover, it seems that the salt of butyric acid, sodium salt or calcium salt also determined the effectiveness of the additive. In this study, calcium salt of butyric acid had lower effect than sodium salts.

Key Words: additives, sodium butyrate, calcium butyrate, tributyrin

T171 Influence of chicory root powder and zinc sulfate on meat quality and histomorphology of muscles in broilers Habib Rehman, Mirza Usman, Hafiz Rehman, Saima Masood, Amber Zafar, Hafsa Zaneb, Saima Ashraf* *University of Vetreinary and Animal Sciences Lahore*

Increase in demand of antibiotic free poultry meat has led the researchers to find alternatives of antibiotic growth promoters for poultry diet. Therefore, different feed additives which can improve the performance and meat quality of broiler are under extensive investigation. Keeping in mind a trial of 35 days was planned to evaluate influence of chicory root powder (CRP) and zinc sulfate ($ZnSO_4$) individually or in combination on meat quality and histomorphology of muscles in broilers. 150 day-old chicks were divided into six groups (5replicates/pen) and raised under standard management. Feeding regimen and groupings were as follows; control group (CONT) received corn-based diet (BD), group 2CRP received BD+2%CRP, group 4CRP received BD+4%CRP, group Zn60 received BD+$ZnSO_4$ 60mg/kg of diet, group 2C+Zn60 received BD+2%CRP+$ZnSO_4$, group 4C+Zn60 received BD+4%CRP+$ZnSO_4$. At 35^{th} day, 2 birds from each replicate were killed to collect samples of muscles for processing. Data analysis was performed by one-way ANOVA followed by DMR. Results were considered significant at $P<0.05$. The pH of all the sampled breast and thigh muscles was in range of 6.26 to 6.56 and 6.21 to 6.4 respectively. An increase in pH and water holding capacity (WHC) of the breast and thigh muscles was observed in BD+2%CRP and BD+4%CRP groups whereas these parameters reduced in the 4C+ Zn60 groups when compared with the CONT groups. Histomorphometric analysis of muscles revealed that the fiber diameters of pectoralis major muscle were increased in all supplemented groups, whereas cross sectional area was increased only in the BD+2%CRP and BD+4%CRP groups when compared with CONT group. Moreover, the fiber diameter of biceps femoris muscle also increased in all the supplemented groups when compared with the CONT group. But, muscle fibers cross sectional area did not vary amongst the groups. In conclusion, results revealed that the dietary supplementation of 2 and 4%CRP has a potential to improve the selected meat quality and histomorphometric parameters in broiler however, its combination with 60mg of $ZnSO_4$ could not produce the expected superior effects so further trails may be needed to evaluate the different combinations of CRP and $ZnSO_4$ in order to get the desired results

Key Words: broiler, chicory root powder, meat quality, histomorphology of muscles, zinc sulphate

T172 The effect of in ovo injection of carbohydrates, amino acids, vitamins, and electrolytes solutions and minerals on performance of broilers Alireza Behnamifar, Shaban Rahimi*, Mohammad Amir Karimi Torshizi, Sedigheh Shahvali *Tarbiat Modares University*

The limitations of nutrient sources in the egg may limit embryonic development. Therefore, it has been thought that infusion of nutrients into the eggs can improve the growth and quality of day old chicks. The purpose of this study was to evaluate the in *ovo* injection of nutrients on some param-

eters of newly-hatched chicks and their performance in the rearing period. Total of 360 fertile eggs from a 50 weeks old commercial broiler breeder flock (Ross 308) at allocated in a completely randomized design including of six treatments with four replicates and fifteen eggs in each replicate. Treatments consisted of: 1- non-injected; 2- injection one mL of sterilized distilled water;, 3-injection of one mL solutions of carbohydrates comprising 2.5% sucrose + 2.5% maltose + 20% dextrin in a 5% saline solution; 4- injection one mL of Aminoven infant 10%; 5-injection one mL of mixture of electrolytes solutions and minerals, 6- injection one mL of mixture of vitamins solution (B complex, vitamin E, vitamin D3, vitamin K). After candling the embryonated eggs on day 18th of incubation, *in ovo* injection into the amnion sac was undertaken from broad end of eggs by 21-guage needle with a depth of 25 mm. After hatching the chicks were transferred to the poultry house for 42 days in the same experimental design. The effects of applied treatments on the weight of hatched chicks and the relative weight of yolk sac of newly-hatched chicks were not significant. At the end of rearing period, FI and FCR were not affected by the treatments. However, the two groups which received the mixture of vitamins solution and amino acids (aminoven infant solution) had a greater body weight than the other groups (P<0.05).

Key Words: In ovo injection, nutrients, newly-hatched chick, broiler, growth performance

T173 Digestible lysine responses of broiler chickens on growth performance and carcass traits from 14 to 35 days post-hatch Sonia Liu*[1], Craig Maynard[2], Samuel Rochell[2], Justina Caldas[3], Mike Kidd[2] *[1]Poultry Research Foundation, The University of Sydney; [2]Center of Excellence for Poultry Science, University of Arkansas; [3]Cobb-Vantress, Inc*

The present study was conducted to evaluate the responses of growth performance and carcass traits in male and female Cobb MV × 500 broiler chickens offered corn and soybean meal (SBM) based diets containing different levels of digestible (dig) lysine (Lys). Eight experimental diets, including six titration treatments and 2 control diets were offered to broiler chickens from 14-35 days post-hatch. The dietary dig Lys levels in titration diets ranged from 0.84 to 1.29 % in increments of 0.09%. The positive control diet was a typical commercial diet based on maize and SBM; whereas, the negative control diet partially replaced SBM with 10% peanut meal to allow aggressive supplementations of feed grade amino acids. Both control diets contained medium level of dig Lys concentration (1.11%). All diets were balanced with essential amino acids by using ideal protein ratio approach. Each diet was offered to both male and female broilers to obtain 16 treatments and each treatment included 6 floor pens with 12 birds per pen. At day 35, birds were processed and various cuts were weighed. Predictably, male birds had significantly higher live BW (2199 versus 1983 g/bird, P < 0.0001), BW gain (1842 versus 1602 g/bird, P < 0.0001), feed intake (2618 versus 2368 g/bird, P < 0.0001) and lower FCR (1.431 versus 1.482 g/g, P < 0.0001) than female birds. Flock uniformity was expressed as the CV of final body weights at the carcass processing plant. There was no difference in flock uniformity between female and male birds but increasing dig Lys levels linearly decreased CV or improved flock uniformity (P = 0.020). The dig Lys requirement was estimated at 1.06% for optimal BW gain, 1.20% for total breast yield and 1.16% for FCR in straight-run birds; it is 1.15% for BW gain, 1.03% for total breast yield and 1.10% for FCR in female birds and 1.03% for WG, 1.23% for total breast meat yield and 1.22% for FCR in male birds. The results suggested that modern broilers are highly responsive to dietary amino acid density, especially breast meat yield.

Key Words: breast, carcass, grower, lysine, response

T174 Energy (ME)-releasing efficacy of graded levels of 6-phytase, Natuphos® E, NSPase, Natugrain® TS, and a combination of phytase +NSPase in 28 day old broilers fed corn/soy diets Robert Jones*, Mike Coelho, Frank Parks *BASF Corporation*

This study evaluated the metabolizable energy (ME) release of graded levels of phytase, NSPase and a combination of phytase+NSPase when fed to 28 day old broilers on mash corn/soy diets formulated to meet or exceed current 1994 NRC recommendations. A total of 1840 Cobb 500 male birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (23 birds/pen x 8 treatments x 10 replications). Birds were blocked by weight. Treatments consisted of T1=PC, T2=NC=PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), -0.02% sodium (Na), -300 kcal/kg, T3=NC +1000 FTU/kg phytase, T4=NC+1500 FTU/kg phytase, T5=NC+2000 FTU/kg phytase, T6=NC+2500 FTU/kg phytase and T7=NC+3000 FTU/kg phytase. NSPase treatments consisted of T1=PC, T2=NC=PC-100 Kcal/kg, T3= NC+50 g/MT NSPase, T3= NC+ 75 g/MT NSPase, T3= NC+100 g/MT NSPase, T3= NC+125 g/MT NSPase, T3= NC+150 g/MT NSPase. Phytase + NSPase treatments consisted of T1=PC , T2=NC=PC- 0.17% Calcium (Ca), - 0.15% non-phytate phosphorus (nPP), -0.02% Na, -300 kcal/kg, T3=NC+1000 FTU/kg phytase+0.125 g/kg NSPase, T4=NC+1500 FTU/kg phytase+0.125 g/kg NSPase, T5=NC+2000 FTU/kg phytase+0.125 g/kg NSPase, T6=NC+2500 FTU/kg phytase+0.125 g/kg NSPase and T7=NC+3000 FTU/kg phytase +0.125 g/kg NSPase. Broilers were blocked by weight. Partial fecal collection was done at day 28 by papering over the pens. Fecal material was freeze-dried via Lypholizer and AME was calculated by the ratio of indigestible marker, TiO_2. ME for phytase treatments was 3175, 2893, 2978, 3025, 3056, 3105 and 3161 Kcal/kg, P=0.02, respectively. ME for NSPase treatments was 3192, 3099, 3119, 3134, 3141, 3174 and 3172 Kcal/kg, P=0.03, respectively. ME for phytase+NSPase treatments was 3169, 2862, 2976, 3022, 3050, 3097 and 3150 Kcal/kg, P=0.02, respectively. Phytase doses released 85, 132, 163, 212 and 268 Kcal/kg, respectively. NSPase doses released 20, 35, 42, 75 and 73 Kcal/kg, p=0.03, respectively. Phytase + NSPase doses released 114, 160, 188, 235, and 288 Kcal/kg. In conclusion, combined phytase+NSPase released up to 288 Kcal/kg ME in broilers. The ME release of combined phytase and NSPase treatments was partially additive.

Key Words: metablizable energy, broilers, phytase, NSPase, corn/soy diets

T175 Effects of corn-expressed phytase, a commercial multi-enzyme product, or combination on broiler performance and tibia ash Jonathan Broomhead*[1], Talman Hylton[2], Phil Lessard[1], Mike Lanahan[1], Michael Raab[1], Joe Moritz[2] *[1]Agrivida; [2]West Virginia University*

An experiment was conducted to analyze the effect of a corn-expressed phytase (CEP) product, alone or in combination with a commercial multi-enzyme (CME) product on broiler performance and tibia mineralization. Treatments included a positive control (PC) or negative control (NC; -0.15% Ca and aP) and NC plus 1500 or 3000 phytase units (FTU)/kg of CEP or NC plus 1500 FTU/kg from CME or NC plus 3000 FTU/kg from combination of CME and CEP. Two additional diets were included, containing either 1500 FTU/kg from CME or 3000 FTU/kg from CME/CEP combination, using NC basal with metabolizable energy (ME) reduced by 68 kcal/kg. Diets were steam conditioned at 80°C for 15 seconds, pelleted, and crumbled for the starter and grower phases or left as a pellet for the finisher phase. Each dietary treatment was fed to 12 replicate floor pens of 23 Hubbard x Ross 708 broilers (n = 2,208) for 42 days. Three birds per replicate were sampled for tibia ash measurements on d 42. Data were analyzed as a randomized complete block design using one-way ANOVA of SAS. Birds fed NC diet had higher (P < 0.05) 10 d feed conversion (FCR) and lower (P < 0.05) 10 d and overall (42 d) live weight gain (LWG) and tibia mineralization compared to birds fed PC diet. Addition of CEP or CME and the combination to the NC basal restored (P > 0.05) FCR, LWG

and tibia mineralization to that of the PC, across both ME levels. Feeding 3000 FTU/kg, either by feeding CEP alone or combining CEP with CME improved ($P < 0.05$) 21-42 d FCR and overall LWG and FCR as compared to feeding CME alone (1500 FTU/kg) within standard ME diet. Feeding 3000 FTU/kg from CEP alone resulted in lower ($P < 0.05$) 10 d FCR compared to birds fed all other diets except ($P > 0.05$) CEP/CME combination (3000 FTU/kg) within the standard ME diet. In summary, addition of CEP and/or CME improved performance and bone mineralization of birds fed reduced Ca and aP diet and the higher phytase level (3000 FTU/kg) improved performance parameters further when feeding a standard ME diet. Addition of CME or CME/CEP combination to a reduced ME, Ca and aP diet recovered performance and bone mineralization to that of birds fed standard nutrient (PC) diet.

Key Words: corn-expressed phytase, mutli-enzyme, broiler, performance, bone mineralization

T176 Influence of graded concentrations of phytase in high-phytase diets on growth performance, apparent ileal amino acid digestibility and phytate hydrolysis in broilers C. Walk*[1], O. Olukosi[2] [1]*AB Vista;* [2]*University of Georgia*

An experiment was conducted to evaluate graded doses of phytase in high-phytate diets. Ross 308, male broilers (n = 600) were assigned to one of four diets, with 10 replicate pens/diet and 15 birds/replicate pen. Diets were a nutrient adequate control (PC), a negative control (NC) diet with a reduction of Ca by 0.22%, available P by 0.20%, energy by 120 kcal/kg, and amino acids by 1-5% compared with the PC. The NC diet was supplemented with 0, 2,000 or 4,000 phytase units (FTU)/kg. Data were analyzed as a one-way analysis of variance in JMP Pro v 14.0. Significant means were separated using non-orthogonal contrasts and post-hoc Scheffe's test. Phytase increased (linear, $P < 0.05$) weight gain. Birds fed the NC + 4,000 FTU/kg ate and gained more ($P < 0.05$) than birds fed the PC. The apparent ileal digestibility (AID) of all nutrients were reduced ($P < 0.05$) in birds fed the NC compared with birds fed the PC. Phytase increased (linear, $P < 0.10$) AID of most nutrients, but AID was lower ($P < 0.10$) in birds fed the NC + 4,000 FTU/kg compared with birds fed the PC. Using daily intake and AID to calculate digestible nutrient intake resulted in no differences between birds fed the PC or NC + 4,000 FTU/kg diets. Digestible intake of methionine or glutamate was better correlated with BWG ($P < 0.0001$) than AID of methionine ($P > 0.10$) or glutamate ($P > 0.10$). Phytase reduced (linear, $P < 0.01$) phytate concentration and increased inositol (linear, $P < 0.01$), phytate hydrolysis (linear, $P < 0.05$) and jejunal expression (linear, $P < 0.05$) of SNAT-1 and LAT-4 transporters. Accretion of P or Ca in the body was reduced ($P < 0.05$) in birds fed the NC compared with birds fed the PC. Phytase increased (linear, $P < 0.01$) carcass accretion of P, Ca and Zn. Birds fed the NC + 4,000 FTU/kg were more ($P < 0.01$) efficient at Ca and P accretion than those fed the PC. Supplementation of high phytate, low-nutrient density diets with increasing doses of phytase improved gain, digestibility, and mineral accretion in the carcass through nearly complete phytate destruction. Digestible nutrient intake may be a better indication of broiler gain than AID, particularly when evaluating enzymes with an impact on endogenous losses or when using AID to define nutrient equivalence of an enzyme.

Key Words: apparent ileal digestibility, broiler, digestible nutrient intake, phytate, phytase

T177 Feeding a combination of xylanase and fermentable oligosaccharide improved feed efficiency of commercial broilers fed corn-soy based diets containing growth promoters and coccidiostats Gilson Gomes*[1], Craig Wyatt[1], Amy Batal[2], Daniel Moore[3] [1]*AB Vista;* [2]*Sanderson Farms;* [3]*Colorado Quality Research*

Recently it was postulated that NSPases may be generating oligosaccharides *in situ* by breaking down dietary fiber and generating prebiotic-like mechanisms. The aim of this study was to evaluate the effects of supplementing a basal diet with a xylanase alone (EC XYL) or a combination of xylanase plus a fermentable oligosaccharide (EC XYL+FermCHO) compared to a multi-enzyme with xylanase and mannanase (HX XYL+H MAN) on broiler performance to 41 days of age. Five hundred and four day-old male Ross 708 broilers were randomly allocated to 3 treatments with 12 replicate pens each. Diets were based on corn, soybean meal and animal by-products, and fed as pellets in three dietary phases. All diets contained a high level of phytase (1,500 FTU/kg, Quantum Blue, AB Vista) and an antibiotic growth promotor throughout the trial. Chicks received cocci-vaccine at day of age and coccidiostat was fed in the grower and finisher diets. Feed and water were available *ad libitum* throughout the trial. Body weight gain (BWG) and feed intake (FI) were measured at 0-14-d, 14-28-d and 28-41-d. Feed conversion ratios were calculated for the same periods after correcting for mortality (mFCR) and adjusting to equal body weights (bwcFCR). Data was submitted to ANOVA, and means separated using Tukey test ($P<0.05$). No differences in liveability were seen throughout the experiment ($P>0.05$). There was no effect of treatment on performance from 0 to 14 days ($P>0.05$), however, at d 28, mFCR and bwcFCR were significantly different between treatments with birds fed EC XYL+FermCHO being more efficient than those fed HX XYL+H MAN ($P<0.05$), and those fed EC XYL diets being intermediate. At 41 days, mFCR of birds fed EC XYL+FermCHO was improved over birds fed HX XYL+H MAN or EC XYL ($P<0.01$) and bwcFCR improved by 3 points. There were no significant improvements in body weights although the EC XYL+FermCHO birds were heavier. In conclusion, the supplementation of the diet with a EC XYL+FermCHO improved broilers FCR when compared to birds fed diets supplemented with EC XYL or HX XYL+H MAN blend. These results indicate that the combination of xylanase and fermentable oligosaccharides may be altering the gut microbiota and enhancing fiber breakdown and fermentation in the ceca.

Key Words: performance, FCR, microbiota

T178 Comparative effects of a xylanase-direct-fed microbial feed additive and a commercial coccidiostat on live performance, intestinal and environmental pathogens, and apparent metabolizable energy of broilers fed corn-soy-based diets and challenged with Clostridium perfringens and Eimeria spp. Sarah Page*[1], James McNaughton[2], Basheer Nusairat[1], James Tyus[1], Jeng-Jie Wang[1] [1]*BioResource International, Inc.;* [2]*AHPharma, Inc.*

The detrimental impact of pathogenic microorganisms and ingredient anti-nutritional factors on production yield offers the opportunity for a multi-faceted feed additive alternative to traditional prophylactic antimicrobials. A study was conducted to compare the efficacy of EnzaPro® (EP; BioResource International), a proprietary blend of *endo*-1,4-β-xylanase (100,000 U/g) and multi-strain *Bacillus spp.* direct-fed microbials (10^9 CFU/g), to a commonly used coccidiostat when supplemented to corn-soy-based diets on the live performance, pathogen load, and nitrogen-corrected apparent metabolizable energy (AME_n) of broilers challenged with *Clostridium perfringens* and *Eimeria spp.* 1,560 day-old mixed-sex Ross 708 broiler chicks were assigned to one of three dietary treatments, with 10 replicate pens per treatment and 52 birds per replicate. The birds were raised to 42 days in floor pens on reused litter. Basal diets were formulated in three phases: Starter d 0-21, Grower d 22-35, and Finisher d 36-42 (2900, 3000, and 3100 kcal/kg ME respectively). Dietary treatments were 1) corn-soy basal control (CON), 2) CON + 100 g/MT EnzaPro (EP), and 3) CON + 100 g/MT monensin (MN). Birds were challenged with *C. perfringens* on d 1, *Eimeria acervulina* and *Eimeria tenella* on d 7, and *Eimeria maxima* on d 10. Data were analyzed via MiniTab 17 One-Way ANOVA. EP significantly ($P < 0.05$) increased 42-d BWG by an average of 52 g/bird and was comparable to MN. EP tended to reduce FCR by 2 points compared to CON ($P = 0.068$) and was comparable to MN. Both EP and MN significantly ($P < 0.05$) reduced intestinal *C. perfringens* and *E. coli* load, as well as *Salmonella* incidence at d 21 and 42 compared to CON. At d 21, EP and MN significantly ($P < 0.05$) reduced litter *C. perfringens* load and

intestinal lesion severity. By d 42, both EP and MN significantly ($P < 0.05$) reduced cecal *E. tenella* load compared to CON. EP had significantly ($P < 0.05$) higher AME_n compared to CON and MN at d 42 by 47 and 41 kcal/kg, respectively. The results of the current study suggest that EnzaPro is an effective solution to limit disease progression, improve energy availability, and improve broiler performance, in addition to achieving results comparable to a commercial anticoccidial.

Key Words: xylanase, DFM, anticoccidial, pathogens, AMEn

T179 Effects of protease treatment on broiler chick ileal amino acid digestibility and apparent metabolizable energy M. Persia*[1], N. Barrett[1], D. Burnham[2], A. Belalcazar[3], F. Mussini[3] *[1]Virginia Tech; [2]House of Raeford; [3]DuPont Industrial Biosciences*

An experiment was conducted to determine the effects of 2,000, 4,000 or 6,000 units of Axtra® PRO) (*Bacillus subtilis*; Pro) supplemented to 8-16 day old broilers on ileal amino acid digestibility (iAAD) and nitrogen corrected apparent metabolizable energy (AMEn). In total, 384 Hubbard x Cobb 500 chicks were selected from a commercial hatchery and allotted to 12 replicate cages (6 male/female with 65.8 in^2/bird) of 8 chicks for the 4 diets (control and 3 Pro). Both a common starter (0-8 d) and experimental diets (8-16 d) were formulated based on corn, soybean meal, and poultry byproduct meal. Analysis of dietary Pro resulted in 3,117, 6,525 and 6,765 units of activity in the diet. Excreta pans were cleaned on d 15 for a 2 d excreta collection to determine AMEn and all remaining birds were euthanized for ileal content collection from Meckel's diverticulum to ileal-cecal junction for iAAD). After ANOVA was established, analysis for interactions between dietary treatment and sex was completed. A lack of interactions (only Arg resulted in an interaction) allowed sex effects to be removed from the statistical model. Dietary treatments were then compared to the control using Dunnett's test with significance accepted at ($P \leq 0.05$). Performance measurements were collected, but due to experiment design for digestibility measurements with no significant differences were noted, although bird performance was typical for age and Pro fed birds resulted in similar or improved performance in comparison to control. Ileal AAD of the Pro treatments were generally increased in comparison with the control: Lys, 3.0 to 4.8%; TSAA, 5.2 to 6.0%; Thr, 4.8 to 8.5; and Trp, 3.6 to 5.2% with the lowest bound generally $P > 0.05$, but the remaining two ileal AAD values (2,000 and 6,000) were $P \leq 0.05$. The AMEn of the diets was improved by Pro treatment resulting in a 48 to 86 kcal/kg uplift in comparison to control. As with iAAD, the lowest response for AMEn was $P > 0.05$, but the two higher responses were $P \leq 0.05$. Additional correlation compared iAAD uplift with the undigested fraction of dietary AA resulting in a positive relationship and generally good fit ($r^2 = 0.644$). Overall, Pro supplementation resulted in consistent improvements in iAAD and AMEn in growing broiler chickens.

Key Words: protease, enzyme, ileal AA digestibility, apparent metabolizable energy, broiler

T180 Evaluation of a specific vectorization of curcumin to reduce inflammatory indicators and improve growth broiler performances Jean-Francois Gabarrou* *PHODE*

Intensive system of production increases oxidative stress and sub-inflammatory status of animals. Curcumin is well known for its anti-oxidant and anti-inflammatory properties, but curcumin is also very fragile and sensitive to oxidation. The main goal of this experiment was to evaluate the effect of the vectorization of curcumin by a technology of delayed release provided by Laboratoires Phodé (France).

84 chicken 10 days old (Ross-308) were randomly distributed in individual cages into 6 groups (n=21).First group receive a standard feed without any plant extract feed additive. The second one receive the same control feed added on the top by 6.66ppm of standard curcumin. The 2 other feeds contained respectively 3.33ppm and 2.20ppm of vectorised curcumin.

Blood interleukins IL6, IL10 and TNF-α were determined on 10 birds per treatment at 35 days. Feed intake (FI), live body weight (LBW) and feed conversion ratio (FCR) were determined at 35 days (n=21).

Statistical analysis was carried out using the General Linear Model procedure of SPSS®

No significant difference was observed between group for pre-inflammatory (IL-6) and pro-inflammatory (TNF- α) indicators. Whatever the dosage or the presentation of the curcumin, curcumin increase significantly IL-10, suggesting that anti-inflammatory effect of curcumin is controlled by anti-inflammatory cytokines' and not pro or pre-inflammatory ones.

The Control group presented significant lower performances compared to curmin's groups suggesting that the anti-inflammatory effect of curcumin permitted a better growth performance.

On the other hand, no significant difference between the 3.33ppm of vectorised curcumin and the standard 6.66ppm was observed: 126.8 vs. 127.9 g/day of FI; 2227 vs. 2263 g LBW; 1.629 vs. 1.612 FCR respectively.

Vectorization of the curcumin permitted animals to exhibit the same level of performance with twice less curcumin.

Key Words: broiler, curcumin, vectorization

T181 Synergistic anti-inflammatory activity of fatty acids, essential oils and plant extracts in a well-proportioned mixture Alireza Khadem*[1,2], Jamal Al-Saifi[2], Ben Letor[2], Markella Al-Saifi[2], Christos Gougoulias[2] *[1]Ghent University; [2]Innovad SA*

Excessive use of antibiotics over the last few decades has led to the current legislation whereby antibiotic use in animal production should be rationalized. As a first step, Antibiotic Growth Promoters (AGPs) were banned from use within the EU. Therefore, it is critical to develop cost effective antibiotic alternative strategies to ensure the long-term sustainability of animal production. Although extensive research has been carried out on ingredients that exert antibacterial effect individually, less work has been done to understand how multiple ingredients may work in combination. Recent findings demonstrate that controlling and reducing inflammation are the main mechanisms behind the growth promoting activity of AGPs (Khadem et al, 2014). A combination of different anti-inflammatory compounds may hold the most promising method to substitute antibiotics in animal feed. Lumance® (obtained from Innovad, Belgium) is a complex product, combining slow release and protection technologies ensuring that fatty acids, essential oils and plant extracts are delivered in a gut active way for an effective anti-inflammatory control. In current study, the anti-inflammatory activity of three main ingredients, fatty acids (FA), essential oils (EO) and plant extracts (PE) individually (similar doses as used in Lumance®) and in a commercial mixture (Lumance®) was tested in an *in-vitro* model using the RAW 264.7 assay, essentially as described by Wu et al 2003. The addition of FA, EO, PE alone (with the same concentration as used in Lumance®) did not show any significant influence on reducing the NO production while the combination of these compounds had a significant effect on reducing the NO production (Fig. 1, A). Figure 1, B shows the measured reduction in NO production by these three ingredients and the sum of them compare to the whole mixture. Although the results showed that these three compounds moderately lowered NO production and inflammation, their combination was more effective. The current study suggests that the anti-inflammatory activity of antibiotic alternatives can be enhanced in a synergistic fashion by combining them with low doses of FA, EO and PE and greater chemo-preventive effect could be achieved by compounds targeting different signaling mechanisms.

Key Words: anti-inflammatory activity, Lumance, Antibiotic Growth Promoters, synergistic, effective antibiotic alternatives

T182 The impact of humic acids and essential oil based blends as alternatives of growth promoter agents in productive performance of broiler chickens in the Middle Tropical Region of Colombia Joaquín Pirateque Castellanos*[1], Blanca Martínez[2], Álvaro Uribe Serrano[2]
[1]National University of Colombia; [2]Promitec Santander

The objective of this work was to assess the impact of phytobiotic based growth promoters at different inclusion levels in the productive performance Ross 308 broilers in a field experiment. One day old birds (n = 2820, half females, half males) were assigned by sex to one of 6 treatments as follows: Treatment 1 (T1) consisted in 100 ppm *Lippia origanoides* Essential Oil (LOEO) + 750 ppm Sodium Humates (SH); T2, 100 ppm LOEO (in a dry emulsified form) + 278 ppm SH; T3, 75 ppm LOEO + 750 ppm SH; T4, 50 ppm LOEO + 50 ppm *Eugenia caryophyllata* Essential Oil (ECEO) + 750 ppm SH; T5, 100 ppm LOEO + 25 ppm ECEO + 750 ppm SH; T6 was a positive control with antibiotic growth promoters (Halquinol and Enramicin) and anticoccidial drugs (Nicarbazin – Narasin for the first 21 days and Salinomicin from 22th day up the end of the experiment); there were 5 replicate pens per treatment (n = 47 birds per pen). Feed Intake, Weight, Feed Conversion Rate (FCR), Productivity Index (PI) European Efficiency Index (EEI) and mortality were recorded. The results were subjected to one-way ANOVA to establish whether were statistically significant (p<0,05) or not; Dunnet and Tukey tests were used for mean comparisons. In males, weight was superior (p<0,05) in T5 (2530,8 g) than control (2439 g) and all treatments were numerically above; FCR was better (p>0,05) in T4 (1,44) than control (1,48), T2 (1,46) and T5 (1,48) were slightly better. In females, weight was superior (p<0,05) in T4 (2140 g) than control (2091 g) and all treatments, except T3 (2084 g) were numerically above. FCR was slightly better in T4 (1,52) than control (1,53) and T1 (1,56), T5 (1,56), T3 (1,56) and T2 (1,54) were slightly higher. At weekly analysis only T2 had a homogeneous behavior; during first 3 weeks T3 reported the best weight gain and FCR for both sex; during fourth week T4 birds had the best performance, in fifth week T5 had the best performance. Overall, T4, T2 and T5 (in that order) were better than controls at the end of the trial. It seems essential oils can have a better performance than conventional growth promoters; and its requirement for broiler chickens seems to differ depending on the age of birds, therefore several additives may be needed within each stage of production.

Key Words: Lippia origanoides, Eugenia caryophyllata, Sodium Humates, phytobiotics, AGP alternatives

T183 Effect of the type of diet and the addition of humic substances as growth promoter in broiler chickens S. Gomez-Rosales*[1], M.L. Angeles[1], Y.R. Lopez-Garcia[1], A. Dominguez-Negrete[2], B. Alvarez[2]
[1]National Center of Disciplinary Research in Animal Physiology/National Institute of Research in Forestry, Agriculture and Livestock; [2]Faculty of Natural Sciences, Autonomous University of Queretaro

The objective was to evaluate the productive parameters, carcass yield, ileal nutrient digestibility, total mesophilic aerobic bacteria (MAB) and E. coli in broilers fed low and high fiber diets (LF and HF) and added with an extract of humic substances (EHS) from a wormcompost. Two hundred and forty Ross 308 male broilers were individually allocated in cages from 28 to 42 days. Broilers were randomly assigned to 3 treatments and 2 diets in a factorial design. The treatments were: Tx1) diet added with an antibiotic growth promoter (Bacitracin methylene disalicylate) and an anticoccidial drug (salinomycin); Tx2) Diet without antibiotic growth promoter and anticoccidial drug; and Tx3) Same as 2) but added with 0.5% of EHS. The LF diet was formulated with corn and soybean meal and the HF was formulated with corn, soybean meal, distillers dried grain with solubles and wheat bran. The jejunal and cecal content and samples of liver were collected at the end of the study for bacterial counts in colony forming units (CFU). The ileal digesta was used to determine the digestibility of nutrients. The results were subjected to ANOVA. The final weight and weight gain tended to be higher and the feed conversion (P < 0.10) tended to be lower in broilers of Tx3, added with ESH, regardless of the type of diet. The weight and yield of the carcass and its components and the digestibility of nutrients were similar among treatments. The CFU of MAB and E. coli were lower (P < 0.01) in the content of jejunum and ceca of broilers on Tx1, added with an antibiotic growth promoter and anticoccidial drug, compared to broilers of Tx2 and Tx3. The BMA and E. coli counts in liver tended to be higher in broilers fed Tx3 and the HF diet. In summary, the addition of the EHS in the diet of broiler chickens improved the productivity, but did not reduce the counts of MAB and E. coli in jejunum and ceca.

Key Words: High fiber diet, Humic substances, Broilers, Production, Bacterial counts

T184 Evaluation of glycerides of organic acids on the performance and intestinal quality of broiler chickens raised in an antibiotic free system. Luiz Demattê Filho*[1,2], Dayana de Oliveira Pereira[2], Gustavo do Valle Pereira[2], Angelo Lauwaerts[3] *[1]Korin Agricultura Ltda.; [2]Fundação Mokiti Okada; [3]Proviron Industries NV*

The objective of this study was to evaluate the efficiency of a blend of glycerides of specific organic acids on the zootechnical performance and intestinal integrity of broilers raised in a system of antibiotic free production and challenged by coccidiosis. 1,080 chickens were fed in five phases, with water and feed supply *ad libitum*. At 21 days of age the birds received live oocysts of *Eimeria* to induce a sanitary challenge to the flock. The evaluated treatments were: T1 - Basal ration (BR) without the additive; T2 - BR supplemented with the additive at 4, 2, 2, 2 and 0 Kg / ton / feeding phase and T3 - BR supplemented with the additive at 2, 1, 1,1 and 0 Kg / ton / feeding phase. Performance variables live weight, daily weight gain, feed intake, feed conversion, mortality and production efficiency index were recorded from 1 to 21 and from 1 to 42 days of age. Additionally, at 26 days of age, intestinal integrity was assessed by a macroscopic (bacterial enteritis score) and a microscopic method (villi height and crypt depth). Therefor, 2 birds per pen were randomly selected, resulting in 24 birds per treatment, from which duodenum samples were taken. A randomised block design was used, with 3 treatments at 12 repeats of 30 birds each. Data were submitted to ANOVA comparing the means with a Tukey test at 5%. The treatments that received the additive showed better zootechnical performance, which was characterised by a better feed conversion in the two evaluated periods. The treatment that did not use the additive presented higher feed intake in the period from 1 to 21 days. Intestinal integrity differed statistically between treatments. Lower bacterial enteritis score was observed in the treatment that used the higher level of inclusion of the product when compared to the treatment that did not receive it. The higher inclusion of the additive also provided significantly higher villi height and lower crypt depth compared to the treatment that used the lowest inclusion. Consequently, the villi to crypt ratio was significantly higher in this treatment. It was demonstrated clearly that the two levels of inclusion are efficient in improving the performance of the birds and that the greater inclusion of the additive provided greater intestinal integrity.

Key Words: coccidiosis, organic acid glyceride, intestinal integrity, antibiotic free

T185 Comparison of Cobb700 broiler performance when fed diets containing organic acid feed amendments from d 0 to d 42 Kristina Feye*[1], Barbara De Almeida Mallmann[2], J.P. Caldas-Cueva[2], Dana Dittoe[1,2], Joshua Jendza[3], Guillermo Tellez-Isaias[2], Casey Owens[2], Michael Kidd[2], Steven Ricke[1] *[1]University of Arkansas, Department of Food Science, Center for Food Safety; [2]University of Arkansas, Department of Poultry Science; [3]BASF*

The removal of most of the antibiotics from poultry diets necessitates the development of alternative feed amendments. These feed amendments must reduce the energy-expensive outgrowth of the microbiota without sacrificing production. Organic acids have become important prophylactic

feed amendments. The introduction of organic acids to the gastrointestinal tract delivers short-chain volatile fatty acids, which can potentially influence the gastrointestinal innate barrier function and preferentially support healthy microbial populations. A common feed amendment used by the poultry industry includes formaldehyde. Significant drawbacks exist in the use of formaldehyde-based feed amendments, including worker safety and negative effects on nutrient availability, particularly amino acids. Therefore, a production trial using Cobb700 broilers was conducted in order to compare Amasil NA (0.25% and 0.5% inclusion rate) and Silo Health 104L (0.25%) with formaldehyde (0.5%) feed amendment. Cobb700 broilers were hatched, sexed, and 864 males were placed at day of hatch (d 0) in 48 floor pens (5 treatments, 10 replicates for feed treatments, 9 replicates for NC, with 18 birds per pen). All three stages of feed (starter, grower, and finisher) contained the feed amendments and were prepared by the University of Arkansas feed mill. Water and feed were supplied ad libitum. Performance data were collected at all three feed changes, starting with d 0 (day of hatch), d 14, d 28 and d 42. Statistical analysis was conducted using JMP 14.0 comparing all of the means against the formaldehyde group using a one-way ANOVA, followed by analysis with the Dunnett's test ($P \leq 0.05$). Statistically significant effects of the treatment were demonstrated throughout the study. Treatment of feed with formaldehyde reduced 14 to 28 d ADG (67.0 g/bird) vs the untreated feed (71.8 g/bird) and feed treated with Amasil NA (73.2 and 70.7 g/bird). Concerning ADFI, NC + SILO Health 104L and NC + 0.25% Amasil birds consumed more feed that the birds consuming formaldehyde amended feed (34.5 and 34.4 vs 32.4g/bird; d 0 to 14). In conclusion, treatment of broiler feed with formaldehyde exhibited negative effects on growth and feed intake when compared with alternative feed amendments like Amasil NA.

Key Words: Organic Acids, Amasil NA, Broiler Performance, Silo Health 104L, Feed Additive

T186 Yeast cell wall immunomodulatory and intestinal integrity effects on broilers challenged with Salmonella Enteritidis Breno Beirão[1], Max Ingberman[1], Melina Bonato*[2], Liliana Borges[2], Ricardo Barbalho[2] *[1]Imunova Análises Biológicas Ltda ME.; [2]ICC Industrial Comércio Exportação e Importação Ltda.*

The objective of this study was to evaluate the immune effects and the dynamics of intestinal integrity in broilers challenged with *Salmonella* Enteritidis (SE) and treated with yeast cell wall (YCW). 100 birds were housed in isolators at 1 d and divided into 4 treatments: G1-Birds challenged with SE; G2-Birds not challenged and supplemented with YCW (*Saccharomyces cerevisiae*, IMW50® from ICC Brazil, at 0.5 kg/MT); G3-Birds not challenged and not treated and; G4-Birds challenged with SE and supplemented with YCW (same dosage). The challenge was administered orally at 2 d with 10^8 CFU per bird. Circulating lymphocyte and monocyte subsets, as well as phagocytic cells, were evaluated at 8, 14 and 21 d. Samples of ileum, ceca, and liver were collected at 14 d (8 birds/treat.) for histopathology. Specific IgA for SE in feces was evaluated at 14 d. Intestinal mucosa permeability was assessed in 8 birds/treat. at 4, 8, 14 and 21 d by the passage of a marker (Dextran-FITC, 3-5 kD) from the intestinal lumen to blood. The data were analyzed by ANOVA and the means compared by Tukey's test at 5% of significance. At 4 d, G1 presented the highest intestinal permeability (significantly different from the treated group [G4]). Circulating leukocytes counts were higher in the non-SE challenged groups (G2 and G3). Despite this, challenged groups consistently presented higher numbers of various cell subtypes, especially at 14 d (APCs, monocytes, suppressor monocytes, and the series of helper T lymphocytes and cytotoxic T lymphocytes). Treatment was effective in controlling leukopenia and in preventing some of the immune subset fluctuations provoked by the challenge, such as for APCs and cytotoxic cells. The number of phagocytic cells was increased by the challenge at 8 d, while the YCW decreased this effect. G4 presented the highest number of reactive animals, as well as the highest level of anti-salmonella IgA. The challenge induced marked inflammatory responses in the intestine and liver. Treatment was effective in improving tissue inflammatory signs such as lymphocyte infiltration in the cecum, but not liver. The challenge with SE induced changes in all evaluated systems; however, intestinal integrity and some immune parameters were improved by dietary YCW in challenged birds.

Key Words: Saccharomyces cerevisiae, Poultry, Intestinal permeability, IgA

T187 Engineered probiotics to reduce Salmonella enterica and Clostridia perfringens in broilers Yiannis Kaznessis* *General Probiotics*

New technologies are needed to replace antibiotics in poultry production and to prevent gastrointestinal (GI) infections in animals and humans caused by bacterial pathogens.

At General Probiotics, we develop a technology that reduces *Salmonella enterica*and *Clostridia perfringens*in chicken.

We modify probiotic bacteria to produce antimicrobial peptides and to deliver these potent antibiotic proteins inside the gastrointestinal tract of poultry. Probiotics are beneficial feed additives but have been marred by inconsistent performance. Modern biological engineering and synthetic biology techniques can modify probiotics and boost them for consistent performance.

We first targeted *Salmonella enterica*. We built a prototype, and tested it in a field trial at Southern Poultry Research Group. We observed a reduction in salmonella prevalence by 50% compared to controls. We then re-designed, re-engineered and optimized a second generation of modified antimicrobial probiotics. We tested the lead candidate in a new field trial and observed a reduction in salmonella prevalence by 90%. SE prevalences in ceca samples were compared using generalized estimating equations logistic models. These models were estimated using robust standard errors and an exchangeable working correlation structure.

With synthetic biology techniques we built and tested over 200 new modified probiotics in eight months, by combining antimicrobial peptides, DNA promoters, ribosome binding sites and probiotic organisms. We have now developed a platform that enables the design, building and testing of 50 distinct systems per month.

More recently, we turned against *Clostridia perfringens*. In a first design cycle, we have built 60 systems. We have identified numerous candidates with strong activity against *C. perf.*

These results constitute a sound foundation for the hypothesis that live biotherapeutic bacteria can reduce the carriage of pathogenic bacteria in pre-harvest chickens. We will present systems we have built and tested against salmonella and clostridia. We will also discuss the scientific, technological, market and business risks of this new technology.

Key Words: antimicrobial peptides, probiotics, Salmonella enterica, Clostridia perfringens, alternatives to antibiotics

T188 In-vitro evaluation of a novel 3 strain Bacillus probiotic. Tom Hashman*[1], Thomas Gaydos[2], Tadele Kiros[2], alain riggi[3] *[1]Envera; [2]Phileo-USA; [3]Phileo-France*

Many species of *Bacillus* are used as probiotics in animal feed not only due their proven effect on health and performance of animals, but also the resistance of the spores to the high temperature of the feed manufacturing process. After the spore is ingested by the host it needs to germinate to be metabolically active and provide a benefit to the animal. The germination time of a spore varies significantly depending on the environmental conditions that the spore is subjected to. Germination of *Bacillus* spores can be inhibited by low pH, amino acid analogues, fatty acids, alcohols, phenols, enzyme inhibitors, and sub-optimal germinants. The priming of Bacillus spores by coupling individual spores with optimized germinants enables

the sporulated bacteria to resist environmental constraints and germinate at higher levels.

In this study, a blend of 3 bacterial spores, hereafter Probiotic 1, containing *Bacillus pumilus (BP), Bacillus licheniformis (BL), and Bacillus amyloliquefaciens (BA)* was compared to 4 commercially available probiotics, hereafter Probiotic 2, containing *B. subtilis (BS), and B. amyloliquefaciens, Probiotic 3 (B. licheniformis), Probiotic 4 (B. subtilis) and Probiotic 5 (B. licheniformis).*

The germination rate of Probiotics 1 after 105 minutes incubation at pH 7 was 79.7% for BP, 71.9% for BA and 47.5% for BL. In contrast the germination rate for all the other probiotics was 0.0% except for probiotics 4 (BS) which germinated at 3.5% at pH 7. Similarly, the germination rate after 105 minutes at pH 3 for, Probiotic 1 was 29.1%, 6.9%, 61.0% for BP, BA and BL, respectively; while none of the other probiotics germinated at pH 3. The ability of *Bacillus* spores to germinate rapidly is important for animals like poultry that have very fast gastro-intestinal transit times. This novel probiotic blend could lead to improved consistency in field applications by increasing the consistency and speed of spore germination, leading to faster bacterial growth and metabolism.

Key Words: Bacillus, Germination, Probiotics, Spores

T189 Bacillus subtilis 29784 strengthens barrier function in vitro, and rescues inflammation-dependent reduction of barrier function during trans-epithelial electrical resistance analysis. Adam Nelson*[1], Stina Rikke Jensen[2], Sonja Christian[2], Karoline Sidelmann Brinch[2] *[1]Novozymes Biologicals; [2]Novozymes A/S*

Understanding how probiotic feed additives contribute to animal performance requires complex mode-of-action studies. *Bacillus subtilis* strain 29784 is a probiotic feed additive that has shown consistent *in vivo* performance in broilers during both performance and challenge trials. We investigated the impact of 29784, and other *Bacillus* isolates, on the host intestinal barrier *in vitro* by tracking real-time measurements of trans-epithelial electrical resistance (TEER) of Caco-2 cell monolayers with a Cell Z-scope. In our studies, 29784 showed a larger and more prolonged increase in TEER over 24 hours compared to other *Bacillus* examined. To further understand gut modulation of the inflammatory response, additional testing was done with inflammatory conditions triggered by IFNγ and TNFα. In these tests, 29784 could rescue the inflammation-induced drop in TEER in Caco-2 cells, suggesting that 29784 has potential anti-inflammatory effects in intestinal epithelial cells. By demonstrating that 29784 can improve barrier function *in vitro*, both with and without inflammatory conditions, these results suggest improved barrier function may be one contributor to performance *in vivo*.

Key Words: Probiotics, Feed Additives, Nutrition, Mode of Action, Barrier Function

T190 A scientific approach to elucidate in vitro properties of the efficacious probiotic strain Bacillus amyloliquefaciens CECT 5940 Lorena Stannek-Goebel[1], Jessica Kleinboelting[1], Stella Molck[1], Álvaro Ortiz Garcia[1], Kiran Doranalli[1], Adriana Barri[1], Claudia Borgmeier[2], Guido Meurer[2], Werner Katzer[3], Pelzer Stefan*[1] *[1]Evonik Nutrition & Care GmbH; [2]BRAIN AG; [3]AnalytiCon Discovery GmbH*

Commercial livestock production is challenged by numerous pathogens, such as *E. coli, Salmonella* spp, *Clostrida* spp which all have the potential to impair growth performance and health of animals and potentially humans. The push to reduce the use of antimicrobials in feed increases the need for alternative feed additives like probiotics. These are ideal due to their multiple modes of action. *B. amyloliquefaciens*CECT 5940 based probiotic products have previously been shown to improve broiler performance under various feeding conditions when potential gut health challenges exist. The current study will highlight unprecedented *in-vitro*lab research to explain the observed *in-vivo*properties of *B. amyloliquefaciens* CECT 5940 products.

The combination of modern microbiological, biochemical, genetic and bioinformatic technologies revealed new insights into the properties of *B. amyloliquefaciens*CECT 5940. This strain has the ability to inhibit the growth of numerous field pathogens, like *E. coli,Salmonella* sppand *C. perfringens*. Analyses of the fermentation supernatant combined with genetic knockout experiments resulted in the molecular identification of different pathogen inhibiting secondary metabolites, e.g. several surfactins, bacilysin and macrolactin. In addition, it was shown that with respect to plant polymer degrading enzymes CECT 5940 displays a 10 times higher amount of xylanase activity and more than 15 times higher cellulase activity compared to control strains. Moreover, production of lactic acid [appr. 1.5 g/l] as well as quorum quenching properties, supported by the degradation of different N-Acyl homoserine lactones, complete the unique portfolio of beneficial properties reducing the risk of pathogen mediated gastrointestinal disorders.

This study provides unique molecular insights providing the scientific basis to explain the consistent *in vivo*performance of *B. amyloliquefaciens*CECT 5940 based probiotics in animal farming. The ability to display multiple modes of action is the key to confer benefits for animals reared under different conditions.

Key Words: probiotics, DFM, mode of action, Bacillus, pathogen

T191 Effect of Bacillus amyloliquefaciens CECT 5940 on performance of broiler chickens: a meta-analysis of 8 independent research trials Anita Menconi*[1], S. Maria Mendoza[1], Alvaro Ortiz[2], Chavalit Piriyabenjawat[3], Kiran Doranalli[4] *[1]Evonik Corporation; [2]Evonik Nutrition & Care GmbH Sucursal en España; [3]Evonik (SEA) Pte Ltd; [4]Evonik Nutrition & Care GmbH*

Direct-Fed Microbials (DFMs) have been extensively studied and used in broiler chickens for improving intestinal health and overall performance. However, under research or commercial conditions, the outcome or effect of DFMs on performance can be affected by several variables. Therefore, it is imperative that several trials are performed in order to evaluate the consistency of the effects of DFM strains on the performance of tested animals. A meta-analysis of 8 independent research trials was conducted with the objective of evaluating the effect of *Bacillus amyloliquefaciens* CECT 5940 (*Ba*) on body weight gain (BWG), feed conversion ratio (FCR), and feed intake (FI) of broilers. The studies were conducted between 2001 and 2018 in Spain, Thailand, Romania, Czech Republic, and Brazil (replicates per treatment per trial ranged from 6 to 25). The statistical analysis utilized fixed and random effects to estimate the mean effect size (MES) of the difference between control and *Ba*-fed broilers, the 95% confidence interval (CI) of MES, and the probability of MES being different from zero (Ho: MES = 0). A meta-regression was performed to evaluate MES variance (heterogeneity) among studies (Ho: studies share a common effect size). The statistical analysis was performed using the robust variance estimation strategy by a SAS Macro. Dietary *Ba* tended to increase BWG (MES = 1.025, 95% CI = -0.149 to 2.198, $P = 0.078$), reduced FCR (MES = -1.667, 95% CI = -2.353 to -0.980, $P = 0.001$), and did not affect FI (MES = -0.322, 95% CI = -0.943 to 0.300, $P = 0.269$). The occurrence of heterogeneity ($P \leq 0.002$) demonstrated that the magnitude of the effect size varied among studies for all the parameters. Some of this heterogeneity can be attributed to differences in the studies (e.g., experimental design, diet composition, genetics, environment, etc.). Overall, dietary supplementation of *Ba* significantly improved FCR and improved BWG in a large manner, since the absolute value of MES was greater than 0.8. Additional studies are being conducted to further evaluate the range of response in growth performance associated with *Ba* in broiler chickens.

Key Words: direct-fed microbials, meta-analysis, performance, broiler

T192 Effect of supplementation of a Bacillus-based probiotic on broiler growth performance Alain RIGGI*[1], Anis HEDHLI[1], Douglas CURRIE[2], Eric AUCLAIR[1], Ruth RASPOET[1] *[1]Phileo - Lesaffre Animal Care; [2]Roslin Nutrition Ltd*

Since the ban of using antibiotic growth promoters (AGPs) in livestock in several countries, probiotics have been considered as possible alternative. Spore forming bacteria that belong to Bacillus species are one of those probiotics selected for their ability to resist adverse environmental and nutritional conditions such as, low pH in the stomach and high temperature of the feed manufacturing process. Our study aims at comparing the effects of two doses of a Bacillus-based-probiotic product (BP) on broilers growth performance. A total of 840 broiler chickens (1-day old, Ross 308, male) were randomly allocated to one of 3 dietary treatment groups, with 8 replicate pens per treatment and 35 birds per pen. The birds were fed one of three dietary treatments: (T1) control with no BP, (T2) control + log 4.5 CFU/kg BP, (T3) control + log 5 CFU/kg BP, for a period of 42 days divided in 3 phases: (i) starter (0-14), (ii) grower (15- 28) and (iii) finisher (29-42). During the starter phase, both birds from T2 or T3 gained significantly more weight (14.5% and +6%, respectively) and consumed more feed (+10% and +4%, respectively) than birds from T1 ($P < 0.0001$). During the grower phase, birds from T3 gained more weight (1003,5 g) than T1 (814.2 g) and consumed significantly also more feed (1459.9 g) than control group (1261.3 g). During the finisher phase, no significant difference in weight gain or in feed intake was recorded between treatments. Overall (d 0-42), birds fed with BP resulted with higher body weight gain ($P < 0.0087$) and consumed more feed compared to control group T1 ($P < 0.0121$) without any difference between T2 and T3. No effect was observed on FCR during the overall period. To conclude, supplementation of diets with either log 4.5 or log 5 CFU/kg of feed of a Bacillus-based probiotic increased body weight gain of broilers and feed intake over the 42-day study as compared to its control.

Key Words: Probiotic, Bacillus, Broiler, Feed Intake, Growth

T193 Compatibility of a live-attenuated Salmonella vaccine and a new multi strain probiotic in chickens Dorthe Sandvang[1], John Schleifer*[2], Roland Koedijk[1], Anée Kehlet[1] *[1]Chr. Hansen A/S; [2]Chr. Hansen Inc.*

Vaccination of chickens and probiotic supplementation in feed are nowadays regarded as relevant measures to increase the resistance of birds against *Salmonella* exposure and decrease shedding. A pilot study was undertaken to assess the compatibility of a new multi strain probiotic with a live-attenuated *Salmonella* Typhimurium (ST) vaccine. Experimental units were individual birds. There were three experimental treatments: control group without ST-vaccine administration or probiotic in the diet (T1), ST vaccinated group without probiotic *Bacillus subtilis* DSM32324 (8×10^5 CFU / g of feed), *Bacillus subtilis* DSM32325 (5×10^5 CFU / g of feed) and *Bacillus amyloliquafaciens* DSM25840 (3×10^5 CFU / g of feed) (T2) and ST vaccinated group with probiotic in the diet (T3). On day 3 of test chicks were challenged with 2.5×10^6 CFU *Salmonella* Heidelberg/bird by oral gavage. Prior to challenge, four chicks from each group were randomly selected and the vaccine was able to be re-isolated from the spleens from treatments receiving the vaccine, indicating the probiotic did not affect the vaccine's initial colonization. At study termination (day 39) caeca were sampled for *Salmonella* prevalence and number. There was no significant difference in *Salmonella* prevalence among experimental treatments. However, the vaccine alone or with probiotic showed numerically lower MPN log10 of *Salmonella* than the challenge control. This study was primarily designed to demonstrate that the tested probiotic would have no negative effect on the ability of the live *Salmonella* vaccine to replicate and induce protection. This study clearly demonstrated that this *Bacillus*-multistrain probiotic does not have any detrimental effect. In addition, the study indicated there may be an additive effect to having both the vaccine and probiotic.

Key Words: Salmonella vaccine, Probiotic, Bacillus, Challenge

T194 Modification of a limestone solubility method and potential to correlate with in vivo calcium digestibility Seon-Woo Kim[1], Roselina Angel*[1], Peter Plumstead[2], Wenting Li[1] *[1]Department of Animal and Avian Sciences, University of Maryland; [2]Chemunique*

Work was done to develop a limestone solubility assay to improve predictions of *in vivo* Ca digestibility in broilers and to predict impacts on P digestibility. Limestones (LIME) were obtained from 3 commercial sources. LIME-1 (0.633 mm mean diameter (dgw)); LIME-2 (a sub-sample of LIME-1, ground to pass through a 0.075 mm sieve, d_{gw}=0.063 mm); LIME-3 (d_{gw}=0.326 mm) and LIME-4 (d_{gw}=0.831 mm). LIME solubility was determined at 5, 15 and 30 min of incubation using either a 0.2N HCl (S1) or a pH 3 HCl (S2) solution buffered with 3M glycine. An apparent ileal digestibility (AID) trial was done with 320 Ross 708 male broilers. Treatments (TRT) were no added LIME, or added LIME-1, 2, 3, and 4 to achieve 0.65% added Ca, to a basal diet (corn/SBM based with no added inorganic P, 0.04% Ca). Trt diets were fed for 36 h from 22 to 23 d of age (n=8, 4 birds/n). Distal ileal digesta was collected from all birds and pooled by pen. Solubility was analysed as a factorial arrangement of LIME sample and solution main effects and their interaction (Proc Mixed, SAS 9.4). AID Ca and P were analysed using TRT as the fixed effect and pen as random effect (Proc Mixed, SAS 9.4). Relationship between AID Ca or P and solubility parameters was analysed using Multiple Regression Model Fit test (SAS 9.4). An interaction between LIME sample and solution was seen only at 5 min (P<0.05). Irrespective of interaction, LIME solubilized quicker and more complete with S1 vs. S2 at all time points (P<0.05). Among all LIME, LIME-2 solubilized quickest, LIME-3 had the lowest solubility through all incubation time (P<0.05). AID Ca was 20, 66, 47, 20 and 66% for no added LIME, LIME-1, 2, 3 and 4, respectively (P<0.05). AID P dig was highest in no LIME added diet (75%). Adding LIME reduced (P<0.05) AID P to 23, 13, 65 and 37%, for LIME-1, 2, 3 and 4, respectively. Regression analysis showed that d_{gw}, 5 and 15 min solubility are critical for AID Ca predictions based on S1 (RMSE=2.125) or S2 (RMSE=2.120). In conclusion, LIME source and d_{gw} can affect AID Ca and AID P in plant based ingredients differently. The solubility dynamics including speed and extend of solubilization, rather than a single time solubility, yield better predictions of *in vivo* Ca digestibility of LIME.

Key Words: limestone particle size, digestibility, solubility, broiler

T195 Evaluation of different limestone sources used by the US poultry feed industry Franco Mussini*, Kenneth Wilson, Janet Remus, Elizabeth Kim, Milan Hruby *Dupont Danisco Animal Nutrition*

A survey to evaluate different characteristics of limestone used by the US poultry industry was conducted to provide an overview of current market situation related to this important source of calcium. Additionally, several recently published studies have suggested that limestone characteristics could impact digestibility of Ca, P and efficacy of phytase. Seventy-four samples from 26 different quarries in 14 states were analyzed for calcium, additional 8 minerals, average particle size (calculated as geometric mean diameter) and solubility (as per method of Kim *et al*, 2019). Samples included fine and grit limestone ranging from 59 to 2803 microns. Average Ca level was 37.52% with a CV of 4.46%. Forty-seven samples were classified as fine with a particle size ranging from 59 to 495 um while 27 grit samples ranged from 848 to 2803 um. Calcium solubility of the samples was measured at a pH of 3.0 at three different time points. Fine limestone solubility was measured at 5, 15, and 30 minutes while grit limestone sample solubility was measured at 30, 90 and 150 minutes. Solubility of fine limestone showed high variability between samples at 5 and 15-minute time points, ranging from 32.83% to 92.25% at 5-minute and from 64.25% to 97.01% for the 15-minute time point. For fine limestone, solubility

showed a reasonable correlation with particle size at 5 minutes (R^2=0.641) reducing to R^2=0.41 at 15 minutes. Grit limestone showed much lower solubility at 30 minutes ranging from 17.47% to 70.18%. At 90 minutes, solubility ranged from 47.27% to 93.19%. Correlation between the average particle size of grit limestone and solubility was very low compared to fine limestone at 30 (R^2=0.3003) and 90 minutes (R^2=0.3609). For both limestone types, solubility at the third time point, 30 minutes for fine and 150 minutes for grit, was very similar among the samples and in the range of 87% to 99%. The data suggest that fine limestone solubility at 5 minutes is to some degree influenced by particle size although inherent characteristics of the limestone rock were likely important as well. Results of this survey supported by previously published data suggest that some limestone currently used in poultry diets could negatively impact digestibility of Ca, P and efficacy of phytase.

Key Words: Limestone, Calcium, Solubility, Particle size

POSTER SESSIONS

Teaching, Pedagogy, Extension

P197 Piloting a Rwandan smallholder broiler production model: Leveraging public-private partnerships Emily Urban*, Tom Gill, Dave Ader, Michael Smith *University of Tennessee Institute of Agriculture*

Despite Rwanda's recent rapid economic growth, there is a limited supply of and access to animal-sourced protein nationally. The USAID Feed the Future *Tworore Inkoko, Twunguke* (Kinyarwanda for "Let's raise chickens and make a profit") project leverages public-private partners to support intensive broiler production for smallholder farmers in northern Rwanda in order to: (a) increase incomes of rural smallholders and (b) improve household nutrition outcomes through the consumption of chicken meat. This three-year project (2017-2019) is jointly funded by USAID/Rwanda and the African Sustainable Agriculture Project (ASAP), a private, US-based foundation. Implementing partners include The University of Tennessee Institute of Agriculture (UTIA) and Zamura Feeds, Ltd., a private feed mill located in Rwanda. Additionally, Tyson Foods and Cobb-Vantress are providing valuable knowledge and technical support through two Tyson Foods Fellows. *Experimental design*: Using an innovative private-sector-based train-the-trainer model, the project aims to train 750 smallholder farmers in efficient broiler production. Customized for the Rwandan context, the project utilizes a 100-bird (modern genetics) model. The hypothesized benefits of the model are being tested over the course of three years. *Results*: After one year of grow-outs, flock data shows an average liability of 91%, an average feed conversion rate of 2.32 (without pelleted feed and the utilization of charcoal brooders) for a 2.62 Kg bird, and the majority of growers doubling their original income. *Conclusion*: Growers in Rwanda have the potential to be successful through this model; however, collaborators are addressing the outlying challenges within the Rwandan poultry value chain, such as securing a reliable and quality chick source and developing post-harvest marketing strategies. Lessons learned from the rollout of this pilot project will be used to inform scale-up of smallholder broiler production across Rwanda and the region.

Key Words: Rwanda, smallholder, international, broiler, private-sector

P198 Correlation between flock manager personality assessment and flock productivity John Brunnquell*[1GS], Anthony Pescatore[2], Sarah Davidson[1], Joe Russell[1] *[1]EGG INNOVATIONS LLC; [2]University of Kentucky*

With small flocks, manager quality has little impact (Zimmerman et al, 2006). As responsibility and flock size grows, personality attributes of a flock manager have also shown bias in managerial success, due to the impact of human-animal interactions (Hemsworth et al. 2011). Poultry science can be taught but having the proper personality to execute is equally important. This research objective was to define certain personality trait(s) of flock managers that increase or decrease the manager's ability to elicit optimal performance from a flock of hens. Organization Analysis and Design has developed a personality profile assessment that meets international standards for providing validity and reliability ("Job Validity Studies – Organization Analysis and Design", 2018). The measurement of success used is delta from standard Hen Housed production at 70 weeks, comparing actual flock performance to projected flock performance. Approximately 40 growers with similar free-range layer barns were ranked according to this standard and had profile testing conducted. Correlations were drawn between personality traits and flock productivity. Statistically, histograms were used for normality and the remaining data was submitted to linear regression. Variables of mortality, feed conversion, and feed consumed were analyzed with linear regression. They explained 67.76% of the variation of delta to standard. Linear regression using personality variables alone was able to explain 41.01% of the variation of delta to standard. The relationship between the delta from standard production and the versatility level (VL), emotional control (EC) and detail minus assertiveness (D- A) contributed the newest information linear regression model and there is statistical evidence that the X variables contribute to the Y response; P=.003. The final regression model including feed consumed (X1), feed conversion(X2), mortality(X3), EC(X4) and D-A(X5) was able to explain 81.85% of the variation in the performance delta. The final model equation is:

Delta = 26.7 + 0.659 X1 - 24.10 X2 + 4.21 X3 + 0.843 X4 - 5.56 X5 - 0.585 X3*X4 + 0.666 X4*X5

Personality traits of flock managers have direct impact on flock productivity. Personality Profile testing may have potential for screening new flock managers

Key Words: Personality Profile, Emotional control, assertiveness, Flock performance

P199 The Animal Food and Nutrition Consortium Established to Fund Pre-competitive Research, Outreach, and Career Development Adam Redhead*, Peter Ferket *North Carolina State University*

The Animal Food & Nutrition Consortium (AFNC) is a new industry-member partnership with the mission to promote innovation and advances in the nutrition and feed technology of production and companion animals. The research focus is on emerging issues, driven by industry needs and an entrepreneurial mindset.The AFNC brings industry partners together with an interdisciplinary team of over 200 food animal faculty distributed across at least three NC State University colleges (College of Agriculture and Life Sciences, the College of Veterinary Medicine, and the College of Sciences). The consortium sponsors pre-competitive research and solutions that encompass the food and nutrition of production and companion animals including, but not limited to: the potential value of feed ingredients and additives; digestive physiology; gut health and the enteric microbiome; impact on environmental sustainability and climate change, efficiency of nutrient utilization and minimization of environmental emissions; animal food quality and safety; integration of nutrition and animal welfare and well-being; big data analytics; and other emerging issues relevant to livestock, aquaculture and companion animals. Benefits accrue to all AFNC partners in the form of resources for faculty research, leveraged research funding for industry, and developing the next generation of industry employees and policy makers. Ultimately, the consumer wins too, as research results are expected to improve the value and quality of food

animal products and companion animal foods, improve environmental sustainability by lowering nutrient emissions, and enhance animal health and welfare. The AFNC provides defined engagement rules for members from competing companies with an opportunity to leverage their investments through one of 3 membership fee tiers: $50,000 for Full Membership with the privilege of 2 votes at Advisory Board meetings, $25,000 for Associate Membership with one vote, and $10,000 for Non-profit Membership (no voting rights). Any intellectual property generated from the consortium will be available for non-exclusive license to Full or Associate Members in good standing. The AFNC will promote member interests through precompetitive research, workshops, symposia, and scholarships.

Key Words: Industry Consortium, funding model, industry-academic collaboration, research, career development

Physiology, Endocrinology and Reproduction

P200 Effect of cold water on the GH, IGF-1, IL-2 and immune responses of laying hens during high ambient temperature in summer Jong Hoon Kim, Chun Ik Lim, Muhamad Masud Rana, Kyeong Seon Ryu, Chang Won Kang* *Chonbuk National University*

The objective of the present study was to examine the effect of provision of drinking water on IL-2, IGF-1 GH and immune responses of laying hens during extreme heat exposure in summer period. Thirty 50-week-old brown type laying hens (Hy-Line) were divided in three groups of 10 hens (one hen/cage). The treatment consisted of a control (ordinary tap water) when thermo-neutral temperature in room was maintained approx. 25°C and other two treatments of: 25°C (warm water, WW) and 15°C (cold water, CW) where hens subjected to heat stress (HS) during the hot summer average at 35°C with 60% relative humidity. Peripheral blood mononuclear cells (PBMC) were isolated from the blood of each group at the commencement of experiment and thereafter 15th and 30th days were performed. CD3, CD4 CD8 and dls BU-la were analyzed by flow cytometry. The immune-cytokines mRNA levels of IL-2 and IFN-gamma of isolated PBMC were determined by RT-PCR. The plasma concentration of IL-2, IGF-1, IFN-gamma, GH and IgG were analyzed using ELISA kit. Results showed that the distribution of B-lymphocyte Bu-la began to decrease gradually after 15 d and 30 d of exposure to high temperature in HS + WW group compared to the control but in case of HS+CW this decrease was restored to the control. In addition, similar change was noticed in CD4, a surface factor of T-lymphocytes. CD8, a surface factor of cytotoxic T cells, was not changed among the treatments at 0 d, 15 d and 30 d. The IL-2 and IFN-gamma mRNA expression were altered significantly among the treatments at 15 d and 30 d but persistent compared to control and HS+CW treatment group. The concentration of plasma IL-2, IFN-gamma and IgG linearly decreased of HS + WW group at the 30 d of experiment and also these expressions restored in case of HS+CW group with control group. In case of heat stress observation, GH and IGF-1 concentration in plasma was significantly lower in HS + WW treatment at the 30 d of experiment but no statistical difference was observed between control and HS+CW. Therefore, the effect of cold water on cellular and humoral immunity were influenced when hens were maintained at a prolonged high environmental temperature.

Key Words: cold water, high temperature, growth hormone, cellular immunity, laying hens

P201 Investigation of the relationship between humoral and egg yolk antibodies in broiler breeder hens. Talaat Al-Alwani*[GS], John Carey *Texas A&M University*

The role of maternally derived antibodies is important to the health of newly hatched chicks. Common vaccination programs attempt to increase the titer of these antibodies in fertile eggs by robust immunization programs for breeder hens. While it is intuitive that there would be a strong relationship between broiler breeder hen humoral titers and egg yolk antibodies, there are no recent data to evaluate the nature of this relationship. The objective of this study was to evaluate the relationship between broiler breeder hen humoral and egg yolk antibody titers. Fifteen mid- production (40-50 weeks of age) broiler breeder hens were acquired from a commercial poultry company and housed in individual laying hen cages. These hens had previously been subjected to a commercial vaccination schedule in accordance with the health program of the commercial poultry company. This program included multiple vaccinations for Newcastle disease. Hens were fed the TAMU Poultry Research Center standard laying hen diet. Blood samples were collected from the wing vein or jugular vein of each hen seven times (weekly) throughout the experiment. Blood samples were collected in EDTA tubes and sera were separated by centrifugation. Eggs were sampled from the hens on the day following blood collection. Yolk antibodies for Newcastle disease were purified. Titers to Newcastle disease (NDV) in blood sample and eggs yolk samples were evaluated by ELISA test. Feed consumption and body weight were monitored throughout the experiment. Egg yolk NDV- specific antibody titers were significantly higher than serum antibodies. The correlation coefficient between sera and yolk titers was highly significant with an R^2 over 0.5. In conclusion, this data demonstrates a strong relationship between humoral and egg antibody titers for NDV in broiler breeder hens.

Key Words: Broiler Breeder, Egg Yolk Antibodies, Newcastle Disease, Serum Antibodies

P202 Delayed access to feed impairs amino acid and carbohydrate utilization in newly hatch broiler chicks 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, NEA*

Post-hatch delays in access to feed occur within the broiler industry and can negatively impact growth, development, and feed efficiency. Despite the importance of sugars and amino acids in these processes, effects of delayed access to feed on their utilization in metabolically important tissues remains unexplored. This study sought to characterize effects of delayed access to feed on expression of genes associated with sugar and amino acid uptake and metabolism in liver and muscle. Broilers were given access to feed within 3h of hatch (FF) or delayed access to feed for 48h (DF). Birds were weighed through day (D) 14 post-hatch, with muscle and liver samples collected from 4 males at 4h, D1, D2, D4, and D8 for RT-qPCR analysis. Data were analyzed using two-way ANOVA, and *ad hoc* means comparisons were conducted using the test of least significant difference, with differences considered significant at $p \leq 0.05$. Birds in the DF group weighed less than FF birds from D8 onwards. In FF birds, carbohydrate response element binding protein (*CHREBP*) mRNA decreased after hatch in muscle and transiently decreased on D2 and D4 in liver. In contrast, *CHREBP* mRNA remained elevated on D2 in both tissues and did not increase on D8 in liver in DF birds, indicative of a transitional delay from lipid to carbohydrate metabolism in these birds. Insulin receptor (*INSR*) mRNA in the liver of FF birds was down-regulated starting on D2 but remained elevated in DF birds until D4, and a similar pattern was observed in muscle. Glucose transporter 12 mRNA began to increase on D2 in FF muscle, and this was delayed until D4 in DF birds. In muscle of FF birds, cationic amino acid transporter (*CAT*) 1 mRNA was transiently elevated on D2 and large neutral amino acid transporter 1 tended to be elevated on D1 and D2 as compared to DF birds (p=0.06). Levels of *CAT1* mRNA in liver of DF birds remained elevated above FF birds on D1 but were significantly lower on D2, while DF *CAT2* expression tended to be higher on D2 (p=0.08). These data suggest that delayed access to feed al-

ters transport and metabolism of amino acids and sugars in muscle, and, to a lesser extent, liver. This is likely an underlying cause for delayed growth and development of broiler chicks not receiving feed immediately upon hatching.

Key Words: delayed feeding, amino acid, carbohydrate, mRNA

P203 Consequences of genetic selection on metabolic regulation of skeletal health in broiler chickens. Laura Ellestad*, Jason Payne, Brett Marshall, Grace Boothby *University of Georgia*

Improvements in broiler growth performance resulting from commercial selection are associated with poor skeletal health. Vitamin D_3 plays an important role in Ca and P homeostasis and is essential for bone strength. Several studies have demonstrated that feeding biologically active metabolites of D_3 [$25(OH)D_3$ and $1,25(OH)_2D_3$] can improve bone health. There remains a lack of data regarding conversion of dietary D_3 into these active forms and subsequent regulation of Ca and P metabolism in broilers; therefore, impacts of genetic selection on these processes were determined in this study. On D21 post-hatch, liver, kidney, and jejunum were collected from Cobb 500 broilers and Athens Canadian Random Bred (ACRB) birds that are representative of broilers from the mid-1950s (n=8). Levels of mRNA for enzymes involved in conversion of D_3, hormone receptors associated with Ca and P homeostasis, and transporters of Ca and P were measured by RT-qPCR. Levels of circulating D_3 metabolites were determined using mass spectrometry. Data were analyzed with a student's T-test and considered significant at $P \leq 0.05$. Levels of mRNA for *CYP2R1*, the enzyme responsible for initial activation of D_3 to $25(OH)D_3$, were higher in the ACRB liver, while mRNA levels of *CYP24A1*, the enzyme responsible for deactivating $1,25(OH)_2D_3$ in the kidney were higher in Cobb. Circulating levels of these metabolites were consistent with this differential mRNA expression. Levels of mRNA for *RXRG*, a transcriptional partner of vitamin D receptor, were higher in the liver and jejunum of ACRB, and expression of mRNA for calcitonin and parathyroid hormone receptors were higher in all tissues in ACRB. Jejunum mRNA levels for calbindin-28, a Ca-binding protein, and the P transporter *NaPiIIb* were higher in Cobb. Modern broilers have a reduced ability to activate dietary D_3 and increased de-activation of its hormonally active form. Parathyroid hormone sensitivity also appears reduced in the liver and intestine of modern birds. Results indicate that altered D_3 metabolism and related hormonal signaling have been compromised by genetic selection and could contribute to skeletal weakness observed in modern broilers.

Key Words: vitamin D3, calcium, phosphorus, bone health, hormone

P204 Enteric proteomic endocrine pathway changes to day of hatch chicks after in ovo bacterial inoculation. Michael Trombetta*[1UG], Kim Wilson[1], Denise Rodrigues[1], Whitney Briggs[1], Kaylin Chasser[1], Audrey Duff[1], Liwen Zhang[1], Walter Bottje[2], Lisa Bielke[1] *[1]The Ohio State University; [2]University of Arkansas*

Microbial populations within the gastrointestinal tract (GIT) have the potential to alter physiological performance of a variety of systems within poultry. From a production standpoint, the ability to manipulate this population can provide an invaluable way to influence health and performance of poultry. The objective of this study was to compare the effects of *in ovo* inoculation with a commercially available LAB-probiotic (L) and two different Gram-negative strains for their influence on endocrinological profile in day of hatch (DOH) chicks. At d18 of embryogenesis, eggs were inoculated with sterile saline (S), 10^2 CFU of one of two species of *Citrobacter* (C1 or C2), or L. Eggs were hatched by treatment and, on DOH, the GIT, duodenum to rectum, was collected from 10 chicks. Extracted protein was subjected to separation by fusion mass spectrometry and identified via Sequest with a *Gallus gallus* reference in Uniprot. Data was then entered into Ingenuity Pathway Analysis (IPA) and analyzed for differences. All experimental treatments were compared to S in IPA to determine effects on canonical pathway activation and inhibition at $p<0.05$, with a minimum z-score of +/-1.5. The C1 group showed inhibition of pathways related to insulin and cholesterol regulation and production including HNF1A (z-score -1.969) and HNF4A (z-score -2.000). This is in contrast to C2 which expressed opposite results of these pathways, with activation of insulin and cholesterol regulation (ex: HNF1A z-score +1.982), plus activation of pathways and receptors for sex hormones (Progesterone receptor z-score +1.982) and the precursor to the hormones related to angiogenesis, angiotensinogen (z-score +1.898), among other endocrine-related proteins. The L group showed activation of sex and steroid hormone pathways including corticosterone (z-score +1.949) and steroid hormone receptor ERR1 (z-score +1.948) as well as Insulin-1 (z-score +1.49), and inhibition of calcitriol (z-score -1.709). These changes suggest that neonatal colonization of poultry can affect significant physiological pathways, especially those related to sex and steroid hormones, insulin, and other glucose-regulating pathways, and emphasize the role of pioneer colonizing bacteria in growth and development of perinatal poultry.

Key Words: Endocrinology, proteome, pioneer colonizers, gastrointestinal

P205 Influence of enteric pioneer colonizers on canonical metabolic pathways in day of hatch chicks Kaylin Chasser*[1GS], Kimberly Wilson[1], Denise Russi-Rodrigues[1], Walter Bottje[2], Audrey Duff[1], Whitney Briggs[1], Lisa Bielke[1] *[1]Ohio State University; [2]University of Arkansas*

Pioneer colonizing bacteria are recognized to impact gastrointestinal tract (GIT) colonization, with potential to influence tissue well beyond the gut. The objective of this study was to introduce apathogenic Gram negative chicken isolates or lactic acid bacteria (LAB) via *in ovo* inoculation to measure effects on enteric canonical metabolic pathways, by proteomics analysis, in day of hatch (DOH) chicks. On d18 of embryogenesis, embryos received either 200µL saline (S) or $1x10^2$ CFU of *Citrobacter freundii* (C1), *Citrobacter sp.* (C2) or LAB (L) into the amnion. At DOH, GIT from duodenum to ceca was collected (n=10/treatment). Protein was extracted and subjected to fusion mass spectrometry, then identified in Sequest, with *Gallus gallus* reference in Uniprot, then uploaded to Ingenuity Pathway Analysis (IPA) software for Canonical pathway evaluation of effects on metabolism. Pathway activation and inhibition was considered significant at $p<0.05$. Treatment L showed increased activation ($p<0.05$) of TCA cycle, NAD salvage, tRNA charging, thiosulfate disproportionation, and aspartate degradation. In contrast, C1 resulted in inhibition ($p<0.05$) of oxidative phosphorylation, tetrahydrofolate salvage, and purine nucleotides *de novo* biosynthesis. Treatment C2 showed intermediate changes, with activation ($p<0.05$) of sorbitol degradation, and citrulline-nitric oxide, urea, and γ-glutamyl cycle and inhibition ($p<0.05$) occurred in adenosine nucleotide, histamine, and purine nucleotide degradation pathways. Nearly all metabolic canonical pathways improved protein and energy synthesis with activation, and depressed synthesis with inhibition. Introduction of LAB resulted in improved metabolic expression with unique proteins on DOH compared to C1 and C2. Pioneer colonizers have been shown to influence poultry health beyond microbial population changes, with influences on protein expression. Further studies on downstream protein metabolism due to pioneer colonizers may enable enhanced poultry production, health, and welfare.

Key Words: lactic acid bacteria, metabolism, proteomics, pioneer colonizer

P206 The effect of genetic selection on gene expression in the developing somatotropic axis of broilers Lauren Vaccaro*[1], Panpradap Sinpru[2], Kristen Brady[3], Yupaporn Chaiseha[2], Tom Porter[3], Laura Ellestad[1] *[1]University of Georgia; [2]Suranaree University of Technology; [3]University of Maryland*

Improved growth and feed efficiency in modern broilers are the result of genetic selection, but the biological basis behind such performance is not fully understood. The objective of this study was to evaluate effects of

commercial selection on gene expression in the somatotropic axis during embryogenesis. Gene expression in a commercial line (Ross 708) was compared to an unselected legacy line, the Athens Canadian Random Bred (ACRB). Hypothalami, anterior pituitary glands, and liver were collected from 12 embryos of each line on embryonic days (e) 10, 12, 14, 16, and 18, and weights recorded. Sex was confirmed using PCR molecular sexing, and total RNA was extracted four male and four female embryos for each tissue (n=4). Expression levels of hormones and their receptors regulating growth were measured using real-time quantitative PCR and normalized to GAPDH (hypothalamus, liver) or PGK1 (pituitary). Data were analyzed for significant effects of line or line-by-age interaction by sex using a two-way ANOVA followed by a test of least significant difference. In males, Ross embryos were heavier than ACRB on e14-e18 ($P<0.01$), while in females this was not apparent until e18 ($P<0.01$). In the hypothalamus, growth hormone releasing hormone (GHRH) mRNA was higher in Ross females on e12 and higher in ACRB females on e18 ($P<0.05$), and somatostatin mRNA was higher in ACRB in both sexes ($P<0.05$). There was no significant difference in expression of pituitary growth hormone (GH), but levels of GHRH receptor were higher in Ross embryos regardless of sex ($P<0.01$). In females, GH secretagogue receptor was higher in ACRB at earlier ages (e12-e14) and Ross at later ages (e16-e18), and pituitary adenylate cyclase-activating polypeptide receptor was higher in Ross embryos at later ages (e16-e18; $P<0.05$). In female embryos, liver insulin-like growth factor 1 mRNA was higher in Ross ($P<0.05$), and they also tended to have higher levels of thyroid hormone receptor beta ($P=0.0665$). In male embryos, GH receptor mRNA appeared higher in ACRB than Ross ($P=0.0718$). These results suggest that gene expression of the GH axis during development have been altered due to commercial selection, and these may contribute to the improved performance seen in modern broilers.

Key Words: growth, feed efficiency, genetic selection, gene expression

P207 Structural differences in the F1 preovulatory follicle between low and high egg producing turkey hens Kristen Brady*[1], Julie Long[2], Tom Porter[1] *[1]University of Maryland; [2]USDA ARS Beltsville Agricultural Research Center*

Within the turkey industry, low egg producing hens (LEPH) and high egg producing hens (HEPH) exist in commercial breeding flocks, with LEPH costing the industry through lost poult production. Previous studies have shown that LEPH displayed lower expression of genes related to progesterone production as well as decreased progesterone production in response to *in vitro*gonadotropin treatment. Within the ovary, progesterone production occurs mainly in the granulosa layer of the largest preovulatory follicle (F1 follicle). The preovulatory follicles are the most mature class of follicle in the ovary and are comprised of an ovum, yolk, and a follicle wall consisting of three cell types. The innermost layer of the follicle wall is the granulosa layer, which is responsible for progesterone production. The outer layer of the follicle wall is the thecal layer, further designated into theca interna and theca externa layers, responsible for androgen and estrogen production, respectively. As a follicle progresses through the preovulatory hierarchy, the follicle wall thickness decreases. Histological sections of the F1 follicle wall were compared in LEPH and HEPH (n=3) to examine structural differences related to steroid production capacity. The F1 follicle was formalin fixed to excise the follicle wall and the sections were hematoxylin- and eosin-stained for examination by light microscopy. Thicknesses of individual follicle wall layers and of the total follicle wall were calculated using ImageJ software and were compared using the mixed models procedure of SAS. LEPH displayed a thinner granulosa layer but a thicker thecal layer, when compared to HEPH ($p<0.05$). The thickness of the total follicle wall was also higher in LEPH in comparison to HEPH ($p<0.05$). Structurally, the follicle wall of F1 preovulatory follicle differs significantly in the thickness of both the granulosa and thecal layers as well as in total follicle wall thickness between LEPH and HEPH, with HEPH displaying follicle wall characteristics consistent with a follicle of higher maturity. Further studies will be necessary to tie structural differences to steroid production capacity and egg production rates.

Key Words: turkey, egg production, granulosa layer, thecal layer, steroid production

Processing and Products

P208 Different combinations of enrichment and plating media for detection of naturally occurring Campylobacter from retail chicken livers Tori Thompson*[UG], Nelson Cox, Mark Berrang, Arthur Hinton, Hung-Yueh Yeh, Jodie Plumblee Lawrence, Susan Brooks *U.S. National Poultry Research Center*

Foodborne campylobacteriosis has been traced to undercooked chicken liver. The objective of this study was to compare the effectiveness of two liquid media: Bolton (B) and Neogen (N) formulation and three solid plating media: RF *Campylobacter* agar (RF), Campy Cefex agar (CCA) and Campy Line agar (CLA) for recovery of *Campylobacter* from chicken liver. Twenty-seven replicate plastic tubs of liver were purchased at retail outlets. From each tub, three samples of exudate and five intact liver lobes were collected. Exudate samples (0.1 mL) were direct streaked onto 3 separate plates (RF, CCA and CLA). An additional 1 mL of exudate was transferred into 9 mL each type of enrichment broth. Each lobe was rinsed in 100 mL of saline for 60 s; 10 mL of rinsate was transferred into 90 mL of B and another 10 mL into 90 mL of N. All plates and tubes were incubated at 42°C for 48 h in re-sealable bags flushed with microaerophilic gas. After incubation, enriched samples were streaked on RF, CCA and CLA plates and were similarly incubated. Following incubation, 3 typical colonies were selected from each plate and confirmed to be *Campylobacter* by observation of cellular morphology and motility under phase contrast microscopy. There were a total of 117 direct plated samples for each plating medium. *Campylobacter* recovery was compared using Chi Square test for independence. *Campylobacter* was detected from 30.8%, 29.1% and 28.2% of CLA, RF and CCA, respectively, for an overall 29.3 % positive samples. For enriched exudate samples, media combination did not have a significant effect on *Campylobacter* recovery ($P>0.05$). Recovery rate ranged from 77.8% (49/63) for the combination of B to RF to 65.1% (41/63) for N to CLA; overall recovery for enriched weep was 73%. Likewise, the combination of media used for enriched rinse did not significantly affect recovery which ranged from N to RF had the highest recovery rate at 76.2% (48/63) for N to RF to 63.5% (40/63) for B to CLA. Neither did plating medium have a significant effect on recovery from directly plated rinses which ranged from 22.2% (12/54) for CCA to 14.8% (8/54) for CLA. More than 50% of chicken livers purchased at retail are *Campylobacter* positive and choice of recovery media did not influence detection.

Key Words: Campylobacter, media comparison, chicken liver, neogen broth

P209 Mild heat treatment of chicken livers to lessen Campylobacter contamination Hannah Huff*[UG], Mark Berrang, Nelson Cox, Richard Meinersmann, Hong Zhuang, Brian Bowker *USDA-AgriculturalResearch Service-U.S. National Poutlry Research Center*

Undercooked chicken liver and chicken liver paté has been linked to foodborne campylobacteriosis. In previous studies, we have detected *Campy-*

lobacter from retail chicken livers with high frequency. Therefore, a prepackaging treatment to minimize *Campylobacter* on chicken livers may be helpful to lessen risk to consumers. The objective of this study was to test a mild heat treatment to decrease the numbers of *Campylobacter* on the surface and inner tissues of chicken livers. Eighteen replicate tubs of chicken livers, each representing a unique combination of plant number and sell by date, were purchased at retail. Three whole liver lobes (18-25 g each) were selected from each tub, placed in separate sterile plastic bags and subjected to 0, 1 or 5 min immersion in a 60°C circulating water bath. After treatment, liver lobes were covered with 60 mL of 4°C *Campylobacter* enrichment broth and placed on ice. All liver lobes were rinsed by hand for 60 s; 10 mL of liver rinsate was removed for analysis. The liver lobe and remaining broth were then macerated for 30 seconds in a paddle blender. Total aerobic bacteria and *Campylobacter* were enumerated from the liver rinse and whole blended liver by plating on plate count agar (PCA) and Campy-Cefex (CC) agar respectively. Bacterial numbers were $\log_{10}$ transformed and compared by Students T test. One minute treatment at 60°C did not cause a significant decrease ($P>0.05$) in either total aerobic bacterial or *Campylobacter* numbers from rinses or stomached liver. However, livers subjected to 5 min at 60°C (internal temperature reaching a high of 55°C) had 1.4 - 1.5 log fewer CFU *Campylobacter* and 1.1 - 1.7 log fewer CFU total aerobic bacteria per liver lobe than did untreated livers ($P<0.05$). These data suggest that a mild heat treatment prior to packaging could lessen consumer exposure to *Campylobacter*.

Key Words: Campylobacter, chicken liver, heat treatment

P210 Segmental bioelectrical impedance analysis of broiler breast fillets to detect the areas of the fillets affected with woody breast myopathy Courtney Keeble*[UG], Avery Smith, Laura Garner, Brock Herron, Nickie Johnson, Olivia Brahms, Amit Morey *Auburn University*

Poultry processors are using a subjective hand-palpation to sort out breast fillets affected with woody or wooden breast myopathy (WB). Previously, we demonstrated that bioelectrical impedance analysis (BIA) can be used as an objective method to detect normal and severe WB meat but not between the mild and moderate WB severity levels. Further research was conducted to determine if segmental BIA can detect differences between normal, mild, moderate and severe WB fillets.

Freshly deboned broiler breast fillets (8-9 lbs live wt.) were procured from a local poultry processor. The fillets were subjected to hand-palpation to categorize them in to normal, mild, moderate and severe WB categories. Each fillet was divided into three segments (cranial, medial and caudal) and further subjected to hand-palpation to determine if it is woody or normal. The segments were then analyzed using BIA attached to two pairs of spring-loaded compression electrodes (Seafood Analytics, MI) to collect the data on resistance and reactance (Ohms). Each segment was ground and subjected to moisture analysis using hot air oven and freeze dryer. Data was collected on 4 WB severity fillets x 3 segments x 3 fillets per trial x 4 trials. Data was analyzed using ANOVA with significant differences between each severity level at $p<0.05$.

Results indicated that there is an overall significant difference ($p<0.05$) between the reactance values of the fillets at various severity levels, however no differences were found in the resistance readings. Differences in reactance between segments of individual breast fillets indicate presence of WB myopathy in those fillets. Segments without WB had a higher reactance (>40 ohms) compared to the ones with WB (<40 ohms).

Segmental BIA can be used to further classify WB fillets as per the severity levels. Moreover, detection of segments with WB can help processors to trim out the segment without downgrading the entire fillet.

Key Words: woody breast, meat quality, bioelectrical impedance

P211 Effects of functional ice (FICE) made with common poultry processing aids to actively eliminate spoilage microorganisms during storage Olivia Brahms*[UG], Jasmine Kataria, Avery Smith, Laura Garner, C. Herron, Ana-Cecilia Rush, Courtney Clay, Courtney Keeble, Elizabeth Johnson, M. Black, Amit Morey *Auburn University*

Raw poultry is generally stored on ice during storage and transportation, however, ice does not actively eliminate the microorganisms. Functional Ice or "FICE" was developed by freezing solutions of commonly used poultry processing chemicals and ingredients which have antimicrobial properties. Research was conducted to study the effects of FICE on the spoilage microorganisms of raw chicken meat during refrigerated storage for 3 days.

Freshly deboned, raw chicken thigh meat was procured from a local poultry processor. Aqueous solutions of 0.5% acetic acid and 0.5% acetic acid-1% sodium tripolyphospate (STP) were frozen to make FICE and potable water was frozen to make control ice. Chicken thighs (n=168) were divided between three FICE treatments, control, 0.5% acetic acid, and 0.5% acetic acid-1% STP (FICE:Meat:: 2:1). Microbiological sampling (n=5 thighs/treatment/day/trial) was conducted on day 0, 1, 2 and 3. Individual thigh was aseptically collected in a whirl-pak bag and rinsed for 1 min with phosphate buffered saline (0.1%), each rinsate was serially diluted, spread plated on de Man, Rogosa and Sharpe agar (MRS) and two sets of standard methods agar (SMA) plates. MRS plates were placed in anaerobic chambers in the 37°C incubator for 48 h to measure the growth of *Lactobacillus* spp. Each set of SMA plates was incubated at 37°C for 24-48 h to measure the growth of aerobic bacteria and at 4°C refrigerator for 7-10 d to measure the growth of psychrotrophs, respectively. Viable colonies were counted and reported as log CFU/mL of rinsate. Data was analyzed using ANOVA with Tukey's HSD ($p<0.05$) to determine the significant differences between the treatments.

At the end of 3-day storage, FICE treatments, 0.5% acetic acid and 0.5% acetic acid+1% phosphate had lower aerobic plate count (1.45 and 1.70 log CFU/mL of rinsate, respectively) compared to the control (2.78 log CFU/mL of rinsate). FICE did not have a significant effect on lactobacilli counts while psychrotrophic plate counts were below the detection limit during the 3-day storage.

FICE can offer an innovative solution to actively reduce spoilage microorganisms in raw poultry, however more studies are warranted.

Key Words: functional ice, storage, transportation, food safety, poultry

P212 Quality effects of Functional Ice (FICE) manufactured with acetic acid and phosphate on raw chicken thighs during a 3-day storage period Ana-Cecilia Rush*[UG], Olivia Brahms, Jasmine Kataria, Laura Garner, Avery Smith, C. Herron, Courtney Clay, Courtney Keeble, Elizabeth Johnson, M. Black, Amit Morey *Auburn University*

Meat quality is of prime importance to consumers and can be affected due to several factors such as processing parameters, antimicrobial applications, and storage conditions. Antimicrobial treatment of raw poultry can eliminate microorganisms thus improving food safety and shelf-life. However this can affect meat quality attributes such as color and water holding capacity. Hence any antimicrobial treatment must be evaluated for its impact on meat quality. We have developed a novel antimicrobial treatment termed "Functional Ice" or FICE made with acetic acid and phosphate which reduces spoilage microorganisms during storage. A study was conducted to determine the effects of FICE on quality attributes of raw chicken thigh meat during a 3-day storage period.

Aqueous solutions of 0.5% acetic acid and 0.5% acetic acid-1% sodium tripolyphosphate (STPP) were frozen to make functional ice (FICE) while tap water was frozen to make control ice. Chicken thighs (n=168) were equally divided into separate plastic coolers (3 coolers/treatment x 3 sampling days) and mixed with FICE or control treatments (FICE:Meat:: 2:1). The batch weight of chicken and ice in each cooler was recorded. The

coolers were stored in a walk-in refrigerator maintained at 4˚C for 3 days. On each sampling day, one cooler per treatment was selected and five thighs from each cooler were used for quality analysis. The temperature and batch weight of the thighs and ice after draining melted water was recorded. All the samples were analyzed for color (Minolta colorimeter), total moisture (drying oven method) and expressible moisture (5 kg for 5 min). Data was analyzed using ANOVA with Tukey's HSD at $p<0.05$ to detect significant differences among the treatments.

The 0.5% acetic acid FICE melted slower compared to the control ice throughout the 3-day storage. Expressible moisture of the control treatment (41.32±3.95) was significantly higher than FICE treatment (0.5% acetic acid + 1% phosphate) (37.40±1.55). FICE did not have any effect ($p>0.05$) on meat color (L*a*b*).

FICE manufactured with acetic acid and phosphate can influence quality of raw chicken thighs during storage.

Key Words: functional ice, storage, shelf-life, poultry, quality

P213 Characterization of Salmonella inhibition by ε-polylysine using growth curve analysis Laney Froebel*[UG], Tri Duong *Department of Poultry Science, Texas A&M University*

ε-Polylysine is a naturally occurring homo-polymer of L-lysine widely used as a food additive. It has been reported to have antimicrobial activity against a wide range of microorganisms including fungi, yeasts, Gram-positive and Gram-negative bacteria, and bacteriophages. *Salmonella* is an important human foodborne pathogen commonly associated with poultry and poultry products. Analysis of microbial growth using a microplate reader is less labor intensive than traditional methods and allows rapid high-throughput characterization of growth under a wide range of conditions. The objective of this study was to develop a high throughput assay for characterizing the inhibition of microorganisms by ε-polylysine. Growth of *Salmonella* was evaluated in various culture media and over a range of ε-polylysine concentrations. *Salmonella* was cultured at 37°C in an incubated microtiter plate reader, and growth was monitored using absorbance ($O.D._{600}$). Growth curves were prepared from absorbance data and fit to a modified Gompertz equation for determination of growth characteristics including growth asymptote, maximum growth rate, and lag time. ε-Polylysine inhibition of *Salmonella* was determined to be dose dependent. The results of this study were used to define an initial set of standard experimental conditions for use in future assays. Future studies will evaluate ε-polylysine inhibition of microorganisms important in the poultry production chain including environmental contaminants and human foodborne and poultry pathogens.

Key Words: ε-polylysine, antimicrobial, Salmonella, bacterial growth

P214 Hardness distribution within raw broiler fillets with woody breast condition during short-term storage Xiao Sun*[1], Casey Owens[2] *[1]Chuzhou University; [2]University of Arkansas*

Woody breast (WB) continues to be a problematic defect in the broiler industry. Fillets can be evaluated by human palpation. Compression force (CF) has been used as an instrumental method of evaluating fillet hardness and high correlations ($r_s=0.79$) between CF and WB score have been reported. Normally, multiple CF are recorded and averaged per fillet. The objective of this study was to determine the hardness distribution of raw broiler fillets with different WB characteristics. A total of 180 breast fillets were collected from commercial processing line and classified into normal (NORM), mild (MILD) and severe (SEV) categories (n=60/category). Fillets were subjected to a compression test on processing day (d 0) where all breast fillets were compressed to 20% of the fillet height 3 times in cranial region using a 6mm flat probe on a Texture Analyzer (Texture Technologies, Inc.). Fillets were aged in a 4°C cooler until d 8 and then CF was measured again on each fillet. The locations of compression testing were located in the cranial region in a shape of a triangle consisting of a top point (CF2) and two base points (CF1, CF3). Height at each point (H1, H2 and H3) was also recorded. CF and H increased as categorizes of WB increased ($P<0.05$, NORM<MILD<SEV), and decreased from d 0 to d 8 storage (d 0>d 8, $P<0.05$). CF2 was higher than CF1 and CF3 which were similar ($P<0.05$), while H1 was higher than H2 and H3 which were similar ($P<0.05$). There was an interaction between woody category and CF measurement ($P<0.05$). CF2 was higher than CF1 and CF3 in SEV which were higher than all measurements in MILD ($P<0.05$). CF2 was higher than CF1 and CF3 in MILD which were higher than all measurements in NORM ($P<0.05$), while no difference among measurements were observed in NORM. Variation among measurement points increased as WB severity increased though categories were different ($P<0.05$) within each point. Compressing fillets at multiple points is recommended to obtain compression force that is representative of the fillet. Further optimization is needed to determine the range of CF associated with each category using multiple or single point compression using the 6 mm probe.

Key Words: woody breast, broiler, compression force, hardness, fillet height

P215 Dietary Supplementation of MSP™ on the incidence, gene expression and physiochemical parameters of breast muscle myopathies Rebecca Delles*[1], Katie Eastridge[1], Rijin Xiao[1], Donavyn Coffey[1], Mike Ford[2], Karl Dawson[1], Ronan Power[1], Anthony Pescatore[2], Tuoying Ao[1] *[1]Alltech, Inc; [2]University of Kentucky*

White striping (WS) and wooden breast (WB) meat are detrimental myopathies that affect the appearance and physicochemical quality of the *pectoralis major*. The objective of this study was to investigate the occurrence and severity of breast muscle myopathies and the gene expression and physiochemical attributes of breast muscle from birds fed MSP™, a new proprietary formulation containing plant extracts and antioxidants (Alltech Inc., KY) in the grower (d19-30) and finisher (d31-49) phases. One-day old Cobb 500 male broiler chicks were randomly placed in 20 pens with 24 chicks per pen. All chicks were placed on a corn-soy starter diet for the first 18 days, and at day 19 pens were randomly assigned to either one of two dietary treatments, a corn-soy control diet or control diet supplemented with 1 kg/MT MSP™. On day 49, 2 birds per pen were processed to evaluate occurrence and severity of WB myopathy in the *pectoralis major*, along with lipid oxidation (TBARS) and cooking loss. On day 51, 6 birds per treatment were randomly sampled for gene expression assay. Meat quality data were subjected to least square differences (LSD) to determine the significance of dietary treatments. Differentially regulated genes were subjected to functional and association analysis. Under the conditions of this study dietary supplementation with MSP™ reduced the severity of WS and WB by 15 and 22%, respectively, compared with control. However, lipid oxidation as measured by (TBARS, mg MDA/ kg meat) did not differ between dietary treatments. Cooking loss was lower ($P=0.0631$) in breast meat from birds fed MSP™ compared to birds fed control diet. Microarray results of the MSP™ treatment group revealed an increased expression of AMOT and CD44 ($P=0.0332$ and $P=0.0556$, respectively), which play a role in lung function, angiogenesis and hematopoiesis, potentially supporting tissue oxygenation. The results of this study indicate that dietary supplementation with MSP™ in the grower and finisher phases has the potential to reduce the severity of WS and WB myopathies, improve cooking loss, and upregulate genes associated with tissue angiogenesis and lung function which are likely beneficial in preventing the severity of WS and WB syndromes due to improved overall health status of the birds.

Key Words: Wooden Breast, White Striping, Gene Expression, Dietary Supplementation

P216 Soybean oil and antioxidants in meat color of broiler Bruno Lala*, Nara Delbem, Carolina Santos, Ana Beatriz Faitarone, Evelyn Brito, Luciana Mena, Roberto Roça *São Paulo State University - UNESP - FMVZ*

Lipid oxidation process is the main cause of decrease in quality of food or feed, affecting in addition to other parameters in the quality of meat, such as color, the main issue of choice at the time of purchase by the consumer. In the present study, 720 Cobb male broilers fed diets supplemented with soybean oil and with antioxidants butylhydroxytoluene (BHT) and vitamin E were evaluated for meat coloring parameters. Animals were distributed in six replicates of four treatments: 1) without inclusion of oil or antioxidants; 2) inclusion of soybean oil; 3) inclusion of soybean oil + BHT; 4) inclusion of soybean oil + vitamin E. Meat color evaluations were performed on 30 breasts per treatment. The color of the Pectoralis major muscle was determined in the CIELab system, where the parameters L* (luminosity), a* (red) and b* (yellow) were evaluated. For these determinations, samples were exposed to air for 30 minutes prior to color readings. Data were submitted to analysis of variance by the GLM procedure, means were adjusted by the LSMEANS method and compared by Tukey test at 5% significance. Significant differences were observed between the experimental treatments studied only for brightness (L*) and yellow (b *) coloring patterns. Less brightness (L* = 53.21) was observed in broiler chicks that were not supplemented with oil or antioxidants. For yellow the results were opposite, since the treatment in which animals were not supplemented with oil and antioxidants registered the highest values (b* = 17.42). The results found for the parameter L* are higher than values considered normal, considering L* value between 45 and 50. One possible cause for high brightness may have been the cooling method used which eventually prevented the absorption of water and resulted in a pale meat. Selective absorption of light by myoglobin and other important components, such as muscle fibers and their proteins, as well as the amount of free liquid present in the meat are responsible for the color observed on meat surface. The use of supplementation of oil + antioxidants in feed for broilers promotes an increase in luminosity of the meat of these animals.

Key Words: meat quality, vitamin E, luminosity, brightness

P217 Effectiveness of two commonly used antimicrobials with processed broiler samples for reduction of Salmonella and Campylobacter Melissa Landrum*[1], Nelson Cox[2], Jeanna Wilson[1], Mark Berrang[2], Gary Gamble[2], Mark Harrison[1], Brian Fairchild[1], Woo Kim[1], Arthur Hinton[3] *[1]University of Georgia; [2]U.S. National Poultry Research Center; [3]USDA ARS*

The Food Safety and Inspection Service has begun enforcing stricter performance standards for *Salmonella* and *Campylobacter*. The purpose of this study was to evaluate two antimicrobials presently being used in broiler processing plants using practical concentrations and exposure times for both *Salmonella* and *Campylobacter.* Current line speeds allow immersion treatment to be between 6 and 15 s and concentrations based on effectiveness, cost, employee safety and comfort. Thighs (n=3/treatment) were inoculated with either a *C. coli* marker strain or *S.* Typhimurium marker strain (10^8) and each dipped into bags containing 1 L of treatment for either 6 or 15 s. Thighs were allowed 5 s drip time, each was placed in a bag with 150 mL buffered peptone water and hand shaken for 60 s; untreated had no treatment, but same rinse procedure using water. Rinsates tested for *Campylobacter* were serially diluted, plated onto Campy Cefex agar with 200 ppm gentamicin and incubated microaerobically for 48 h at 42°C. Rinsates tested for *Salmonella* were serially diluted, plated onto Brilliant Green Sulfa agar with 200 ppm nalidixic acid and incubated for 24 h at 37°C. Results were compared using a Tukey's Honest Significant Difference test. A 6 s and 15 s immersion in PoultrypHresh (pH 1.3) significantly ($P<0.05$) reduced a marker strain of *Salmonella* Typhimurium 86.2% and 91.7% when compared to water immersion, respectively. For *Campylobacter*, the reduction was significant ($P<0.05$) for the 6 and 15 s dip was 85.5% and 96.1%, respectively. For PAA (600 ppm), a 6 and 15 s immersion significantly ($P<0.05$) reduced *Salmonella* 86.2% and 98.3% when compared to water immersion. For *Campylobacter*, a 83.4% and 92.2% reduction was observed. All experiments were replicated 4 times. As one might expect a longer contact time produced slighter greater reductions but are not practical. The use of surfactants and agitation are being researched to possibly reduce concentrations using the shortest possible exposure time. Additional research with these two chemicals may further enhance their effectiveness and use in commercial processing plants. The two chemicals tested had acceptable reductions of both *Salmonella* and *Campylobacter* with realistic contact times and concentrations.

Key Words: Salmonella, Campylobacter, chicken thighs, peracetic acid, PoultrypHresh

P218 Texture analysis of cooked woody breast meat by the temporal dominance of sensation (TDS) method Hong Zhuang*, Debolina Chatterjee, Brian Bowker *US National Poultry Research Center*

Chicken is the most consumed meat per capita in the US. The woody breast condition (WBC) is an emerging quality defect observed in broiler breast fillets (pectoralis major). Experiments have shown that WBC can affect texture properties of both raw and cooked broiler breast fillets. Raw meat with the WBC is palpably hard and has poor functionality. Standard descriptive sensory tests with end-point attribute assessment have shown that cooked meat with the WBC can feel gummy and harder to chew. However, sensory data has varied and data on the effects of WBC on the complete eating experience with cooked breast fillets is lacking. The purpose of this study was to evaluate the effect of WBC on the sensory texture properties of cooked broiler fillets using the descriptive temporal dominance of sensation (TDS) method. Broiler fillets (removed from bone 2-3 h post-mortem) were collected from a commercial plant and sorted into normal (no WBC) and severe WBC categories based on palpable hardness and rigidity throughout the fillets. Samples were cooked to 76°C and evaluated by a 9-member trained sensory panel using the TDS method for texture attributes of springiness, cohesiveness, hardness, juiciness, and chewiness. Regardless of the WBC category, dominant sensations showed similar patterns during the testing evolution. Springiness was the initial dominant attribute, followed by juiciness and cohesiveness. Chewiness became the dominant sensation at the end. A few differences in the dominant sensations were noted between normal and WBC samples. For hardness, significant dominance was only detected in the WBC fillets. For springiness and juiciness, percentages of dominance were higher in the WBC samples than normal samples. Chewiness was the dominant attribute of normal fillets prior to 0.4 min of the sampling evolution. However, chewiness was not dominant for the WBC fillets until after 0.5 min. These data demonstrate that WBC can significantly affect the dominant sensory attributes experienced during the eating of cooked chicken breast meat.

Key Words: sensory descriptive analysis, poultry, broiler, hardness, chewiness

P219 Salmonella survival in filter sterilized carcass rinse with various concentrations of cetylpyridinium chloride Douglas Cosby*, Gary Gamble, Susan Mize, Mark Berrang, Jonathan Frye, Arthur Hinton, Jr. *U.S. Dept of Agriculture, Agriculture Research Service, U.S. National Poultry Research Center*

Control of *Salmonella* in the processing plant continues to become more important as processors work to ensure regulatory standards are met. Cetylpyridinium chloride (CPC, a commonly used biocide) has proven to be effective *in vitro* in the control of *Salmonella.* This study evaluated the recovery of *Salmonella* after overnight storage in 4°C filter-sterilized carcass rinsate with concentrations of CPC ranging from 0.5 to 1000 ppm. Ten *Salmonella* serotypes commonly isolated from poultry products were grown in Bacto Trypticase Soy Broth (TSB) overnight at 37°C and enumerated to determine the number of colony forming units (cfu) present. Serial dilutions of a CPC/propylene glycol (PG, included to maintain CPC in solution) solution were prepared in plastic 24-well tissue culture plates

containing filter-sterilized carcass rinsate. Approximately 10^7 cfu of each *Salmonella* serotype was added to the appropriate wells. Inoculated plates were stored overnight in plastic zip lock bags at 4°C. After storage, triplicate 10 µL drops of the contents of each well were placed onto Brilliant Green Agar with Sulfapyridine (BGS) plates and incubated at 37°C for 24 hrs. Three replications were conducted on separate days. Presence of typical *Salmonella* colonies on BGS plates was recorded as growth and verified through further biochemical and serological testing. Of the ten serotypes chosen for this study, *Salmonella* Dublin and *S.* Enteritidis were the least resistant to the concentrations of CPC used with growth only at or below 62.5 ppm (average of 41.67 ppm); *S.* Typhimurium and *S.* Reading demonstrated the most resistance with growth at or below 250 ppm (average of 187.5 ppm 145.83 ppm, respectively). These data when compared to a previous *in vitro* study, indicate that *Salmonella* species may better survive the presence of CPC when exposed in caracass rinsate than when in pure microbiological media. Residual CPC potentially remaining attached to a carcass after processing may adversely affect the recovery of *Salmonella* serotypes from carcass rinses used for regulatory purposes, resulting in false-negative observations and providing a false sense of security to consumers.

Key Words: Salmonella, cetylpyridinium chloride, biocides, survival, processing

P220 Determination of functionality of liquid smoke fractions as all-natural antimicrobial against foodborne Salmonella spp. Douglas Cosby*[1], Susan Mize[1], Mark Vanderbleek[2], Sara Llamas-Moya[3], Roshan Adhikari[2] *[1]Poultry Microbiological Safety & Processing Research Unit, USDA-Agriculture Research Service; [2]Kerry Inc; [3]Kerry, Global Technology and Innovation Centre*

Smoke has been used historically to preserve meat, provide desirable colour, flavour and aroma. Different levels of carbonyl, phenolic, and organic acid compounds generated during wood pyrolysis exhibit its antimicrobial properties. *Salmonella*, a gram-positive, non-spore forming bacterium is of great concern to the food industry causing foodborne illness. An *in vitro* study was conducted to evaluate the antimicrobial effect of nine different fractions of natural liquid smoke against 10 different strains of foodborne *Salmonella* species. *Salmonella* strains were used and maintained from the frozen culture on brilliant green agar (BGA). The strains were grown in tryptic soy broth overnight at 37°C, optical density was determined at 540_{nm} and serially diluted to the appropriate dilutions for enumeration. Approximately 1 x 10^{6-7} CFU/mL was used for the inoculation. The growth study was conducted in the 24 well tissue culture plates. One mL of buffered peptone water was added to each well except the first well. Two ml of the 1% stock solution of smoke was added to the first well and serial 1:2 dilutions were made. Concentration of the liquid smoke fractions (v/v) were 1%, 0.5%, 0.25%, 0.13%, 0.06%, 0.03%, 0.016%, 0.008% and 10 µL of the appropriate *Salmonella* cultures were added to the appropriate wells. A positive and negative control for each strain was maintained. The plates were incubated at 37°C for 24h and the contents of the wells were streaked onto the BGA plates and incubated at 37°C for 24h. Maximum concentration of the smoke product which allowed growth of the *Salmonella* strains was determined. Positive results were verified by biochemical reactions and serological agglutination assays for *Salmonella* strains. The experiment was repeated 3 times for each smoke fraction and strain. Two fractions of smoke containing a higher carbonyl concentration were able to inhibit the growth of all strains tested at 0.5%. A mixture of a different fraction of smoke in unfiltered form at 1% concentration was able to reduce all strains. The carbonyl fraction of smoke has the potential to serve as a natural anti-microbial solution to reduce salmonellae in foodborne/feed application where the smoke flavour is desired.

Key Words: liquid smoke, Salmonella, Grown Curve, Inhibition

P221 Improving value and utilization of breast fillets with the wooden breast myopathy through further processing into hotdogs Harshavardhan Thippareddi*[1], Edward Moore[1], Taylor Krause[1], T. Dean Pringle[1], Alexander Stelzleni[1], Hong Zhuang[2], Brian Bowker[2] *[1]University of Georgia; [1]University of Gerogia; [2]National Poultry Research Center, USDA Agricultural Research Service*

The woody breast myopathy reduces the product utility and value of broiler breast meat. The effect of including woody breast fillet meat with normal breast fillet meat in chicken hotdogs on hotdog quality (instrumental texture, color and sensory) was evaluated. Five treatments: (i) beef, (ii) beef and pork, (iii) chicken (normal), (iv) chicken with 25% moderate woody breast meat, (v) chicken with 25% severe woody breast meat were formulated with pork fat (to provide 25% fat content), water, sodium nitrite (156 ppm), sodium erythorbate (550 ppm), and a standard hotdog spice mix. The batter for each treatment was prepared following a standard procedure. The batter was stuffed into cellulose casings, cooked in a smokehouse following a standard hotdog schedule, cooled, and peeled. Hotdog instrumental texture and color were evaluated. The cook yield for the hotdogs was similar (92 – 94%), regardless of the meat source. Hotdog sensory quality profiles were evaluated by a trained panel. Hotdogs formulated with chicken had greater L* values ($P \leq 0.05$) and decreased a* and b* values ($P \leq 0.05$) compared to those with beef or beef and pork. The beef hotdogs had greater ($P \leq 0.05$) instrumental hardness values compared to those formulated with chicken breast meat. Incorporation of woody breast meat did not affect ($P > 0.05$) the instrumental hardness of the hotdogs formulated with chicken meat. Increases ($P \leq 0.05$) in instrumental resiliency, cohesion, springiness, gumminess, and chewiness were observed when moderate and severe woody breast meat was included in the hotdog formulation containing chicken breast meat. Hotdogs formulated with chicken (normal) or chicken with woody breast meat were scored as having lower ($P \leq 0.05$) saltiness and meaty profile compared to those formulated with beef or beef and pork. All other sensory characteristics (brothy, cohesiveness, hardness, juiciness, grittiness and chewiness) were similar ($P > 0.05$) for all the hotdogs, regardless of the meat (beef, pork or chicken) used in the formulation as well as the inclusion of woody breast meat in the formulation. Broiler breast meat with the woody myopathy can be included into formulations of emulsion type sausages with minimal impact on sensory characteristics of the product.

Key Words: Woody breast, broiler, hotdogs, texture, sensory

P222 Effects of corn kernel hardness and grain drying conditions on particle size when grinding using a roller mill or hammermill Hernan Cordova-Noboa*, Edgar Oviedo-Rondón, Ivan Ospina-Rojas, Adam Fahrenholz, Viviana San Martin, Miguel Chico, Yilmar Matta-Lozano, Andres Ortiz-Diaz, Lina Peñuela-Sierra *Prestage Department of Poultry Science North Carolina State University*

Corn kernel hardness and grain drying conditions may affect particle size and distribution subsequent to grinding, which could affect feed mill productivity and animal performance. In the present study, the effects of two corn hybrids (Dekalb 68-05 and Dekalb 65-20) with differing kernel hardness (average and hard respectively) and three grain drying conditions (35, 80, and 120°C) on the geometric mean (Dgw) and standard deviation (Sgw) of particle size were assessed by the sieving method. For roller-milling, four combinations of roll gap settings were studied (0-0, 25-20, 30-30, and 35-35.) For hammer-milling, three hammermill speeds were evaluated (900, 2,400 and 3,600 rpm) in each case grinding through a 12-12 screen combination. Treatments were arranged in a 2 x 3 factorial design with two corn hybrids and three grain drying temperatures. There were three replicates per treatment combination. Data were analyzed in a completely randomized design and mean separation was done using Tukey's test. Results obtained by roller milling with 0-0 and 35-35 roll gaps showed that harder corn kernels yielded higher ($P < 0.001$) Dgw values in comparison to corn with average kernel hardness when dried at 35°C. Similar response was observed when the corn was dried at 120 °C.

In addition, the Sgw was reduced ($P < 0.001$) when using corn with harder kernels that was dried at 80 and 120°C and milled at 0-0 (2.39 *vs*. 2.53), 30-30 (2.41 *vs*. 2.65), and 35-35 (2.34 *vs.* 2.69), respectively. In contrast, no effects ($P > 0.05$) were observed on Sgw when corn dried at 120°C was ground by the hammer mill. Higher ($P < 0.001$) Sgw was detected in the corn with average hardness that was dried at 35 and 80°C and milled at 2,400 and 3,600 rpm (3.84 *vs*. 3.45; and 3.21 *vs.* 3.03 respectively). The Dgw was higher ($P < 0.001$) in corn with harder kernels dried at 120°C when ground at 2,400 (744 *vs*. 594 µm) and 3,600 rpm (556 *vs*. 424 µm) respectively. Generally, corn with harder kernels and dried at 120 °C had higher Dgw and corn with average hardness had greater Sgw independently of the grinding method. In conclusion, kernel hardness of corn and grain drying temperatures affected the particle size and distribution subsequent to grinding in both a roller mill and hammermill.

Key Words: roller mill, hammermill, grinding method, Sgw, Dgw

P223 Effect of Spaghetti Meat abnormality on broiler chicken breast meat quality GIULIA TASONIERO*, HONG ZHUANG, BRIAN BOWKER *USDA-ARS*

Spaghetti Meat (SM) is an emerging muscular abnormality of broiler chicken *Pectoralis major* muscles that is macroscopically characterized by an extremely soft consistency and stringy/mushy appearance. The aim of the study was to provide information on the effects of SM myopathy on proximate composition and protein functionality of affected chicken breast meat. For this study, 30 severe SM and 30 unaffected (normal, N) fillets were collected during two sampling sessions. Drip loss, ultimate pH, salt-induced water uptake, and proximate composition were assessed. Muscle collagen content (soluble and insoluble) and muscle protein solubility (total and sarcoplasmic) were also quantified. Analysis of variance was carried out using a mixed model that included group (N and SM) as a fixed effect and sampling repetition as a random effect. Means were separated using Bonferroni adjustments and $P < 0.05$ was considered significant. Spaghetti Meat affected fillets were heavier (N = 483 vs SM = 564 g; $P < 0.0001$) and lost a higher amount of drip compared to their normal counterpart (N = 1.22 vs SM = 1.44 %; P = 0.0131) despite exhibiting a higher pH (N = 5.84 vs SM = 5.99; $P < 0.0001$). Differences in salt-induced water uptake, cook loss and final yield between N and SM groups were not statistically significant. However, the values obtained suggested a potential impairment of water-holding capacity in affected meat. As for proximate composition, a higher moisture content (N = 75.4 vs SM = 76.8%; P = 0.0012) was detected in SM affected meat, together with a lower protein level (N = 22.2 vs SM = 20.4%; $P < 0.0001$). The two groups exhibited similar fat and ash percentages, as well as similar soluble and insoluble collagen amounts. SM affected fillets were characterized by lower total protein solubility (N = 229 vs SM = 203 mg protein/g muscle tissue; $P < 0.0001$), while the values of sarcoplasmic protein solubility remained statistically similar between SM and N groups. These data indicate that the Spaghetti Meat abnormality adversely impacts chicken breast meat nutritional value and some functionality attributes fundamental for further processing.

Key Words: broiler chicken meat, Spaghetti Meat abnormality, meat proximate composition, meat protein quality

Pathology

P224 Effect of different challenge models to induce necrotic enteritis on the intestinal microbiota of broiler chickens Cristiano Bortoluzzi*[1], Bruno Serpa Vieira[1], Ana Villegas[1], Jun Liu[1], Charles Hofacre[2], Todd Applegate[1] *[1]University of Georgia; [2]Southern Poultry Research Group*

The objective of this study was to evaluate the diversity and composition of the intestinal microbiota of broiler chickens raised under three different conditions and methods to induce NE. The chicks in experiment 1 and 2 were vaccinated against coccidiosis on d 1. Experiment 1: non-challenged and challenged birds were raised on floor pens with new litter and 58 birds/pen. The challenge consisted of *Eimeria maxima* inoculation on d 14, and *Clostridium perfringens* via water on d 18-19. Cecal microbiota was evaluated on d 18, 21, and 28. Experiment 2: non-challenged and challenged birds were raised on floor pens with recycled litter and 50 birds/pen. The challenge consisted of challenged with *C. perfringens* via feed from d 18-20. Ileal and cecal microbiota were evaluated on d 21. In experiment 3, non-challenged and challenged birds were raised in battery cages with 8 birds/cage. Challenged birds were inoculated with *E. maxima* on d 14, and *C. perfringens* on d 19-21. Ileal and cecal microbiota were evaluated on d 21. In the three experiments DNA was isolated from the ileal and/or cecal digesta, and the microbiota analyzed through 16S rRNA sequencing. The FCR of the birds was impaired by 5.2, 11.1, and 30.1%, and the lesion score characteristic of NE (0 to 3) averaged in 0.50, 1.20, and 1.29 in experiments 1, 2, and 3, respectively ($P < 0.05$). In experiment 1, the cecal microbiota did not significantly vary due to challenge. In experiment 2, α-diversity indices were lower in challenged vs. non-challenged birds in both ileal and cecal microbiota. The induction to NE reduced ($P < 0.05$) the frequency of *Firmicutes* and increased ($P < 0.05$) *Bacteroidetes* in the cecal microbiota. Yet, the challenge increased ($P < 0.05$) bacteria belonging to the genus *Bacteroides* and decreased *Ruminococcaceae* and *Ruminococcus* related bacteria. In experiment 3, Chao index (α-diversity) increased in the challenged birds. There was a trend (P = 0.08) towards increased frequency of *Enterobacteriaceae* in the cecal microbiota of challenged vs. non-challenged birds. In conclusion, the largest variations on the microbiota was observed in experiment 2, when birds were raised on floor pens with recycled litter, vaccinated against coccidiosis, and challenged with *C. perfringens* on d 19-21.

Key Words: broilers, microbiota, necrotic enteritis

P225 Evaluation of deoxycholic acid as a prophylactic treatment to prevent Histomoniasis in turkeys Lesleigh Beer*[GS], Christine Vuong, Juan Latorre, Samuel Rochell, Xiaolun Sun, Guillermo Tellez, Billy Hargis *University of Arkansas*

Histomoniasis, also known as blackhead, is an important disease of turkeys. Caused by the protozoan parasite *Histomonas meleagridis*, mortality can approach 80-100% of the flock with significant economic damage incurred. Deoxycholic acid (DCA) is a naturally occurring secondary bile acid, originating from intestinal bacterial metabolic conversion of cholate, a primary bile acid. DCA has been shown to have anti-*Histomonal* properties *in vitro*, leading to our hypothesis that DCA inclusion within the feed may prevent Histomoniasis in turkeys. Selected concentrations of DCA within a basal starter diet were evaluated for effects on BWG, lesions, and mortality of Histomoniasis-challenged turkeys. Treatments consisted of Non-Challenged Controls, 1% DCA diet, 0.5% DCA diet, 0.25% DCA diet, or Positive-Challenged Controls. The basal turkey starter diet was fed to all groups until d7, at which time DCA diets were administered to respective groups. Via intracloacal inoculation, $2x10^5$ *H. meleagridis* cells/turkey were administered on d14, and lesions were evaluated d13 post-challenge. Pre-challenged d0-14 BWG in the 0.25% DCA group was higher ($p<0.05$) than the 1% DCA group. There were no significant differences in pre-challenge d0-14 BWG between any of the other groups. Histomoniasis-related mortality was significantly higher in all groups as compared to the non-challenged controls. No histomoniasis-related lesions were observed at any time in the non-challenged controls. Presence of classic histomoniasis-related liver lesions was statistically higher in the 0.5% DCA diet as compared to the 1% DCA diet and the challenged con-

trols. Classic histomoniasis-related cecal lesions were higher in the 0.5% DCA diet as compared to the 1% DCA diet. Taken together, these data suggest DCA inclusion within the feed at these concentrations and under these experimental conditions does not prevent Histomoniasis. Although DCA treatment reduced *H. meleagridis* cells *in vitro*, the *in vivo* trial resulted in no reduction of histomoniasis-related mortality or lesion presence within the DCA diets as compared to the positive challenge controls.

Key Words: blackhead, deoxycholic acid, Histomoniasis, Histomonas meleagridis, turkey

P226 Acute fowl cholera in a flock of Ring-necked Pheasants Laura Chen*[1], Doug Anderson[2], Oscar Fletcher[1] *[1]North Carolina State University; [2]Georgia Poultry Laboratory Network*

Two juvenile ring-necked pheasants (*Phasianus colchicus*) from a mixed age flock with elevated mortality presented for postmortem evaluation. On gross examination, the 12-week-old male had an enlarged and mottled tan-red spleen; a segmental band of hemorrhage at the junction of the esophagus and proventriculus; and an enlarged liver with multifocal to coalescing hemorrhage. The 8-week-old female had an enlarged and mottled tan-red spleen. Swabs of bone marrow and liver were collected from both birds for aerobic culture. *Pasteurella multocida* serotype 4 and/or 12 was isolated in pure culture from all samples. Histologically, both birds had severe fibrinoheterophilic splenitis with myriad intralesional, coccobacilli bacterial organisms and systemic vasculitis with fibrin thrombosis and intravascular organisms. In the 12-week-old male, there was also necrotizing hemorrhagic hepatitis and proventricular hemorrhage associated with vasculitis. Though not commonly reported in the literature, acute fowl cholera can be an important disease in flocks of ring-necked pheasants and overlap in clinical signs and gross lesions with other important diseases.

Key Words: fowl cholera, Pasteurella multocida, pheasant, bacteria, pasteurellaceae

P227 Changes to gut microbiome through ten days of age affected by pioneer colonizing bacteria Emily Winson*[UG], Denise Rodrigues, Kimberly Wilson, Whitney Briggs, Audrey Duff, Kaylin Chasser, Lisa Bielke *The Ohio State University*

Microbial populations that initially colonize the gastrointestinal tract (GIT) play a long lasting role in health and welfare, and hatcher cabinet exposure to bacteria can affect these pioneer colonizers (PC) in poultry. In commercial hatcheries, chicks are exposed to a wide range of bacteria, which can include pathogenic species and studies have shown that they can affect disease susceptibility and growth performance. However, little is known about the influence of different PC on the pattern of gut microbial colonization during the development of mature microbiota in chickens. The objective of this study was to identify the impact of two apathogenic Gram negative chicken isolates or lactic acid bacteria (LAB) as PC on the GIT microbiome through d10. On d18 of embryogenesis, embryos were inoculated with either saline (S) or $1x10^2$ CFU of *Citrobacter freundii* (C1), *Citrobacter* spp. (C2), or LAB (L) into the amnion. GIT samples were collected at DOH, 3d, and 10d for microbiome analysis. Ilumina MiSeq library preparation and sequencing were completed, and Pearson correlation coefficients between relative abundance of bacterial communities were performed via R software. *Enterobacteriaceae* was negatively correlated through d10 in the ceca, upper ileum (UI), and lower ileum (LI) among all groups. *Ruminococcaceae* abundance in UI was positively correlated with time in S (r= 0.56) and C1 (r= 0.89), while in L (r= -0.39) and C2 (r= -0.67) had negative correlation with the time-course. In addition, *Clostridiaceae* abundance decreased in LI across 10d in S (r= -0.72), while in L (r= 0.94), C1 (r= 0.33), and C2 (r= 0.95) its population increased as birds aged. *Lactobacillaceae* was positively correlated with time in ceca, UI, and LI among all groups, with exception in L (r= -0.99) and C1 (r= -0.14) treatments, where population decreased in the ceca through d10. These results suggest that different pioneer colonizers in the gut may alter microbiome patterns during the establishment of the mature microbiota. Better understanding of these interactions would provide valuable information and tools for improving overall gut health in broilers.

Key Words: microbiome, pioneer colonizers, gastrointestinal tract, enteric health

P228 Development of chicken intestinal organoid as a model to study function and disease Mohan Acharya*[1], Narayan Rath[2] *[1]University of Arkansas; [2]USDA/Agriculture Research Service*

Tissue organoids can simulate various functional aspects of real organs making them promising models to understand the effects of nutrients, pharmacological and toxicological agents, and host-pathogen interactions. In the current study, we developed a method to culture chicken intestinal organoids using intestinal villi harvested from day old broiler chicks. The villi were sedimented by low speed centrifugation, washed with antibiotics supplemented Dulbecco's modified Eagle's medium (DMEM), and plated in same medium containing antibiotic/antimycotic, fetal bovine serum, bovine pituitary extract, insulin transferrin selenium (ITS), and polyamine supplements. The villi undergo self-repair leading to organ spheroids with trapped vascular tissues that can be preserved in liquid nitrogen, and revived. We tested the effect of some selective chemicals using these organoids following their effect on organoid morphologies and their effect on permeability to FITC labelled dextran. We incubated the organoids with or without the following chemical additives: dextran sulphate (inducer of colitis), retinoic acid (vitamin A), propranolol (a β-adrenergic blocker), 2,4 dinitrophenol (a weight reducing agent), deoxycholic acid a (bile acid), capsacin and lipopolysaccharide, both, inflammatory agents, phorbol 12-myristate 13-acetate (a protein kinase C activator), β-glycophate (a pesticide), indomethacin (a prostaglandin inhibitor), and hydroxytryptamine (serotonin), in 96-well plate cultures containing 5-10 organoids/0.1 ml medium, tested at the concentration of 1 µg/ml. Dextran-FITC was introduced into culture for 1 h prior and the organoids fixed on to the slides by cytocentrifugation prior to their examination using a fluorescent microscope. Based on the results which show some chemicals that increase permeability of the organoids and cause their damage whereas others show differential changes in the enterocytes, we surmise that this model may be able to screen chemicals and nutrients that can alter gastrointestinal physiology.

Key Words: Chicken, intestine, organoids, screening chemicals, tissue permeability

P229 Gut microbiota-mediated suppression of multi-drug resistant Salmonella Reading and Braenderup by Diamond V Original XPC in an in vitro poultry model Andrea Binnebose*, Tom Weigand, Cole Reedy, Joan Butler, William Chaney, Hilary Pavlidis *Diamond V*

In poultry*, Salmonella* is typically avirulent, but it can become a formidable food safety hazard when if poultry products become contaminated during processing. Infeed pathogen mitigation strategies designed to reduce *Salmonellosis* is of great importance in the poultry industry, especially with recent outbreaks, new federal regulations, and oversight designed to control contamination in poultry products. The objective of this study was to evaluate the effects of the functional metabolites of Diamond V Original XPC™ (XPC) on intestinal volatile fatty acid (VFA) production and reductions in *Salmonella* Reading (SR) and Braenderup (SB) in an *in vitro* avian intestinal model (IAMM). Fresh fecal excreta were collected from up to twelve Hy-Line W-36 layers. The fecal slurry was homogenized, filtered and added to a buffered salts medium. Aliquots of the inoculated medium were added to replicate culture tubes containing a substrate and 0.15 g of XPC. In separate experiments, either a multi-drug resistant SR or SB strains were challenged at 1×10^4 CFU/ml in each culture tube. For each experiment, tubes were incubated for 24h and either live SR or SB was measured using standard serial dilution culture techniques. Short chain VFA concentrations were analyzed by gas chromatography. The ad-

dition of XPC significantly ($P < 0.05$) reduced CFU/mL of SR and SB by nearly 0.5 and 1.0 logs, respectfully. The inclusion of XPC resulted in a significant ($P < 0.05$) increase in both individual and total VFA production by nearly 23% compared to Control. The increase in VFA's can be attributed to an increase in feed conversion and availability of nutrients necessary in maintaining epithelial cell growth, blood flow, and the normal secretory and absorptive functions of the intestine. Research has also shown the effectiveness of VFA's in inhibiting the growth of the family *Enterobacteriaceae*. These data along with previously reported data with a multi-drug resistant *S*. typhimurium demonstrated that the observed effects were not entirely dependent on intestinal host factors. These findings suggest that use of XPC in poultry feed can significantly reduce *Salmonella* numbers as well as increase VFA production, and may contribute to reducing consumers' risk of developing a foodborne illness.

Key Words: Original XPC, Salmonella Reading, Salmonella Braenderup, Volatile Fatty Acids, Poultry

P230 Autogenous, inactivated vaccine and research techniques developed for use against inclusion body hepatitis. Christine Vuong*[1], Makenly Coles[1], Lucas Graham[1], Kyle Teague[1], Brittany Graham[1], Mikayla Baxter[1], Thaina Barros[1], Juan Latorre[1], Guillermo Tellez[1], Holly Sellers[2], Chip Garritty[3], Billy Hargis[1] *[1]University of Arkansas; [2]University of Georgia; [3]OK Foods*

Historically, inclusion body hepatitis (IBH) has not been a disease of high concern for the poultry industry, initially suspected as only occurring as a secondary infection after depression of the immune system by other causes, such as infectious bursal disease or chicken infectious anemia. For this reason, very little research has been completed on this disease, resulting in very sparse information available on IBH in poultry. In recent years, there has been increased rates of IBH-related disease and mortalities in commercial poultry operations of the southern midwest region, as well as Delmarva and Frazier Valley regions. In response to this renewed need, we report the development of various techniques and reagents for use in IBH research. An IBH challenge model was developed for use in naïve day-of-hatch chicks, both broilers and SPF layers, as well as older 4 weeks-of-age broilers. Chicks intraperitoneally-injected with infective liver homogenate from field mortalities averaged 25-50% mortality between 5-9 days post-challenge. Older broilers exhibited 10-15% mortality 3 days post-challenge intravenously. Non-challenged birds obtained from the same source and housed in the same room, but physically separated by pen, exhibited no disease morbidity/mortalities. An agarose immunodiffusion assay, as well as an ELISA, were also developed for qualitative detection of the virus or seroconversion against virus. The avian adenovirus 8b genotype was detected and confirmed as the primary circulating genotype for this region. Field sources of this 8b virus was used as a parent stock to develop an autogenous, inactivated oil-emulsion vaccine. Subcutaneous immunization into ~18 weeks old pullets from the affected region with this autogenous vaccine have demonstrated ongoing protection against the disease/have not broken with IBH disease (neither the vaccinated pullets nor their progeny) since May 2018. The techniques presented in this report serve as a preliminary platform to further IBH research in poultry, particularly if disease prevalence increases or spreads geographically.

Key Words: inclusion body hepatitis, avian adenovirus, fowl adenovirus, challenge model, autogenous vaccine

P231 Early chick immunity after in ovo vaccination of F strain Mycoplasma gallisepticum and the influence of sex on the response to the vaccination Katie Elliott*[1], Scott Branton[2], Jeffrey Evans[2], Edgar Peebles[1] *[1]Mississippi State University; [2]USDA-ARS Poultry Research Unit*

Commercial layer chickens are currently vaccinated during pullet rearing to combat the negative effects of *Mycoplasma gallisepticum* (MG) infections. Previous trials in which layers were *in ovo* vaccinated against strain F MG (FMG) showed that nearly 50% of the birds produced IgM antibody against FMG by 6 wk of age (woa). Standard MG vaccination techniques, which utilize eye drop or spray delivery at 9 woa, result in this percentage at approximately 15 woa. In this study, it was investigated as to when FMG *in ovo*-vaccinated birds initiate a humoral immune response prior to 6 woa, and if male vaccinates are more susceptible to MG infections. Hy-Line W-36 embryonated eggs were either not vaccinated (controls) or *in-ovo* vaccinated with a 50 µL volume of a 10^{-6} dilution of Poulvac MycoF vaccine (Zoetis, New York, NY). For each treatment group, 384 straight-run chicks were reared. At hatch and at 2, 3, 5, 7, 14, 21, and 28 d post-hatch, 54 birds per treatment were individually weighed and a blood sample was collected for MG IgM antibody detection. In those birds that tested positive for IgM antibody production, they were further tested for an IgM to IgG antibody production shift. At each age, BW was not different between vaccinated and control chicks (all $P > 0.19$). Males, however, outweighed females starting at d 5 ($P = 0.02$). Mortality was 1.0% for the control birds and 12.2% for the FMG birds during the first 2 wk. The majority (72.3%) of the mortalities in the FMG group were male. The percentage of control and FMG *in ovo*-vaccinated birds with IgM antibody production was 0% and 1.9% on d 7, 0% and 31.5% on d 14, 1.9% and 55.9% on d 21, and 0% and 60.6% on d 28, respectively. A shift in IgM to IgG antibody production in the FMG *in ovo*-vaccinated birds was 0.0% at 2 wk, 2.9% at 3 wk, and 21.2% at 4 wk of age. All control birds tested negative for FMG-related IgG production. In conclusion, the earliest detection of MG antibodies after *in ovo* vaccination with live FMG occurred at 7 d of age. In this study, it was confirmed that male poultry are more susceptible to the effects of FMG than females. Future research will evaluate how *in ovo*-vaccinated pullets perform during a lay cycle in comparison to those that receive a standard vaccination.

Key Words: Mycoplasma gallisepticum, layer, in ovo, sex, antibody

P232 The effect of sodium bisulfate and coccidiostate on intestinal lesions, GUT microbiota, and growth performance of broilers Mayam Talghari, Shaban Rahimi*, Mohammad Amir Karimi Torshizi *Tarbiat Modares University*

In the first experiment, 240 day-old broiler chicks (Ross 308) were randomly divided into four experimental groups of 15 chicks with four replicates. The first group was negative control (fed basal diet), and the second to fourth groups were supplemented with feed additives including: Sodium bisulfate, Coccidiostate (Monensin), Sodium bisulfate plus Monensin (Monensin + Sodium Bisulfate), respectively. The second trail was same as the first experiment except that all groups at day 14, were orally inoculated with 200 µl of suspension containing oocysts mixture of four species of *Eeimeria* (*E. tenella* (3.5×10^4) *E. maxima*, *E. necatrix* and *E. acervulina* (7.5×10^3). From fifth day after challenge for 10 days, fecal samples were taken to determine the number of oocysts excreted per gram of the feces. At day 42, two chicks from each replicate were humanely euthanized in order to investigate the intestinal lesions. The measured factors included BW, FI, FCR, intestinal lesions and intestinal morphology, microbial counts of ceacal contents, blood biochemistry, oocyst shedding count, carcass characteristics and relative weight of internal organs. Analysis of data were done by SAS software and comparison of means carried out by Duncan test. The results showed that the highest density of the goblet cells and the highest villi height to crypt depth ratio were related to sodium bisulfate. The lowest total number of colibacilli and the highest number of Lactic acid bacteria were related to sodium bisulfate treatment. The results of the second experiment showed that the highest body weight gain in the starter period (before challenge) and the grower period (after challenge) were related to monensin + sodium bisulfate treatment. The highest FI was related to monensin + sodium bisulfate treatment at the starter period, and the lowest FCR in the finishing period was related to the monensin and sodium bisulfate treatment. The lowest total number of colibacilli was related to monensin treatment. The lowest incidence of intestinal lesions were seen in monensin treatment. According to the result of this experi-

ment, administration of sodium bisulfate plus monensin improve growth performance and reduce intestinal lesions in broiler chickens.

Key Words: Broiler chickens, sodium bisulfate, coccidiostate, intestinal lesions, oocytes count

SCAD

P233 Evaluation of long-term immunity and protection against Salmonella spp by orally administrated inactivated vaccine. Emanuel Gumina*[1], Gonzalo Almaraz[1], Ariel Sugezky[1], Igor Praxedes-Campagnoni[2,3], Sherry Layton[1,4] *[1]Vetanco SA; [2]Vetanco Brazil; [3]University of Santa Catarina State, Department of Animal Health; [4]BV Science*

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

Key Words: immunity, vaccine, Salmonella, poultry, immunoglobulins

P234 Evaluating the efficacy of spray-dried plasma in mitigating intestinal colonization by Salmonella Typhimurium in broiler chicks Candice Blue*[1], Yasin Jababu[1], Devendra Shah[2], Yewande Fasina[1] *[1]North Carolina Agriculture and Technical State University; [2]Washington State University*

Salmonella Typhimurium (STyph) is a food borne pathogen which adapts, enters to and alters the gastrointestinal environment. It employs multiple defense mechanisms to resist environmental stressors like the acidic pH of the stomach, and uses virulence mechanisms to invade the intestinal epithelium and disseminate throughout the host. An experiment was conducted to evaluate the efficacy of spray-dried porcine plasma as an immunoenhancer against cecal colonization and invasion by STyph in broiler chicks. Day-old (192) Ross 708 male chicks were randomly weighed and placed in 16 pens at 12 chicks per pen with 4 treatments. Chicks placed in the CX treatment were given the control unmedicated diet, and SDP treatment consisted of chicks given the unmedicated diet into which spray dried plasma (30 g / kg diet) was added. The ST and PST treatments consisted of chicks that were respectively given diets similar to those of CX and SDP, in addition to being gavaged 10^8 CFU of ST / mL. Chicks had ad libitum access to feed and water throughout the 14-day experiment. On d 2, 4, 7 and 13, ceca and livers were aseptically collected and contents were plated for the enumeration of STyph on xylose lysine tergitol 4 (XLT4) agar supplemented with 50 µg/mL nalidixic acid. Results showed that the ceca of control chicks (in CX and SDP) were negative for STyph throughout the experiment. Among challenged treatments (ST and PST), PST had higher cecal STyph levels throughout the experiment. In addition, STyph invaded ($P < 0.05$) the livers of ST and PST chicks. It was concluded that dietary SDP was not effective in reducing colonization by STyph within the ceca.

Key Words: Salmonella Typhimurium, Spray-dried plasma, Nalidixic acid, Cecal colonization, Liver invasion

P235 Model development: day of challenge of Salmonella heidelberg in broilers Nathaniel Ollis*[1], Charles Hofacre[1], Jennie Baxter[1], Roy Berghaus[2] *[1]Southern Poultry Research Group; [2]University of Georgia*

Salmonella from poultry is a significant cause of foodborne illness in people. There are increasing efforts to find interventions to reduce salmonella in broilers before they reach the processing plant. To evaluate these products in pen trials, we must first have an understanding of the salmonella challenge model. One variable in challenge models can be a constant dose (CFU/chick) and variable age of challenge. A comparison of control groups in four similar studies consisting of 50% seeder birds that were tagged and orally gavaged with 0.1ml of a ~1x108 dose of a nalidixic acid resistant Salmonella heidelberg resulting in a dose of ~1x107 per bird on day of age 0, 1, 2, and 4. On days 42-44 the birds were necropsied. Ceca samples were collected and cultured for prevalence and enumeration of S. heidelberg using the most probable number (MPN) method of Berghaus et al. 2013. Tetrathionate with iodine was added to each sample after stomaching. Each sample was set up for the MPN method, where 10-fold dilutions were performed in triplicate from 1x100 to 1x105 in a 96-well deep well block. Each sample and block were incubated for 24 hours at 42°C and plated to a 96 well plate with xylose lysine tergitol-4 (XLT-4) agar that contained 25µg/ml of nalidixic acid and incubated for 24 hours at 37°C. In testing prevalence, the samples were struck to XLT-4 agar that contained 25µg/ml of nalidixic acid and incubated for 24 hours at 37°C, then again 7 days later for secondary testing. Comparing the estimated mean log10 MPN/g of each study shows a reducing trend the older the bird at challenge date in the directly challenged birds (0.54-0.21 MPN/g). No trend was observed in the horizontally challenged birds. The prevalence results of both direct (66.6- 33.3%) and horizontally (56.6-27.7%) challenged birds tend to have a lower trend as the age of inoculation increased. These results may be due to competitive exclusion in the gut flora as the birds age. In conclusion, the younger the bird at the day of challenge showed higher rates of colonization of S. heidelberg. This suggests that when testing products to inhibit and reduce salmonella development in broilers, these different challenge models will lead to a better understanding on how to run tests for products.

Key Words: Salmonella Heidelberg, challenge model development, day of age

P236 Screening for potential avian pathogenic E. coli (APEC): surveillance data from two broiler flocks Nicolle Lima Barbieri*, Estefania Rama, Manpreet Singh, Harshavardhan Thippareddi, 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 at 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. In 2008, Johnson et. al. described a rapid PCR diagnostic tool which consists of a nine gene panel (cva, *iroN*, *ompT*, *hlyF*, *etsB*, *iss, iutA, ireA, papC*) that defined the minimal predictors of APEC virulence.

In this work, two broiler farms (one conventional and one antibiotic free (NAE)) were sampled during grow out. Samples were collected on day 1, 19, 30, 44, 51, 56 and 57. At every sampling day the cecum and ileum were collected from 15 birds. In addition, environmental samples of litter, feed and water were also collected. All samples were homogenized in peptone water and inoculated into MacConkey agar for *E. coli* isolation. A total of 416 *E. coli* were isolated and screened for the presence of the minimal predictor genes of APEC. Differences in the prevalence and detection of APEC genes between the two farms was observed. On the conventional farm, APEC-like positive samples were detected at the first sampling and decreased over time to slaughter, while samples collected on the NAE farm detected APEC-like isolates from the second sampling day (day 19) and increased in prevalence until slaughter. This study allows us to investigate the distribution of APEC-like strains across two rearing systems, and to evaluate the role of production practice on APEC selection as well as the potential role of prophylactic measures that could be taken in order to control the presence of this potential pathogen and reduce production loss.

Key Words: avian pathogenic E.coli, colibacilosis, antibiotic free, broiler, Escherichia coli

P237 Characterizing avian pathogenic Escherichia coli (APEC) associated with cellulitis in production turkeys from Iowa Aline de Oliveira*[GS], Nicolle Barbieri, Catherine Logue *University of Georgia*

Turkey cellulitis (TC) is the second greatest concern among turkey producers nationally, and Avian pathogenic *Escherichia coli* (APEC) is one of the pathogens implicated in the disease after *Clostridium spp*. TC is characterized by locally extensive inflammation of subcutaneous tissues of the inguinal, tail, and breast regions, often striking production birds at or near market age resulting in increased mortality and carcass condemnation at slaughter, leading to multi-million dollar losses for the turkey industry. Information on the role of *E. coli* in cellulitis-associated disease in turkeys is still limited.

Here we aimed assess the relationship between the source of APEC and the pathology of the disease by examining the association between APEC isolated from lesions of turkeys diagnosed with cellulitis and litter quality of barns.

Three turkey production barns located in Iowa were monitored for TC from 10 weeks to market age (approximately 18 weeks) among farms with or without a history of cellulitis. Weekly samples were collected from litter and birds showing signs of cellulitis during field or diagnostic necropsy. All samples collected were cultured for *E. coli* and screened for the presence of APEC. Additional analysis included screening for 64 virulence and antimicrobial resistance genes, phylogenetic analysis and serotyping of 240 litter and 54 cellulitis isolates.

A relatively high prevalence of genes involved in resistance to heavy metals, such as copper (*pco*), arsenicals (*ars*) and silver (*sil*) was observed (≥30%). Additionally, a high frequency of genes encoding resistance to macrolides (*etsA*, *etsB)* was detected in cellulitis and litter isolates (56%). Interestingly, plasmid-associated genes *aerJ* and *traT* were also detected at a high frequency (>45%), suggesting virulence plasmids may be contributing to virulence of APEC in cellulitis.

Phylogenetic typing results found most litter isolates were classified as A (40%) and B1 (32%), whereas most cellulitis samples were also classified as A (41%), followed by B2 (22%). Serotypes detected among these isolates included O2, O8, O25, and O50.

Overall, this data suggest that APEC plays a significant role in turkey cellulitis.

Key Words: Cellulitis, APEC, turkey production, pathology

P238 Avian Pathogenic Escherichia coli (APEC) Diagnostics in Georgia – Comparison of Gene Profiles with Tissue of Isolation. Catherine Logue*, Darby Newman, Lisa Nolan, Nicolle Barbieri *University of Georgia*

Colibacillosis caused by Avian Pathogenic *Escherichia coli* (APEC) is a significant cause of morbidity, mortality and carcass condemnation to the poultry industry worldwide resulting in significant economic losses. Here, we assess the use of a multiplex PCR panel for classifying APEC from diagnostic cases and further characterize these isolates using gene profile analysis.

A total of 48 *E. coli* isolates, collected between August and October of 2018 through the PDRC diagnostic lab, were used in this analysis. All isolates were screened using a multiplex PCR panel that targets genes associated with APEC chromosomal and plasmid virulence genes. Isolates were also assessed for the relationship between their gene profile and host tissue of origin.

Overall, isolates met the criteria for definition as well-developed pathogens with more than 90% of isolates being positive for the genes *iroN*, *ompTp*, and *hlyF*; 78% were positive for *aerJ* and 67% positive for *iss*. A significantly lower prevalence was observed for *cvaC*, *etsB*, *ireA* and *papC* (range 5-36%). When overall gene prevalence in our isolates was examined in regard to their sites of origin, we found that APEC from the ovary, bone marrow, pericardium and lung had higher average numbers of genes compared to isolates recovered from the skin and yolk sac. Genes associated with the ColV virulence plasmid (*iss*, *iroN*, *hlyF* and *ompTp*) were detected in 43 of 48 isolates (89.5%) further confirming that the ColV plasmid is the defining trait of the APEC subpathotype.

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. The path panel diagnostics is currently available through the PDRC diagnostic lab and provides significant value to APEC screening.

Key Words: Escherichia coli, Disease, Disgnostics, Molecular tools, Pathogenesis

P239 Effect of select Gram negative enteric strains on inflammation in broiler chicks Kate McGovern*[UG], Kaylin Chaser, Audrey Duff, Whitney Briggs, Denise Rodrigues, Emily Winson, Mike Trombetta, 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. Wild-type Gram negative poultry GIT isolates were tested for potential to cause inflammation and affect BWG to determine feasibility as research strains. Two independent studies consisted of 144 birds, divided equally into four treatment groups with three replicates per treatment, each, which included non-challenged control (NC), and three challenge levels, a low (L), a medium (M), and a high (H) group. In both experiments, body weights (BW) were measured on d0, d7, and d14. In exp 1, on DOH, all birds in the L, M, and H groups received ~10^2CFU, ~10^3CFU, or 10^4CFU per chick, respectively, of a mixed dose of two species of *Citrobacter* and *E. coli*. Subsequently, exp 2 consisted of ~10^3CFU, ~10^4CFU, or ~10^5CFU *E. coli* administration by

oral gavage on DOH. BW was measured on DOH, d7, and d14, plus d14 blood samples were collected for chicken serum alpha-1 acid glycoprotein (AGP) ELISA analysis. In exp 1, d7-d14 %BWG statistically increased ($p<0.05$) between NC and M, with no other differences observed. In both experiments, AGP analysis revealed significantly increased ($p<0.05$) APG expression for M in exp 1, and L in exp 2, compared to NC. Although there was an absence of change in BW, inflammation was increased, as evidenced by AGP, which suggests these bacterial isolates may successfully induce GI inflammation up to d14. Despite a lack of change in BWG, they may serve as a predisposing factor for opportunistic disease, and future studies are warranted.

Key Words: Gram negative, E. coli, inflammation, opportunistic disease

P240 Timing of challenge model for Campylobacter jejune colonization in broiler chickens Virginia Baxter*[1], Charles Hofacre[1], Roy Berghaus[2] *[1]Southern Poultry Research Group; [2]University of Georgia*

The CDC estimates *Campylobacter*affects more than 1.3 million people every year. Most human illness is caused by one species, *Campylobacter jejuni.* In 2014, National Antimicrobial Resistance Monitoring System (NARMS) testing found *Campylobacter* on 33% of raw chicken bought from retailers. Most *Campylobacter* infections are associated with eating raw or undercooked poultry or from contamination of other foods by these items. If *Campylobacter* is present in the intestinal tract, broiler carcasses become extensively contaminated during the slaughter process. In order to properly evaluate interventions we must first understand the peak of *Campylobacter* colonization in order to appropriately design studies to measure preharvest interventions that can lower the load of bacteria that can potentially enter the processing plant, or take necessary steps during processing to lower the contamination of carcasses. In a controlled environment pen study *Campylobacter jejuni* was inoculated at 14 days of age, and ceca aseptically collected at 35 and 41 days of age. After collection, ceca were weighed and stomached. Boltons Broth was added to ceca, 10 fold serial dilutions were performed in Maximum Recovery Diluent (MRD) and plated to Campy Cefex Agar. On day 35, none of the ceca samples was classified as having colonies that were too numerous to count (TNTC). On day 41, 6 out of 40 (15%) ceca had *Campylobacter*counts that were classified as TNTC. The mean (SE) $\log_{10}$*Campylobacter*counts (CFU/g) in culture-positive ceca was 8.23 CFU/g on day 35, and 9.50 CFU/g on day 41. The results of this study suggest that *Campylobacter* colonization and load is the highest at processing age (41 days).

Key Words: Campylobacter

P241 Speciation of Campylobacter spp. isolates from poultry sources with end-point PCR and real-time PCR Hung-Yueh Yeh*, Jodie Plumblee-Lawrence, N. Cox, T. Thompson, M. Berrang, A. Hinton, Jr., S. Brooks *USDA ARS USNPRC*

Statement of the purpose of the experiment: *Campylobacter* is the leading foodborne pathogen that causes human acute gastroenteritis worldwide. Accurate, rapid identification of *Campylobacter* at the species level is essential for surveillance and effective intervention. Because of their fastidious growth and sophisticated biochemical requirements, speciation of *Campylobacter* by traditional methods is problematic. Polymerase chain reaction (PCR) is an alternative for *Campylobacter* speciation. The purpose of this communication was to compare end-point PCR and BAX® System Real-Time PCR Assay for *Campylobacter* speciation.

Description of the 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 in duplicate. Next day, colonies from one plate were examined using the BAX® System Real-Time PCR Assay, and colonies from the other plate were examined using the end-point PCR assay. Three sets of primers as previously described were used in the end-point PCR assay. *Campylobacter* genomic DNA used in the end-point PCR assay was isolated by boiling or by using a commercial kit.

Results: The results show 181 isolates and 69 isolates were identified as *Campylobacter jejuni* and *Campylobacter coli*, respectively, from both assays. We also found that boiled DNA from seven isolates were not detected in the end-point PCR assay, but were detected using DNA templates isolated with a commercial kit, indicating that unknown inhibitors may exist in the DNA preparation with the boiling method.

Conclusion: Based on our results, we concluded both PCR assays were suitable for speciation of *Campylobacter* isolates. Further evaluation of DNA preparation for PCR assays is needed.

Key Words: Campylobacter, PCR, speciation

P242 Efficacy of trace mineral-amino acid complexes in a necrotic enteritis challenge model Charles Hofacre*[1], Greg Mathis[1], Brett Lumpkins[1], Marco Rebollo[2], John Smith[3] *[1]Southern Poultry Research Group; [2]Zinpro Corporation; [3]Southern Poultry Research Group*

Zinc and copper are known to be important in maintaining intestinal integrity, especially under challenge situations, but there is little information regarding their role in ameliorating the effects of necrotic enteritis (NE). Two trials utilized the NE challenge model of Hofacre et al. 1998. Battery cages with six treatments and ten replicates were used in the first trial: NRC diet unchallenged; NRC diet challenged; 100 ppm each $ZnSO_4$/$MnSO_4$ challenged; 60 ppm $ZnSO_4$/ 40 ppm Availa-Zn/ 100 ppm $MnSO_4$ challenged; 60 ppm $ZnSO_4$/ 60 ppm Availa-Zn/ 100 ppm $MnSO_4$ challenged; 60 ppm $ZnSO_4$/ 40 ppm Availa-Zn/ 60 ppm $MnSO_4$/ 40 ppm Availa-Mn challenged. The second trial was a floor pen study with five treatments and ten replicates: 100 ppm $ZnSO_4$/ 100 ppm $MnSO_4$/ 20 ppm $CuSO_4$ challenged; 60 ppm Availa-Zn/ 100 ppm $MnSO_4$/ 20 ppm $CuSO_4$/ 40 ppm $ZnSO_4$ challenged; 40 ppm $ZnSO_4$/ 100 ppm $MnSO_4$/ 60 ppm Availa-Zn/10 ppm Availa-Cu challenged; 60 ppm $ZnSO_4$/ 60 ppm $MnSO_4$/ 20 ppm $CuSO_4$/ 40 ppm Availa-Zn/ 40 ppm Availa-Mn challenged; 50 g/ton BMD challenged. Results: Battery NE mortality: 15%^{a} challenge control, sulfate 2.5%^{b}, Availa Zn low 0.0%^{b}, Availa Zn high 2.5%^{b} and Availa Zn/Mn 1.3%^{b}. Results: Floor pen NE mortality: sulfate 12.7%^{a}, Availa Zn 5.1%^{b}, Availa Zn/Cu 8.6%^{ab}, Availa Zn/Mn 3.9%^{b}, BMD 7.6%^{ab}. These studies demonstrated that replacing a portion of the dietary zinc, copper and manganese with metal-amino acid complexed minerals can be effective in reducing the effects of *E. maxima* and *C. perfringens* in an NE challenge model.

Key Words: necrotic enteritis, amino-acid complexed minerals

P243 Comparison of the impact of monobutyrin, other glycerides, and glycerol (SiloHealth 104), sodium formate (Amasil NA) and bacitracin methylene disalicylate (BMD) on performance and necrotic enteritis when fed to broiler chickens challenged with mild coccidia and clostridium perfringens (Cp). Mike Coelho*[1], Peter Ader[2] *[1]BASF Corporation; [2]BASF SE*

A total of 3,744 Ross 708 birds were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (52 birds/pen x 8 treatments x 9 replications). Birds were blocked by weight and sex. The treatments were T1=PC, no additive, no Cp; T2=NC, no additive, Cp; T3=NC +4/4/4 kg/MT SiloHealth 104 (SH) in starter/grower/finisher; T4=NC+ 4.0/2.5/1.5 kg/ MT SH; T5=NC+2.5/1.50/0.75 kg/MT SH; T6=NC+8/8/8 kg/MT Amasil NA (NA); T7=NC+2.5 kg SH + 4.0 kg/MT AN/ 2.5 kg SH + 4.0 kg/MT AN/2.5 kg SH + 4.0 kg/MT AN; and T8=NC+ Coccidiostat+50 g/MT BMD. Least significant difference was used to compare means of treatment groups. T1-8 had litter inoculated on D0 with 2,500 oocytes of E. acervulina and E. maxima/bird, and T2-8 had litter inoculated with $5*10^4$ CFU/bird of E. coli and $5*10^4$ CFU/bird of Cp. Birds and feed were

weighed on D0, 14, 21, 35 and 42, and performance parameters were measured for each period. On D21 and 42, three birds from each pen were sacrificed and examined and scored on the degree of severity of necrotic enteritis lesions (0-3). D14 avg body weight gain/bird (BWG) (346.39, 331.06, 340.29, 340.07, 336.20, 343.62, 344.38 and 341.37 g, P<0.05, respectively; FCR corrected (1.036, 1.075, 1.051, 1.059, 1.042, 1.040 and 1.047, P<0.05, respectively; mortality (1.07, 4.27, 1.92, 2.35, 1.28, 1.07 and 1.71%, P<0.05, respectively; D21 BWG (789.84, 751.62, 775.16, 775.32, 764.59, 781.57, 787.17 and 777.78 g, P<0.05, respectively; FCR corrected (1.283, 1.334, 1.304, 1.303, 1.317, 1.293, 1.289 and 1.297, P<0.05, respectively; mortality (1.50, 4.91, 2.78, 2.56, 3.42, 1.92, 1.28 and 2.14%, P<0.05, respectively; D42 BWG (2443, 2320, 2395, 2395, 2361, 2415, 2433 and 2409 g, P<0.05, respectively; FCR corrected (1.76, 1.84, 1.81, 1.79, 1.79, 1.81, 1.78, 1.77 and 1.79, P<0.05, respectively; mortality (1.932, 6.763, 3.865, 3.866, 4.589, 2.657, 2.174 and 3.140%, P<0.05, respectively; D21 SI lesion scores (0-3) (0.204, 1.667, 1.019, 1.037, 1.333, 0.778, 0.667 and 0.796, P<0.05, respectively): D42 SI lesion scores (0-3) (0.178, 1.233, 0.656, 0.722, 0.889, 0.556, 0.456 and 0.644, P<0.05, respectively). In conclusion, monobutyrin, sodium formate and BMD significantly improved BWG, FCR and mortality.

Key Words: necrotic enteritis, monobutyrin, sodium formate, clostridium perfringens

P244 Evaluation of effectiveness of three probiotic products and a prebiotic to control Clostridium perfringens induced necrotic enteritis in broilers Charles Hofacre*[1], Anis Hedhi[2], Ruth Raspoet[2], T.J. Gaydos[3], Tadele Kiros[3], Eric Auclair[2] *[1]Southern Poultry Research Group; [2]Phileo - Lesaffre Animal Care - France; [3]Phileo - Lesaffre Animal Care*

Necrotic enteritis (NE) caused by *Clostridium perfringens* (CP) is a devasting bacterial disease responsible for economic losses, by increasing mortality because of damage to the broiler small intestine. Since ban of antibiotics use as growth promoters (AGPs) in some markets like in Europe and no antibiotic ever programs (NAE) in U.S., economic losses have become more prevalent. To prevent NE, supplementing probiotics and prebiotics in feed has been considered as an alternative to AGPs and seems to improve both bird health status andanimal welfare.

The present study assessed the effect of four products administered in the feed (prebiotic product A: 250g/t, and 3 probiotic products: B, C and D at 5, 6.2 and 5.5 log CFU/g of feed, respectively) for reducing the effects of CP in a moderate NE challenge.

Three-thousand day-of-hatch chicks were assigned to six groups: (i) two control groups, negative control without NE challenge (NCtrl) and positive control (PCtrl) with NE challenge; (ii) four treated groups (A, B, C, D) with ten-replicate pens per group and 50 birds per pen.

The trial lasted 42 days, and NE was induced by adding CP to the water at dose $1x10^8$ CFU/ml on d 18 and 19.

After the NE challenge, at d 21, no significant difference in small intestine lesion scores was recorded. On d 35, treated groups had lower FCR and heavier body weight than both NCtrl and PCtrl (10% and 16% on average respectively). At d 42, group B had the lowest mortality adjusted FCR (1.72) compared to D (1.76). Animals from groups A, B, C, D had higher body weight compared to PCtrl, showing that all products were successful in alleviating subclinical effects of CP.

Although probiotic B was used at the lowest concentration in feed it outperformed the other probiotic products by significantly reducing mortality: 8.2% versus 11.4% and 9.6% for product C and product D, respectively.

Key Words: Necrotic enteritis, probiotic, Clostridium perfringens, broilers performance

P245 Histomonas meleagridis oral inoculation of fed and fasted turkeys fails to cause blackhead disease. Catherine Fudge*[UG], Elle Chadwick, Robert Beckstead *North Carolina State University*

Histomonas meleagridis is a protozoal parasite that causes blackhead disease in gallinaceous birds. Blackhead disease manifests in turkeys with lesions in the ceca and liver resulting in up to 100% flock mortality. Turkeys become infected with *H. meleagridis* by consuming *Heterakis gallinarum* eggs carrying the parasite to the ceca or by direct cloacal infection. Recent reports have challenged this idea, suggesting that turkeys can also be infected by orally consuming *H. meleagridis*. The objective of this study was to determine if *H. meleagridis* oral inoculation of turkeys during various feed withdrawal times would lead to blackhead disease. We hypothesized that as feed withdrawal time is extended, blackhead disease rate would increase in *H. meleagridis* orally inoculated turkeys. To test this hypothesis, 140 nine-day old turkey poults were subjected to the following treatments: negative control (no inoculation), 0 hour oral inoculation, 0 hour cloacal inoculation, 4 hour oral inoculation, 4 hour cloacal inoculation, 12 hour oral inoculation, 12 hour cloacal inoculation. The cloacal inoculation acted as the positive control for each time period. Water was provided *ad libitum* throughout the study. On day 9, all inoculated poults were fasted for 12 hours. During this feed withdrawal, poults were orally or cloacally inoculated with 85,000 histomonads per bird at one of the three time periods. The trial was terminated 10 days post infection and poults were scored for signs of blackhead disease. No control birds showed clinical signs of infection. The cloacal inoculation infection rate for the 3 time periods were as follows: 72% (0 hour), 82% (4 hour), and 67% (12 hour). For the oral inoculation, there was a 0% infection rate at all three time periods. Infection rates were compared using JMP Pro 13 and differences of means were determined using Tukey HSD ($p \leq 0.05$). There was no significant difference in infection rate for the three time periods when analyzing a specific inoculation route, suggesting that feed withdrawal does not affect direct inoculation rates. The oral inoculation route failed to cause blackhead disease indicating that once a poult has ingested feed the digestive tract is not a suitable environment for passage of *H. meleagridis* to the ceca.

Key Words: Feed withdrawal, blackhead disease, Turkey

P246 Establishing monoclonal Eimeria spp. isolates in immunosuppressed chickens Miranda Carrisosa*[GS], Kenneth Macklin, Ruediger Hauck *Auburn University*

Due to their life cycles, it is impossible to isolate *Eimeria* spp. *in vitro*. However, a single oocyst can replicate and produce thousands of oocysts within its host. This can be used to establish monoclonal isolates by single oocyst purification. In the first part of the study, it took 24 attempts to establish two monoclonal isolates. In the second part, we tested if immunosuppressing the chicks would make the process more effective. Five chicks were given a dose of cyclosporine to suppress their immune system, specifically to reduce T-cells, and five were left as a control. All chicks were given a single oocyst isolated from a fecal sample containing *Eimeria maxima*. After 6 days post infection, feces from each bird were collected and examined using the McMaster Method. Oocysts present in the same fecal samples were concentrated and purified. DNA was extracted, and PCR was used to confirm presence of *Eimeria*spp. Three of ten bird samples – 1 immunosuppressed and 2 non-immunosuppressed – were positive for *Eimeria* spp. by PCR, no oocysts were visualized by microscopy. In further passages of the samples that were positive by PCR, no oocysts were shed in the feces. The results indicate that immunosuppression may not increase the chances of obtaining monoclonal *Eimeria*isolates.

Key Words: Eimeria, coccidia, immunosuppression, infection

P247 Protection efficacy of a recombinant Herpesvirus of turkey infectious laryngotracheitis (rHVT-LT) vaccine when administered at double the commercial dose Daniel Maekawa*[GS], Sylva Riblet, Maricarmen García *University of Georgia*

Infectious laryngotracheitis (ILT) is an economically important respiratory disease that affects the poultry industry worldwide. The disease is caused by Gallid alpha herpesvirus I (GaHV-1) commonly known as Infectious laryngotracheitis virus (ILTV). Control of the disease is built on biosecurity and vaccination. A new generation of recombinant herpesvirus of turkey (rHVT) vector vaccines expressing antigens of ILTV are being used worldwide. The rHVT-LT vaccines are characterized by their lack of transmission and reversion to virulence. Experimental evidence has shown that rHVT-LT vaccines induce partial protection because challenge virus replication still occurs in the trachea of vaccinated chickens. However, it is conceived that protection efficacy of rHVT-LT vaccines would be improved by increasing the number of plaque forming units (PFUs) each embryo receives. The objective of this study was to evaluate the protection efficacy of a rHVT-LT vaccine when its administered *in ovo* at a 2x commercial dose of 13000 PFUs in specific pathogen free (SPF) chickens. The protection induced by the rHVT-LT vaccine was assessed by the ability of vaccinated chickens to prevent clinical signs of the disease, to lessen challenge virus replication in the trachea, and to avoid body weight loss after challenge. Independently of the dose administered, 6500 or 13000 PFUs, both vaccinated-challenge groups of chickens showed significant decrease in clinical signs, body weight gain was maintained after challenge and the challenge virus genome load in trachea was reduced between 69 and 72%. Therefore, increasing the rHTV-LT dose from 6500 to 13,000 PFU did not enhanced the level of protection against ILTV challenge.

Key Words: rHVT-LT, PFUs, Protection, Recombinant

P248 Effect of dietary spray-dried porcine plasma on humoral immune response to Newcastle disease virus vaccination in broiler chicks Candice Blue*, Yewande Fasina *North Carolina Agriculture and Technical State University*

The early detection of Newcastle disease virus (NDV) infection and the assessment of the immune status induced due to vaccination are important for effective control measures. This study was conducted to evaluate the effect of spray-dried porcine plasma, an immunomodulatory feed additive, on humoral immune response to Newcastle disease virus vaccination in broiler chicks. Day-old (240) Ross 708 male chicks were obtained from a commercial hatchery, weighed, and randomly assigned to six dietary treatments. Treatment 1 (CX) consisted of chicks fed unmedicated corn-soybean meal (SBM) basal without SDP. Treatment 2 (MX) consisted of chicks given unmedicated corn-SBM basal into which Bacitracin methylene disalicylate (BMD) was added at 0.055g/kg diet. Treatments 3 (SP1), 4 (SP2), 5 (SP3), and 6 (SP4) consisted of chicks given unmedicated corn-SBM basal into SDP was added at 10, 20, 30, and 40 g/kg diet, respectively. Each treatment consisted of 4 replicate pens, with each pen housing 10 chicks. During the experiment, growth performance (i.e. body weight, weight gain, feed intake, feed conversion ratio (FCR), and mortality were recorded weekly. On d 21, blood was collected, and serum was harvested after centrifugation at 12,000g for 10 minutes. The serum was subjected to enzyme-linked immunosorbent assay (ELISA) for the detection of NCD-specific antibody titers. Results showed that SP2 (0.5707) and SP4 (0.4570) had higher (P<0.05) levels of NCD-specific antibodies compared to MX, while those of SP3 and SP1 treatments were similar to MX. It was concluded that dietary inclusion of SDP enhanced humoral immune response to NDV.

Key Words: Newcastle's Disease Virus, Spray-dried plasma, Antibody titers, Broiler chicks

P249 Evolution of Mexican H7N3 highly pathogenic avian influenza viruses Sungsu Youk*[1], Dong-Hun Lee[1], Helena Ferreira[2], Claudio Afonso[1], Angel Absalon[3], David Swayne[1], David Suarez[4], Mary Pantin-Jackwood[1] *[1]Agricultural Research Service, U.S. Department of Agriculture; [2]University of Connecticut; [3]University of Sao Paulo; [4]Vaxbiotek S.C.*

Outbreaks of H7N3 highly pathogenic avian influenza (HPAI) have been occurring in poultry in Mexico since 2012 and vaccination has been used to control the disease. We conducted full genome sequencing on eight recent H7N3 HPAI viruses from 2015 to 2017 and compared them with ancestral sequences to characterize the virus evolutionary dynamics. Phylogenic analyses indicated that the Mexican H7N3 HPAI virus had diverged into at least three clusters by 2015 (A1, A2, and B). There were three isolates with heterologous clustering for the PB2, PB1 and NS genes but no reassortment event with non-H7N3 viruses. The HA protein of each cluster showed a different number of basic amino acids (n=5-7) in the cleavage site, and six different patterns at the predicted N-glycosylation sites. By calculating and comparing the nucleotide substitution rate of North American H7N3 influenza viruses in time (wild bird origin viruses and chicken origin viruses from Mexico), we found that the nucleotide substitution rates in PB2, PB1, PA, HA, NP, and NS genes greatly increased once the virus introduced into poultry in Mexico (the highest increase 3.7 folds in PB1). In contrast, no significant increase was observed in NA and M genes. The global nonsynonymous and synonymous ratios imply strong purifying selection driving the virus evolution. Forty nine positively selected sites were detected in the Mexican H7N3 viruses, several of these amino acid changes also found in other studies as associated with adaptation of influenza virus in poultry. Under the selective pressure on the virus as it adapted to a new host and under immune pressure due to vaccination, the H7N3 HPAI virus has branched into distinct genetic clusters, still remaining highly pathogenic for terrestrial poultry. Continuous monitoring of the H7N3 HPAI virus is important for better understanding of evolutionary dynamics and further implementing biosecurity measures including vaccination.

Key Words: Avian influenza, H7N3, Mexico, Evolution

Environment, Management and Animal Well-Being

P250 Effect of dual light warmth on broiler distribution, feeding and drinking behavior Douglas Aldridge*, Colin Scanes, Michael Kidd *University of Arkansas*

A choice system was employed to determine the preference of Cobb 700 broilers for two commonly used light emitting diodes (LED) of differing warmth: cool- 5000 K and warm- 2700 K, each at 20 lx. The lighting regiment was set at 16:8 at d14. The choice system consisted of two pens (1.22 x 1.22 m) separated by a raised divider along a common sidewall allowing birds to pass between pens. Each system (n=6) contained a pen illuminated by a cool and warm bulb with feed and water available in each. A remote video system was used to observe the number of birds within each pen as well as feeding and drinking. Observations were made on d16 d24, d33 in trials 1 and 2 together with d41 (trial 1) and d44 (trial 2). On each of the days samples were collected during 4 time periods: 1- the first hour of the photophase, 2- a random hour during the first half of the photophase, 3- a random hour during the second half of the photophase, and 4- during the final hour of the photohase. Scan sampling methods were used and the average of observations every 15 minutes during the sampling hour were used as the value. In trial 1, a greater percentage of broilers (p<0.05) were observed under warm than cool LED sources during periods times 1 and 4 for each day sampled. In addition, at three other sample days (d16 sample 2, d33 sample 2 and d41 sample 3), more birds were also observed under

warm light than cool. For trial 2, a similar pattern was seen with more birds were observed under warm at sample time 1 and 2 but not 3 or 4. This study suggest that broiler chicken exhibit a preference for warm LED lighting at some but not all the times of day.

Key Words: Light, LED, Choice

P251 Behavioral response of broilers chickens fed with probiotic Ianê Almeida*[1], Leonardo Vega[2], Ibiara Almeida Paz[1], Mariana Borges[1], Gustavo Chaves[1], Marconi Silva[1], Caio Ouros[1] *[1]UNESP - FMVZ - DPA; [2]F & S Consulting*

The aim of this study was evaluated the behavior and welfare in broilers chickens suplemented with probiotic, whereas animals fed probiotics have an improvement in intestinal health. For this, were used 500 male chicks one day old of the Cobb slow strain, equally distributed in the treatment. The experimental design was a randomized blocks with two treatments (T1:control without commercial probiotic administration and with use of antibiotic; T2: with commercial probiotic supplementation, by feed, and without use of antibiotic) and 5 repetitions with 50 birds each. The birds of treatment 2 were also inoculated with the commercial probiotic still in the hatchery. The welfare was evaluated by two tests: approach test (an evaluator went into pen and waited 3 minutes, after that time stretched his arms and counted how many animals could touch) and catch test (the birds were taken in groups of 3 for a calm region, surrounded and with a diameter of 1 meter, after 3 minutes the evaluator tried to hold the birds, simulating the catch, scores are assigned varying from 1 to 5). The data were analyzed using the statistical program SAS 9.2. There was a improvement for welfare with de probiotic intake. For the approach and catch tests, the birds that was fed diets containing antibiotics presented worse results, showing that they were less calm. In conclusion, the use of commercial probiotic in the broiler diet provided less stressed birds, consequently, in better welfare conditions, which can reduce losses in the quality of the final product, such as carcass scrapes or broken wings, since these birds are less stirs.

Key Words: Poultry Production, Welfare, Antibiotic

P252 BEHAVIORAL RESPONSE OF BROILER TO ENVIRONMENTAL ENRICHMENT Gustavo Chaves*, Ibiara Almeida Paz, Ianê Almeida, Marconi Silva, Caio Ouros, Mariana Borges, Bruno Lala *UNESP - FMVZ*

In this study, 400 one day old broiler chickens were housed in experimental facility, divided into two parts, equipped with drinkers and automatic feeders. At 7d old, 200 animals were submitted to the Environment Enriched (EE) with metallic strips hanging at the height of the back and a plastic floor with 1200cm² disposed 4cm from the ground. The other 200 broilers were in an Environment Not Enriched (ENE). At 35d old broilers were submitted to three well-being tests, Latency to Lie (LTL), approach test and catch test. LTL is a method in which the time spent by broiler to sit when exposed to an uncomfortable situation. For this, 3cm of water was placed at room temperature in a plastic box, where 6 broilers were allocated at same time. The time was recorded to bird took until the first attempt to lie down. If the broiler was still standing after 370s, the test was stopped. In approach test an examiner entered boxing and waited 3min, after this time stretched his arms and counted how many animals could touch. In catch test, the animals were taken in groups of 3 broilers to a calm place, surrounded and diameter of 1m, after 3min the evaluator tried to hold the birds, simulating the catch, using score 1 (S1): animal doesn't move; S2: animal walks but does not vocalize or run; S3: animal walks and vocalizes; S4: animal runs and vocalizes; S5: animal jumps to try to avoid catching. LTL data and approximation test were submitted to F test ($P<0.05$) and to the catch test data using the chi-square test ($P<0.05$). Broilers raised in the EE had better responses to behavioral tests. Referring to the plastic floor, animals could climb and exercise their paws, LTL test showed that they were able to stand longer than those who didn't have access to the floor. In approach and catch tests, broilers that were in EE had better results, demonstrating that they were calmer. There are reports that the quality of life of animals is increased when plastic floor and objects are provided to reduce the monotony of physical environment, with reduced expression of fear and increase the exploratory activity. EE provided less frightened broilers and more adapted to the daily management. This improves broilers welfare and can reduce quality losses of the final product (FAPESP 2018/07801-2).

Key Words: Poultry Production, Welfare, Well-being

P253 In ovo injected Bacillus subtilis probiotic serotypes alter broiler hatchability and intestinal microflora Josie Gamble*[UG], Claudia Castaneda, Kelley Wamsley, Christopher McDaniel, Aaron Kiess *Mississippi State University*

Probiotics traditionally provided in the feed have been administered *in ovo*. However, recent research in our lab has proven that *Bacillus subtilis,* a probiotic commonly provided in feed, has demonstrated that unlike other probiotics, it can be detrimental when *in ovo* injected. Therefore, the objective of this research was to determine if serotype of *B. subtilis* influences hatchability, chick performance or intestinal microflora. . On d18 of incubation, 540 fertile broiler eggs were *in ovo* injected for each of 4 treatments: T1=Marek's Disease (MD) vaccination, T2=MD + *B. subtilis* (ATCC 6051), T3=MD + *B. subtilis* (ATCC 8473), and T4=MD + *B. subtilis* (ATCC 9466). Eggs were then transferred to 3 hatchers/treatment. It should be noted, T2 was detrimental to hatchability in a previous study. At hatch, % hatch of transfer, average chick weight, and hatch residue was obtained. A total of 10 chicks/battery cage and 10 cages/treatment were used. Growth performance characteristics (BW, BW gain, feed intake, and feed conversion) and microbial samples from the ileum and ceca were collected on d 0, 7, 14, and 21. Data were analyzed using a completely randomized design for hatchability and a randomized complete block design for live performance and bacterial counts. No differences were observed for % mid dead, cracked, and cull chicks ($P>0.05$). However, % hatch of transfer was greater for T1, T3 and T4 compared to T2 ($P<0.001$). T2 had significantly higher % late dead and % pips when compared to T1, T3, and T4 ($P=0.002$ and $P<0.001$). Chicks hatched from T2 were not vigorous and, thus, not used for the grow-out trial. No differences were observed for growth performance characteristics ($P>0.05$). However, ileum T3 and T4 had equal or fewer overall bacterial counts when compared to T1 on every sampling day, except for d21 where T4 had higher bacterial counts ($P<0.05$). For the ceca, T3 and T4 had equal or fewer bacterial counts than T1 on every sampling day, except on d14 where T4 had higher aerobic counts ($P<0.05$). In conclusion, the serotype of *B. subtilis in ovo* injected can have a direct impact on hatchability. In addition, each *B. subtilis* serotype may modify the intestinal microflora with potential to reduce pathogenic bacteria present in young broilers.

Key Words: Probiotics, in ovo, Bacillus subtilis, hatchability, microflora

P254 Investigating temperature and humidity in a commercial poultry transport trailer. Benjamin Zuniga*[GS], Trever Crowe *University of Saskatchewan, College of Agriculture and Bioresources, Department of Animal and Poultry Science*

The objective of this study was to characterize temperature and relative humidity within a fully-loaded, modified broiler transport trailer during cold ambient conditions. The trailer incorporated solid tarps, with openings in the floor and along the midline of the trailer's roof. Temperatures were recorded, using 192 miniature data loggers positioned in groups of 16 in 12 locations in one-half (left-hand side) of the load. At each location within the truck, the data loggers were organized into four groups of sensors, where all sensors within each group were the same distance from the tarp. The data was collected for four ambient temperature ranges: -23°C to -20°C, -20°C to -10°C, -10°C to 2°C, and 2°C to 15°C. For each range, ambient temperatures and trailer internal temperatures were recorded during transportation from the farm to the processing plant. Data recorded

during the interval 25 to 15 minutes prior to plant arrival were used for the analysis. Delta temperature values were calculated by subtracting the ambient temperature from the temperatures recorded internally. Mean delta temperature values were then added to an assumed ambient temperature to generate representative mean conditions. A similar procedure to the temperature data was used to analyze relative humidity. Data was analyzed using SAS 9.4 in a 12×4×4 factorial design, with frame location and tarp distance as main effects on each temperature range. Differences were significant when $P \leq 0.05$. Positions closest to the tarp at all locations and ambient temperature ranges were coldest, compared to temperatures at other positions within the same location. The next coldest location was near the midline of the trailer ($P \leq 0.01$). Generally, temperatures were stratified, with temperatures at lower locations being lower than temperatures in locations directly above ($P \leq 0.01$) for all locations except the most rearward position when ambient temperatures were between -10°C and -20°C. The temperature trends from the tarp to the middle of the trailer increased, reaching a peak approximately one-quarter of the way across the trailer. The temperature then tended to decrease toward the middle of the trailer due to the openings in the floor and ceiling.

Key Words: Broiler, Transport, Temperature, Relative humidity

P255 Improving brooding efficiency for small holder broiler farms in rural Rwanda Robert Mihelic*[GS], Emily Urban, Brynn Voy, Tom Gill *The University of Tennessee, Knoxville*

Purpose: Rwanda has experienced rapid economic growth over the past two decades, but still faces the dual challenges of poverty and malnutrition. Poultry farming is a viable option to increase family income and dietary protein. Rearing chicks requires constant use of a fuel, usually charcoal burned in clay ovens, as a heating source during the brooding period. Brooding with charcoal constitutes 1/6 of the cost of poultry rearing in Rwanda. Charcoal prices are increasing, which threatens the potential for poultry farming to provide economic and public health benefits to Rwandans. The purpose of this study was to develop a poultry brooding method to reduce the need for charcoal for broiler farmers in Musanze, Rwanda. *Experimental Design:* Day-old broiler chicks were obtained from a local hatchery and brooded in coops (100 chicks/coop). Four methods to reduce charcoal consumption were tested during the brooding period (day 1- 21): i) Metal Hood Heat Reflector - Corrugated sheet metal with a surface area of 2463cm^2 was suspended 30cm above the clay oven inside the coop; ii) Mylar Hood Heat Reflector – Mylar was fastened to a 90cm diameter circular wire and suspended 60cm above clay oven; iii) Biomass Briquettes – Biomass briquettes made of compressed sawdust (60%) and rice hulls (40%) were used as charcoal replacement; iv) Half-house Brooding – The coop was divided in half by a plastic curtain creating a designated brooding area. Charcoal was weighed daily to determine the amount required to maintain brooding temperature. Chicks were weighed weekly to assess growth rate and ambient coop temperature was taken daily while brooding. Data[Voy, Bryn1] were analyzed statistically by ANOVA and mean separation. *Results:* The coop temperature variation did not differ between the experimental groups. The control coop, Mylar hood and metal hood coops used 55.3kg (3 bags), 29.6g (2 bags) and 25.3g (2 bags) [Voy, Bryn2] of charcoal, respectively. Half-house brooding used 1.5 bags. The biomass group was terminated due to significant smoke production and stunted growth rates; growth rates did not differ from control for other treatments ($p<0.05$). *Conclusion:* Poultry farmers in Rwanda should adopt a half-house brooding method as it resulted in a 50% reduction in fuel consumption.

Key Words: Broiler, Brooding, Sustainable, Africa, Efficiency

P256 Evaluation of thermal conditioning during incubation and dietary fatty acids on broiler performance during heat stress in late rearing. Kelly Brannan*[1,2], Kimberly Livingston[3], Teresa Herman[2], Lisa Wilson[2], Herbert Ledford[2], Christine Jansen van Rensburg[1] *[1]University of Pretoria; [3]North Carolina State University; [2]North Carolina Department of Agriculture and Consumer Science*

Heat stress continues to be an issue for broilers, especially in developing countries where environmentally controlled housing is limited. Even mild heat stress can result in oxidative damage on a cellular level that can hinder bird performance. The use of thermal conditioning during incubation is a developing heat management strategy, however it's unclear to what degree this treatment may limit bird performance as high temperatures during incubation are well associated with poor chick quality and later broiler performance. Increasing dietary fat is another heat management tool and there may be an advantage in feeding specific fatty acid profiles during heat exposure. This study will evaluate the influence of thermal conditioning on chick quality as well as broiler performance in combination with dietary fatty acid profiles under heat stress during late rearing. Embryos were exposed to a thermal conditioning (TC) profile by increasing setter temperature to 39.5C for 12h daily from incubation day 7-16 or maintained at a control (CN) profile. Dietary treatments consisted of finisher diets supplemented with either soya oil (S), poultry fat (P), or olive oil (O), formulated to energy equivalent levels. A period of heat stress of 32C for 4h was applied at 43d. Production parameters were evaluated weekly and oxidative stress was evaluated by TBARS assay at 0, 42, and 43d. Data were analyzed using JMP (2013). TC did not alter chick BW, residual yolk, or TBARS at hatch. Body weight (BW) was significantly influenced by incubation only after 14d of age, with TC consistently exhibiting lower BW until 43d. Dietary treatment was not demonstrated to interact significantly with incubation treatments. TBARS were noted to be higher in TC birds at 42d, however the difference disappeared after the heat stress. TC did not appear to be beneficial to bird growth past 14d and heat stress increased TBARS for both groups similarly.

Key Words: Thermal conditioning, Fatty acid, Heat stress, TBARS, Incubation

P257 N-acyl-phosphatidylethanolamines (NAPEs) producing probiotic improves young broiler response to stress caused by individual housing. Reagan Leger*[1], Kristin Moncada[1], Tara Price[1], Sohini Bhatia[1], Sean Davies[2], Suresh Pillai[1], Rosemary Walzem[1] *[1]Texas A&M University; [2]Vanderbilt University*

Feed consumption, immune function and behaviors are key variables in poultry performance and welfare. Neuroactive N-acylethanolamines (NAE) are active in appetite control, behavior and immunity. We found tissue specific RNA expression of key NAE metabolizing enzymes was responsive to feed intake in breeder hens. The effects of these compounds in young birds are unknown. N-acyl-phosphatidylethanolamines (NAPEs) are immediate NAE precursors and can be produced by probiotic E. coli Nissle 1917 carrying expression plasmid for the A. thaliana NAPE acyltransferase. We used this probiotic (pNAPE-EcN) and empty vector control bacteria (pEcN) for bulk probiotic production and live bird trials.

Thirty Cobb 500 broiler chicks were housed in individual 1'x1' pens, with 12 never handled chicks housed in groups of four in 1'x1' pens. Chicks received a daily 0.5mL oral dose for two weeks to either pNAPE-EcN (10^7 CFU), pEcN (10^7 CFU) or 0.125% gelatin vehicle. Bodyweights were recorded daily, blood and livers were harvested at week 2. Serum corticosterone was measured by ELISA. Liver NAPE content used LC/MS (https://doi.org/10.1172/JCI72517). Growth rates for individual birds were found to best fit $y = 0x^p$ where p = power function. Individual values for each group were averaged and difference in mean values compared using a t-test. Mean values for p and R^2 for each group and significance was seen when comparing pNAPE-EcN treated chicks to all other groups ($p<0.01$).

Corticosterone concentrations among singly housed chicks were similar and ~ 5-fold higher than group housed chicks being 25.8 ± 3.4 ng/ml vs. 6.80 ± 0.69 ($p < 0.05$). Daily 1 minute restraint while oral dosing combined with individual housing can be considered a high stress situation for young poultry. Probiotics in this setting did not provide a universal benefit, albeit provision of the pNAPE-EcN probiotic appeared to provide benefit compared to pEcN alone as birds were 34g (10%) heavier (NS) or gelatin as birds were 19.8g (5.6%) heavier (NS). Housing and handling methods must be carefully considered when evaluating probiotics aimed at improving welfare.

AgriLife Research project #8738

Key Words: Stress, Broiler, Probiotics

P258 Development of on-farm euthanasia methods for individual turkeys and broilers Caitlin Harris*[1,2GS], Leonie Jacobs[3], Dianna Bourassa[4], L Bartenfeld Josselson[2], R Buhr[2] *[1]University of Georgia; [3]Virginia Tech; [4]Auburn University; [2]USDA-ARS, US National Poultry Research Center*

Because of the difficulty of euthanizing larger older birds (i.e. turkeys and broiler breeders) via manual cervical dislocation, alternative on-farm euthanasia methods need to be developed. The goal of this research was to develop and assess methods of euthanasia for larger older birds that would be safe to administer by a single operator. The assessed methods included: mechanical cervical dislocation, captive bolt, carbon dioxide (CO_2) inhalation, and electrocution (head-to-cloaca). In order to allow for each method to be performed by a single operator, a mobile bird euthanasia apparatus (MBEA) was constructed. A plastic traffic cone was attached to a gas cylinder cart and mounted at a 45º, which allowed for a bird to be inserted into the cone with the head exposed. Utilization of a plastic cone allowed for customization of the head opening based on bird size (5 and 6 wk old broilers to turkey hens and toms) as well as the non-conductivity for use during electrocution euthanasia. Use of a MBEA allowed the bird to be properly positioned for euthanasia by a single operator and minimized the clonic/tonic convulsions that may occur with these euthanasia methods. The Koechner euthanizing device (KED) was used for mechanical cervical dislocation by separating the skull from the spinal cord. Captive bolt euthanasia was administered with a turkey euthanasia device (TED) to induce brain concussion. Both KED and TED are large bird euthanasia devices currently on the market. In addition to these methods, a portable CO_2 inhalation euthanasia device was developed using a face mask to perform head-only gas stun/kill with a maximum concentration of 30% CO_2 at 1 min increasing to 70% at 3 min. Euthanasia by electrocution (120 V AC for 15 s) induced unconsciousness and cardiac fibrillation, resulting in hypoxia. For all euthanasia methods, birds were monitored for 4 mins to ensure absence of induced brain stem reflexes (nictitating eye membrane and toe pinch). In conclusion, the MBEA successfully allowed for the implementation of each euthanasia method by a single user. Of the four methods tested, TED and electrocution were the easiest to administer by one user, followed by KED and the CO_2 method requires additional modification to achieve a consistent death within 4 min.

Key Words: euthanasia, cervical dislocation, captive bolt, electrocution, carbon dioxide

P259 Is scatter feeding enriching for broiler chicks? Brittany Wood*[GS], Maja Makagon, Richard Blatchford *University of California, Davis*

The use of scatter feeding as enrichment is often recommended by animal welfare certification programs, however, there is little evidence of its efficacy. The aim of this study was to determine if scatter feeding would stimulate foraging behavior. Five individually marked broiler chicks were housed in each of 30 pens with ad libitum access to feed and water. Six pens were assigned to each of three scatter feeding enrichment treatments: dried mealworms (MW; known to be a high value feed item), whole wheat (WW; a recommended foraging enrichment item), or shavings (SH; control). The enrichment items were scattered on the pen floor three mornings per week from 2-6 woa. Chicks were observed for 30 minutes after the enrichments were first scattered on weeks 2, 4 and 6. Using a 1-0 sampling strategy, observers recorded whether or not each of the chicks within a pen engaged in foraging during 1 minute every 5 minutes (total of 6 observations per chick * 5 chicks per pen * 6 pen replicates per treatment). Within the MW treatment all birds were observed foraging during at least 1 of the 6 observation periods, and 78% (week 2), 83% (week 4) and 89% (week 6) of the chicks foraged on at least 3 of the 6 observation periods. SH chicks engaged in less foraging: 47% (week 2), and 8% (weeks 4 and 6) birds never foraged, and only 10% (week 2), 44% (week 4) and 33% (week 6) of chicks were observed foraging during 3 or more observations. Within the WW treatment, 6% (2 week), 8% and 6% of chicks never foraged, and 53% (week 2), 33% (week 4) and 44% (week 6) foraged during at least half of the observations. A repeated measures ANOVA on data summarized by pen (experimental unit) revealed that chicks in the MW treatment spent a larger proportion of time foraging during the half hour after feed delivery than SH or MW. The results suggest that scatter feeding can promote foraging behavior at 2, 4 and 6 woa, but the value of the scattered item matters. Further research is needed to evaluate whether foraging triggered by the scattering of feed leads to improvements in other aspects of chicks welfare.

Key Words: broiler, enrichment

P260 Structural changes of eggs from hens receiving reduced Calcium diet at peak lay Lauren Nolan*[GS], Anthony Pescatore, Michael Ford *University of Kentucky*

Structural changes of eggs from hens receiving a reduced Calcium diet at peak lay were determined by standard egg quality measurements and scanning electron microscopy. Twelve hens were divided into two groups and were fed either a diet sufficient in calcium (4.96% Ca), treatment 1, or a calcium deficient diet (0.88%), treatment 2. Hens were housed individually in laying cages with feed and water supplied ad libitum. At 26 week of age (peak lay) eggs were collected for a 28-d period. Eggs were weighed and subjected to breaking strength, Haugh unit calculation from albumen height, dry shell weight (g), and percent shell was calculated. Eggshells were soaked in a bleach solution overnight, rinsed with water, and dried for 48 hrs., to remove shell membranes. Randomly selected membrane free shells were imaged using the scanning electron microscope (SEM). Data were analyzed using ANOVA in SAS. It was determined that there was a significant difference in egg weight, with heavier eggs from treatment 1 (56.9g), than eggs from treatment 2 (50.17g) ($P < 0.10$). As expected, significant differences in breaking strength were found between treatments. Eggs from treatment 1 had an average breaking strength of 3.860kgf, while treatment 2 were 2.976 kgf ($P < 0.10$). Eggs from treatment 1 had significantly higher percent shells at 9.26% compared to 7.10% shell from treatment 2. It was determined that hens on treatment 1 produced significantly lower Haugh unit (76.49HU) compared to treatment 2 (78.24HU). Although, Haugh unit was significantly different, both treatments created AA eggs (HU > 72). From the SEM images of the eggs, eggs from treatment 1 had a narrower mammillary cap (107.04μm) and had a thicker palisade layer (233.8 μm), compared to the wider (146.72 μm) mammillary cap on a thinner palisade layer (180.57 μm) of eggs from treatment 2. Reduction of Ca resulted in microscopic level changes in shell structure that ultimately impacted shell integrity. These changes to mammillary caps and palisade layer maybe similar to the effect on shell integrity that occur at later stages of lay.

Key Words: egg quality, Calcium, Scanning electron Microscopy

P261 Use of sound stimulus, analgesic and its effect on the gait score of broilers chickens Ibiara Almeida Paz*, Ianê Almeida, Mariana Borges, Caio Ouros, Gustavo Chaves, Marconi Silva, Kauan Alves *Universidade Estadual Paulista - UNESP*

This experiment was carried out aiming to assess walking manner and speed of broiler chickens with different gait scores (GS), with or without sound stimulus, and with or without administration of analgesic. To that end, 1,000 birds were evaluated by the GS test and 74 were selected for walking speed analyses. Weight at slaughter were obtained for comparisons. Walking speed analyses, both with and without analgesics and with and without stimulus were performed. Non-parametric statistics was applied to the GS data that did not meet the assumptions of the statistical model using Fisher's exact test ($P<0.05$). The analyses of data on speed, weight at slaughter were evaluated by ANOVA and compared by Tukey's test ($P<0.05$). Walking speed differed after acoustic stimulus with or without administration of metamizole sodium. Body weight was also different in each GS. It is thus concluded that the birds may feel discomfort when their GS is higher than 0, but that such discomfort may be suppressed when they are stimulated to walk. The walking speed of birds in each gait score was different and improved after analgesic administration, mainly for birds with GS higher 0, which allows concluding that their discomfort decreases with medication. However, acoustic stimulation of the birds, whether or not associated with analgesic use, also led to improved acceleration of the birds in each course. That leads to the conclusion that the fear caused by this stimulus overcomes the discomfort caused by locomotion issues. (FAPESP PROC 2015/19225-8).

Key Words: Poultry production, Welfare, Body Weight, Metabolic syndrome

P262 Relationship between weight gain and bone quality in broiler chickens Ibiara Almeida Paz*, Mariana Borges, Ianê Almeida, Caio Ouros, Gustavo Chaves, Marconi Silva, Kauan Alves *Universidade Estadual Paulista - UNESP*

The aim of this study was to evaluated the relationship between body weight, walking ability and frequency of black bone syndrome in commercial broilers strain. For the experiment, 1000 broiler chickens from a commercial strain were housed 1 day old in a blue house, with negative pressure, equipped with nipple drinkers, automatic helical feeders and a new bed. The gait score was evaluated in 100% of the birds weekly, and 144 birds was individually weighed and slaughtered like a commercial method. For the black bone syndrome evaluation, the left thighs and drumstick were used. The data were submitted to the statistical program SAS 9.2 and evaluated according to their behavior by ANOVA and Tukey's test ($P <0.05$) or by Chi-Square test ($P <0.05$). There was a linear increase in the worsening of the walking ability of the birds. It was also found that the heavier the bird, the higher the incidence of black bone syndrome. The birds that presented worsening walking ability up to approximately 28 days old had a lower body weight at 42 days, causing a loss of productivity and that birds with higher body weight also present a higher frequency of black bone syndrome. (FAPESP PROC 2015/19225-8).

Key Words: Poultry production, Welfare, Body Weight, Metabolic syndrome

P263 Effect of different drinking water temperature on performance, egg quality, serum biochemical profile and yolk fatty acids of laying hens during summer period Chun Ik Lim, Ho Sung choe, Kyeong Seon Ryu*, Muhamad Masud Rana *Chonbuk National University*

The aim of this study was to investigate the drinking water temperature effects on performance, egg quality, serum characteristics and yolk fatty acids of laying hens in summer season for a period of 41 weeks on 23th April to 9th September 2018. Five hundred and forty, Hy-Line brown hens were arranged in a completely randomized design of 3 treatment groups with 5 repetitions (2 hens/cage and dimension of cage 30.5 × 40.6 cm^2). One of the following treatments: Control T1 (ordinary tap water), two fixed water temperature which was maintained by automated heating panel T2 (15.0±0.5^{o}C) and T3 (25.0±0.5^{o}C) respectively. Hens were fed a commercial layer diet of 2750 kcal kg^{-1} ME, 16% CP, 4.0% Ca and 0.30% NPP. The average temperatures were recorded in house 17.5^{o}C, 21.5^{o}C, 25.0^{o}C, 33.0^{o}C and 27.4^{o}C respectively, for each 28 days period. Parameters including performance and egg quality were evaluated total five times after end of each four weeks. Serum characteristics and yolk fatty acids were determined only during on the period of 16th July to 12th August 2018, when house temperature was recorded 33.0^{o}C that considered extreme hot environmental weather. The data were analyzed using one-way ANOVA procedure of SAS (9.1) and when significant means were compared with Duncan's test p-value at 0.05. During extreme hot environmental temperature (16th July to 12th August), egg production and egg mass were significantly increased when hens given 15.0±0.5^{o}C (T2) water temperature compared with those water temperature of ordinary tap water (T1) and 25.0±0.5^{o}C (T3). While, no statistical difference between the treatments groups T2 and T3 on feed intake and egg weight was noticed. Albumen height, haugh unit, egg shell breaking strength and thickness had no influenced among the treatments of observation periods. There was no effect of drinking water treatments on albumin, total cholesterol, HDL, protein, glucose, triglycerides and AST in serum. Likewise, total mono, poly, saturated and unsaturated fatty acids in the eggs yolk of the hens were not affected by drinking water temperature. From the present results, it can be concluded that offering water temperature 15.0±0.5^{o}C during extreme hot weather in summer has positive effects on performance of laying hens.

Key Words: performance, egg quality, serum, water, laying hens

P264 Carriage of multidrug resistant Salmonella Apeyeme in Nigerian commercial breed broilers raised antibiotic-free Adelumola Oladeinde*[1], Akinniyi Dare[2] *[1]USDA-ARS; [2]Obafemi Awolowo University, Ile - Ife*

Non-typhoidal *Salmonella* remains a major food-borne pathogen associated with poultry consumption in developed and developing countries. Furthermore, their increasing resistance towards critical antibiotics used for the treatment of invasive infections caused from *Salmonella* consumption poses significant public health risks. In this study, we investigated the effect of imported versus commercially available feed on the occurrence of antibiotic resistant *Salmonella* in Nigeria Arbor Acre (AA) broilers. Sixty day-old AA chicks were randomly divided into 2 equal groups (A and B) of 30 chicks/pen group in 8 x 6 feet per floor pen. Birds were allowed ad libitum access to feed and water. Group A were fed an Imported Commercial Single diet feed (ICs-USA) while Group B were fed a Local Commercial phases feed (LCp-Nigeria) for 25 days. After which Group A and Group B broilers were switched to a National Research Council (NRC) formulated phases feed for 31 days. Live Lentogenic (LaSota) Strain BP (Vet) was administered intraocular to the birds at age 7 and 21. AMPRol® 25% Feed Premix at a dose of 0.0125% was added to the LCp-Nigeria feed to match the ICs-USA amprolium dose. No other medication was used throughout the experiment. At 48 days, fresh fecal droppings were collected as grab samples from each pen and shipped to the USDA, ARS facility in Athens, GA, USA for *Salmonella* isolation and characterization. The majority (75 %) of the *Salmonella* isolates recovered from Group A and Group B feces displayed resistance to multiple classes of antibiotics including the fluoroquinolones (ciprofloxacin, nalidixic acid, enrofloxacin) that are the first line of treatment for invasive infections. Further, whole genome sequencing revealed that these *Salmonella* isolates were *enterica* serovar Apeyeme (sequence type 3755) and carried 3 transferable mobile DNA called plasmids. These plasmids denoted IncHI1, IncFIA, and Col440I carried metal and antibiotic resistance genes known to confer resistance to copper, zinc, beta-lactams, tetracyclines, quinolones, sulfonamides, aminoglycosides and trimethoprim. This preliminary study

suggests that even in the absence of antibiotics in the feed or water used during grow-out, antibiotic resistant *Salmonella* could still persist.

Key Words: Salmonella, Feed, multidrug resistance, antibiotic-free, Arbor-Acre broilers

P265 The reliability of palpation, x-ray, and ultrasound techniques for the detection of keel bone damage Linnea Tracy[1], Mieko Temple[2], Darin Bennett[2], Kim Sprayberry[2], Maja Makagon[3], Richard Blatchford*[3] *[1]University of Pennsylvania, Philadelphia; [2]California Polytechnic State University; [3]University of California, Davis*

As housing for laying hens has transitioned away from conventional cages, an increase in keel bone damage has been observed. The major method of measuring keel bone damage on live birds has been through palpation, yet this method is often subjective, and requires thorough training to perform correctly. The aim of the current study was to examine the intra- and inter-rater reliability of evaluators when immediate feedback of accuracy was available, along with evaluating the accuracy of two new methods, portable radiography and sonography. Immediately following euthanasia, 50 103-week old Lohmann LSL-lite hens were palpated by 4 evaluators. The birds were then radiographed, sonographed, and palpated a second time. The keel bone of each bird was then dissected and examined for damage. The presence of deviations (DEV), fractures (FR), and tip fractures (TFR) was scored for each method. Reliability of palpation was analyzed using Cronbach's Alpha (intra) and Fleiss' Kappa (inter) tests. Sonography and Radiography scores were compared against the dissection scores for sensitivity and specificity. Initial inter-observer reliability was 0.39 DEV, 0.53 FR, and 0.12 TFR, with similar scores for the second round of palpation. Scores for intra-observer reliability ranged from 0.58-0.79 DEV, 0.66-0.90 FR, and 0.37-0.87 TFR. Both sonography and radiography showed relatively high sensitivity for FR (75.0, 85.7%, respectively) and TFR (90.9, 84.4%, respectively), but low for DEV (50.0, 60.9%, respectively). On the other hand, specificity was generally high across all damage types: DEV (75, 72.7%, respectively), FR (78.6, 81.5%, respectively), and TFR (67, 100%, respectively). Reliability was low and highly variable, both within and between evaluators, even when immediate feedback on accuracy was available. These results provide clear evidence that palpation is a poor method of detecting keel bone damage. However, portable sonography and radiography show promise for detecting fractures, but perhaps not deviations.

Key Words: laying hen, keel bone damage, palpation, radiography, sonography

P266 Variability in Enterobacteriaceae enumeration from poultry feed Andra Powell*, Kurt Richardson, Cheryl Weller, Nicole Holcombe *Anitox Corp*

The agriculture sector uses *Enterobacteriaceae* levels as an indicator of feed microbial quality. Current validated methodology for human foods utilizes buffered diluents and pour plating with Violet Red Bile Glucose agar to enumerate *Enterobacteriaceae*. However, this method has not been validated for animal feeds/ingredients. In preliminary studies, a high degree of variability (>80%) was observed in current *Enterobacteriaceae* enumeration methodology from poultry feed. Sample preparation/subsampling, microbe adhesion to feed particles, and *Enterobacteriaceae* sensitivity to agar temperature can contribute to this variability. Experiments were conducted using a liquid broth culture and a dry inoculum of *Enterobacteriaceae* (E. coli ATCC 8739 served as a representative of the *Enterobacteriaceae* class) to evaluate these possible causes. Broth and feed samples were plated via pour plate, spread plate, and 3M Petrifilm™ methodology. A surfactant was used in the diluent media to promote bacteria detachment from feed particles and prevent cell aggregation while in solution. The use of a liquid culture permitted the evaluation of each plating methodology while limiting subsampling variability (10% variability for liquid cultures on Petrifilm™) as microbial distribution in feed is not uniform. To assess variability associated with subsampling of feed, a dry inoculum was used to contaminate ground poultry feed at a 10^5 cfu/g challenge level. Grab sampling and mechanical subsampling were used to obtain feed samples. With surfactant added to the diluent, results revealed a ~50% reduction in variability with mechanical subsampling compared to grab sampling. *Enterobacteriaceae* levels in liquid and dry samples were 0.5 log units lower when determined by the pour plate methodology and demonstrated increased variability compared to values obtained by spread plate and Petrifilm™ methodology. In summary, eliminating the impact of agar temperature when using Petrifilm™, utilization of a surfactant in the diluent, and proper sample preparation reduces the variability from 81% to 16%. Method improvement for enumeration of *Enterobacteriaceae* in feed/ingredients allows feed producers to make reliable decisions with respect to microbial quality and intervention strategies.

Key Words: Enterobacteriaceae, Feed, Methodology

P267 Field study to control inclusion body hepatitis in broilers by addition of spray-dried plasma in prestarter feed Thomas Cherian[1], Joy Campbell*[2], Saif Balios[1] *[1]Rossdown Farms; [2]APC*

The objective was to determine if feeding 2% spray-dried plasma (SDP) in the broiler prestarter feed would impact broiler performance and mortality associated with inclusion body hepatitis (IBH) in farms with previous history of IBH. Two field trials were conducted using Cobb 500 broilers. Experiment (Exp.) 1 was conducted in a double deck commercial barn with average 33000 birds per barn and replicated twice. Exp. 2 utilized commercial barns consisting of three single barns and 3 double deck barns ranging with 4400 to 26,000 birds per barn. In both trials, broilers were housed on new wood shavings with average market age of 40. Treatments were prestarter feed with 2% SDP fed from d 1 to 10 or control with no SDP. After d 10, the feed program was common between treatments. Feeds were formulated to meet broiler requirements for the different growing phases. Barn close out production parameters of mortality, feed efficiency, and gain were measured. The initial evaluation in Exp. 1 resulted in lower mortality (4.49 vs. 7.02%) and higher ($P = 0.16$) average daily gain (57.5 vs. 55.5 g) when broilers consumed SDP in prestarter compared to control feed. Following up with further field evaluation and replication in Exp. 2 resulted in reduced ($P = 0.0089$) mortality (3.36 vs. 5.99%) in flocks consuming SDP in prestarter feed compared to control feed in farms with history of IBH. Production performance of the flocks in Exp 2 were 54.9 vs. 49.8 g daily gain and 1.72 vs. 1.79 feed efficiency for SDP vs. control fed groups, respectively. Overall, farms with a history of IBH had lower mortality and increased daily gain when consuming SDP during the prestarter phase compared to control feed.

Key Words: Inclusion body hepatitis, spray-dried plasma, broilers

P268 Early-life thermal conditioning alleviates effects of heat stress in broilers Ronique Beckford*[1], Linda Farley[1], Laura Ellestad[2], Monika Proszkowiec-Weglarz[3], Kristen Brady[1], Roselina Angel[1], H-C Liu[4], Tom Porter[1] *[1]University of Maryland; [2]University of Georgia; [3]USDA ARS; [4]North Carolina State University*

Thermal conditioning (TC) during brooding, exposes young chicks (3-5-d old) to high ambient temperature for a set time period. This exposure imparts long-term resistance to heat stress (HS) allowing birds to survive higher temperatures as they get older. Two trials were conducted to determine the efficiency of TC on alleviating the effects of HS in broilers. Firstly, in a battery trial, 900 1-d old male broiler chicks (Ross 708) were placed in floor pens prepared for brooding. At d 3, chicks were weighed and divided into 2 pens in each of 3 rooms. Half of the chicks were subjected to TC (37.8°C) for 24h while the remaining half were not thermally conditioned (NTC) (33°C). On d 24, chicks from each pen were allocated into 4 grower batteries. On d 31, half of the birds were HS (35°C) for 8h, while the other half were not (22°C). During the HS challenge, birds were euthanized and blood collected. Blood samples were analyzed for

pH, pO_2, pCO_2, HCO_3^-, glucose, Na^+, and K^+. Subsequently, plasma was obtained and assayed for levels of T_3, T_4, and corticosterone. Secondly, in a growout trial 1,440 1-d old chicks were placed in 12 floor pens for TC. On d 3 half of the birds were subjected to TC for 24h. At 4 d, chicks were distributed into 60 pens in the broiler house. Feed and water were provided *ad libitum*, and performance was monitored. Blood samples were collected at d 13, 29 and 35 for plasma corticosterone, T_3 and T_4 analysis. On d 31, all the birds were subjected to HS for 8h. Data were analyzed using a mixed model ANOVA (SAS v9.4, Cary NC). Results from the battery trial showed that TC birds had a lower ($p<0.05$) FCR, plasma glucose and corticosterone compared to NTC birds. In addition, during HS, NTC birds had a higher ($p<0.05$) mortality rate (26.7%) than TC birds (6.7%). In the growout trial, there were no differences in performance between the TC and NTC birds. Similar to the battery trial, the mortality rate was higher ($p<0.05$) for NTC birds (49.7%) than the TC birds (38.3%). These results indicate that TC reduces stress levels and has a protective effect during heat stress. Follow-up studies are being conducted to better understand the mechanisms that regulate the response to heat stress and how TC can be better used to alleviate the impact of heat stress.

Key Words: Thermal Conditioning, Heat stress, Battery cages, Growout trial

P270 Effect of a direct-fed microbial on broiler performance, paw score and carcass traits when supplemented to a feed program containing enzymes. S. Watkins[1], J. Remus*[2] *[1]Center of Excellent for Poultry Science, University of Arkansas; [2]Danisco Animal Nutrition/ DuPont Industrial Biosciences*

This investigational effort was designed to measure the impact of a *Bacillus*-based direct fed microbial, Enviva® Provalen Plus (P+), on various aspects of broiler production when supplemented to a feed regime containing enzymes. Parameters measured included bird performance, paw score and carcass traits. Day-old Cobb 500 male broiler chicks were weighed and allocated to 24 pens (30 chicks/pen; 8 pens/treatment) and grown to 42 days of age under summer conditions. Treatments consisted of a control, which was a 3 phase program wherein feed contained 200 units alpha-amylase/kg feed, 4000 units protease/kg feed, 2000 units xylanase/kg feed and 500 units phytase/kg feed or the control program supplemented with P+ at either 0.5 or 1 kg/MT feed. Bird performance was measured at 15, 31 and 42 d. Data were analyzed via a one-way ANOVA with means being deemed significantly different at $P\leq0.05$. Addition of P+ to the control feed program with enzymes reduced foot pad lesions at 42 days with scores of 0.534 for the 0.5kg, 0.525 for the1 kg and 0.932 for the control. Live performance and livability were not affected by treatment. Carcass parameters of percent breast ($P<0.07$) and percent breast plus tender ($P<0.10$) tended to increase with increasing P+ addition. Despite feed costs increasing with addition of supplement, the economic return over feed cost was the most advantageous for supplementation of 1kg P+ at 42 d. These results suggested supplementing P+ to standard broiler feed regimes containing enzymes decreases foot pad lesions and may benefit breast meat yield and profitability.

Key Words: feed enzymes, direct-fed microbial, broiler, paw score, breast meat

P271 Occurrence of mycotoxins in Latin America in 2018 Alexandro Marchioro*, Ursula Hofstetter, Gerd Schatzmayr, Verena Starkl, Anneliese Mueller *BIOMIN Holding GmbH*

Climate changes is a worldwide recognized issue and geographical blocks are involved on this change leading to different problems in food and feed safety. Mycotoxin occurrence in different kind of commodities is a worldwide phenomenon that affects animal health. The aim of this survey is to evaluate the extent of mycotoxin contamination in raw materials and finished feed samples in the Latin America countries (LATAM).

From January to September 2018, over 6,677 samples (mainly corn, soybean/soybean meal, wheat, finished feed and other grains) were sourced from LATAM and analyzed by high performance liquid chromatography (HPLC) and Enzyme-Linked Immunosorbent Assay (ELISA). The samples were analysed for aflatoxins (Afla), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM), T-2 toxin (T-2) and ochratoxin A (OTA). For the purpose of data analysis, non-detect levels are based on the quantification limits (LOQ) of the test method for each toxin.

In 68% of all animal feed and ingredient samples more than one type of mycotoxin was found and only 24% of all samples contained at least one of the five main mycotoxins. Afla was present in 25% of all samples at an average concentration of 8 µg/kg. ZEN was present in 49% of the samples at an average level of 135 µg/kg. T-2 was detected in 21% of all samples at an average of 44 µg/kg. OTA was detected in 5% of all samples at an average of 10 µg/kg. DON was found in 67% and FUM in 72% of all samples. These results show that DON and FUM are the most prevalent groups of mycotoxins in LATAM with an average contamination of 1.002 and 1.977 µg/kg, respectively. Overall, the average levels of DON and FUM pose a potential health threat to poultry production due to their impact on the gastrointestinal tract and immune system.

The survey results indicate that mycotoxins are a serious concern in agricultural production. Multi-mycotoxin occurrences continue to be a global and regional threat, as they may result in additive or synergistic effects. Therefore, the results underline the necessity of constant mycotoxins monitoring in feedstuffs and a proper mycotoxin risk management.

Key Words: Mycotoxins, Prevalence, Deoxynivalenol, Fumonisins

P272 Can Flue Gas Desulfurization Gypsum be used as a Bedding Material for Broiler Production Dexter Watts*[1], Joseph Purswell[2], H. Torbert[1], Jeremiah Davis[3] *[1]USDA-ARS National Soil Dynamics Laboratory; [2]USDA-ARS Poultry Research Unit; [3]Department of Biosystems Engineering, Auburn University*

There is an abundant supply of flue gas desulfurization (FGD) gypsum being produced at utilities as a byproduct of power generation. This synthetic gypsum source may be a viable alternative bedding material for broiler production. Thus, a study was conducted to evaluate the feasibility of using FGD gypsum as a bedding for raising 56 day old broilers in five successive trials. The bedding was placed in pens at a 3“ depth (7.6 cm) and managed to simulate commercial broiler production throughout the five growout cycles. Body weight, feed conversion, and mortality were determined at harvest and foot pad lesions were evaluated after processing. Litter caking scores were determined after each flock, while ammonia concentrations from the litter were evaluated on flocks four and five. No differences were observed for body weight, feed conversion, or foot pad lesions among the litter treatments from any of the five growouts. However, there was a tendency for the FGD gypsum material to have higher litter caking, while ammonia concentrations tended to be lower. Overall, the FGD gypsum has shown good potential as an alternative bedding material for rearing broilers.

Key Words: Broilers, Bedding material, FGD gypsum, Ammonia concentrations

P273 Impact of water sanitation on broiler chicken production and welfare Leonie Jacobs*[1], Mike Persia[1], Nisana Siman-Tov[1], Jesse McCoy[2], Lindsay Good[2] *[1]Virginia Polytechnic Institute and State University; [2]Neogen Corp.*

The study’s aim was to assess the impact of three water sanitation treatments and fresh or used litter for broilers on performance and welfare outcomes. Seventy-two pens housed 1,944 male broilers (Hubbard*Ross 708) for 35 days. Treatments included tap water (control) or water with a dilution of fully-activated chlorine dioxide (full-ClO_2; 1.5mL:10L), partially-activated ClO_2 (part-ClO_2; 1.5mL:10L) or iodine (iodine; 0.42%

iodine at 13mL:10L). Half the birds were housed on used shavings from a previous flock (20 birds/pen) provided Coccivac-B via starter feed (ca. 1250 oocytes/bird). Those birds were housed for 21 days to allow for oocyst shedding. Thereafter, litter was moved the middle of the facility, mixed and returned to the pens. Body weights (BW), mortality-corrected feed conversion (FCRm), drinking frequency, water intake (WI), foot pad dermatitis (FPD), hock burns (HB), and chronic stress (adrenal gland weight asymmetry (AGA) relative to BW) were recorded. Data were analyzed using mixed models and Tukey-Kramer tests.

Used litter resulted in worsened FCRm (day 0-14: 1.103 vs. 1.100; P=0.049) and reduced BW (day 14: 476g vs. 491g; P<0.001) compared to birds raised on fresh litter, validating the experimental model. At day 28, full-ClO_2 (1.61kg) and part-ClO_2 treated birds (1.61kg) were heavier compared to control birds at 1.56kg (post-hoc P=0.018 and P=0.025 respectively). At day 35, full-ClO_2 treated birds (2.38kg) tended to be heavier (P=0.095) than iodine birds (2.32kg; P=0.038) and control birds (2.33kg; P=0.053). Total WI did not differ among treatments, but iodine-treated birds showed fewer drinking bouts compared to other treatments (P≤0.036).

Iodine-birds tended to show better FPD scores compared to full-ClO_2 birds (P=0.084). HB did not differ. Iodine-treated birds (P=0.034) and full-ClO_2 treated birds (P=0.036) showed lower AGA compared to control birds, suggesting reduced chronic stress in those groups. FPD scores were better in birds on fresh litter compared to birds on used litter (0.80 vs. 1.31 on a 0-4 scale; P<0.0001).

Fully-activated chlorine dioxide treatment appeared to positively affect production (BW) and welfare (AGA). Iodine provided over the duration of the experiment seemed to improve welfare outcomes (FPD, AGA), but reduce BW.

Key Words: water line sanitation, broiler chickens, animal welfare, production

P274 Effect of stocking density and dietary antimicrobial inclusion on blood physiological variables of male broilers grown to 35 days of age. Hammed Olanrewaju*, Joseph Purswell, Stephanie Collier, Scott Branton *USDA-ARS, Poultry Research Unit*

Significant expansion in conventional and antibiotic-free (ABF) broiler production is expected to continue as demand for poultry products continues to increase and feed prices remain stable. Much concern nowadays is expressed about stocking density as it relates to the well-being and welfare of broilers. This study investigated the effects of stocking density and dietary antimicrobial inclusion of male broilers grown to 35 days of age on blood physiological variables. A total of 1952 1-d-old Ross x Ross 708 male chicks were randomly distributed into 32 pens based on 4 assigned stocking density treatments. Conventional (AGP+) and antibiotic free (ABF) diets were equally assigned to each pen with feed and water provided ad libitum. The treatments consisted of 4 densities (27, 29, 33, 39 kg/m^2) and 2 diets (AGP+, ABF) arranged in a 4 × 2 factorial. Blood samples were collected from the brachial wing vein of 3 birds per pen on d 15, 28, and 35, which were then analyzed immediately for whole blood physiological variables. Blood plasma samples were analyzed for corticosterone. Results show there was no effect of stocking density on any of the selected physiological variables. However, in comparison to broilers fed with ABF diet, broilers with AGP+ had higher levels of pO_2, sO_2, SaO_2, and K^+ which were within physiological ranges. In addition, blood glucose and plasma corticosterone concentrations were not affected by treatments, suggesting an absence of physiological stress. In conclusion, stocking density up to 39 kg/m^2 in the presence or absence of antibiotic in the diets may be suitable for both poultry integrators and contract growers to enhance broilers production efficiency without compromising the welfare of male broilers grown to 35 days of age.

Key Words: Stocking-density, antimicrobial, acid-base balance, broilers, welfare

P275 Developing artificial hatching with natural hatching (maternal care) of broiler chickens and its effect on the behavioural and hormonal traits of the embryos. Salwan Abdulateef*, Adel Al-Hamadani, Ahmed Majid *University of Anbar*

The most important feature of hens behaviour towards chicks is the releasing of a sound that is recognized by the chicks and attracted to the hen and that this sound can stimulate the embryos, this study conducted to investigate the physiological and behavioural changes of the embryos and to compare the industrial incubation and the natural incubation, and who is more welfare on the chicks, to reach incubation methods are successful. The first experiment, 120 eggs hatching were used divided into two treatments as follows: Artificial Incubation (AI) and Natural Incubation (NI), In the AI, incubator a type (Cimuka) was used and 60 eggs divided into three replicates. On the other hand, 60 eggs were used in NI, divided into three replicates, each replicate is contained two hens chicken (local chicken) brooded in a separate nest put 10 eggs (each nest = one hen+10 egg). After that, the date of the hen's sound releasing and recording and the date of touching of body hen with the eggs and temperature of the body were observed. In the second experiment, used 60 eggs divided into three replicates by using AI, the traits of incubation were mixed NI with AI the found in the NI and not present in the AI, as the sound of a hen and touch the hen body for eggs, two treatments were used as follows The first, control treatment, without any treat (CO) and second treatment, Chicks embryo were heard sound hen (obtained from the first experiment) from10-day incubation until hatching and touch with toy-like hen body (semi-temperature of body hen) (HT). This experiment analyzed by using Complete Randomized Design. The results show: Significantly increase (p<0.01) in embryonic development for embryo weight, chick body weight, Hatchability and embryo Index (EI) for HT compare with CO. Significantly increase in behavioural traits as the percentage of tucking and the number of motility of chick embryo for TH compare with CO. Significantly increase, in hormone level prolactin and decreasing in corticosterone in embryo chicks for HT compared with CO. So we recommend adding these traits (hen sound and touching the body) to modern hatcheries.

Key Words: artificial hatching, natural hatching, maternal care, behavior, hormones

P276 In ovo feeding dose response of probiotic Clostridium species on hatch performance and hatchling quality of broilers. Shaymaa Abousaad*[1], Peter Ferket[1], Ramon Malheiros[1], Shawn Jones[2], Bryan Tracy[2] [1]*North Carolina State University;* [2]*White Dog Labs, Inc.*

Due to current public concern about antimicrobial resistance, the use of direct fed microbials to manage enteric health is becoming a feasible practice, especially if administered early in a broiler's productive life. *Clostridium*, butyric acid-producing, spore-forming anaerobes found in the intestines of healthy animals was demonstrated by researchers to promote growth performance, improve gut health. White Dog Labs (New Castle, DE) has developed gastrointestinal tract derived *Clostridium* libraries (CL) with suitable protocols to manage the growth and prepare spore suspensions of these species. The objective of this study was to examine the dose response of several CL strains delivered by *in ovo* feeding (IOF) on hatch performance. Defined or selected Clostridia (DCL and SCL) culture libraries were injected into the amnion of fertile eggs at ~17 d of incubation with 100 µL of either saline (9%) as a control (Cont) or 10^3, 10^5, 10^9 of DCL and SCL. All treatments adversely affected hatch rate due to egg explosion and embryo death, which was attributed to excessive gas production. Therefore, two new consortiums were developed and tested: the A1 library, identified as the acetogenic library consisting of 3 gas consum-

ing microbes, and the D1 library consisting of a combination of the same 3 gas consuming microbes as in A1, along with 3 gas producing microbes. A series of experiments were done to determine the IOF delivery of 10^1, 10^3, 10^5, 10^7 and 10^9 cfu/egg of A1 and D1 culture libraries on hatchability, hatch window and hatchling quality. In comparison to saline-injected controls, IOF delivery of $>10^3$ cfu A1 library significantly improved the hatchability rate by 8 – 9%, and finished hatching time window 4 hours earlier. In contrast, the D1 library significantly decreased hatchability and increased hatch time window as IOF dose increased. No significant differences in hatchling quality parameters were observed among treatments as compared to Cont. *In ovo* feeding gas-consuming acetogenic microbe consortiums of late-term embryos is a novel application of defined probiotic clostridia cultures for broiler chickens.

Key Words: in ovo feeding, Clostridium, probiotics, hatch performance, broilers

Metabolism and Nutrition

P277 Energy value of hydrolyzed feather meal for broiler chickens, determined by regression method Ayodeji Aderibigbe*[GS], Olayiwola Adeola *Purdue University*

The energy value of hydrolyzed feather meal (HFM) was determined with Cobb-500 broiler chickens. A common broiler starter diet was fed to the birds from d 0 to d 21 and experimental diets were introduced from d 21 to d 26 post-hatching. A total of 192 birds were allotted to 3 dietary treatments in a randomized complete block design with 8 replicate cages per diet and 8 birds per cage. Experimental diets consisted of a corn-soybean meal reference diet (RD) and two test diets which had the HFM added at 50 or 100 g/kg to partially replace the energy sources in the reference diet. The analyzed DM, gross energy and CP of HFM were 958 g/kg, 5860 kcal and 877 g/kg DM respectively. There were linear (P=0.006) and quadratic (P=0.044) effects of HFM addition on body weight gain of birds. Linear and quadratic responses (P<0.01) with increasing HFM concentration was observed on ileal DM and energy digestibility coefficients. Diet IDE were 3561, 3575 and 3469 kcal/kg DM for RD, 50g/kg HFM and 100 g/kg HFM, respectively. Also, both linear and quadratic effects (P<0.01) of increasing HFM levels were observed on metabolizability of DM, energy and N-corrected energy. Calculated N-corrected ME were 3521, 3534 and 3445 kcal/kg DM for RD, 50 g/kg HFM and 100 g/kg HFM respectively. The respective IDE, ME and MEn of HFM determined by the regression method were 3014 kcal/kg DM, 3190 kcal/kg DM and 3124 kcal/kg DM. The current study shows that between 51 and 53% of the gross energy of HFM is utilized by broiler chickens.

Key Words: hydrolyzed feather meal, ileal digestible energy, metabolizable energy, chicken

P278 Effect of dietary protein level on performance and tail feather length of male ring-necked pheasant chicks from 3 to 9 weeks of age. Christopher Delfelder*, Richard Beyer *Kansas State University*

Pen raised ring-necked pheasants must be well feathered and healthy for restocking programs. A research trial was conducted to evaluate the effect of graded levels of protein on male pheasant chick performance and tail feather length in the 3 to 6-week and 6 to 9-week growth periods. Pheasants were housed in interior netted pens for the first 3 weeks and then, at 6 weeks of age, allowed outdoor access in a forage-covered exterior exercise pen for the remainder of the trial. Groups of pheasants were fed diets with 3 protein levels (24%, 26%, 28%) after the first 3 weeks of growth on a commercial 28% protein starter. The crude protein content of all 3 diets was reduced by 4% (20%, 22%, 24% respectively), for the final 3 weeks of the 6-week trial. Feed and water were provided *ad libitum* throughout the trial. Body weight gain and feed consumption were determined weekly for 6 weeks and tail feather lengths were measured weekly for the first 3 weeks and then once more at the end of the 6-week trial. Data were analyzed as a completely randomized design using the PROC GLIMMIX procedure of SAS (9.4). No significant differences (P≤0.05) in feed intake, tail feather length or overall growth of the tail feathers were observed. A significant difference (P≤0.05) in body weight was observed for the first 3 weeks of the trial between the 28% and 24% protein diets but not between the 28% vs 26% or the 26% vs 24% protein diets. This difference in body weight was observed in the second phase with the 28% chicks staying significantly heavier than the birds receiving the other 2 diets during the second 3-weeks of the trial. No significant difference (P<0.05) in feed conversion was detected for the first 3 weeks of the trial but during the second 3-week period the feed conversion ratio of the 28% birds was lower than the ratio of the other 2 treatment diets. The data indicates that pheasant chick performance is affected based on starter ration protein levels but the length of the tail feathers of the male pheasant chick are not affected by the addition or subtraction of protein into the diet. More research is needed to determine whether there may be potential for slower growth programs so that growers holding birds for long periods may reduce feed costs.

Key Words: ring-necked pheasant, reduced protein, feather quality, growth performance, tail feathers

P279 Effect of production rate and knife distance on pellet quality Reid Beeman*[UG], Marut Saensukjaroephon, Caitlin Evans, Chad Paulk, Charles Stark *Kansas State University*

Longer pellet lengths may lead to increased pellet breakage, resulting in decreased pellet durability index (PDI). Thus, the objective of this experiment was to determine the effects of production rate and knife distance on pellet length and subsequent pellet quality. Treatments were arranged in a 2 x 3 factorial design with two production rates (7.3 and 15.0 kg per minute) and three knife distances (6.4, 12.7 and 19.1 mm). The treatments were pelleted at 82°C using a CPM pellet mill (Model 1012-2 HD) equipped with a 4.8 mm × 31.8 mm die. The production rate (PR) and knife distance (KD) were randomized to minimize the effects of pelleting and sampling orders. There were 3 replicates per treatment. Samples were analyzed for pellet length, %fines, and PDI using the standard tumble box method (STD) and Holmen NHP100 (NHP) for 60 sec. Data were analyzed using the GLIMMIX procedure of SAS (Cary, NC). There was no interaction between PR and KD for all analyzed variables ($P > 0.23$). The low PR yielded a longer pellet ($P \leq 0.05$) compared to the high PR (8.7 and 7.9 mm, respectively). The PR had no effect on %fines and PDI regardless of analytical method ($P \geq 0.06$). Pellet length and %fines were impacted by KD ($P < 0.01$). Increasing KD resulted in lower %fines (66, 11, and 6%, respectively) and longer pellets (4.9, 8.6, and 11.5 mm, respectively). Reducing KD to 6.4 mm reduced PDIs (STD = 40%; NHP = 14%) as compared to 12.7 mm and 19.1 mm treatments, which yielded similar PDI results (STD = 70 and 75%; NHP = 55 and 63%, respectively). In conclusion, a majority of the improvement in pellet quality was observed when increasing the pellet length from 4.9 to 8.6 mm (KD 6.4 and12.7 mm, respectively).

Key Words: Pellet quality, Pellet length, Knife distance, Production rate

P280 The effect of course corn inclusion in broiler diets on pellet quality Aundria Ogles*[GS], Caitlin Evans, Courtney Truelock, Charles Stark, Chad Paulk *Kansas State University*

Previous research has demonstrated the benefits of including large grain particle size in diets on gizzard muscling and gastrointestinal tract development. However, including these larger particles may influence pellet

integrity. Therefore, the objective of this study was to evaluate the effect of coarse corn inclusion level on pellet quality. A corn soybean meal-based grower broiler diet with 1.5% mixer added fat was used in this experiment. Treatments were created by replacing 600 µm corn with 1200 µm coarse rolled corn at 0, 10, 20, or 30% of the diet. Mash treatments were pelleted using a CPM pellet mill (Model 1012-2 HD) equipped with a 4.8 mm × 31.8 mm die. Pelleting order was randomized with 3 replicates per treatment. Target conditioning temperature was 85˚C for 30s at a 15.4 kg/min production rate. Samples were analyzed for hot pellet temperature (HPT), fine to pellet ratio (%fines) and pellet durability index (PDI) using the standard tumble box (STD) and Holmen NHP100 (NHP) for 60 sec. Data were analyzed using the GLIMMIX procedure of SAS (Cary, NC). There were no differences among analyzed variables as the inclusion level of coarse corn was increased ($P > 0.05$). There was trend for increased ($P = 0.06$) HPT when coarse corn was added to the diet at 10, 20, or 30% (82, 86, 87, 85°C, respectively). The %fines remained similar across treatments (9.1, 9.8, 8.7, and 9.5%, respectively) as well as PDI, regardless of method (STD: 60, 60, 64, and 60%; NHP: 65, 65, 66, and 63%, respectively). The lack of difference is potentially attributed to retention of the large corn particles on the #5 sieve used to separate the fines from the pellets. However, increasing the concentration of coarse corn from 0 to 30% did not influence PDI.

Key Words: Pellet Quality, Coarse Corn, Particle Size, Pelleting

P281 The effect of filter type and warm-up time on pellet durability index using the Holmen NHP100 portable pellet tester Rachel Kort*[UG], Caitlin Evans, Marut Saensukjaroephon, Haley Wecker, Chad Paulk, Charles Stark *Kansas State University*

The Holmen NHP100 (TekPro Ltd, Norfolk, UK) is a portable forced air pellet tester commonly used by the industry to determine pellet durability index (PDI). The objective of this study was to determine the effect of filter type and machine warm-up time on PDI. A corn-soybean meal-based broiler grower diet was conditioned at 85˚C for 30 s and subsequently pelleted using a laboratory pellet mill (Model CL5 California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.0 mm x 12.77 mm die. Production rate was 54.5 kg/hr. Once cool, pellets were analyzed for PDI using the NHP100 with a 60 sec run time. Air temperature and pressure within the NHP100 were recorded throughout the experiment. Treatments were arranged in a 3 × 8 factorial with varying filters (none, factory filter, or commercial paper towel) and machine warm-up time (0, 3, 6, 9, 12, 15, 18, or 21 min). There were 3 replicates per treatment. Pellets were sifted pre and post analysis for separation of fines and pellets using a U.S. #6 standard sieve. There was a filter × warm-up time interaction ($P \leq 0.05$) for air temperature. Increasing warm-up time from 0 to 9 min increased the air temp with no further increase from 9 to 21 min when using none, factory, or paper towel as a filter. However, the air temperature without warm-up time (0 min) was greater with the factory filter and paper towel compared to no filter. There was a filter × warm-up time interaction ($P \leq 0.05$) for air pressure. At 0 and 3 min warm-up time there were no differences in air pressure between none, factory and paper towel filters. At 6 to 21 min warm-up time, air pressure was greater with no filter paper compared to using factory and paper towel filters. There was a filter × warm-up time interaction ($P \leq 0.05$) for PDI. For no filter, increasing warm-up time from 0 to 6 min increased PDI with no further increase from 6 to 21 min. However, there were no differences in PDI with increasing warm-up time when using the factory filter or paper towel. Using the factory filter or paper towel had similar PDI, but resulted in greater PDI compared to no filter. In conclusion, warm-up time did not influence PDI when using a filter. Greater PDI were observed when using either filter compared to no filter.

Key Words: PDI

P282 Changes to the poultry microbiome may mediate the anti-Salmonella effects demonstrated by Diamond V Original XPC Kristina Feye*[1], Peter Rubinelli[1], Hilary Pavlidis[2], J. Byrd[2], W. Chaney[2], Don McIntyre[2], Steven Ricke[1] *[1]University of Arkansas, Department of Food Science, Center for Food Safety; [2]Diamond V Mills, Inc*

The functional metabolites of Diamond V Original XPCä (XPC) added to commercial poultry feed has been shown to reduce *Salmonella* prevalence, load, and antibiotic resistance. In order to evaluate whether or not differing inclusion rates of XPC modulate the microbiome, and in turn produce anti-*Salmonella* effects, the following study was conducted. Day of hatch male Cobb 500 broilers were randomly assigned to one of four diet groups: non-infected control (NICON), infection control (ICON), 0.125% XPC/infected (XPCA), and 0.200% XPC/infected (XPCB). Birds in the XPC treatment groups were fed XPC starting from hatch. The broiler chicks were experimentally infected with a nalidixic acid resistant *Salmonella* UK-1 at D1. Broilers were raised using conventional methods and were euthanized and ceca aseptically harvested and analyzed for *Salmonella* prevalence and load on D42. The ceca contents were expressed, the DNA was extracted, and sequenced using the Illumina MiSeq platform, with data analyzed using the QIIME2 pipeline (Version 8). Briefly, sequences were imported, trimmed with DADA2, filtered *de novo* for chimera sequences, and then evaluated for compositional differences. Filtered data and networks were also analyzed for compositional alpha and beta diversity differences using standard pipelines. Taxonomy was assigned via sk-learn using SILVA. Evidence suggests that group XPCA resulted in significant changes to the microbiome in both alpha and beta diversity, as well as the underlying microbiome networks and composition as compared to NICON, ICON, and XPCB (PERMANOVA, $P < 0.05$). These changes in the microbiome of XPC-A groups resulted in a more significant reduction in recovered *Salmonella* Typhimurium UK-1 as compared to the controls (NICON, and ICON), and XPC-B groups (ANOVA, $P < 0.05$). Prevalence in the ceca colonization of *Salmonella* was also significantly reduced in the XPC-A group (69%) vs. XPC-B (85%) and the controls (ICON= 92% and NICON=20%). Trends in the microbiome paralleled the statistically significant trends of the *Salmonella* data. Therefore, evidence suggests that XPC-mediated changes to the microbial ecology of the poultry gastrointestinal environment likely play significant roles in the established anti-*Salmonella* effects of Original XPC.

Key Words: XPC, Salmonella, Microbiome, Gut Health, Broilers

P283 Distribution of trypsin Inhibitor and urease activity in soy products in different countries and world areas Juxing Chen*[1], Cindy Atwell[1], Karen Wedekind[1], Jeffery Escobar[2], Mercedes Vazquez-Anon[1] *[1]Novus International Inc; [2]Elanco Animal Health*

Trypsin inhibitors (TI) and urease activity (UA) are the two most relevant quality measurements for soybean products as feed ingredients for animals. TI were reported to be correlated with UA, so feed processing plants use UA as an indicator of TI in soybean meal (SBM). The objectives of this study were to determine the levels of TI and UA in 414 SBM samples from 19 different countries in 5 world areas, and to validate whether TI and UA are correlated. TI in solvent-extracted SBM were 2-6 mg/g with 80% of them 3-5 mg/g. UA was 0.01-0.20 pH unit with 98% of them ≤ 0.1 pH unit. TI and UA results varied by country and by world area. By country, TI were highest in Germany, UA was highest in India, and both TI and UA were high in South Africa. By world area, both TI and UA were high in Africa, TI were low in Asia and North America, and UA was low in Europe and Latin America. Full-fat-extruded-SBM, heat-inactivated full-fat SBM and expeller-SBM had higher TI than solvent-extracted SBM. TI were poorly correlated with UA in solvent-extracted SBM samples, suggesting that UA should not be used as a surrogate indicator for TI content in soybean products.

Key Words: Trypsin inhibitor, urease activity, soybean meal

P284 Effects of dietary ViligenTM on the performance and ceca VFA concentration of broiler chicks Tuoying Ao*, Marquisha Paul, Anthony Pescatore, Hui Zhao, Lizza Macalintal, Mike Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

Viligen™, a proprietary blend of fatty acids, prebiotics and essential trace elements can support tissue health and activity in gastrointestinal tract. One study was conducted to evaluate the effect of dietary Viligen™ on the performance and ceca volatile fatty acids (VFA) concentration of broiler chicks fed a wheat-soybean meal diet. One-day old Cobb 500 male broiler chicks were raised in floor pens with a free access to the feed and water. Dietary treatments consisted of feeding a wheat-soybean meal mash control diet alone or supplemented with 0.05, 0.10 or 0.15% Viligen™. Five replicate pens of 22 chicks per pen were randomly assigned to each of four dietary treatments and were fed treatment diets for 42 days. Body weight and feed consumption were measured weekly. On day 42, one bird from each replicated pen was randomly selected and euthanized for ceca content collection. The VFA concentration of ceca content was analyzed. Data were subjected to ANOVA using the linear model of Statistix V. 9. Mean differences were determined using Fisher's LSD test and linear trend was determined using polynomial contrast. A linear effect ($P<0.05$) of dietary Viligen™ on the weight gain and feed to gain ratio in starting period (d1-21) was found. With the increasing level of dietary Viligen™, weight gain of chicks was linearly increased ($P<0.05$) and feed to gain ratio was linearly decreased ($P<0.05$). The ceca content from chicks fed diet with 0.10% Viligen™ had higher ($P<0.05$) acetate, propionate and total VFA concentration compared with those from chicks fed diets with 0.00 or 0.05% Viligen™. The ceca content from chicks fed diets with 0.10 or 0.15% Viligen™ had higher ($P<0.01$) butyrate concentration than those from chicks fed diets with 0.00 or 0.05% Viligen™. Results from this study indicate that dietary Viligen™ can improve growth performance and increase VFA concentration in the ceca content of chicks.

Key Words: broiler, performance, ceca content, VFA, butyrate

P285 Diet mediated changed in fecal microbiome of broilers chickens consuming a combination of rye and corn based diets. Guillermo Tellez-Isaias[1], Billy Hargis[1], Juan David Latorre[1], Peter Rubinelli[1], Sang In Lee, Stephen Bickler[2], Ruben Merino-Guzman[3], Si Hong Park[4], Steven Ricke[1], Kristina Feye[1], Nick Anthony[1], Mikayla Baxter*[1] *[1]University of Arkansas; ; [2]University of California, San Diego; [3]National Autonomous University of Mexico; [4]Oregon State University*

Diet plays a major role in influencing the microbiome. The purpose of this study was to evaluate changes in the fecal microbiome after the consumption of a low digestible diet (rye) and/or a highly digestible diet (corn) in broiler chickens. There were four dietary treatments: 1) corn-based diet (corn-corn); 2) rye-based diet for 10 days, and then switched to the corn diet (rye-corn); 3) rye-diet (rye-rye); and 4) corn diet for 10 days, and then switched to the rye diet (corn-rye). The same chickens from each treatment (n=5) were temporarily isolated to collect daily individual fecal samples for microbiome analysis. Bacterial genomic DNA was extracted from the samples, and the V4 region of 16S rRNA gene was amplified. Amplicons were sequenced on Illumina MiSeq, and microbial communities were analyzed using QIIME2. Results were analyzed using non-parametric ANOVA, comparing microbial population between diets and over time. Due to the daily fluctuation in fecal microbiome population, samples collected from day (D) 0-10 and D11-20 were combined. Regardless of age, feces isolated from chickens fed a rye-based diet had a significantly ($P < 0.05$) higher abundance of the genera *Lactobacillus* and *Clostridium* and the family *Lactobacillaceae* and *Clostridiaceae*. Fecal samples isolated from chickens fed a corn based diet had a significantly higher abundance of the genera *Wautersiella* and *Francisella* and the family *Peptostreptococcaceae* and *Francisellaceae*. From D11-20, corn fed chicks had a significantly higher abundance of Gram positive bacteria like *Corynebacterium*, *Curtobacterium* and *Turicibacter*. There was age related shift in microbiota population during D1-10 in rye fed chickens which showed increased abundance of the putatively beneficial bacteria *Pediococcus,* and from D11-20 there was a higher abundance of potentially opportunistic pathogenic bacteria of the genus *Streptococcus.* Regardless of diet, the abundance of *Enterococcus* decreased as chickens aged. In conclusion, it's clear that switching diets results in a shift in the fecal microbial population specifically the genera *Lactobacillus*, *Clostridium*, *Wautersiella* and *Francisella.* Unfortunately due to the daily fluctuations in fecal microbial population we were unable to detect when exactly this shift occurred.

Key Words: Fecal Microbiome, Broiler, Diet

P286 In ovo delivery of mannose-based oligosaccharides in fertile broiler eggs facilitate early intestinal cell development Lizza Macalintal*, Rijin Xiao, Anthony Pescatore, Ronan Power, Tuoying Ao, Michael Ford, Ryan Goettl, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

A previous study indicated that *in ovo* delivery of mannose or mannose-based polymer (Alltech, Inc) in fertile broiler eggs at 18d of incubation (DOI) did not negatively affect hatchability. In the current study, the influence of mannose-based polymer on small intestine morphology was investigated. Four different mannose polymer extracts (MPE) in saline solution were used; MPE13, MPE14, MPE16, MPEO. Saline-injected eggs were included as control eggs. At 18 DOI, 15 viable eggs were allocated per treatment group. A 0.6ml volume of the test compounds was injected into each egg, following disinfection and drilling of injection site on the egg shell surface. Injection sites were sealed and eggs were returned into the incubator. Hatch parameters were recorded at hatch. Jejunum samples were collected from 6 chicks/trt group at day of hatch for histological analysis. Histomorphometry was assessed on jejunum samples that were prepared using Periodic Acid-Shiff-Alcian Blue differential staining procedure. All the data were subjected to one-way ANOVA using R-studio software. Hatchability of fertile eggs injected with MPE13, MPE 14, MPEO and saline were 100% whereas MPE16 was 86% respectively. These injections did not result in any chick abnormalities nor did it affect hatchability. In this sudy, early small intestinal cell development *i.e.* jejunum was observed on all MPE treated eggs. Taller villi contribute to the enhanced efficiency of digestion and absorption of nutrients. At day of hatch, villi height were significantly longer for the MPEs compared to the control ($P<0.0001$; MPE16 > MPE14 > MPE13 > MPEO >control). More goblet cells were present along the mucosal epithelium for the MPEs ($P<0.001$; MPE16 = MPE13 > MPE14 > MPEO = control). Higher goblet cell count is associated with increased production of mucin in the intestinal lumen, which can promote intestinal mucous membrane protection. These results demonstrated that MPEs can positively alter the intestinal cell differentiation at day of hatch to better prepare the gut as the bird transitions to coarse diet. Taken together, *in ovo* delivery of MPEs facilitate early small intestine cellular development which can possibly lead to better gut health in the growing chick.

Key Words: in ovo, mannan oligosaccharide, hatchability, jejunum, goblet cell

P287 Differences in Brazil, India, China and USA-origin corn AMEn estimation using near infrared transmittance technology directly linked to broiler AMEn and the Monte Carlo simulation Mauricio Cunha*[1], Milan Hruby[2] *[1]Dupont Industrial Biosciences; [2]Dupont Danisco Animal Nutrition*

A large percentage of corn harvested globally is fed to livestock. Its ME results show disparities between trials conducted at different locations impacting published energy values . This variation in energy is multifactorial including used methodologies. Practically, one corn AMEn reference value provides less useful information to a nutritionist looking for a more precise information when formulating poultry feeds in a specific market. A quick reliable prediction of corn energy value is a key requirement for today's efficient and profitable animal protein production. It is crucial to

use prediction models based on a large and varied data set of corn samples. Corn samples from different markets were evaluated in several *in vivo* AMEn trials with 21-day old broilers to develop a near infrared transmittance-based (NIT) calibration PLS model for analysis of whole corn grain. The model has been validated with an independent sample set and shown to be highly predictive of corn AMEn. In the follow up step, AMEn was determined for 2684 commercial corn samples from Brazil, USA, China and India collected during 2017 and 2018 (some corn samples from China were stored since 2015) using the developed NIT AMEn model. A regression analysis of the entire dataset was conducted for each country corn using the Minitab® 18.1. software. Furthermore, each variable from the regression analysis (starch, oil, protein, moisture) was fitted according to the data characteristics (the @RISk software, Palisade Corp.). A simulation model was done using @RISK with a Monte Carlo sampling method. All AMEn data obtained from the simulation were subjected to one-way ANOVA followed by Tukey HSD test for pairwise comparisons. The evaluation of dataset showed that corn samples from Brazil, USA, China and India had average AMEn of 3418^{c}, 3354^{d}, 3428^{b} and 3438^{a} kcal/kg (as fed basis), respectively at a level of significance α=0.05. This suggests that using one table value does not account for incoming corn variability across different markets. For example, Brazilian tables (4^{th} edition, 2017) recommend corn energy value of 3364 kcal/kg. The chance of achieving this approximate corn energy value in Brazil, USA, China and India is 95%, 40%, 85% and 100%, respectively, based on this research finding.

Key Words: Corn AMEn, Brazil, USA, India, China

P289 Effect of the dietary inclusion of black soldier fly larvae meal on broiler performance and carcass traits Mireille Arguelles-Ramos*[1], Christian Hernandez-Gracia[2], Gabriela Ramirez-Diaz[2], Tayna Carrasquillo-Santiago[2], Nashalisse Fernandez-Otero[2], Karla Rodríguez-Camacho[2], Jose Fonseca-Martinez[2], Aixa Rivera-Serrano[2], Lizmarie Camareno-Lozada[2] *[1]DEPARTMENT OF ANIMAL AND VETERINARY SCIENCES, CLEMSON UNIVERSITY; [2]DEPARTMENT OF ANIMAL SCIENCE, UNIVERSITY OF PUERTO RICO - MAYAGUEZ CAMPUS*

Black soldier fly larvae meal (BSLM) has a desirable nutritional profile for poultry diets, being an excellent source of protein and energy. The objective of this study was to evaluate different inclusion rates of BSLM in broiler diets on growth performance, and carcass traits. A total of 400, 1 d-old CobbxHubbard chicks were randomly distributed among 20 floor pens and fed one of three dietary treatments. The starter and grower feeds were fed (mash) from 1-14 and 15-42d, respectively. The dietary treatments were as follow: control (0% BSLM), 5% BSLM and 10% BSLM inclusion. Feed intake (FI), body weight (BW), mortality and adjusted feed conversion ratio (FCR) were determined on a weekly basis. At 42d, 3 males per pen were randomly selected and weighted for carcass traits determination. A one-way ANOVA using a completely randomized block design with 6 replicate pens per dietary treatment was employed. The GLM procedure of SAS was used to analyze live performance and carcass traits, and means were partitioned by LSMEANS. Statements of statistical significance were based on $P<0.05$. Until 21d, broilers fed 5% BSLM had similar BW gain (BWG) to the 0% BSLM birds. After that period, the inclusion of the BSLM caused a reduction in the BWG, being more accentuated at the highest inclusion level. In fact, 10% BSLM birds gained less weight than their counterparts from 14d until the end of the trial. FI was similar for all groups on the first three weeks of the trial. Broilers fed 0% and 5% BSLM had higher FI than 10% BSLM birds, from 22-28 d and 29-35 d. On the last week of the trial, the 0% BSLM had the highest FI for all groups. FCR was similar for all groups from 1-7d and 8-14d. However, on the subsequent weeks, 0% BSLM broilers were more efficient, followed by the 5% BSLM treatment, being the 10% BSLM birds the least efficient. The control group showed a higher percentage of dressed carcass and *Pectoralis major* than 10% BSLM group. No significant differences among treatments were observed on the percentage of thighs, wings or *Pectoralis minor*. It was concluded that under the conditions of this study, a 5% inclusion of BSLM could be used as a source of protein and energy in poultry diets.

Key Words: black soldier fly, broilers, alternative feed ingredients

P290 Titration of spray-dried plasma in broiler starter feed on early broiler performance Joy Campbell*[1], Ricardo Gonzalez-Esquerra[1], Javier Polo[1], Dan Moore[2] *[1]APC, Inc.; [2]Colorado Quality Research, Inc.*

Spray-dried plasma (SDP) has been utilized in poultry and other livestock species for the beneficial effects of improved growth and feed efficiency and reduced mortality in both, production and challenge conditions. The objective of the current study was to determine if feeding titrated levels of spray-dried plasma (SDP) in the broiler starter feed would impact early broiler performance and mortality when housed in simulated production conditions. The experimental design consisted of 3 treatments of titrated levels of plasma of 0, 0.75, or 1.5% SDP formulated into the starter feed fed from d 0 to 14. Diets were corn/SBM-based, crumbled and formulated with nutrient density like commercial practice in North America. All diets were formulated accounting for SDP nutrient content and level of inclusion so that all diets had similar nutrient profile. The trial was terminated at 14 d of age and birds were disposed of in agreement with the animal welfare protocol in place. Prior to placement, broilers were cocci vaccinated via spray at 1 day of age using the commercial dose. The broilers were housed in floor pens with built up used litter with humidity and temperature maintained to simulate production field conditions. The broilers were housed as 25 birds per pen with 10 pens per treatment. Average body weight (BW), gain, mortality adjusted feed conversion ratio, feed intake, and mortality were determined on days 0, 7, and 14. Data were analyzed by ANOVA with linear contrast and significance determined at $P<0.05$. From d 0 to 14, as plasma inclusion increased from 0 to 1.5% in the starter feed, BW (0.33, 0.35 and 0.37 kg), average gain (0.287, 0.303 and 0.325 kg), and average daily feed intake (27.2, 27.9 and 29.1 g/d) increased linearly ($P<0.001$) for 0, 0.75 and 1.5% inclusion level of SDP respectively. However, feed conversion (1.333, 1.298 and 1.291) and adjusted feed conversion (1.327, 1.286 and 1.282) decreased linearly ($P<0.05$) for 0, 0.75 and 1.5% inclusion level of SDP respectively. Mortality was unchanged ($P>0.10$) between treatments. In conclusion, these results indicate that increasing levels of SDP up to 1.5% in the starter feed from d 0 to 14 positively impacted early broiler performance by improving gain and feed conversion linearly.

Key Words: spray-dried plasma, broilers, performance

P291 Evaluation of dried black soldier fly larva as a feed ingredient for poultry production Matthew Jones*, Adam Davis *University of Georgia*

Female black soldier flies deposit their eggs near decaying organic matter such as animal manure, vegetative waste, carrion, and municipal waste. Once the eggs hatch the larva consume the organic material for about 18 days, and thus effectively convert this waste material into larval protein and fat deposits which sustain them through the pupa and adult fly stage. Nutrient dense larva can be harvested, dried and included in diets to support animal production. The goal of the present research was to establish the nitrogen corrected true metabolizable energy value and digestible amino acid values of dried black soldier fly larva (BSFL) and prove that BSFL could be incorporated in broiler diets to replace part of the dietary corn and soybean meal without compromising growth and feed efficiency. Cecectomized and intact adult Single Comb White Leghorn roosters were used to determine amino acid digestibility and TMEn, respectively of dried BSFL samples from 6 different suppliers. In 2 experiments, BSFL was incorporated into broiler starter diets at 0, 6 or 12% and fed from 0 to 21 days of age. The TMEn values on as is basis ranged from 3,611 to 5,142 kcal/kg. The variability in TMEn values reflected in large part the fat content of BSFL which varied from 20 to 44%. The lower fat content of BSFL is associated with a larva harvest a few days earlier than occurs with larva

containing a higher fat content. The crude protein content varied from 28 to 46%. The average digestibility coefficient of the essential amino acids in the 6 samples ranged from 85 to 92%. This variability in part, appears to be associated with drying technique, as a modification to a less harsh drying technique by the manufacturer that produced the BSFL with an essential amino acid digestibility coefficient of 85% resulted in an improvement of this value to 92%. Interestingly, the mean digestibility coefficient of valine across all samples was only 82%. Broilers fed starter diets containing 6 or 12% black soldier fly larva had body weight gains that were equivalent to those fed a control diet containing no black soldier fly larva. Overall, the results suggest that as uniformity increases in the production of BSFL, it has excellent potential as a feed ingredient for poultry.

Key Words: true metabolizable energy, digestible amino acids

P292 Tolerance of Trans-2-Hexenal in Broilers Kurt Richardson*[1], Phil McQuire[1], Carlos Millán[2], Inmaculada Ramírez[2], Marta Gracia[2] *[1]Anitox Corp; [2]Imasde Agralimentaria, S.L. C*

Aldehydes have biological activity, including inhibition of metabolism in eucaryotic and procaryotic organisms, antitumor effects, cell division, and activity against various bacteria, fungi, and viruses. Trans-2-hexenal, an aldehyde present in fruits and grains, has been demonstrated to exhibit antimicrobial activity against both gram positive and gram negative bacteria including Salmonella at relatively low inclusion rates. The purpose of these experiments were to determine the level of trans-2-hexenal tolerated by broilers and study the effect of long term of feeding trans-2-hexenal treated feed to broilers. In Trial 1, a total of 192 Cobb 500 male one-day-old broilers were placed in 24 Petersime battery cages with 8 broilers/pen. Six treatments were applied based on the inclusion of trans-2-hexenal in the diets at 0, 250, 500, 1000, 2000 and 4000 ppm. Body weight gain, feed intake and mortality were recorded. Feed efficiency was calculated at 21 days. No significant differences ($P < 0.05$) were observed in growth and feed intake trans-2-hexenal up to 2000 ppm. At 4000 ppm, feed intake and body weight gain were reduced, but there was no significant on feed conversion. In Trial 2, a total of 440 Ross 308 one-day-old broilers, half male and half female, were placed in 20 floor pens with 22 broilers/pen. Two treatments were applied based on the inclusion of trans-2-hexenal in the diets at 0 and 500 ppm. Observations of body weight gain, feed intake and feed efficiency were recorded at 7, 21 and 42 days. From 0-7d and 0-21d, broilers receiving trans-2-hexenal exhibited significantly ($P < 0.05$) improved feed efficiency compared to control birds. No other significant differences between treatments were observed during the finisher period (22-42d). For the long term feeding experiment, trans-2-hexenal supplementation at 500 ppm significantly improved European Production Efficiency Factor (EPEF) over the non-supplemented (+3.8%, 316 vs 328; $P = 0.017$). It is concluded that, at dosages up to 2000 ppm, trans-2-hexenal was well tolerated by broilers. Improved feed conversion and EPEF was observed with trans-2-hexenal supplementation and no significant adverse changes were detected.

Key Words: trans-2-hexenal, tolerance, broilers

P293 Development of consolidated feed composition tables for poultry species Andres Schlageter-Tello[1,2], Melissa Hannas[3], Julianna Jespersen[4], Kaylee Hahn[4], Muhammed Shameer Rasheed[4], Maci Oelschlager[4], Ashley Bigge[2], Dani-el Hanna[2], Ryan Dilger*[1,4] *[1]National Animal Nutrition Program; [3]Universidade Federal de Viçosa; [4]University of Illinois; [2]University of Nebraska*

The process of formulating diets for poultry species requires an accurate knowledge of the nutrient composition of feedstuffs. One of the most important sources of information on feed composition for poultry species are tables published in the Animal Health and Nutrition series by the National Academies of Sciences, Engineering, and Medicine (NASEM; formerly the National Research Council or NRC). The last version of the consensus study report for poultry species was published in 1994, but a currently convened poultry committee is working on an updated report to be published in2020. With this context, our objective was to describe a process that is in development to potentially be used in the creation of feed composition tables that will be referenced by the 10th edition of the poultry consensus study report. The new feed composition tables will exist largely as an online database linked to the NASEM report and accessible through a website maintained by the National Animal Nutrition Program (NANP, http://animalnutrition.org). The NANP is a National Research Support Project (NRSP-9) supported by the USDA that is specifically focused on curating large databases involving analytical and performance data useful in modeling outcomes pertinent to animal agriculture. As such, the NANP feed composition database allows a user to access verified compositional data on more than 400 feedstuffs with 255 analytical targets, plus nutritive values as generated from more than 2 million feed samples, all in a mobile-friendly format. Moreover, the database is easily filtered to allow for the display of composition data for a feedstuff given a specific moisture content, published within a given timeframe, and originating from various sources. Data for the NANP feed composition database are extracted from peer-reviewed literature sources as well as academic and commercial analytical laboratories. Nutrient composition data pertinent to poultry species were obtained from consolidating feed composition tables for from the 11th edition of the swine NASEM report and a systematic literature review of feed composition data collected from more than 30,000 peer-reviewed articles published between 2011 and 2018 in 20 peer-reviewed journals.

Key Words: feed composition, nutrients, database, analytical data, NANP

P294 Effect of levels and sources of methionine change gene expression of sulphur amino acids pathway in the kidney of chickens Silvana Lima Dos Santos, Patricia Naves Givisiez, Danila Campos, José Filho, David Pereira Da Silva, Alexandre Moreira Filho, José Vilar Da Silva* *University Federal of Paraiba*

The role of levels and DL-Methionine (DL_Met) and DL-Methionine Hidroxy-Analogue-Free Acid (DL-MHA-FA) in regulating the activity of the complex gene network of metabolism of sulfur amino acids (SAAs) still need to be better elucidated. A total of 450 Cobb-500 male chicks at 8 d of age were randomly distributed in factorial arrangement (3 levels of Met+Cys x 2 Met sources), resulting in 6 diets, with 6 replicates of 15 birds. Three diets (deficient, requirement and excess in Met) were formulated to 8-21 d (0.63, 0.88 and 1.13%), 22 to 33 d (0.58, 0.83 and 1.08%) and of 33 to 49 d (0.52, 0.77 and 1.02%). The chickens were slaughtered at 49 d of age and liver samples were collected and frozen in liquid N. Total RNA was extracted with Trizol and purified. Afterwards, RNA were transcribed till cDNA. This was taken as template in mixture with primers (forward and reverse) and Fast SYBR Green Master Mix. RT-qPCR analyzes were performed on 4 samples, one/animal, in triplicates. The data were analyzed by the method $2^{-\Delta\Delta Ct}$ and normalized by β-actin expression. In the Met requirement, MHA-FA reduced ($P<0.05$) the expressions of the MTRR, CBS and GSS genes. The MTRR gene encodes the enzyme that converts Hcys to Met in the presence of folate and B_{12}, whereas the CBS gene encodes the enzyme that converts Hcys + Ser into Cystathionine and then Cys. The GSS gene encodes the enzyme that converts Cys to Glutathione (GSH). The highest expression of MTRR, CBS and GSS genes in chicken liver in the deficiency or excess of Met ($P<0.05$) show that MHA-FA in the deficiency or excess of Met acts in the protection of the organism from oxidative stress and in the improvement of immunity. Excess of Met, regardless of source, increased the expression of the BHMT gene encoding the enzyme that catalyzes the conversion of betaine + Hcy to Dimethylglycine and Met. The higher MTRR, CBS and GSS gene expression in the liver of broilers fed with deficient diets or with excess SAAs suggest previously unknown role of MHA-FA in stimulating Met and GSH synthesis as a possible compensatory homeostatic mechanism. DL-Met and MHA appear to interact with different genes from the route

of AAS metabolism. MHA in deficient or in excess of Met diets increases the expression of the Met and GSH synthesis genes.

Key Words: Animal nutrition, Molecular biology, Nutrigenomics, Amino acids metabolism

P295 Effect of feeding spray-dried plasma in the starter diet for 4, 7 or 10 days at different doses on the overall performance of broilers at 42d of age Ricardo Gonzalez-Esquerra*[1], Joy Campbell[1], Javier Polo[1], Sergio Vieira[2], Liris kindlein[2], Andre Favero[2] *[1]APC, Inc.; [2]Universidade Federal de Rio Grande do Sul*

Spray-dried plasma (SDP) is a functional ingredient that has demonstrated beneficial effects in improving growth, feed efficiency and reduced mortality either in production or under challenge conditions in poultry and livestock. The objective of the current study was to determine if feeding titrated levels of SDP for different durations in the broiler starter feed would impact broiler performance and mortality at 42d when housed in pens simulating commercial production conditions. Ninety floor pens of 25 male Cobb 500 birds per pen were housed in pens within in a commercial barn of 25,000 chickens with reused litter. Treatments were: 1) No SDP; 2) 2% SDP for 4d targeting intake of 1.5 g/bird [4d-1.5g]; 3) 4% SDP for 4d targeting intake of 3 g SDP/bird [4d-3g]; 4) 1.8% SDP for 7d targeting intake of 3 g SDP/bird [7d-3g]; 5) 3.6% SDP for 7 d targeting intake of 6 g SDP/bird [7d-6g]; 6) 1% SDP for 10 d targeting intake of 3 g SDP/bird [10d-3g] and 7) 3.6% SDP for 10 d targeting 6 g SDP/bird [10d-6g]. SDP was formulated into all diets that were Corn/SBM based. Within the same feeding phase, all diets had similar nutrient content across treatments. From d 10 to 42, all birds were fed common grower and finisher diets. In general, SDP intakes were close to target (+3.8%). Differences at 10 and 28d of age were numerical for all performance traits. At 42d of age, the BWG of all SDP treatments was significantly higher vs Ctrl (3143 vs 3269g; $P<0.05$) surpassing the targets suggested by the Cobb Management Guide in 2018 by about 165g per bird. Feed conversion improved by feeding all SDP treatments (1.747 vs. 1.686; $P<0.05$) and feed intake was unchanged ($P>0.05$). Live weight per m2 was also increased by feeding SDP (35.2 vs 37.5 kg for Ctrl vs SDP-groups, respectively; $P<0.05$). Mortality was reduced (7.2 vs 4.86%) and uniformity improved (8.06 vs. 8.50% CV of d 42 BW) by feeding SDP vs Ctrl, respectively. Feeding SDP to broilers in the starter diet improved most performance parameters from 0 to 42d still, no differences were noted across level or duration of feeding. Conclusions: Feeding SDP in the starter feed significantly improved BWG, FCR, Uniformity, Livability, and kg of live BW per m2 in broilers from 0 to 42d housed in a simulated commercial environment.

Key Words: Broilers, Spray Dried Plasma, Performance, Functional Ingredients, Early feeding

P296 Evaluating the effects of saponin and essential oils on performance of meat type quail production Chandler Keck*[GS], Zack Lowman, Chris Ashwell *NC State University*

As population around the world has increased exponentially, so has the need for protein sources such as poultry products. Although laying hens, broiler and turkey meat production dominate the poultry industry, quail meat and egg production provide a possible alternative to these. Due to the current size of the industry, research on the efficacy of feed additives specific to quail production is limited compared to other commercial poultry. The objective of this study was to evaluate the effects of two different feed additive products (a saponin based additive and an essential oil blend) on all phases of quail production (grow-out, egg production, and hatchability). 180 Birds were brooded together until d21 and then randomly assigned to 1 of 3 treatment diets: Control (Basal), Treatment 1 (Saponin 500g/ton), or Treatment 2 (Saponin 500g/ton and essential oil blend 500g/ton). The birds were tagged and placed in three levels of a brooder, one for each treatment. Individual body weights were measured weekly starting at d14. At d21 the birds were moved to 30 battery-style cages with 10 replicate cages per treatment with six birds each. At d42, 62 females and 31 males were transferred to 31 layer cages where they were fed layer diets with equivalent treatment levels to the grow out diets. Egg production was recorded daily for six and a half weeks. Eggs were collected and saved for 10 days, incubated and hatched in order to determine the effects of the treatments on hatchability. All data was analyzed using one-way ANOVA of JMP 13. The addition of Saponin alone showed significant increased body weights at early ages while the addition of both Saponin and essential oil demonstrated significant increases ($P<0.05$) in body weight throughout grow-out as well as increased egg production. No significant differences were observed in fertility or hatchability. These results warrant further research in order to test different inclusion rates of each product to determine optimal levels for quail.

Key Words: Quail, Saponin, essential oil, meat production, egg production

P297 Intermittent administration of probiotics during feed changes promotes beneficial microbiota and improves intestinal morphology and growth performance of broiler chickens Timothy Broderick*[1GS], Ida Hindrichsen[2], Jason Lee[1], Tri Duong[1] *[1]Texas A&M University; [2]Novozymes A/S*

Lactic Acid Bacteria (LAB) have been used widely as probiotics in poultry production because of their health and performance benefits. Shifts in feed ingredient profiles across diet phases alter fermentation substrates in the gastrointestinal (GI) tract and perturb the microbiota. Administration of LAB during diet changes may mitigate perturbations in the GI microbiota and improve growth performance. In this study, we evaluated the effect of intermittent administration of probiotic LAB through drinking water during diet changes on growth performance, intestinal morphology, and GI microbiota in broiler chickens through 28 d of production. Broiler chickens were fed the same diet and administered either untreated water or LAB-treated water at 10^6 CFU bird^{-1}day^{-1} for 1 d pre-diet change (Low) or 10^8 CFU bird^{-1}day^{-1} for 2 d pre- and post-diet change (High). Growth performance, intestinal morphology, and bacterial counts were analyzed using ANOVA and correlated using Pearson's *r*. FCR over d 0-27 post-hatch of broilers administered probiotic-treated water was lower when compared to the untreated control ($P = 0.013$). Recovery of LAB at d 15 post-hatch was greater from broilers administered the high probiotic dose than from the other treatments ($P < 0.001$). Additionally, the decrease in total LAB from d 11 - 15 observed in the untreated group was reversed by intermittent probiotic administration ($P = 0.012$). Crypts were shallower at d 15 in the probiotic treated groups as compared to the untreated control ($P = 0.007$). Increases in LAB counts over d 11 – 15were correlated with lower grower ($r = -0.307$, $p = 0.013$) and cumulative ($r = -0.470$, $p = 0.009$) FCR and shallowing of crypts ($r = -0.570$, $p = 0.001$). Shallowing of crypts from d 11 – 15 was correlated with lower grower ($r = 0.428$, $p = 0.018$) and cumulative ($r = 0.451$, $p = 0.012$) FCR. Our results suggest that intermittent administration of probiotic LAB in drinking water during diet changes promotes positive shifts in the GI microbiota, decreases crypt depth, and improves feed efficiency in broiler chickens.

Key Words: probiotics, microbiota, intestinal morphology, growth performance, Lactic Acid Bacteria

P298 Effect of probiotic metabolite as an antibiotic replacement on performance, digestibility and immunity in broilers fed different energy diets Fernanda Castro*[1GS], Sean Yang[2], Elizabeth Wozniak[2], Woo Kim[1] *[1]University of Georgia; [2]Cytozyme Laboratories*

The aim was to investigate the effect of Lumensa™ for Poultry (Lumensa; AAFCO 36.11 Dried *Lactobacillus acidophilus* Fermentation Product) as a replacement of bacitracin methylene disalicylate (BMD-50) on performance, nutrient digestibility and immunity in broilers. A total of 640-d-old male chicks (Cobb 500) were distributed randomly in 4 treatments, 8 replicates of 20 birds, allocated to floor pens with used litter and grown for

42 d under a normal condition. The diets were two energy levels (Positive control – PC: recommended energy level, and Negative control – NC: reduction in 100 kcal/kg ME) and the addition of either BMD-50 (0.05%) or Lumensa (0.1%). At 28 and 42 d, feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) were measured, and at 42 d cecal tonsil was collected for immune gene expression and ileal digesta for dry matter (DM), protein (CP) and nitrogen-corrected apparent metabolizable energy (AMEn) digestibility. One-way ANOVA was performed and means were compared by Tukey's test (P<0.05). From 1-28 d, BWG and FI were not different between the treatments, while FCR was higher for birds fed NC+Lumensa compared to PC+Lumensa (P=0.027), with no difference in FCR between BMD-50 and Lumensa groups. Similar results were obtained from 1-42 d with no differences found for BWG and FI, and FCR was higher for NC+Lumensa compared to PC diets (P=0.006). There was no significant difference in FCR between NC+BMD-50 and NC+Lumensa. At 42 d, CP digestibility was lower for NC+BMD-50 compared to PC+BMD-50 (P=0.012), and no differences between BMP-50 and Lumensa groups were significant. AMEn was lower for birds fed NC diets compared to PC+BMD-50, and PC+Lumensa was higher than NC+Lumensa (P=0.003). The expression of Interleukin-6 (IL-6) was upregulated in NC+BMD-50 compared to PC+BMD-50 and NC+Lumensa (P=0.003), indicating that Lumensa in NC diet has an immune modulation effect. The expression of IL-1β was upregulated in birds fed NC+BMD-50 compared to PC+BMD-50 (P=0.008). In conclusion, the inclusion of 0.1% of Lumensa resulted in similar performance, nutrient digestibility and immune responses compared to antibiotic BMD-50 at 0.05%, suggesting that Lumensa is a great candidate product to replace a growth-promoting antibiotic treatment.

Key Words: Antibiotic, BMD-50, Broilers, Lumensa, Immunity

P299 Effects of Butyric Acid on performance parameters, egg quality, and nutrient utilization in young White Leghorn hens Daniel Hanna*[GS], Sheila Purdum, Napoleon Vargus-jurado *University of Nebraska-Lincoln*

The purpose of this trial was to evaluate the effects of Prophorce Butyric Acid on the performance of young White Leghorn laying hens. A total of 180 White Leghorn hens were placed in 60 conventional cages (3 birds/cage) using a Randomized Complete Block Design. The hens were 35 weeks of age at the start and the trial spanned 40 weeks. The two treatments were the Control (TRT 1) and Butyric Acid (TRT 2). Egg production and hen mortality were recorded daily. Egg shell strength, egg weight, and egg mass were recorded biweekly. Hen weights and egg components were measured monthly. Calcium (Ca) digestibility, Phosphorus (P) digestibility and apparent metabolizable energy (AME) were calculated when the hens were 55 weeks (Col.1) and 65 weeks of age (Col.2). There was no significant treatment effect on average egg production, egg weight, egg mass, mortality, feed intake, egg components or hen weights. There was a significant treatment effect on egg shell strength (p=0.0493) in favor of butyric acid. There were numerical differences seen in mortality and hen weights in favor of butyric acid. Both time collections showed significant differences between the two treatments for AME (p<0.0001) as well as a significant trt*time interaction (p=0.0002). Numerical improvement in Ca digestibility from the first to the second collection were shown for both treatments, but there were no significant differences in Ca digestibility between the two treatments. P digestibility had no significant treatment effects. Time had a significant effect on both Ca and P digestibility (<0.0001 and 0.0004 respectively) with P digestibility having a significant trt*time interaction (p=0.0410). In summary, Prophorce Butyric Acid significantly improved the egg shell strength and metabolizable energy. Improving these factors would thereby increase the efficiency of energy utilization for egg production and producing a high-quality egg while reducing the amount of energy that is lost in the feces.

Key Words: Butyric Acid, Laying Hens, Performance Parameters, Digestibility, Metabolizable Energy

P300 Effect of different doses of curcumin on growth performance and gut health in Eimeria-challenged and non-challenged broiler chickens Sudhir Yadav*[1GS], Po-Yun Teng[1], Tatiane Santos[2], Lorraine Fuller[1], Woo Kim[1] *[1]University of Georgia; [2]São Paulo State University*

Supplementation of broiler diets with feed additives such as chemotherapeutic drugs, vaccines, and antibiotics has side effect, meat residue, and antibiotics resistance complications. Plant-derived natural compounds could be a safe and easy substitute for chemical additives. One of the natural compounds is curcumin, the extract from the herbal plant *Curcuma longa*, known for its anti-oxidant and anti-microbial properties could be effective in eliminating coccidia in poultry birds. The objective of this study was to evaluate the effect of curcumin on *Eimeria* challenged and non-challenged Cobb 500 broilers. A total of 360 one-day-old male chicks were housed in 36 cages in a completely randomized design with six replicates of 10 birds each treatment diet. The corn-soybean meal based treatment diets were fed from d12 to d20 to challenged and non-challenged birds in three by two factorial set up: Non-challenged control (NC), Non-challenged 100mg/ kg curcumin (NC+ 100CRM), Non-challenged 200mg/ kg curcumin (NC+ 200CRM), Challenged control (CC), Challenged 100mg/ kg curcumin (C+ 100CRM), and Challenged 200mg/ kg curcumin (C+ 200CRM). Broilers in challenged groups were inoculated orally with 50,000 oocyst of *E. Maxima* and *E tenella* and 250,000 oocysts of *E. acervulina* on d14, and birds in non-challenged groups were inoculated with the same amount of phosphate buffered saline. The growth performance parameters, intestinal permeability, and intestinal lesion scores were recorded on d20. The means were subjected to ANOVA and, significant were compared by Duncan test ($p < 0.05$). The growth performance was higher ($P < 0.0001$) in non-challenged compared to the challenged group. Although, no interaction was observed between curcumin doses and cocci challenge on growth performance parameters. The intestinal permeability increased ($P < 0.0001$) in challenged birds compared to non-challenged. Results from lesion scores showed a reduction ($P < 0.0001$) in cecal lesions of birds fed C+ 200CRM compared to birds fed C+ 100CRM. However, curcumin had some positive responses in the non-challenged group such as decreased FCR, permeability and lesion score. In conclusion, this study suggests short-term supplementation of curcumin did not show any anti-coccidial property.

Key Words: curcumin, growth performance, intestinal permeability, intestinal lesion scores, anti-coccidia

P301 Efficacy of Buttiauxella phytase on broiler performance, tibia ash, and processing parameters when using a phytase matrix which includes minerals, energy, and amino acids. Hunter Walters*[1GS], Dalton Dennehy[1], Jim Wilson[2], Craig Coufal[1], Jason Lee[1] *[1]Texas A&M University; [2]Danisco Animal Nutrition, DuPont Industrial Biosciences*

The objective of the current study was to evaluate the impact of a *Buttiauxella* phytase matrix value which includes minerals, energy, and amino acids on broiler performance, tibia ash, and processing parameters when fed in corn-soybean diets. experimental design consisted of 3 dietary treatments with 10 replicates per treatment and 40 birds per replicate for a total of 1,200 male broilers. All diets were corn-soybean meal based and contained xylanase with treatments including a positive control (PC), negative control (NC) with a reduction in energy (-71 kcal/kg), minerals (-0.17 avP, -0.19 Ca), and amino acids (-1.9% CP), and NC with 1000 FTU/kg of *Buttiauxella* phytase (NC+1000 FTU/kg). Average body weight (BW), mortality adjusted feed conversion ratio (FCR), feed consumption (FC), and mortality (%) were determined on days 12, 26, 40, and 48. On d 21, 5 birds per replicate were euthanized and tibias were removed, dried, defatted, and ashed to determine tibia ash percent. Following an 8 hour feed withdrawal period, 5 birds per replicate were selected and processed on d 49 for carcass, breast, and tender weight and yield. Data were analyzed via a one way ANOVA with means being deemed significantly different at $P \leq 0.05$. Reducing nutrients in the NC decreased ($P<0.05$) BW and FC while increasing FCR throughout the trial when compared to the

PC. Supplementing *Buttiauxella* phytase in the NC yielded the highest (P<0.05) FC amongst treatments resulting in the heaviest (P<0.05) broiler BW beyond that of the PC through d 26. addition of phytase in the NC yielded BW comparable to that of the PC on d 40 and d 48 while reducing (P<0.05) FCR compared to the NC. The nutrient reduction in the NC decreased (P<0.05) bone ash percent compared to the PC with NC+1000 FTU/kg yielding a bone ash percent similar to that of the PC diet. The removal of nutrients in the NC negatively impacted processing parameters with the NC decreasing (P<0.05) individual live weight and carcass weight compared to the PC. Supplementing *Buttiauxella* phytase in the NC increased (P<0.05) carcass and breast weight beyond the PC. These data confirm the use of a *Buttiauxella* matrix value which includes minerals, energy, and amino acids for phytase inclusion without negatively influencing performance or yield.

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

P303 The effects of medium chain fatty acids in mash and pelleted diets on growth performance of broilers. Gage Nichols*[GS], Charles Stark, Cassandra Jones, R.S. Beyer, Christopher Delfelder, Michaela Braun, Haley Wecker, Caitlin Evans, Chad Paulk *Kansas State University*

The objective of this experiment was to determine the effects of medium chain fatty acids (MCFA) in mash and crumbled diets on broiler growth performance. A total of 400 male chicks (Cobb 500; initial BW 41.8 g) were used in an 18-d study. Broilers were housed in 4 Petersime batteries with ad libitum access to feed and water. Treatments were randomly assigned to 80 cages within location block, resulting in 8 cages per treatment with 5 chicks per cage at placement. Treatments were arranged in a 2 × 5 factorial with main effects of feed type (mash or crumble) and lipid addition (0.5% soy oil control or 0.5% C6:0, C8:0, C10:0, or C12:0). Feed was pelleted with a 85°C conditioning temperature and 20 sec retention time using a California Pellet Mill model CL5 equipped with a 4×22 mm die, then crumbled. Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (Cary, NC). Results were considered significant at $P \leq 0.05$ and marginally significant between $P > 0.05$ and $P \leq 0.10$. There were no feed form × MCFA interactions for growth performance. From d 0 to 12, broilers fed crumbles had improved ($P < 0.001$) BW, ADFI, and FCR when compared to those birds fed mash diets. Broilers fed C6 had increased ($P = 0.036$) FCR compared to those fed the control or diets containing C8 or C10. For the overall treatment period, chicks fed crumbled diets had improved ($P < 0.001$) BW, ADFI, and FCR when compared to those fed mash diets. There was no measured impact of MCFA ($P > 0.10$) on broiler performance over the 18-d experiment. Pelleting and crumbling pellets improved broiler growth performance and final body weight regardless of MCFA inclusion. However, MCFA inclusion did not measurably impact broiler growth performance.

Key Words: Medium-Chain Fatty Acids, Broiler, Poultry

P304 Effectiveness of canola meal on N-3 fatty acid deposition in breast and thigh meat of broiler chickens. Josephine Foley*[GS], Ryan Sedlacek, Napoleon Vargas-jurado, Sheila Purdum *University of Nebraska - Lincoln*

N-3 fatty acids, or Omega-3 fatty acids, are important for human health. Benefits range from improved cardiovascular health, blood pressure and cholesterol numbers to anti-inflammatory, antioxidant, anticancer, and antiarthritis effects. Therefore, foods enriched with Omega-3's have been increasing in popularity. Previously, it was believed that fish oil, or fish meat, is the best source of these fatty acids, but this is a more uncommon diet staple. Without specifically supplementing for N-3 fatty acids, most people are deficient in these important nutrients. In 2013, chicken meat became the most consumed meat in the United States. If N-3 fatty acids could be found in chicken meat, it would dramatically increase the amount of N-3 fatty acids eaten by Americans. This study sought to investigate the effect of 2 canola meal feed additives on the amount of N-3 fatty acid deposition in the breast and thigh meat of conventional broiler chickens. A total of 90 Ross broiler chickens were housed in 9 conventional cages and 12 floor pens for a total of 6 weeks. Each cage or pen was assigned to one of 3 treatment groups. Group 1 was assigned as the control group and the birds were fed a traditional broiler diet. Group 2 was supplemented with GM Canola Meal. Group 3 was supplemented with Defatted Isoline Canola Meal. Body weight gain was measured weekly, feed intake was measured weekly, mortality was tracked daily, feed conversion was calculated weekly, and breast and thigh meat were collected at the end of the trial. Breast and thigh meat samples were sent to a lab for analysis of the total N-3 fatty acid content, with focus directed at deposition of C18:2, C18:3, C20:5, and C22:6 fatty acid content. Overall, this study found improved performance in Groups 2 and 3. A higher feed intake was found for Group 3, supplemented with Defatted Isoline Canola Meal ($p<0.01$). Bird weights reflected this, with a higher overall bird weight in Group 3 ($p<0.01$). This study did not find significant differences in N-3 deposition in Group 1 versus Group 2 or Group 3. Based on these results, canola meal supplementation may be a good addition to improve performance of broilers. However, canola meal supplementation does not greatly affect the amount of N-3 fatty acids deposited in the meat.

Key Words: Broiler chicken, feed additives, poultry meat

P305 Effect of dietary mixture of Fenugreek (trigonella foenum-graecum), Garlic (allium sativum) and Coriander (coriandrum sativum) on performance of broilers Said Ali*, Abdul Hafeez, Junaid Akhtar *Agriculture University of Peshawar*

Phytogenic Feed Additives (PFAs) may be considered as alternative of antibiotics in broiler feed. The present study conducted at Department of Poultry Science, The University of Agriculture Peshawar, the effect of dietary mixture of fenugreek, garlic and coriander was evaluated on growth performance and economic efficiency of broiler chicks. A total of 128, day-old broiler chicks purchased from local market, were randomly divided to 4 groups (A, B, C and D) contained four replicates with 8 birds. Group-A was kept control. 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 along 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. Data was analyzed through standard procedure of Analysis of Variance using SPSS software. Results revealed that feed intake (FI) was significantly lower for group B compared to other groups at 1st week. Feed intake at finisher phase and overall FI was lower for group C compared to other groups. The overall weight gain was significantly higher for group B compared to other groups. Overall FCR was significantly lower in group D as compared to other groups. Live body weight at day 21 was significantly higher in group C. Carcass weight was significantly higher for group B at day 21 and day 42. No significant effect of dietary treatments on weight of giblets and dressing percentage was found. At finisher phase, cost of feed intake per bird was significantly lower for group D. Significantly lower feed intake cost $Bird^{-1}$ was recorded for birds in group C. Total revenue $Bird^{-1}$ and profit $Bird^{-1}$ was higher for group B. It was concluded that inclusion of PFAs (mixture of Fenugreek, Garlic and Coriander at 0.33% each) at 1.0% in broiler feed resulted in overall lower feed intake and FCR, and higher weight gain, live weight, carcass weight and dressing percentage. Moreover, the use of PFAs at 1.0% was economically feasible. Therefore, dietary mixture of these PFAs at 1.0% may be used for better growth and feed conversion in broilers. The use of these PFAs mixture with different concentrations is recommended in broiler as well as in layer feed.

Key Words: Broilers, Phytogenic Feed Additives, Growth Performance, Economics

P306 Evolution of the ileal flora affected by phytogenic or ionophor feed supplementations on industrial broilers Claire Girard, Thibaut Chabrillat, Sylvain Kerros* *PHYTOSYNTHESE*

Microbiote understanding through metagenomics analyses emerges as a key science to explain how antibiotic alternatives, as phytogenics, may affect the gut health. This study used this new technology to compare the ileal flora modulation effect between phytogenic alternative, ionophor antibiotic program and negative control. 3 batches of 660 day-old chicks Ross PM3 were reared until 35 days and fed with 3 comparative programs: feed supplemented with Maxiban (D1-21) and Monensin (D21-32) (Group M), feed supplemented with Primactiv, a phytogenic additive (Group P), and a control feed (Group C). Each treatment were divided in 10 repeats of 22 boilers places in a 1m² pens. All pens were put in a line on a side of a broiler production unit of 22 000 birds. All birds, per group of 5, were weighed every week. At day 17 and 23, 5 broilers per group were sacrified: ileal contents were collected and bacterial population was determined using MiSeq sequencing of variable regions of the 16S rRNA bacterial gene. Operationnal Taxonomic Units (OTUs) were produced and taxonomic assignment was performed in order to present relative abundance of most important phyllas (% OTUs). LDA Effect Size (LEfSe) was used to identify taxonomic features that were statistically different ($p < 0.05$) between groups. We observed significant differences on live weight at days 14, 22 and 28 whereas at day 35 they were not different. On average of all groups ileum were composed of 73% firmicutes, 22% bacteroidetes and 5% proteobacteria. Between day 17 and day 23, the evolution of several bacterial taxa in ileum was different depending of the group ($p<0.05$). 16 S sequencing show that C had the most important evolution with 48 taxa (46 increased, 2 decreased). Group P had 21 taxa for which proportion increased (including ruminococcaceae and bifidobacteriales), and Group M got only 4 taxa which increased. Moreover, these taxa evolutions were different between M and C groups. This study shows how feed additive programs may influence the broiler ileal flora at the crucial transition period of 17-23 days old.

Key Words: metagenomic, phytogenic, coccidiosis, ileal flora

P307 Effect of target dosing phytase, Natuphos E, levels on apparent metabolizable energy in corn-soy diets fed to female turkeys Mike Coelho*, Stuart Ferry, Ken Parsons *BASF Corporation*

Recent studies demonstrated that higher doses of phytase can break down most of the phytic acid releasing energy from the inositol ring, and decrease in phytic acid increases endogenous enzymes, such as amylase activity (Liu, 2008). This study evaluated the metabolizable energy (ME) release of graded levels of phytase when fed to 28 day old female turkeys on mash corn/soy diets formulated to meet or exceed current NRC (1994) recommendations. A total of 960 female Big 6 turkeys were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (12 birds/pen x 8 treatments x 10 replications). Birds were blocked by weight. Treatments consisted of T1=PC, T2=NC=PC - 0.17% Calcium (Ca), - 0.15% nonphytate phosphorus (nPP), -0.02% sodium (Na), -300 kcal/kg ME, T3=NC +1000 FTU/kg, T4=NC+1500 FTU/kg phytase, T5=NC+2000 FTU/kg phytase, T6=NC+2500 FTU/kg phytase, T7= NC + 3000 FTU/kg phytase and T8=NC+3500 FTU/kg phytase. Partial fecal collection was done at day 28 by papering over the pens. Fecal material was freeze-dried via Lypholizer (FreeZone Freeze Dry Systems) and AME was calculated by the ratio of indigestible marker, using partial excreta collection. TiO_2 was used as the undigestible marker. AME was 3256, 2967, 3060, 3106, 3145, 3174, 3245 and 3248 Kcal/kg, P=0.03, respectively. The NC reduced the ME by 289 Kcal/kg versus the PC. 1000, 1500, 2000, 2500,3000 and 3500 FTU/kg released 93, 139,178, 207, 278 and 281 Kcal/kg, respectively. In conclusion, Natuphos E, by breaking down phytic acid, released up to 281 Kcal/kg ME in turkeys.

Key Words: Metabolizable energy, Turkeys, Phytase, corn/soy diets, AME

P308 Effects of PrimaLac and prototype probiotic on broiler chicken performance and body composition during a naturally occurring necrotic enteritis challenge model Nima Khodambashi Emami*[1], Ali Calik[1], Mallory White[1], Aaron Oxendine[1], Mark Young[2], Rami Dalloul[1] *[1]Avian Immunobiology Laboratory, Department of Animal and Poultry Sciences, Virginia Tech; [2]Star Labs/Forage Research, Inc.*

Ban on the use of antibiotic growth promoters has led to higher risk of enteric disease outbreaks in poultry production. Among these, necrotic enteritis (NE), caused by *Clostridium perfringens,* has a high negative impact on poultry producers' revenue, leading to $5-6 billion loss annually. This study examined the effects of probiotics on performance and body composition of broiler chickens under a naturally occurring NE model. A total of 1,440 day-old male Cobb 500 broilers were allocated to four treatments, each with 12 pens of 30 birds/pen. Dietary treatments included: 1) Negative control (NC): corn-soybean meal basal diet; 2) Positive control (PC): NC+ 20 mg Virginiamycin/kg diet; 3) NC + Prototype at the rate of 0.3 and 0.2 kg/ton from 1-21 and 22-42 days, respectively; and 4) NC + PrimaLac at the level of 1.36 and 0.91 kg/ton from 1-21 and 22-42 days, respectively. One day post-placement, all birds were challenged by a commercial live oocyst coccidia vaccine as a predisposing factor to naturally occurring NE. Body weight and feed intake (FI) were measured at the onset of NE (d8) and end of starter, grower, and finisher periods with average daily gain (ADG), average daily FI (ADFI) and FCR calculated accordingly. At d42, body composition of protein and fat was measured by dual energy X-ray absorptiometry (DXA) from two birds/pen. Data were analyzed using JMP (Pro13) and significance between treatments identified by LSD ($P < 0.05$). Results showed that compared to NC (11.39%), supplementation of PrimaLac and Prototype reduced d1-42 mortality (6.94% and 7.87%, respectively); however, PC (1.94%) was the only group with significantly lower mortality. Despite significantly higher ADG in PC (77.69 g/bird) compared with NC (74.99 g/bird), ADFI was similar in all the treatments. FCR was significantly improved in the PrimaLac, Prototype and PC groups for the entire experimental period. Body composition data showed that the Prototype group had the highest lean and lowest fat percentages, which were significantly better than PC (79.49 and 23.65 *vs.* 73.50 and 25.08, respectively). Collectively, these data indicate that in a naturally occurring NE model, supplementation of PrimaLac and Prototype could have positive effects on broiler performance and body composition.

Key Words: Necrotic enteritis, broilers, performance, body composition

P309 Effect of steam pressure and conditioning temperature on phytase stability. Courtney Truelock*[1], Nelson Ward[2], Jonathan Wilson[2], Charles Stark[1], Chad Paulk[1] *[1]Kansas State University; [2]DSM Nutritional Products*

An experiment was designed to evaluate the effects of steam pressure and conditioning temperature on the stability of microbial phytase. Treatments were arranged as a 2 × 3 factorial of steam pressure (24 and 44 psi) and conditioning temperature (77, 82, and 88ºC). Phytase was added to a corn-soybean meal-based diet and 10 mash samples were collected for phytase analysis. The diet was pelleted via steam conditioning (245 mm × 1397 mm Wenger twin staff pre-conditioner, Model 150) and using a pellet mill (CPM Model 1012-2) with a 4.8 mm × 31.8 mm pellet die. Conditioner retention time was 30 s and production rate was 900 kg/h. All treatments were replicated on 3 separate days. Five pellet and 3 condition mash samples were collected and immediately placed in an experimental counter-flow cooler for 15 min. For each treatment, pellet and condition mash samples were composited such that 2 samples of each were analyzed

for phytase activity. Conditioning temperature and hot pellet temperature (HPT) were recorded throughout each processing run. Data were analyzed using the GLIMMIX procedure in SAS 9.4, with pelleting run as the experimental unit and day as the blocking factor. There was no evidence ($P > 0.54$) for a steam pressure × conditioning temperature interaction for HPT or phytase stability of condition mash. Increasing conditioning temperature from 77 to 88ºC increased ($P < 0.01$) HPT linearly (81.8, 85.6, and 89.1ºC, respectively), and there was no evidence of difference ($P = 0.68$) in HPT between steam pressures. Phytase stability of condition mash decreased linearly ($P < 0.01$; 115.7, 74.0, and 38.3%, respectively) with increasing conditioning temperature. There was a tendency ($P = 0.08$) for a steam pressure × conditioning temperature interaction for phytase stability in pellets. There was no evidence of difference in phytase stability of pellets conditioned at 24 (60.1%) or 44 psi (58.9%) at 77ºC. However, at 82 and 88ºC phytase stability was greater in pellets conditioned at 44 psi (36.7 and 19.1%, respectively) compared to 24 psi (28.1 and 12.3%, respectively). Microbial phytase stability of condition mash and pellets was reduced by increasing conditioning temperature, although the amount of reduction in cooled pellets was dependent on steam pressure.

Key Words: conditioning temperature, pelleting, phytase stability, steam pressure

P310 The effect of Poulk (Stachys schtschegleevii) on performance and immune system of broiler chickens challenged with Infectious Bronchitis Virus Ahad Mozaffari, Shaban Rahimi*, Mahdi Ayyari *Tarbiat Modares University*

This experiment was conducted to evaluate the effects of Poulk (*Stachys schtschegleevii)* on performance, intestinal morphology, microbial population, serum lipids, immune system and improvement of respiratory anomalies in broiler chickens challenged with an infectious bronchitis vaccine virus. The experimented performed for six weeks using 300 male broiler chickens Ross 308 in a completely randomized design with 5 treatments including control group, oxytetracycline 50% antibiotic group 150 g / ton of feed and three groups containing 0.5, 1 and 1.5% extract of the Poulk in drinking water. Each treatment included 4 replicates and 15 chicks per replicate. On day 28, all challenged groups received infectious bronchitis vaccine 10 times of standard dose via the eye drops. On day 21 and 35, three chicks from each pen were selected and the SRBC solution injected through the chest muscle. One week after each injection, the blood sample from three broiler chicks was taken and serum was isolated for the antibody titer against the SRBCs. On day 42, blood samples were taken to determine the serum lipids and antibody against the infectious bronchitis from one bird per replicate. After slaughter and autopsy of one bird from each replication, the trachea with larynx were isolated and transmitted to the lab for histopathological examination. Data analysis was performed by SAS software and comparisons of the averages performed by Tukey test. At 42 days of age, BW, carcass yield, abdominal fat, liver, gizzard, proventriculus, bursa of Fabricius, triglyceride, cholesterol, hematocrit and hemoglobin percentages, and number of the goblet cells were not affected by experimental treatments ($P < 0.05$). Also, FI, FCR, heart weight, antibody titer against SRBC, spleen weight, HDL, LDL, heterophile to lymphocyte ratio, antibody titer against infectious bronchitis virus, laryngeal injuries, Lactic acid bacteria in ileum, villus height, villus width, villus depth and villus surface area of the ileum were significantly affected ($P < 0.05$).

Key Words: Poulk, broiler, infectious bronchitis virus, respiratory tract function, immune response

P311 Carbohydrases or prebiotic oligosaccharides influenced growth performance and the expression of genes related to immune function in 28-day old broilers raised on clean or dirty litter. Allison Craig[1], Farina Khattak[1], Peter Hastie[2], Michael Bedford[3], Oluyinka Olukosi*[4] *[1]Scotland's Rural College; [2]University of Glasgow; [3]AB Vista; [4]University of Georgia*

aim of this experiment was to evaluate the effect of carbohydrase or xylo-oligosaccharide (XOS) supplementation on growth performance, energy utilization and the expression of immune-related genes in broilers. Six hundred and forty Ross 308 broilers were used in this 28-d study. All of the diets were marginally deficient in energy by 239 kcal/kg and protein by 20 g/kg. The study employed a randomized complete block design to investigate 2 litter environments (dirty or clean litter) and 4 additives (control, xylanase alone (XY), xylanase and β-glucanase (XY+BG) or xylo-oligosaccharide (XOS)) in a 2×4 factorial arrangement. birds were allocated to 8 treatments; each treatment had 8 replicates and 10 birds per replicate. Feed and birds were weighed on d 0, 14 and 28. On d 28, two birds per pen were euthanized; tissue samples from the caecal tonsil and bursa of fabricius were collected and fixed in RNA*later*. remaining birds were euthanized and ileal digesta was collected. On d-14, body weight gain (BWG) and feed intake were greater ($P < 0.05$) in birds on clean litter compared with those on dirty litter. It was noted that performance was poor with 28d BWG achieving only 825g (less than half of the target weight for this breed) on average and an gain:feed of 389 g/kg which will have influenced the results. On d 28, BWG was ($P < 0.05$) lower in diets supplemented with XOS or the XY+BG compared with the control. Ileal digestible energy (IDE) was greater ($P < 0.001$) in birds receiving the XY+BG compared to other treatments irrespective of litter type. IL1β expression was greater ($P < 0.05$) in birds raised on clean litter compared to those raised on dirty litter. TLR4 expression was greater ($P < 0.05$) when diets were supplemented with XY compared to XY+BG. re were no treatment effects on IL10 or TLR21 expressions. In conclusion, the lack of positive effect of additive supplementation on growth performance in the current study, is contrary to our previous and subsequent studies which may be related to whatever caused the poor growth rate in this study. In addition, the effect of additive supplementation on the expression profiles of immune-related genes shows potential for carbohydrases or XOS to positively modify immune responses in broilers.

Key Words: Broiler, xylanase, beta-glucanase, xylose-oligosaccharide

P312 Effect of the adition of humic acids and essential oils based blends as growth promoters in broiler chickens in Colombian Middle Tropical Region Joaquín Pirateque Castellanos*[1], Blanca Martínez[2], Álvaro Uribe Serrano[2] *[1]National University of Colombia; [2]Promitec Santander*

The objective of this work was to assess the productive performance in broiler chickens supplemented with *Lippia origanoides* Essential Oil (LOEO), *Eugenia caryophyllata* essential oil (ECEO) and Sodium Humates (NaH) blends at different inclusion rates in a field experiment. One day-old Ross 308 broilers (n = 3060, half males, half females) were grouped by sex and them randomly to one of 5 treatment groups: T1 consisted of 50 ppm LOEO + 50 ppm ECEO + 1320 ppm SH; T2, 75 ppm LOEO + 1350 ppm SH; T3, 3000 ppm SH; T4, 1300 ppm; and T5 as a positive control with antibiotic growth promoters (Halquinol and Enramicin) and anticoccidial drugs (Nicarbazin – Narasin for the first 21 days and Salinomicin from 22th day up the end of the experiment). There were 6 replicate pens in each treatment group (51 birds per pen). Weight, Feed Conversion Rate (FCR), Productivity Index (PI) European Efficiency Index (EEI) and mortality were recorded. Results were subjected to one-way ANOVA to establish whether there were statistically significant differences between groups ($p<0,05$) or not; Tukey and Dunnett test were used for mean comparisons. In females, weight was superior ($p<0,05$) in T2 (2222 g) than in control (2166 g); FCR was superior ($p<0,05$) in T2 (1,60) than control (1,72); in males, weight was superior in T8 (2404 g) and T7 (2398,3 g) than control (2384 g); and FCR was superior ($p<0,05$)

in T2 (1,55) than control (1,63). Overall, birds in T2 developed the best performance at the end of the study. In conclusion, the results indicate that broilers fed diets added with antibiotic growth promoters and anticoccidial drugs; according with the results of this research work a mixture of NaH and EOLO is more effective as a growth promoter additive and that dietary inclusion of 1300 and 300 ppm of NaH alone did not benefited the growth performance of broiler chickens.

Key Words: Lippia origanoides, Eugenia caryophyllata, Humic Acids, phytobiotic additives, Growth performance

P313 Effect of dietary lipase supplementation on energy utilization of broiler chicken Basheer Nusairat*[1], Belal Obeidat[1], Kamel Mahmoud[1], Serhan Haddad[1], Ahmad Al-Azzeh[2], Walaa Al-Yonis[1] *[1]Jordan University of science and Technology; [2]Shenzhen A.Y.A Co., Ltd*

Two trials were conducted to evaluate the effect of adding lipase and/or emulsifier to broiler diet on energy utilization of broilers. Growth performance, carcass characteristics, intestinal morphometric measurements and ileal digestibility of crude fat (CF) were investigated using a total of 840 one-day-old commercial mixed sex broiler chicks. Basal diets of starter, grower, and finisher were formulated according to the recommendations of commercial broiler chicks and designated as positive control (PC). Negative control (NC) diets were formulated with a lower 50 kcal of metabolizable energy (ME) compared to PC diets. In experiment 1, 600 broiler chicks were randomly assigned into five treatment groups of PC, NC, NC plus lipase (NCL), NC plus emulsifier (NCE), and NC plus lipase and emulsifier (NCLE). Each treatment was represented with six replicates with 20 broiler chicks per replicate. In experiment 2, 240 broiler chicks were assigned randomly into four groups of PC, PC plus lipase (PCL), PC plus emulsifier (PCE), or both (PCLE). Each treatment was replicated two times with 30 chicks per replicate. The results of experiment 1 showed that lipase supplementation did not affect broiler growth performance. No differences were observed in feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) as cumulative data whereas numerical improvements in FCR values were reported for broiler chicks fed diets supplemented with both lipase and emulsifier. Lipase supplementation increased (P<0.05) pancreas percentage from the body weight but no differences were recorded for carcass characteristics parameters at 35 days of age. The gut morphology examination showed that lipase and/or emulsifier had no effect on jejunum morphology. Experiment 2 results showed that lipase supplementation to PC diet improved (P<0.05) CF digestibility at the end of 8 days of age. It may be concluded that adding both of lipase and emulsifier positively improved CF ileal digestibility which reflected on a better FCR values.

Key Words: Broiler, lipase, emulsifier, growth performance, ileal digestibility

P314 Effect of Clostridum sp. nov. and Lactobacillus sp. nov. on broilers challenged with Clostridium perfringens Grant Gogul*, Gavin John, Adam Taylor, Allison Wells, James Gaffney, Shea Grenier-Davis, Michael Pham, Jennifer Cao, Mallory Embree *Ascus Biosciences*

Concerns over decreased broiler performance due to *Clostridium perfringens*-induced necrotic enteritis (NE) has amplified in the poultry industry. The objective of this study was to evaluate *Clostridium perfringens*-induced necrotic enteritis (NE), growth performance, feed conversion, and mortality in broilers supplemented with an endomicrobial supplement (EMS) consisting of native bacteria isolated from the chicken gut microbiome. The study consisted of 10 treatment (TRT) groups (n=180/TRT): TRT 1 and 2 received feed mixed with calcium carbonate while TRT 3-10 received feed mixed with an EMS comprised of different combinations/doses of 2 *Clostridium* sp. nov. and a *Lactobacillus* sp. nov ranging from 10^4 to 10^7cells/bird. On d 17, TRT groups 2-10 were challenged with *C. perfringens* ~10^8cfu/ml via a broth culture mixed with feed (~25 g/bird). All treatment diets were a standard formula representative of commercial broiler diets and feed were provided *ad libitum*. Feed conversion ratio (FCR), body weight gain (BWG), necrotic enteritis (NE) lesion scores, and mortality were evaluated at d 0-17, d 0-28, d 0-35, d 0-42, d 17-28, d 17-35, d 28-35, and d 35-42. Data were analyzed using a one-way ANOVA and performance means were compared using Tukey's Post Hoc Test. On d 16, 21 and 42, 2 birds from each TRT were removed, weighed, and sacrificed for collection of ileal, cecal contents to confirm colonization of the administered bacteria by sequencing the 16S rRNA V1-V3 hypervariable regions on the Illumina MiSeq Platform. On d 21 TRT 4, 5, and 8 had lower mean NE lesion scores compared to TRT 2 (NE lesion score<1.0 vs. score>1.5); on d 28, TRT 4, 6, 8, and 9 had significantly reduced NE lesion scores (p<0.05). Although the mean NE lesion scores for TRT 3, 7, and 10 is not significant on d 28, there is a trend of lowering in the lesion scores (score<1.0) as well as lower mortality across TRT 4-10. There were no significant trends observed for FCR, BWG, or mortality. Data demonstrate that the endomicrobial supplement containing all 3 bacteria may help prevent the development of necrotic enteritis in birds challenged with *C. perfringens* but further research is needed to extend these findings.

Key Words: Clostridium perfringens, endomicrobial supplement, performance, mortality, microbiome

P315 Can phytogenic additives improve the performance of broilers and replace antibiotics? A meta-analytic approach. Gustavo Polycarpo*, Gabrieli Andressa de Lima, Thaís Ávida, Valquíria Cruz-Polycarpo, Polyana Giacomini, Mariana Lira, Jonas Camporezi, Fernanda Braga *São Paulo State University - UNESP*

Large quantities of scientific articles have been published about the use of phytogenic additives, and many of them have conflicting results among each other. So, meta-analysis is necessary to organize the information, considering the heterogeneity between the experiments, transforming the research results into an applicable knowledge. The aim of this work was to evaluate the phytogenic additives as an alternative to growth-promoting antibiotics on the performance of broiler chickens, through a meta-analysis. The main criteria for the articles selection were: a) *in vivo* experiments with phytogenic supplementation for broilers, b) evaluation of performance variables, c) no antibiotic or anticoccidial in the diets (except for the antibiotic treatments), and d) no combinations of phytogenic additives with other additives. At the end of searches and filtering, 376 experiments published from 1997 to the first half of 2018 were used. A control group without additives and two other groups with phytogenic additives or antibiotics were studied in the absence or presence of microbial challenge (inoculation). Mixed procedures in SAS were used in the statistical analysis, with the experiments as a random-effect class variable and the age of the birds as fixed-effect covariate. The additives presented an interaction with microbial challenge on ADG and FCR (P < 0.01). Without challenge, the ADG and the FCR were improved by the phytogenic additives [+3.02% (P < 0.01) and -2.76% (P < 0.01), respectively], and no differences between phytogenic and antibiotics were observed (P > 0.05). However, under high microbiological challenge, despite the phytogenic additives again had improved ADG and FCR [+8.00% (P < 0.01) and -7.38% (P < 0.01), respectively], the results obtained in the phytogenic group were worse than those observed with antibiotics [-4.36% (P = 0.02) and +3.49% (P = 0.01), respectively]. ADFI was not influenced by the additives (P > 0.05). In conclusion, phytogenic additives improve the performance of broiler, and may substitute growth-promoting antibiotics without impairing performance under low microbiological challenge conditions. Nevertheless, the results fall short of those obtained with antibiotics if broilers are subjected to a high microbiological challenge.

Key Words: broiler, meta-analysis, microbiological challenge, performance, phytogenic additive

P316 Dose response evaluation of OceanFeedTM Poultry seaweed blend on growth performance, breast meat yield, and plasma biochemical profile in broiler chickens Mohsen Mohammadigheisar[1], Victoria Shouldice[1], Jason Sands[2], Elijah Kiarie*[1] [1]*University of Guelph;* [2]*Ocean Harvest Technology*

Considering restrictions on antimicrobial growth promoters, strategies to improve gastrointestinal tract health are needed. We evaluated dose response of OceanFeed™ Poultry seaweed blend (OFP) on growth, breast yield and plasma biochemical profile in 864 Ross x Ross 708 male broiler chickens. Diets were formulated for a 3-phase feeding program: starter (d 0-10), grower (d 11-24) and finisher (d 25-42). There were 4 dietary treatments: 0, 0.5, 1, and 2% of OFP. Birds were housed in 48 floor pens (n=12) and had free access to diets and water. Body weight and feed intake (FI) were monitored by phase and serum samples were taken on d 42. In starter phase, OFP linearly ($P \leq 0.01$) improved BW, BWG, and FCR. However, the improvement was also quadratic indicating no further improvement beyond 0.5% OFP inclusion. Growth response to OFP in the grower phase was similar to the starter except for FCR ($P > 0.05$). Linear ($P = 0.07$) and quadratic ($P = 0.08$) increases in BWG were observed in birds fed OFP in the finisher phase. Overall (d 0-42), a linear and quadratic ($P < 0.01$) response was observed for final BW, in that birds fed 0.5, 1, and 2% OFP were heavier than control by 166, 183, and 180 g, respectively. The BWG for birds fed 0.5, 1, and 2 % OFP was higher by 5.9, 6.5 and 6.4%and corresponding FI was higher by 3.5, 3.6, and 4.5%, respectively compared to control birds. As a result, diet effect on FCR was not significant overall ($P > 0.05$). Overall, mortality was 3.2, 1.9, 3.2 and 1.9% for birds on 0, 0.5, 1 and 2% OFP diets, respectively. A quadratic ($P = 0.08$) tendency was observed for breast yield in response to OFP with birds fed 0.5, 1, and 2% OFP showing 6.6,7.8 and 4.6% higher breast yield relative to the control. Glutamate dehydrogenase (GLDH) is a key link between catabolic and anabolic pathways for amino acids utilization. Supplemental OFP linearly ($P = 0.04$) increased plasma GLDH. There were no diet effects on other plasma metabolites, minerals and electrolytes. In summary, the optimal inclusion for OFP was in the range of 0.5 to 1% for improved BWG and breast yield. However, this study was conducted in a high health facility, given the quadratic response to seaweed blend, the higher doses may be applicable under more health challenging environmental conditions.

Key Words: Broiler, growth performance, gut health, seaweed blend, plasma biochemical profile

P317 In vitro degradation of soybean and sorghum anti-nutritional factors (ANFs) by a commercial mono component protease Tine Jensen*, Mads Pedersen, Lærke Haahr *Novozymes A/S*

The quality and processing conditions of feed raw materials such as soybean (*Glycine max*) and sorghum varies. Heat treatment during processing can to a certain extent reduce the content of anti-nutritional factors (ANFs) present in the meal, but the final level varies, which is seldom accounted for in diet formulation. ANFs work by different mode of actions to decrease nutrient uptake and animal growth performance.

The objective of this study was to evaluate the effect of a commercial available protease (RONOZYME® ProAct, DSM Nutritional Products, Basel, Switzerland) by SDS-PAGE for its ability to degrade the soy protein ANFs Lectin, The Kunitz trypsin inhibitor (KTI) and Bowman-Birk trypsin inhibitor (BBI), glycinin and beta-conglycinin under physical conditions relevant for the gastrointestinal tract of mono-gastric production animals regarding pH, temperature and retention time.

Additionally, the potential inhibitory effect of Condensed Tannins (CT) from sorghum on activity of RONOZYME® ProAct was tested using a soybean/maize meal (30:70) diet by an *in vitro* gastrointestinal model (gastric phase with pepsin, pH 3 for 15 min and intestinal phase with pancreatic enzymes, pH 7 for 4.5h).

The results showed that RONOZYME® ProAct completely degrades glycinin and beta-conglycinin to smaller units, which would likely eliminate the antigenic properties of these ANFs. RONOZYME® ProAct also effectively degraded approximately 95% of both lectin and KTI and approximately 65% of the BBI. Only minor degradation of these ANFs was detectable by trypsin and chymotrypsin.

The in vitro analysis of tannic acid showed that it does not affects the activity of RONOZYME® ProAct. There is a negative effect on solubilization of protein by pepsin and pancreatin with addition of tannic acid. However, addition of ProAct to the sample containing tannic acid uplifts the protein solubility to the same level as the reference without tannic acid.

In conclusion, RONOZYME® ProAct has the potential to reduce the pressure on the pancreatic proteases (trypsin and chymotrypsin) by counteracting the negative effect of soybean and sorghum ANFs, and thereby reducing the sensitivity of animal performance to low quality soybean meal and sorghum.

Key Words: Enzymes, Proteases, Digestibility, anti-nutritional factors, Soybean and Sorghum

P318 Evaluation of the Bacillus subtilis DSM32315 strain stability under different feed manufacturing processes Anita Menconi*[1], Adebayo Sokale[1], Mark Daniel[1], S. Maria Mendoza[1], Rose Whelan[2], Kiran Doranalli[2] [1]*Evonik Corporation;* [2]*Evonik Nutrition and Care GmbH*

Pelleting and the use of formaldehyde containing products to decrease pathogenic bacteria are common practices in feed manufacturing. However, those practices can be detrimental to certain feed additives such as direct-fed microbials (DFMs). Spore-forming *Bacillus* have the capability to withstand high temperatures, chemicals, or drying processes; nevertheless, these capabilities are dependent on the genetic potential and the sporulation process used. Therefore, it is crucial to demonstrate stability of DFMs under such feed processing conditions. Two studies were conducted with the objectives of evaluating the stability of *Bacillus subtilis* DSM 32315 (*Bs*) under research and commercial feed manufacturing processes including different pelleting temperatures, application of a commercial formaldehyde containing product, and adverse storage conditions. In both studies, *Bs* spores were mixed in broiler feed at 500 g/MT for a final expected concentration of 1×10^6 CFU/g. In the first study, feed was pelleted at 85 °C, and spore viability was evaluated after pelleting and at 2, 4, 8, and 12 weeks of storage (40 °C and 80% humidity). In the second study, the stability of the strain was tested under commercial conditions. First, *Bs* spores were mixed in feed, conditioned for six minutes, and pelleted at 85.5, 87.8, and 90.5 °C. A formaldehyde containing product was then applied to the pelleted feed at all temperatures at the concentrations of 0.5, 1, and 2 Kg/MT. Additionally, the concentration of 3 Kg/MT was applied to feed pelleted at 85.5 °C. All samples (n=10/group) were analyzed for viable spore count and reported as Log_{10} CFU/g of feed. Results obtained from study 1 showed no significant difference in both *Bs* spores enumerated in pelleted feed and at 0, 2, 4, 8, or 12 weeks of storage, and the overall coefficient of variation was 1.6% for the spore counts in samples analyzed post-pelleting. In study 2, *Bs* spores remained viable in all treatments tested in both phases, and the range of total spore count was between 5.83 and 6.04 $\log_{10}$ CFU/g, with the overall coefficient of variation being 2.9%. The overall percent recovery of *Bs* spores was between 99.7 and 101.8%. All in all, the recovery of viable *Bs* spores was at the expected levels in all treated feed evaluated.

Key Words: direct-fed microbial, Bacillus subtilis, feed, stability

P319 Intestinal morphometry of broilers fed with butyric acid to replace growth promoting antibiotics Gabriela Ventura*, Gabrieli de Lima, Bárbara Barbosa, Henrique Centenaro, Bianca Cunha, Mariana Lira, Barbara Polidoro, Leonardo Zanetti, Gustavo Polycarpo, Valquíria Cruz-Polycarpo *São Paulo State University (Unesp)*

The aim of this research was to evaluate the morphometry of the duodenum and jejunum at 14 and 21 days of age of broilers challenged with *Eimeria* spp., using uncoated and microencapsulated butyric acid to replace growth promoting antibiotics in the diets. A total of 1,320 1-d-old male Cobb chicks were housed, allotted in a completely randomized design with 5 treatments and 6 replicates. Treatments consisted of: T1 - basal diet (BD); T2 - BD + 0.1% uncoated butyric acid (U); T3 - BD + 0.1% microencapsulated butyric acid (M); T4 - BD + 0.05% U + 0.05% M; T5 - BD + 0.005% avilamycin + 0.03% monensin sodium. At 16-d-old, broilers were inoculated with 0.5 mL of oral solution containing a pool of *E. acervulina, E. maxima* and *E. tenella*. Measurements of height (VH) and width (WV) of the villi and crypt depth (CD) in the duodenum and jejunum were performed. It was considered P <0.05 of significance and treatments were analyzed by orthogonal contrasts. There was no significant difference between the groups studied for the duodenum at 14 d. At this age the inoculation had not yet occurred which may be indicative that the mucosa was not undergoing interference processes. In the jejunum at 14 d, the group of additives obtained higher VH compared to the control group, showing that the additives, butyric acid or antibiotic, help maintain the integrity of the intestinal mucosa, providing greater capacity of nutrient absorption. In the duodenum at 21 d, the additive group provided higher VH in relation to the control group, showing that they helped in the recovery of the integrity of the intestinal mucosa after challenge, providing greater capacity of digestion of the nutrients. However, the group with antibiotic presented higher VH and WV, as well as higher CD than the groups with butyric acid, a result of the injury caused by the challenge in order to renew these losses. There was no significant difference between the groups studied for the jejunum at this age. However, the values found are higher than 14 d, which may be associated with the cell proliferation induced by the additives. It is concluded that butyric acids do not improve the intestinal morphometry of broilers, under the challenge conditions imposed in this study, being inferior to the effect of the antibiotics.

Key Words: Birds, histology, intestinal morphology, microencapsulation, organic acid

P320 The efficacy of the probiotic metabolites (LumensaTM: Dried Lactobacillus acidophilus Fermentation Product) in broilers: meta-analysis Sean Yang*[1], Danny Hooge[2], Elizabeth Wozniak[1] *[1]Cytozyme Laboratories, Inc.; [2]Consulting Poultry Nutritionist*

The aim of this meta-analysis was to investigate the effects of probiotic metabolites (Lumensa; AAFCO 36.11 Dried *Lactobacillus acidophilus* Fermentation Product) on the growth performance and gut health in broilers. In the statistical meta-analysis, 8 trials provided results for 29 comparisons from which overall averages for body weight (BW), feed conversion ratio (FCR), mortality, European Poultry Efficiency Factor (EPEF), intestinal lesion score, and microbiota were analyzed. Results were averaged by treatments (all comparisons) using Paired T-test to compare no Lumensa supplement (Control) means with corresponding Lumensa means. Lumensa supplementation increased BW (+1.43%, $P = 0.0001$) and EPEF (+3.56%, $P = 0.0001$), and reduced FCR (-1.67%, $P < 0.0001$), lesion score (-26.7%, $P < 0.0001$), *E. coli* counts (-7.70%, $P = 0.0005$) and *Salmonella* incidence (-27.3%, $P < 0.0001$) at 0-21 d trials. In the 0-21 d trials with 4 different Lumensa supplementation rates (0, 0.025, 0.05, and 0.1%), the product increased BW at 0.1% ($P = 0.0275$) and EPEF at 0.05 and 0.1% ($P = 0.0114$), and reduced FCR at 0.05 and 0.1% ($P = 0.0001$), lesion score ($P = 0.0014$), *E. coli* counts ($P = 0.0023$) and *Salmonella* incidence ($P = 0.0001$) at 0.05% and 0.1% compared to Control. At 0-47.8 d, supplementation of Lumensa increased BW (+2.79%, $P = 0.0014$) and EPEF (+9.18%, $P = 0.0008$), and reduced FCR (-2.57%, $P = 0.0004$), mortality (-28.1%, $P = 0.0052$), lesion score (-31.0%, $P = 0.0028$), *E. coli* counts (-10.4%, $P = 0.0109$) and *Salmonella* incidence (-37.1%, $P = 0.0038$). The average inclusion rates of Lumensa for 0-21 d and 0-47.8 d were 0.060% and 0.067%, respectively. In conclusion, dietary Lumensa supplementation to broiler diets indicated strong beneficial effect on growth performance and gut health in broilers.

Key Words: broilers, Lumensa, meta-analysis, probiotic metabolites

P321 Performance, egg quality, plasma inorganic phosphorus and tibia ash of Hy-Line brown laying hens fed different concentrations of non-phytate phosphorus with or without supplemental phytase from 40 to 60 weeks of age Kyeong Seon Ryu*, Masud M Rana, Chun Ik Lim, Ho Sung Choe *Chonbuk National University*

An experiment was conducted to evaluate the effects of dietary non-phytate phosphorus and phytase enzyme on laying performance, egg quality, mineral retention in plasma and tibia bone in laying hens. A total of 540 Hy-Line brown laying hens aged at 40 weeks were housed in conventional type three-tier cages and assigned into 1 of 6 dietary treatments with 5 replicates each in a completely randomized design for twenty weeks. A replicate consisted of 3 adjacent cages in upper and alike in middle and bottom to minimize cage level effect, and two hens were placed in a cage at dimension of 30.5 cm × 40.6 cm. The house temperature was maintained 22±3°C and lighting provided 16 hours/day. Three iso-energetic (2750 Kcal/kg ME), iso-nitrogenous (16.0% CP) diets were formulated to contain three levels of 0.20, 0.25 and 0.30% non-phytate phosphorus (NPP) and with or without phytase supplementation (30ppm/kg diet). A constant Ca level of 4.0% was maintained in all treatment diets and other nutrients to fulfill as the requirement. Data were analyzed using a one-way ANOVA with the GLM procedure of the Statistical analysis System (SAS 9.1, 2002). The results showed that significantly higher egg production was found in hens fed on diet containing 0.25% NPP with phytase whereas lower value observed in 0.20% NPP. Though, it did not notice consistent difference between dietary treatments of phytase groups and just level 30g/kg of NPP, and hens fed between AP level of 20g/kg and 25g/kg in diet. Egg mass was differed significantly among the treatments and maximum value was observed in 0.25% NPP with phytase. While, supplementation of phytase and different levels of NPP had no influenced on feed intake, egg weight, FCR and egg quality in terms of albumen height, haugh unit, egg shell breaking strength and thickness. In addition, no significant differences were found in serum phosphorus concentration and tibia bone ash percentage among the treatments but tibia ash tended to be numerically higher for hens fed on diet with phytase groups. However, it can be concluded that hens fed diet containing 0.25% NPP with 30ppm phytase per keg diet resulting improved egg production, egg mass and tibia bone ash whereas no effects were found on egg quality and phosphorus concentration in serum.

Key Words: phosphorus, performance, egg quality, mineral retention, laying hens

P322 Enzyme release from granulated products Roberto Maeda[1], Diogo Rosso[1], Elisama Cella[1], Leticia Ulrich[1], Ryan Saller[2], Morten Tovborg*[3] *[1]Novozymes Latin America Ltda; [2]Novozymes North America Inc; [3]Novozymes A/S*

Solid feed enzymes are often granulated and coated products. The right granulation technology provides physical strength and minimizes fines under stress. In addition, it may protect the enzyme in harsh pelleting conditions and maximize product recovery. However, while the solid formulation must give strength and stability it must also be readily released when ingested by the animal. Seven commercial phytases have been studied to compare their product form and rate of enzyme release. The enzyme protein release rates (Abs. 280 nm) fall in two groups: One set of products released the majority of enzyme in less than a minute whereas others require +5 minutes just to release half of the enzyme protein to the

liquid phase. Enzyme protein release from RONOZYME® HiPhos GT is measured in seconds rather than minutes.

Enzyme performance was also assessed by measuring the observed rate for the solid phytase products to decay the turbidity of a phytate-protein complex in solution (Abs. 880 nm). All phytases caused turbidity clearing over time with RONOZYME® HiPhos GT producing the fastest rate of turbidity decay (-2.05 A_{880}/min) of all commercial phytases tested. The slowest rate presented was -0.157A_{880}/min.

Enzyme dissolution rates are shown neither to depend on granule shape nor product stability. Therefore, formulating to get spherical and pelleting stable granulates does not necessarily slow enzyme release or inhibit performance. This is exemplified with the phytase RONOZYME® HiPhos GT which is a spherical salt coated granule where pelleting stability is ensured by a combination of molecule stability and granule coating.

Key Words: granulation, phytase, dissolution rate, pelleting stability

P323 Importance of trace mineral source on digestive protease function Eugene Kelly[1], Mark Gaffney[1], Richard Murphy[1], Rebecca Delles*[2] *[1]Alltech European Bioscience Centre; [2]Alltech Global Headquarters*

This study examined the effects of dietary trace minerals on protease activity *in vitro,* with an emphasis on the interactions between digestive protease activity and mineral source. Feed formulation, particularly with respect to novel protein ingredients, is becoming more complex for broiler diets. This places a greater importance on protease function to improve feed efficiency and digestibility. To ensure feed efficiency, it is important to identify any negative interactions that may occur between proteases and dietary trace minerals that are routinely added to feed.

Inorganic trace minerals (ITMs) are often supplemented to monogastric feeds at levels that exceed the recommended levels by the National Research Council (NRC). Organic trace minerals (OTMs), such as those in the Bioplex® range (Alltech Inc.), can be used at comparatively lower concentrations due to their greater bioavailability. The enzyme activity of three commercial proteases with copper mineral mix were quantified *in vitro*, using the free α-amino nitrogen (FAN) assay.

Results from this study indicated that ITM in the form of copper sulphate, had a negative effect on digestive protease function, while proteinated copper (Bioplex®-Cu®), resulted in preservation of greater digestive protease function when copper was added at 50% NRC. As the concentration of inorganic copper was increased to NRC concentrations and levels commonly used in industry (200% NRC), digestive protease activity decreased ($p \leq 0.05$). On average of the three proteases tested, when $CuSO_4$ was compared to Bioplex®-Cu® at equivalent rates, there was a 2%-9% difference in digestive enzyme function at 50% NRC ($p \leq 0.05$), at NRC concentrations there was a 4%-12% difference in enzyme activity, while at 200% NRC there was a greater 9%-26% difference in protease activity ($p \leq 0.05$).

The current study highlighted the potential impact of trace mineral source on digestive protease function *in vitro*, which may have potential *in vivo* ramifications for reduced feed efficiency. The findings indicate an importance for considering potential interactions between feed ingredients and the effect these ingredients can have on optimal digestive function.

Key Words: Protease, Digestive function, Organic, Inorganic, Copper

P324 Betaine supplementation improves water balance and meat quality of broiler chicks Hosam Al-tamimi, Kamel Mahmoud*, Basheer Nusairat, Anas Al-Swalqeh *Jordan University of science and Technology*

Two studies were conducted to assess the effect of dietary betaine supplemented to broiler diets on productive and thermoregulatory responses of broiler chicks. In experiment 1, a total of 440 one-day-old Indian River broiler chicks were randomly distributed into 2X2 factorial treatment groups of betaine and choline and reared on floor pens through 35 day of age. Birds consuming basal diet acted as the control (CONT) group; second group (BETA) supplemented with betaine (1 g/kg); third group (CHOL) supplemented with choline (1 g/kg); the last group (COMB) a betaine-choline combination. Performance parameters (FI, BWG, and FCR) were reported on a weekly basis, and carcass meat quality (dressing percentage, pH, cooking loss, water holding capacity and shear force) were evaluated at the end of experiment. In the second experiment, a total of 60 broiler chicks were selected from CONT and BETA groups at one week of age and fitted with compact thermosensors (to measure core body temperature; T_{core}). At 31-day of age, 20 chicks from each group were exposed to acute heat stress (A-HS) for 3 hrs (ambient temperature; $T_a = 34.45 \pm 0.20$°C; RH% = 37.60 ± 0.28%), and hemogramic profiles screened before and after. The results showed that betaine supplementation did not affect broiler growth performance. No differences were observed in FI, BWG, and FCR through 5 weeks of age. However birds fed betaine supplemented diets had higher ($P<0.05$) water holding capacity and retained ($P<0.05$) more water during slaughter processing compared to control group. Birds fed on betaine supplemented diet maintained lower ($P<0.05$) T_{core} when exposed to A-HS compared to control group. It may be concluded that betaine improved body water balance by retaining more water during production and slaughtering process.

Key Words: Broiler, betaine, body core temperature, processing, water holding capacity

P325 Application of labeled peptidoglycan to measure the activity of a novel muramidase Jacqueline Chimilovski[1], Mikkel Klausen[2], Roberto Maeda[1], Lars Skov[2], Miriam Sluis*[3], Rual Lopez-Ulibarri[4] *[1]Novozymes Latin America Ltda.; [2]Novozymes A/S; [3]Novozymes North America Inc.; [4]DSM Nutritional Products AG*

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 into the gut environment that could interact with the intestinal wall and impact gastrointestinal functionality. A novel microbial muramidase (EC 3.2.1.17) that hydrolyzes the peptidoglycan (PGN) polymer of bacterial cell-wall fragments from bacterial debris was recently used in broiler feed to improve performance (Lichtenberg J. *et al*., 2017). By hydrolyzing PGN in bacterial cell wall fragments, this enzyme can reduce the impact of excessive cell wall fragments, which optimizes gastrointestinal functionality and results in improved performance of broiler chickens.

In this study, we describe the *in vitro* measurement of the novel muramidase enzymatic activity. A colorimetric dye-release assay was adapted from Zhou R. *et al*. (1988). Cells from *Micrococcus lysodeikticus* ATCC No. 4698 were labeled with the dye Remazol Brilliant Blue (RBB) and used as the substrate for the enzymatic hydrolysis. During careful incubation of RBB with the PGN-rich dead cells of *M. lysodeikticus* under alkaline conditions, the RBB undergoes an elimination reaction and its derivative reacts with glycan hydroxyl groups of the peptidoglycans, producing a dye-labeled substrate for the muramidase.

Muramidase cleaves the b-1,4 glycosidic linkages between the *N*-Acetylmuramic acid (NAM) and *N*-Acetylglucosamine (NAG) components of PGN, which releases soluble, dyed fragments from the substrate. After the reaction, the insoluble fraction was removed by centrifugation and the soluble hydrolyzed products were measured with a spectrophotometer at 600 nm. The signal was enzyme-dependent and dose-dependent. The colorimetric assay was used to accurately measure enzymatic activity ranging from 1 to 750 U/mL with % RSD of less than 5%. An additional advantage of the method is that muramidase-mediated color release can be visually scored without a spectrophotometer. This can be advantageous for use outside of laboratory settings. *In vitro* enzymatic activity measure-

ments complement the understanding of muramidase and its benefits to gastrointestinal functionality in broilers.

Key Words: Muramidase, Peptidoglycan, Gastrointestinal functionality, Microbiota, Broiler

P326 Impact of Balancius Supplementation on Broiler Performance
N. E. Ward*[1], A. W. Levy[1], R. D. Malheiros[2], P. R. Ferket[3] *[1]DSM Nutritional Products; [2]Prestage Poultry Science Dept, NC State University; [3]North Carolina State University*

Balancius™ is a muramidase that uniquely degrades peptidoglycan (PGN) from dead bacterial cell walls in the intestinal tract. The abundance of PGN is theorized to interfere with enzyme-substrate coupling and nutrient absorption. Two experiments were conducted to ascertain the effect of this enzyme on broiler performance.

In both experiments, all chicks were weighed on day of hatch to equalize starting weights in all groups. Chicks were spray vaccinated on day 1 with a commercial coccidia vaccine (Coccivac-B52). Floor pens contained previously used pine shavings litter and were equipped with a tube feeder for ad libitum feeding. Diets were pelleted corn/soybean-based and met Ross 708 formulation guidelines.

In the first experiment, Balancius at 0, 25,000, 35,000 and 45,000 LSU(F)/kg feed was evaluated for 28-day broiler performance. Male Ross 708 chicks (n=1,800) were allocated to 72 pens of 25 chicks/pen in a randomized complete block design (RCBD) with 18 replications/treatment. Body weight (BW), feed intake (FI), and mortality were determined at 14 and 28d. Day 28 BW was increased (P<0.001) over the control with the addition of Balancius. FI was elevated (P<0.059) with Balancius, while FCR was improved (P<0.05) by 45,000 LSU(F)/kg. Mortality was unaffected from day 1 to 28.

The second experiment ascertained Balancius at 0 or 25,000 LSU(F)/kg with 0 or 145 mg/MT of either copper sulfate ($CuSO_4$) or tribasic copper chloride (TBCC) on the performance of 49-day broilers. Ross 708 males and females (n=2,304) were allocated (16 chicks/sex) to 72 pens in a RCBD with 12 replicates per treatment. The basal diet contained 10 mg $CuSO_4$/MT. BW, FI, and mortality were determined on days 14, 28, 42 and 49. Balancius improved male BW on days 14 (P<0.019) and 28 (P<0.044) and increased female BW on days 42 (P<0.022) and 49 (P<0.057). FI and mortality were unaffected by treatment, while day 28 FCR was improved (P<0.013) by Balancius. Day 28 female BW was increased (P<0.012) by TBCC over basal but did not differ (P>0.05) from $CuSO_4$, while day 42 female BW was increased (P<0.020) by $CuSO_4$ over basal but was similar (P>0.05) to TBCC. A Balancius x Cu interaction occurred (P<0.032) for 14d FCR.

Balancius can effectively improve broiler performance.

Key Words: Balancius, muramidase, peptidoglycan, broiler performance

P327 Occurrence of wooden breast in broilers fed diets with reduction of calcium levels and two doses of phytase
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Wooden breast (WB) has been considered one of the most important challenges in the Poultry Industry in the 21st century. The superdosing of phytase can affect metabolic functions in the early life of broilers, and the studies with the interaction of this enzyme with calcium, phosphorus and carcass evaluation must be incremented. Based on this, calcium reduction and phytase supplementation effect on the occurrence of WB in broilers at 42 days of age was evaluated. The experiment was conducted in the experimental facilities of EVZ/UFG, Goiania, Brazil, with 864 Cobb500 male chicks allotted in a completely randomized design and factorial arrangement 2x3, 6 treatments and 8 replications of 18 birds each. Birds were fed corn soybean meal and corn gluten meal diets with 3 levels of calcium, 100% of Brazilian Tables recommendations, and reduction of 18 and 36% (1.011; 0.907; 0.828 e 0.661% of calcium for phase pre-starter, starter, growth and finishing, respectively). Phytase matrix was considered to release phosphorus to 750FTU, and for 1500FTU, the level above 750 FTU was considered on top. On the 42nd day, 72 birds were slaughtered, 12 per treatment, and WB occurrence was evaluated. The scores were classified into 3 levels (0 - normal, 1 - moderate, and 2 - severe). Data were submitted to the Kruskal-Wallis and Bonferroni tests (P <0.05). No diferences were observed for WB occurence, but when phytase levels were considered separately, the score 0 was 52,28% and 47,22%, score 1, 38,89% and 36,11% and score 2, 8,33% and 16,67% for 750 and 1500 FTU, respectively. For calcium levels, the score 0 was 66,67%, 41,67% and 41,67%, score 1, 29,17%, 41,67% and 41,67%, and score 2, 4,17%, 16,67 and 16,67% for 64%, 82% and 100% of calcium levels recommended by Brazilian tables, respectively. The medians were not statistically different when effects of calcium and phytase were considered separately or in the interaction. The reduction of calcium levels were not effective to reduce the WB occurence, even when 750 or 1500 FTU of phytase is used.

Key Words: broiler carcass, enzyme, meat quality, nutritional programs

P328 Effect of essential oil based eubiotic product in improving performance and reducing intestinal lesions as well as mortality in broiler birds under necrotic enteritis challenge
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Necrotic enteritis is one of the major gut health challenges causing huge economic loss to modern poultry producers as a result of removal of antibiotic growth promoters (AGP) from feed formulations. *Clostridium perfringens* is the causative organism of necrotic enteritis, while coccidiosis is a major predisposing factor. Among the alternatives for AGP, essential oils are widely used to reduce the severity of necrotic enteritis. Several studies have shown that essential oils could have a direct antimicrobial effect on *C. perfringens* and also reduce the Eimeria oocyst excretion in birds with coccidiosis. Previous studies have shown that thymol and carvacrol based product NEXT ENHANCE® 150 (NE150) can improve performance in birds with coccidiosis, by modulating the immune/inflammatory response. The objective of the current study was to explore the effect of NE150 in birds challenged with necrotic enteritis. The study included a CON or no additive group, NE150 (30g/MT of feed), and BMD60 (Bacitracin Methylene Disalicylate® @ 500g/MT of feed). All birds were raised in battery cages (8 replicates/treatment; 8 birds/replicate) and challenged with *Eimeria maxima* (~5,000 oocysts/bird) at 14d followed by *C. perfringens* (~10^8 cfu/bird) at 19, 20, and 21d of age. Results from the study revealed that NE150 resulted in reduction (P<0.05) of feed conversion ratio (FCR) post challenge when compared to CON, while BMD60 showed lower (P<0.05) FCR than both CON and NE150. Furthermore, necrotic enteritis lesions and mortality were similar between both NE150 as well as BMD60, and were lower (P<0.05) than CON. In conclusion, the results from the study suggest that the essential oil product NE150 was effective in improving performance in broiler birds under necrotic enteritis challenge, mostly by reducing mortality and lesions, and could be used as an effective alternative to AGPs. It is hypothesized that its mode of action could be by reducing the tissue damage caused by *C. perfringens* and/or coccidia and by modulating the host immune/inflammatory response to the infection.

Key Words: necrotic enteritis, AGP, essential oils, Clostridium perfringens, BMD60

P329 The effect of enzyme supplementation in corn or wheat-based diets to broilers. Cristiane Araújo*[1], Andre Murcio[2], Lucio Araujo[1], Carla Silva[3], Carlos Alexandre Granghelli[1], Fabricia Roque[1], Priscila Zorzetto[1], Brunna Leite[1] *[1]University of Sao paulo; [2]Cobb Vantress Brazil; [3]Agroceres Multimix*

Ingredients of vegetal sources, which compose the diets of broilers, have in its composition an indigestible fraction known as the nonstarch polysaccharides (NSP). These polimeric macromolecules of simple sugars (monosaccharides) are resistant to hydrolisis in the gastrointestinal tract of monogastric animals and are characterized as anti-nutritional factors. The objective of this study was to evaluate the performance and the carcass yield of broilers raised from 1 to 42 days of age, fed with diets based in corn or wheat, with or without inclusion of xylanase and valorization of the energetic matrix. Treatments were arranged in a 2 x 2 x 2 completely randomized block in a factorial design varying in diet type (corn and wheat), xylanase (0 and 100 mg/kg); and energy levels (according to recommendations for each phase and reduced in 60 kcal/kg in relation to the recommendations). One thousand and one hundred and fifty-two male chicks Cobb-500 were randomly allocated to eight treatments with eight replicates having 12 broilers each. Over the entire experiment (d 0-42), it was observed an interaction between energy levels and xylanase on FCR. Xylanase supplementation improved FCR with the standard level of energy. Also, no main effects on growth performance were observed for diet type and xylanase. However, birds fed standard level of energy showed had higher BWG. Regarding carcass traits, broilers fed wheat had higher breast yield than those fed corn. Also, the presence of xylanse decreased carcass, legs and breast yield. In contrast, energy levels did not influence carcass traits. The current study indicated that xylanase supplementation was effective to improve FCR; however, carcass traits were negatively affected.

Key Words: carcass yield, nonstarch polysaccharide, performance, xylanase

P330 The impact of Agaricus bazei mushroom extract water supplement on broiler performance Nuket Acar*, Carolyn Lamb, Paul Patterson *The Pennsylvania State University*

An extract of the mushroom Agaricus blazei (ABE) was provided to commercial broiler chickens as the water supplement Agaricus Bio-CX manufactured by Atlas World USA, Inc. Pasadena, CA. Three levels of supplementation corresponding to 0.5, 1.0 and 3.0 times the manufactures recommendations were provided to 8 replicate cages of birds per treatment and compared to Control birds receiving pure water during a 3 wk study. Body weight, feed intake and water intake were measured weekly and mortality was recorded. The results indicated that the ABE had no significant impact on body weight or weight gain during the 3 wk study ($P > 0.05$). Feed consumption and the feed conversion were not impacted by the ABE supplement at 1, 2, 3 wk or overall ($P > 0.05$). Water consumption was not significantly impacted by the ABE at any period of the study, neither was the water to feed intake ratio ($P > 0.05$). Finally, there was no impact of any of the ABE treatments on bird mortality compared to the Control birds ($P > 0.05$). These results suggest that ABE provided at 0.5, 1.0 and 3.0 times the manufactures recommendations, had no negative impacts on standard measures of broiler performance or mortality to 3 weeks of age, and do not indicate any safety hazard.

Key Words: Agaricus blazei, mushroom extract, broilers, water intake

P331 Evaluation of a specific blend of oleoresins of spices and essential oils to replace an antibiotic growth promoter program on broiler performance Jean-Francois Gabarrou*[1], Michael Sims[2], Danny Hooge[3] *[1]PHODE; [2]Virginia Diversified Research Corp.; [3]Consulting Poultry Nutritionist*

Considering the simple removal of antibiotic growth promoters might have a negative economic impact, search for alternative additives has been incentivised. The main goal of this experiment was to evaluate the effects of a blend of essential oils and oleoresins of spices on performance of broiler between 7 to 42 days compared to usual conditions of production based on antibiotic growth promoter (AGP).

A total of 1,200 newly hatched chicks (Ross-308) were randomly distributed (30/pen) into 3 groups with 12 (Control) or 14 (treatments) replicates in each one. All broilers received from d 0 to d 7 the same non-medicated starter. Thereafter, Control group received only non-medicated feed (starter from day 0 to day 14, grower from day 15 to day 28 and finisher from 29 to 42). Birds from AGP group received 55 ppm of Bacitracin Methylene Disalicylate (BMD®) from d 8 to d 28 shuttled to 22 ppm of Stafac® from 29 to 42 d. In the tested group (OLEO), AGP were substituted by a phytogenic feed additive (Oleobiotec, Laboratoires PHODE - France) at 100 g/MT. Statistical analysis*:* A completely randomized design was used ($P \leq 0.05$), and means were separated by LSD ($P=0.05$). Statistical analysis was carried out using the General Linear Model procedure of Statistix®, Analytical Software, Tallahassee, FL.

The Control group presented significantly lower performances from 0-42 d compared to AGP group (1.906 vs. 2.111 kg BW; 1.933 vs. 1.765 feed conversion ratio or FCR), confirming the negative effect of AGP removal. Both groups (OLEO *vs* AGP) presented statistically equivalent performance on mortality rate (5.48% vs. 6.19%), growth performance (2.061 vs. 2.111 kg live weight) and feed efficiency (1.775 vs. 1.765 FCR).

Diets with the phytogenic feed additive Oleobiotec (Laboratoire PHODE), a specific blend of essential oils and oleoresins, were an effective alternative for antibiotic growth promoter (BMD /Stafac) diets. Further studies need to be done to investigate whether this product improves digestibility of energy and nitrogen to help explain those results.

Key Words: BMD, Broiler, Phytogenic, Stafac, Oleobiotec

P332 The effect of combinations of protease and phytase enzymes on the performance of broiler chicks fed corn or sorghum-based feed rations with limiting nutrients Kaci Merriwether-Hawkins[1], Kenyanna Jones[2], Dan Seitz[2], C Delfelder[2], Scott Beyer*[2] *[1]Tuskegee University; [2]Kansas State University*

Enzymes used as feed additives may actively degrade target substrates to allow nutrient release from complex associations and improve processing and uptake by the digestive system. Some enzymes, such as phytase (PHY), target specific nutrients like P in phytic acid, while others such as protease (PRO) are class specific but tend to target substrates more randomly. Two studies were designed to determine synergistic effects of these enzyme combinations on 0 to 21d old male broiler chick (Cobb 500) performance. The studies used combinations of PHY (DSM Ronozyme) and PRO (Jefo Protease), on either a corn-soy based ration or a sorghum-soy based ration. For each study, 2 diets were formulated: a positive control diet similar to a commercial ration (PC) and second diet with reduced available P with restricted levels of energy, protein and amino acids (NC). The enzymes were added to the NC diet at 0, 1X, and 3X recommended levels, singly and in all combinations. The battery trials were conducted using 70 pens and 420 chicks with 6 chicks per pen and 7 replications of each test ration. Performance data were determined at 21 days and a sample of tibias were removed from each pen to determine tibia mineralization after ether extraction and ashing. Data were analyzed using the GLIMMIX procedure of SAS. The 1X and 3X levels of PHY significantly improved BWG, FC, and tibia ash (TA) compared to the unsupplemented NC while PRO improved BWG and FC but not TA. For corn rations, all combinations of enzymes at 1X and 3X increased performance over the NC or NC with only one added enzyme and were similar to the PC fed chicks. The TA was highest in groups fed enzyme combinations with the best mineralization occurring with 3X PRO and 1X PHY. For sorghum-based rations, both PHY and PRO improved performance and TA, with a synergistic effect when the enzymes were tested together. In both studies, the mortality levels of the nutrient restricted chicks were lowest for

the combined enzymes compared to the negative control as well as when compare to diets with only one of the two enzymes. To optimize nutrient availability in feed rations, there may be synergistic effects of combining enzymes in broiler feeds.

Key Words: protease, phytase, enzymes, broilers, sorghum

P333 Post pellet fat application using fat coater improves pellet durability index (PDI) and does not affect enzyme (phytase) recoveries when applied using post-pellet liquid application (PPLA) system Megharaja Manangi*, Matthew Deeker, Deana Hancock, Mercedes Vazquez-Anon *Novus International, Inc.*

The objective of this study was to examine the effects of different levels of fat added pre- and post- pellet on pellet durability index (PDI) and if varied levels of fat added post-pellet affect enzyme (phytase) recoveries when applied post-pelleting after fat application using post-pellet liquid application (PPLA) system. The three treatment diets consisted of D1 with 4.5% fat in the mixer; D2 with 2% fat in the mixer and 2.5% fat post-pellet, and D3 with 1% fat in the mixer and 3.5% fat post-pellet. Post-pellet application of fat was done using the fat coater built into the PPLA system. For all three diets, the liquid phytase (CIBENZA® PHYTAVERSE®) was sprayed @500u/kg diet post-pellet after fat was applied using PPLA system. The common Basal diet for all three diets was formulated to meet industry specifications with calculated fat levels of 6.84% (including 4.5% supplemental fat). The feed samples were collected at the exit of PPLA system and analyzed for 1) particle size distribution to calculate % fines by sieving using US#6 sieve (sample size, n=4 per diet), 2) PDI (n=8 per diet) using Holmen NHP100 pellet durability tester, 3) crude fat (n=4 per diet), and 4) phytase (n=4 per diet). The data were subjected to ANOVA and significance was tested at $P \leq 0.05$. The fines for 3 diets were 50.8, 37.9, and 20.2%, respectively, and were significantly different from each other ($P<0.05$). The PDI for 3 diets were 46.9, 73.7, and 88.0%, respectively, and were significantly different from each other ($P<0.05$). The fat recoveries for 3 diets were 7.08, 7.82, and 7.75%, respectively, and were not different from each other ($P>0.05$). The enzyme recoveries for 3 diets were 464, 652, and 572 u/kg diet, respectively, and were not different from each other ($P>0.05$). In summary, data indicates that reduction of fat addition in the mixer and adding fat using fat coater post-pellet helped to achieve significant reduction in %fines and increase in pellet durability. Additionally, fat application up to 3.5% using fat coater (post-pellet) did not interfere with post-pellet enzyme application using a PPLA system as recoveries for both fat and enzyme were as expected relative to theoretical values.

Key Words: Fat coater, Phytase, Percent fines, Post-pellet liquid application, Pellet Durability Index

P334 Protected sodium butyrate and BMD effect on broiler chickens performance, a field study Oscar Vazquez*[1], Angelica Ortiz[2], Cinta Sol[3], A.Z.M. Salem[4], Monica Puyalto[3] *[1]Norel México S.A. de C.V.; [2]Colegio de Postgraduados; [3]Norel S.A.; [4]FMVZ-UAEM*

The aim of this field study was to evaluate the effect of combined use of protected sodium butyrate and Bacitracin Methylene Disalicylate (BMD) on chicken broilers performance. A total of 138,400 one-day-old Cobb chickens were housed in 4 sheds which were randomly distributed into two treatments: CON (fed on the basal diet with 30 mg of BMD / kg of feed) and BUT (fed on the same basal diet with BMD plus 700 mg of protected sodium butyrate (Gustor® N´RGY)/ kg of feed) from 0 to 21 d of the experiment. The study lasted 49 d and the recorded parameters were: feed intake (FI), body weight (BW), average daily gain (ADG), feed conversion ratio (FCR), mortality (M), productivity index (PI) and villi height of duodenum at 20 d (VH). The inclusion of butyrate improved BW at 49 d by 2.54% (2752.5 vs 2822.5 g, for CON and BUT, respectively, P=0.011), and the ADG tended (P=0.079) to be 2.58% lower in CON (56.2 g) vs BUT (57.6 g), however no effect (P>0.05) was observed on FI, FCR, PI. Although, M was numerically higher (+0.6%) in CON (10.1%) vs BUT (9.5%) treatment. Regarding to VH the BUT treatment was 38.95% greater (579.5 µm vs 805.15 µm, for CON and BUT respectively, P=0.010) than the treatment CON. Data suggested that the inclusion of protected sodium butyrate combined with BMD could to improve weight gain and duodenum villi height in broiler chickens.

Key Words: Gut health, growth promoters, synergy

P335 Effect of Bacillus sp. nov endomicrobial supplement on growth performance and lesion score of broilers challenged with Clostridium perfringens Gavin John*, Grant Gogul, Adam Taylor, Allsion Wells, Michael Pham, Jennifer Cao, Jordan Embree, Mallory Embree *Ascus Biosciences*

As the poultry industry moves towards antibiotic free systems, *Clostridium perfringens* induced necrotic enteritis (NE) requires alternative solutions to decrease NE mortality in broilers and increase overall performance. This study aimed to evaluate *C. perfringens*-induced necrotic enteritis (NE), growth performance, feed conversion, and mortality of broilers that were administered an endomicrobial supplement (EMS) comprised of a native *Bacillus* sp. nov isolated from the chicken gut microbiome. The study consisted of 7 treatment groups (n=210 per TRT): TRT 1 and 2 received feed mixed with calcium carbonate while TRT 3-7 received doses of a *Bacillus* sp. nov increasing in ten-fold increments per treatment from 10^4 to 10^8cells/bird. TRT groups 2-10 were challenged with *C. perfringens*~10^8cfu/ml via a broth culture mixed with feed (~25 g/bird) on d 17. All treatment diets were a standard formula representative of commercial broiler diet and feed were provided *ad libitum*. Feed conversion ratio (FCR), body weight gain (BWG), necrotic enteritis (NE) lesion scores, and mortality were evaluated at d 0-17, d 0-28, d 0-35, d 0-42, d 17-28, d 17-35, d 28-35, and d 35-42. Data were analyzed using a one-way ANOVA and performance means were compared using Tukey's Post Hoc Test. Two birds were removed, weighed, and sacrificed on d 16, 21 and 42 from each TRT for the collection of ileal, cecal contents. Collected samples were used to confirm colonization of the EMS via Illumina MiSeq sequencing of the 16S rRNA V1-V3 hypervariable regions. There were no trends of significance observed in any of the treatments; however, there is a trend of improved adjusted FCR in TRT 7 and reduction of mortality in TRT 6 and 7. Results of this study suggest that administration of the *Bacillus* sp. could positively impact growth performance benefits during necrotic enteritis infection. Continued investigation is needed to confirm these findings and better to understand EMS dosing requirements.

Key Words: Clostridium perfringens, endomicrobial supplement, Bacillus, performance, microbiome

P336 Evaluation of hydrolysed yeast in the diet of white laying hens. Natalia Koiyama[1], Bruno Santos[2], Ricardo Barbalho[3], Melina Bonato[3], Cristiane Araujo[1], Lucio Araujo*[1] *[1]University of Sao Paulo; [2]Federal University of Grande Dourados; [3]ICC Brazil*

This study aimed to investigate the effects of supplemental hydrolysed yeast (*Saccharomyces cerevisiae*) in the diet of laying hens on productive performance and egg quality as well as to analyse the economic viability of using this additive. A total of 256 laying hens were assigned to four treatments with hydrolysed yeast supplement at the levels 0, 1, 2, and 4 g/kg in a completely randomized design, with eight replicates each of eight hens from 48 to 68 weeks of age. Hens in the 2 and 4 g/kg additive treatments showed higher feed intake, egg production, egg mass, and egg weight as well as improved feed conversion per dozen. Egg quality was higher with inclusion of the additive, resulting in higher albumen height, shell thickness, and resistance in addition to a lower percentage of unsellable eggs, but dietary effects were not observed in the Haugh units and yolk colour. Hydrolysed yeast supplementation for laying hens was economically viable, especially treatment with 4 g/kg of hydrolysed yeast. To achieve the best gross margin, the additive supplementation level may

range from 2 to 4 g/kg of hydrolysed yeast, depending on the price per dozen eggs.

Key Words: Economic Analysis, Mannan-oligosaccharide, Nucleotides, Saccharomyces cerevisiae

P337 The effects of supplementation of Amasil NA in the diets of Cobb 700 broilers from d 0 to 14 on ileal digestibility Kristina Feye*[1], Barbara de Almeida Mallmann[2], Dana Dittoe[1], Blaine Booher[1], Joshua Jendza[3], Guillermo Tellez-Isaias[2], Casey Owens[2], Michael Kidd[2], Steven Ricke[1] *[1]University of Arkansas, Department of Food Science, Center for Food Safety; [2]University of Arkansas, Department of Poultry Science; [3]BASF*

The introduction of organic acids to the gastrointestinal tract (GIT) with organic acid-based feed amendments is an essential tool for producers in replacing feed-based antibiotics. Organic acid supplementation in poultry feeds offers the opportunity to deliver these acids directly to the GIT function. Amasil and Silo Health 104L have been proposed to be more effective feed amendments than formaldehyde, though their effects on digestibility are untested. The current study was conducted in order to determine if energy absorption changed with the inclusion of feed amendments from D0 to D14. A production trial using Cobb700 broilers was conducted to order to determine whether or not Amasil NA (0.25% and 0.5%) and Silo Health 104L (0.25%) were compared to formaldehyde (0.5%) feed amendments. On d 0, day-of-hatch, 864 male Cobb700 broilers were placed in 48-floor pens (5 treatments, 10 replicates for treatment groups, 9 replicates for NC, 18 birds per pen) with untreated pine litter. All experimental starter diets (d 0 to 14) were prepared by the University of Arkansas feed mill according to industry standards and contained the feed amendments and a titanium tracer to determine ileal digestibility. Ileal digesta were collected on d 14 and was analyzed for digestibility, along with the individual feed treatments. Statistical analysis was conducted using JMP 14.0 comparing all of the means individually against the formaldehyde group using a one-way ANOVA, followed by analysis with the Dunnett's test ($P \leq 0.05$). Significant effects of the treatment were demonstrated throughout the study. The overall effect of diet was statistically significant for Cysteine ($P<0.04$), Lysine ($P<0.05$), Methionine ($P<0.01$), and Tyrosine ($P<0.04$). The greatest difference in methionine absorption was between NC+ Silo Health 104L (x=0.846) and formaldehyde (x = 0.942). The significant main effect of diet on the AID of Cysteine and Tyrosine did not result in significant comparisons between formaldehyde and the other groups ($P<0.05$). The NC + 0.5% Amasil group demonstrated a trend of increased digestibility of glycine compared to the formaldehyde group ($P \leq 0.1$). Therefore, Amasil NA as a poultry feed amendment is capable of improving the digestibility of important amino acids.

Key Words: Organic Acids, Digestability, Poultry Production, Feed Amendments, Protein

P338 Effect of the addition of a fermented yeast product and coccidia challenge in broiler chickens R.F. Cortes-Coronado[1], S.F. Gomez-Rosales[2], M.L. Angeles*[2], M.T. Casaubon-Huguenin[3], T. Sørensen-Dalgaard[4] *[1]PANCOSMA MEXICO, S.A. DE C.V.; [2]National Center of Disciplinary Research in Animal Physiology, National Institute of Research in Forestry, Agriculture and Livestock,; [3]Poultry Science Department, University City, National Autonomous University of Mexico; [4]Department of Animal Science, Aarhus University*

The objective of the research was to evaluate the productive performance, tibia mineralization, histopathology of the small intestine and immune response in broiler chickens fed diets added with a fermented yeast product (FYP) and challenged with a coccidia vaccine (COCV). One hundred and eighty, Ross B308 male chicks were allocated in battery cages. From 1 to 20 d of age (phase 1), chicks were randomly assigned to 2 treatments: 1) Control without FYP and, 2) Experimental group with addition of 800 ppm of YFP in the feed. The diet was added with an anticoccidial drug. On day 21, 30 birds from each treatment were killed, and the rest of the broilers were divided in 2 subgroups: one subgroup was vaccinated with a COCV, using a 16x dosage, while the other one was not vaccinated (NCOC). The vaccine was mixed in the feed. From 21 to 28 d of age (phase 2) the anticoccidial drug was withdrawn from the feed. The last day of the trial, all broilers were weight and killed. The right tibia, samples of duodenum and jejunum, duodenum content and blood for determinations of mannan-binding lectin (MBL) in serum were obtained from broilers killed at the end of phase 1 and 2. Results were subjected to ANOVA. In phase 1, the diameter of the lower diaphysis of the tibia was larger, the IgA concentration in duodenum was greater ($P<0.10$) and the hyperemia/hemorrhage in the duodenum was lower ($P < 0.05$) in YFP fed-broilers. In phase 2, the lowest feed intake was observed in COCV broilers without YFP ($P < 0.05$), while lower feed conversion was observed in COCV broilers with and without YFP ($P < 0.05$). The yield of the carcass and breast ($P < 0.05$) were lower in COCV broilers without YFP. The length and concentration of ashes of the tibia ($P < 0.01$) and the weight of the ashes ($P < 0.10$) were lower in COCV broilers compared with the NCOC group. The excretion of oocysts was higher in COCV broilers without YFP ($P < 0.05$). The degree of hyperemia/hemorrhage in duodenum was lower ($P <0.05$) in the NCOC group and broilers fed YFP. COCV and YFP fed broilers had higher serum MBL ($P <0.05$). In summary, the addition of YFP improved the carcass yield, tibia mineralization and the immune response and reduced the oocyst counts in broilers vaccinated against coccidia.

Key Words: Fermented yeast, Coccidia challenge, Broilers, Production, Immune response

P339 Effect of an alpha-galactosidase based enzyme formulation on the performance and apparent metabolizable energy of broiler chickens: a meta-analysis Sara Llamas Moya*[1], Niall Higgins[1], Roshan Adhikari[2], Sean Lacey[3] *[1]Kerry, Global Application Center; [2]Kerry; [3]Dept of Mathematics, Cork Institute of Technology*

The objective of this study was to evaluate the effectiveness of an enzyme formulation based on alpha-galactosidase on the performance and nutrient digestibility of broilers through a meta-analysis of a range of independently run randomized controlled studies. Seventy-seven (77) broiler studies supporting product development, regulatory compliance and practical application were screened, of which 23 experiments assessed the effectiveness of a commercially available alpha-galactosidase derived from *Saccharomyces cerevisiae*in combination with carbohydrases derived from *Aspergillus niger*and *Trichoderma longibrachiatum*(AlphaGal™280P [AGAL]), dosed at 200 g/MT. Sixteen (16) studies evaluated final body weight (BW) and feed conversion ratio (FCR) to an age of 35, 42 or 49 days, encompassing a total of 11,314 broilers distributed in 338 replicates. Seven (7) studies evaluated the effect of AGAL on nitrogen-corrected apparent metabolizable energy (AME_n), using 1,392 broilers distributed in 186 replicates. Corrective actions were undertaken to ensure compliance regarding publication bias and heterogeneity, which were assessed by the Egger test and the Cochran Q test. Results demonstrated that AGAL delivered significant BW improvements compared to the control group, which were measured at +2.3% ($p=0.0002$) and 1.9% ($p=0.0114$) at 35 and 42 days, respectively. Furthermore, AGAL significantly improved FCR ($p=0.0030$), with increased efficiency up to -2.8% compared to the control group. AME_n was also significantly improved in AGAL treated diets ($p=0.0001$), with the effect of AGAL supplementation quantified in additional +58 kcal/kg feed. These results confirm the effectiveness of an alpha-galactosidase based enzyme in improving broiler performance and nutrient digestibility.

Key Words: Alpha-galactosidase, Broilers, Meta-analysis, Performance, Digestibility

P340 Bacillus probiotics has the ability to inhibit E.coli in a chicken feed matrix Dorthe Sandvang[1], Line Skjoet-Rasmussen[1], John Schleifer*[2], Roland Koedijk[1], Anée Kehlet[1] *[1]Chr. Hansen A/S; [2]Chr. Hansen Inc.*

By inhibiting pathogens, Bacillus probiotics may confer health benefits to the host. The objective was to evaluate the efficacy of reduction of *Escherichia coli* contamination by three probiotic *Bacillus* strains in a feed matrix. The test feed was added a multi strain *Bacillus* probiotic containing two *Bacillus subtilis* strains (DSM 32324 & DSM 32325) and a *B. amyloliquefaciens* strain (DSM 25840). The feed was autoclaved prior to *Bacillus* inoculation to reduce natural contaminants. Buffer was added to control and test samples and *Bacillus* probiotic was added to test samples to reach $1x10^5$ CFU/g feed. Samples were incubated aerobically at 37°C for 24 hours. *E. coli* (NCTC 10650) was added to control and test samples to achieve $3x10^4$ CFU/g feed and incubated aerobically at 37°C for 8 hours. Two replicates were analyzed per control and test sample. *Bacillus spp*. and *E. coli* were counted at 0, 4, 6 and 8h. *Bacillus sp.* CFUs were counted on TSA agar, *E. coli* CFUs were counted on selective MacConkey agar plates. In test samples, containing both *Bacillus spp.* and *E. coli*, the *E. coli* count remained stable throughout the experiment. At 4, 6 and 8 hours, respectively, the multi-strain *Bacillus* probiotic inhibited the growth of *E. coli* with 3, 4 and 5 logs, respectively ($p<0.0001$). These result indicate that this multi-strain bacillus product may be used as part of an action to decrease *E.coli* in chickens.

Key Words: E. coli, inhibition, pathogen, bacillus, probiotic

P341 Effect of cholecalciferol vitamin D3, calcium, phytase (Natuphos E) and NSPase (Natugrain TS), on performance, egg quality and bone strength of laying hens in extended production through 110 weeks Robert Jones*, Mike Coelho, Frank Parks *BASF Corporation*

To extend laying hen production to 110 weeks, special care must be given to gizzard and liver health (Bain, 2016). This study evaluates the effect of different levels of vitamin D3 (D3), gizzard health additives (coarse limestone, coarse feed, fat), gut health additives (phytase, Natuphos E, and NSPase (Natugrain TS)) on the lay persistency and egg quality from 80 to 110 weeks (wks). A total of 800 Hy-Line W36 laying hens at 80 wks laying were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (8 hens/cage x 10 treatments x 10 replications). Layers were fed corn/soy meal diets and the PC was formulated to meet or exceed current Hy-Line W36 recommendations. Treatments consisted of T1=PC Hy-line W36 recommendations, except formulated with 4000 IU/kg D3, 4.50% Ca, 400 FTU/kg phytase and 50% coarse limestone (CL); T2=PC+ 4000 IU/kg D3; T3=PC+85% CL; T4=PC+1500 FTU/kg phytase; T5=PC+ 4000 IU D3 + 85% CL+1500 FTU/kg phytase; T6=PC+2% vegetable fat (VF) + 4% soybean hulls (SBH); T7=PC+120 g NSPase; T8=PC+ 2% VF + 4% soybean hulls + 120 g NSPase; T9=PC+4000 IU D3+85% CL + 1500 FTU phytase + 2% VF+4% SBH, and T10=PC+4000 IU D3 + 85% CL+1500 FTU phytase + 2% VF + 4% SBH+120 g/kg NSPase. At 110 wks, feed intake, g/b/d (P=0.03) was highest with T1 and lowest with T9 and T10; FCR (P=0.02) was lowest with T9 and T10; Hen-day egg production, % (P=0.03) was highest with T9 and T10; Egg weight, g (P+0.03) was highest with T9 and T10; Egg shell, % (P=0.03) was highest with T9 and T10; Egg thickness, mm (P=0.02) was highest with T9 and T10; Bone strength, kgf/cm2 (P=0.03) was highest with T9 and T10. In conclusion, T9=PC+4000 IU D3+85% CL + 1500 FTU phytase + 2% VF+4% SBH and T10=PC+4000 IU D3 + 85% CL+1500 FTU phytase + 2% VF + 4% SBH+120 g/kg NSPase significantly increased layer performance, egg quality and bone strength of laying hens at 110 weeks versus the PC.

Key Words: cholecalciferol, phytase, NSPase, calcium, egg quality

P342 Effect of cholecalciferol vitamin D3, 25,hydroxycholecalciferol vitamin D3, calcium and phytase, Natuphos E, on performance, egg quality and bone strength of laying hens in extended production through 100 weeks. Robert Jones*, Mike Coelho, Frank Parks *BASF Corporation*

The industry priority is to increase egg production by breeding for increased persistency in lay and stability in egg quality so that the laying cycle of commercial flocks can be extended to 100 weeks (wks) (Bain, 2016). This study evaluates the effect of different levels of D3, 25(OH), phytase and calcium on the lay persistency and egg quality from 80 to 100 wks laying period of layers. A total of 800 Hy-Line W36, laying hens at 80 wks laying were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (8 hens/cage x 10 treatments x 10 replications). Layers were fed meal corn/soy diets and was formulated to meet or exceed current Hy-Line W36 recommendations. Treatments consisted of T1=PC Hy-line W36 recommendations, except formulated with 4000 IU D3/kg, 4.50% Ca and 400 FTU/kg phytase; T2=PC+ 2000 IU D3/kg, T3=PC+ 4000 IU D3/kg, T4=PC+75 mcg 25(OH)/kg, T5=PC+ 2000 IU D3 + 55 mcg 25(OH)/kg, T6=PC+1000 FTU/kg phytase, T7=PC+2000 FTU/kg phytase, T8=PC+ 3000 FTU/kg phytase, T9=PC+0.5% Calcium (Ca) and T10=PC+1.0% Ca. At 100 wks, feed intake, g/b/d (P=0.04) was highest with T4, T5 and T2 and lowest with T7 and T8; FCR (P=0.02) was lowest with T7, T8 and T3; Hen-day egg production, % (P=0.03) was highest with T7 and T8; Egg weight, g (P=0.03) was highest with T7 and T8; Egg shell % (P=0.04) was highest with T7 and T8; egg thickness, mm (P=0.04) was highest with T7 and T8; bone strength, kgf/cm2 (P=0.03) was highest with T7 and T8. In conclusion, T7=PC+2000 FTU/kg phytase and T8=PC+ 3000 FTU/kg phytase significantly improved laying hen production, egg quality and bone strength at 100 wks versus the PC.

Key Words: cholecalciferol, 25,hydroxy D3, Vitamin D3, phytase, layers

P343 Performance of broilers fed diets supplemented with an experimental direct-fed microbial in the presence of a moderate Clostridium perfringens challenge in a 42-day floor pen trial M. D. Sims[1], T. J. Gaydos[2], T. Kiros[3], Ruth Raspoet[3], E. Auclair[3], D. M. Hooge*[4] *[1]Virginia Diversified Research Corporation; [2]Phileo-LeSaffre Animal Care; [3]Phileo LeSaffre Animal Care; [4]Consulting Poultry Nutritionist*

An experimental direct-fed microbial (DFM) was evaluated in a dose- response trial to determine broiler live performance to 42 d with Coccivac® B at placement (d 0), used litter on d 4, and toxin-producing *Clostridium perfringens* (*Cp*) challenges on d 17-18 (by oral gavage and via water trough daily). There were 8 dietary treatment groups with 10 replicate pens of 30 chicks each per treatment (2,400 Cobb 500 straight- run chicks total). Economical, low-protein mash feeds were used except in a non-supplemented challenged (NS-C) treatment from a contemporary study fed crumbles/pellets. Other treatments were: non-supplemented non-challenged (NS-NC); yeast cell wall 250 ppm (YCW); or DFM at $1x10^4$ [Log 4], $3.16x10^4$ [Log 4.5], $1x10^5$ [Log 5], $3.16x10^5$ [Log 5.5] or $1x10^6$ [Log 6] cfu/g feed. Necrotic enteritis (NE) lesion scores (0=normal to 3= severe) were taken using 3 random birds/pen on d 21. Completely randomized design ($P\leq0.05$) was used with Tukey's HSD test ($P=0.05$) to separate means. The NE scores were higher for NS-C (2.17) than for NS-NC (0.59) ($P=0.028$) with YCW and DFM groups intermediate (1.17 DFM Log 4 to 1.47 YCW). Feed conversion ratio (FCR) 0-28 d was higher ($P<0.01$) for NS-C group than YCW and DFM supplemented diets (statistically equivalent to NS-NC). The 42-d BW were approaching significance (P=0.059) with NS-C (1.580 kg) *vs*. NS-NC (1.850 kg) with other groups intermediate (1.681 to 1.745 kg). The FCR and mortality- adjusted FCR (MAFCR) were higher for NS-C than NS-NC ($P<0.01$), and DFM Log 4 and DFM Log 5 to Log 6 improved FCR, and YCW, DFM Log 4 and DFM Log 5 to Log 6 improved MAFCR *vs*. NS-C. Mortality % from 0-28 d was not different ($P=0.40$) but from 0-42 d ($P=0.013$), NS-C was higher than NS-NC

(18.06 *vs.* 4.44%) and improved with DFM Log 4.5 (8.00%) or DFM Log 5 (7.67%) with other groups intermediate (8.67 to 10.00%). In conclusion, under *Cp* challenge, YCW diets improved 0-28 d FCR and 0-42 d MAFCR *vs*. NS-C, and DFM Log 4 to Log 6 diets improved 0-28 d FCR, and DFM Log 4 and Log 5 to Log 6 diets improved 0-42 d MAFCR *vs.* NS-C showing effectiveness. The DFM Log 4.5 and Log 5 diets lowered 0-42 d mortality % *vs*. NS-C indicating efficacy.

Key Words: broiler, Clostridium perfringens, direct-fed microbial, necrotic enteritis, yeast cell wall

P344 Performance and tibia ash of broilers fed diets reduced in minerals, energy and amino acids from 1 to 20 days of age, and supplemented with different dose rates of 3 commercially available phytases Gilson Gomes*[1], Craig Wyatt[1], Daniel Moore[2] *[1]AB Vista; [2]Colorado Quality Research*

The aim of this trial was to evaluate the effects of 3 commercially available phytases, fed at either 325 or 750 FTU/kg, on performance and bone traits of broilers fed diets reduced on minerals, energy and amino acids. Five hundred and forty day-old Cobb 500 male birds were assigned to 9 dietary treatments with 10 cage replicates each. A positive control diet (PC) was formulated to be nutritionally adequate in all nutrients (PC), and two negative control diets reduced by either 1.2 (NC1) or 1.7 g/kg of avP (NC2). Ca, Na, dLys, dM+C, dThr, dVal (as g/kg) and AME (as kcal/kg) were reduced by 1.32/1.87, 0.27/0.39, 0.14/0.19, 0.32/0.45, 0.27/0.38, 0.17/0.24, 42/60 on NC1/NC2, respectively. Phytases AP, QB and NPE were supplemented at 325 or 750 FTU/kg by top dressing the NC1 or NC2, respectively. Diets corn and soybean meal based and fed as mash, being feed and water provided *ad libtum*. Body weight gain (BWG) and feed intake (FI) were measured at 20 d, and feed conversion ratio calculated correcting for mortality (mFCR). At the end of the trial 3 birds per cage were sacrificed and left tibia excised for bone ash determination (TA). Data was submitted to ANOVA, and means separated using Tukey test ($P<0.05$). Livability was not affected by treatments ($P>0.05$). BWG, FI, mFCR and TA were affected by treatments ($P<0.05$). NC2 had the poorest BWG, and supplementation of phytases, regardless of source or dosage, improved BWG to similar levels as of PC ($P>0.05$). FI was lowest on birds fed NC2, and supplementation of NPE, independently of dosage, improved FI with other treatments being intermediate. mFCR increased as nutrient reductions increased ($P<0.05$). Supplementation of 325FTU/kg of phytase restored mFCR to similar levels of PC, although, at 750FTU/kg, the only phytase to restore mFCR to similar levels of PC was QB phytase. TA decreased as dietary nutrients decreased ($P<0.05$), and all phytases, regardless of source or dosage, restored TA to similar levels as PC fed birds. As conclusions, reduction in nutrients reduced performance and TA of birds. Supplementation of 325 FTU/kg of any tested phytase successfully restored performance, while at 750 FTU/kg differences were more apparent and QB phytase was the only one to restore mFCR to similar level as PC fed birds.

Key Words: bone, weight gain, FCR

P345 Effect of a feed incorporated Silymarin based phytobiotic on the performance of broiler chickens administered a non-defined challenge Bruno Vecchi*[1], Emanuel Gumina[1], Ariel Sugezky[1], Sherry Layton[2] *[1]Vetanco SA; [2]BV Science*

Preoccupation with antibiotic growth promoters (AGP) and their effects on bacterial resistance in poultry production is on rise creating an increased pressure for the development of effective alternative products that generate the same or better production results when compared to AGPs. Silymarin is found naturally in the seeds of *Silybum marianum*. Previously, there have been numerous studies reporting the effects of Silymarin on increased body weight and increased egg hatchability. The objective of this study was to evaluate the effect of a feed incorporated Silymarin based phytobiotic on the productivity parameters of broilers chickens administered a non-defined challenge. 300 day-old chicks (Cobb 500) were individually tagged, weighed and randomly assigned to one of 15 pens (n=20 birds/pen). Pens were randomly assigned to 1 of 3 treatment groups: 3 treatments with 5 replicates each (n=100 birds/treatment). All birds were fed with a 4-phase standard commercial diet with the following additions: T1 non-treated, T2 BMD 11%/tn, and T3 phytobiotic 0.5 kg/tn. On d 7, 14, 15 of life, all birds were challenged by drinking water (5L/pen/day) with a non-defined challenge derived from fermentation and subsequent crude filtration of used poultry litter from a flock that broke with necrotic enteritis. General microbial counts were determined using Petri-film: $6.4x10^7$ (aerobics), $1.5x10^5$ (fungi and yeast), $7.5x10^9$ (anaerobic) and $1.1x10^5$ (E.Coli). Birds were individually weighed again on d42 to determine total body weight gain (BWG). Mortality and feed conversion (FCR) were also recorded and calculated throughout the course of the experiment. Data was analyzed for statistical significance using a one-way ANOVA ($p<0.05$) followed by a Tukey test. Different letters represent difference between means. BWG (kg) was as follows: T1 was 2.425a ± 0.069; for T2 2.697b ± 0.054 and for T3 2.903c ± 0.077. FCR (kg) was 2.213a ± 0.063 for T1; 2.020b ± 0.041 for T2 and 1.786c ± 0.047 for T3. Mortality was 6% for T1 and 5% for T2 and T3. Results indicate that while both BMD and the phytobiotic increased total BWG and improved feed conversion over control results with the phytobiotic exceeded those of BMD; indicating this plant based phytobiotic may prove a good alternative to AGPs.

Key Words: Silymarin, Phytobiotic, Poultry, Antibiotic Growth Promoter, Body Weight Gain

P346 Zinc source in broiler breeder diet affects offspring bone-specific alkaline phosphatase activity at hatch Marquisha Paul*[GS], Anthony Pescatore, Tuoying Ao, Michael Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

Broiler breeder nutrition has an impact on offspring skeletal development. Bone-specific alkaline phosphatase (BSAP) is a chemical biomarker of bone formation and metabolism. This study was conducted to evaluate the effect of supplementing microalgae (FORPLUS™, Alltech, Inc.) and two sources of Zn (ZnO or Bioplex® Zn, Alltech, Inc.) in broiler breeder diets on the BSAP concentration of breeder offspring at hatch. An RCB design with a 2x2 factorial trt structure was used for this study. A total of 240 Cobb500™ broiler breeder hens and 20 Cobb500™ broiler breeder roosters were randomly assigned to a corn-soybean meal diet containing either 0 or 2% microalgae and an additional 40 mg Zn/kg diet as ZnO or Bioplex® Zn for the entire rearing period. Broiler breeders were raised in floor pens with *ad libitum* access to water and nest boxes, 15L: 9D lighting, and controlled access to feed. Each dietary trt consisted of 5 rep floor pens (12 hens + 1 rooster/pen). Fertile broiler breeder eggs were collected and set in ten-week intervals at three time points: 19-20 wk of lay (peak lay), 30-31 wk of lay (post-peak lay), and 41-43 week of lay (post-peak lay). At hatch, the serum of breeder offspring was collected and evaluated for BSAP concentration using competitive ELISA. Data were analyzed using the GLM procedures of SAS v9.4 with mean differences determined using Fisher's LSD test. No interaction effect of broiler breeder dietary microalgae level and Zn source on offspring BSAP concentration was observed. However, a main effect of broiler breeder dietary Zn source on offspring BSAP was observed. Offspring from breeders fed Bioplex® Zn during peak lay had higher ($P<0.01$) serum BSAP concentration than offspring from breeders fed ZnO. No effects of breeder diet on offspring serum BSAP were observed during post-peak lay. In conclusion, dietary supplementation of Bioplex® Zn during the peak lay phase of broiler breeders increased the serum BASP of broiler breeder offspring at hatch.

Key Words: Broiler breeder, zinc, peak lay, breeder offspring, bone-specific alkaline phosphatase

P347 Effects of Availa®Zn and Availa®Z/M and Availa®ZMC on progeny performance of 0- to 16-week-old male turkey poults, from turkey breeders fed Availa®ZMC Colwayne Morris*[1GS], Leonardo Linares[2], Jeffre Firman[1], Marco Rebollo[2], Duarte Neves[2] *[1]University of Missouri; [2]Zinpro Corporation*

This study evaluated efficacy of dietary supplementation of amino acid complexed (AAC) trace minerals on performance and bone health of 0- to 16-week-old male turkey poults, from breeders fed inorganic minerals (IM) or Availa®ZMC (AvZMC). Day-old Hybrid Converter male poults (n=1,248) were randomized in a factorial design with 2 breeder × 4 progeny treatments, 12 replicate pens and 13 birds/rep. Birds were fed corn-SBM based diets: Starter (S) 0-21d; Grower (G)1 22-49d; G2 50-70d; Finisher (F)1 71-91d; F2 92-112d. Progeny S and G1: T1, 125ppm Zn, 125ppm Mn, 7ppm Cu from sulfates; T2, 60ppm Zn from Availa®Zn+65ppm Zn, 125ppm Mn, 7ppm Cu from sulfates; T3, 40ppm Zn, 40ppm Mn from Availa®Z/M+85ppm Zn, 85ppm Mn, 7ppm Cu from sulfates; T4, 40ppm Zn, 40ppm Mn, 7ppm Cu from AvZMC+85ppm Zn, 85ppm Mn from sulfates. G2 diets were reduced by 25ppm Zn and Mn from sulfates for all treatments in S diets; F1 and F2 diets were reduced by 50ppm Zn and Mn from sulfates for all treatments in S diets. Data were analyzed by ANOVA to determine statistical differences among treatments. A performance effect from 0- to 13-weeks-of-age (P=0.02) occurred, where poults from AvZMC breeders had lower mortality adjusted feed conversion (adjFCR) than poults of breeders fed IM (2.243 vs 2.287). A progeny effect (P=0.07) was noted, where sulfates had the highest adjFCR (2.306, 2.265, 2.240 and 2.248 for T1, T2, T3 and T4, respectively). From 10 to 13 weeks, poults from breeders fed AvZMC gained more weight than poults from breeders fed IM (3.494 vs 3.327kg) with an adjFCR of 2.788 and 2.917, respectively (P=0.02). There was no difference (P>0.05) in tibia weight, ash, or cross-sectional measurements among breeder or progeny treatments. Progeny of breeders fed AvZMC had significantly higher Mn tibia ash concentration (P<0.10). Histological analysis of the tibia noted presence of rickets, suggesting changes shown as increased length of proliferative zone of growth plates, which was lower in turkeys fed Availa®Zn from breeders fed AvZMC (P<0.05). In conclusion, feeding supplemented AAC trace minerals to turkey breeders impacted progeny bone characteristics and performance both statistically and numerically throughout the experimental period, compared to progeny of breeders fed inorganic minerals.

Key Words: Ash, Copper, Manganese, Turkey, Zinc

P348 Evaluation of increasing levels of manganese hydroxychloride in 45 week old white leghorn laying hens on yolk and shell Mn content Austin Jasek*[1GS], Kyle Brown[1], Terri Parr[2], Craig Coufal[1], Jason Lee[1] *[1]Texas A&M University; [2]Micronutrients USA LLC*

The objective of the current study was to evaluate increasing levels of manganese hydroxychloride in 45 week old white leghorn laying hens on yolk and shell Mn content as a potential marker for trace mineral requirements. A total of 84, 45 week old white leghorns were assigned to 6 dietary treatments each consisting of 14 individually caged laying hens. The experiment consisted of a reference diet that contained 70 ppm of supplemental inorganic manganese (Mn) in the form of Mn oxide and 5 experimental treatments each containing 0, 15, 30, 60, and 90 ppm supplemental Mn hydroxychloride. Prior to feeding the experimental diets, all of the experimental birds were subjected to a 21 day depletion phase in which no supplemental Mn was included in the diet; however, during this time the reference fed birds were fed the control diet (70 ppm Mn). After the 21 d depletion phase, the birds were fed experimental diets for a 35 d evaluation period. During the depletion phase, yolk and shell Mn content and shell breaking strength were analyzed on D 0, 7, 14, and 21 days. During the experimental phase, yolk and shell Mn content, egg weight, and shell breaking strength were analyzed on d 5, 9, 15, 21, 25, 30, and 35 days and feed consumption was measured on d 10, 20, 30, and 35 days. Daily lay rate was evaluated throughout the duration of both the depletion and experimental phase. During the experimental phase, Mn was replenished into the yolk and shell in all experimental treatments, however, dose and time impacted the rate of replenishment. The yolk tended to be more sensitive to variations in Mn level as increases in Mn inclusion significantly (p<0.05) increased concentration. These data demonstrate the ability to deplete and replenish Mn, and the use of egg yolk Mn concentration as measurement for determining changes in dietary Mn. At the conclusion of the experiment at 35 d, 60 ppm of Mn hydroxychloride seemed to be adequate in replenishing Mn to the level of the reference.

Key Words: Manganese, Layers, Egg, Yolk

P349 Response of broiler chickens to supplemental chelated copper, zinc and manganese or their corresponding inorganic salts Abiodun Ayoola*[1GS], Adeboye Fafiolu[1,2], Oluseyi Oluwatosin[1,2], Oluwatomide Ariyo[2], Oluremi Osinowo[1], Luis Azevedo[3] *[1]Centre of Excellence in Agricultural Development and Sustainable Environment, Federal University of Agriculture Abeokuta; [2]Department of Animal Nutrition, Federal University Agriculture Abeokuta; [3]Novus International Inc.*

This trial evaluated the effect of supplemental Cu, Zn and Mn chelated with 2-hydroxyl-4-methyl thiobutanoic acid (HMTBa) and its inorganic sources on growth performance, trace mineral digestibility and trace minerals concentration in tissues (liver and kidney) of broiler chickens. A 42-day feeding trial was conducted with a total of 300 Arbor Acre broiler chickens as experimental birds. The AA broiler chickens were allocated to five dietary groups. Individual dietary group was replicated six times with ten birds each. The dietary groups were control (basal diet), 100% inorganic trace minerals (ITMs) supplemental level (15, 100, 100 mg/kg for Cu, Zn, Mn respectively), 50% ITMs supplemental level (7.5, 50, 50 mg/kg for Cu, Zn, Mn), 50% chelated trace minerals (CTMs) supplemental level (7.5, 50, 50 mg/kg for Cu, Zn, Mn) and 25% CTM supplemental level (3.75, 25, 25 mg/kg for Cu, Zn, Mn respectively). Data collected were subjected to one – way Analysis of Variance using 5% significant level in a Completely Randomised Design. Results showed the birds fed diet with 100% supplemental level of ITM and 50% supplemental level of CTM had improved (P<0.05) feed conversion ratio between 0 and 21 days of the experiment. The group fed 50% supplemental level of CTM had a lower (P<0.05) daily feed intake than the group fed 100% supplemental ITM level. Daily feed intake and daily weight gain were significantly higher (P<0.05) for the birds fed 100% ITM level and 50% supplemental CTM level between 21and 42 days. The trace mineral (Cu, Zn and Mn) excreted was slightly lower for the group fed basal diet (BD) than the other groups fed supplemented ITM and CTM. Birds fed 50% supplemental diet of CTM had a significantly lower excreta Zn than the group fed 50% ITM level. The liver-Zn and –Mn and kidney-Cu for the birds fed 25% supplemental diet of CTM were significantly higher (P<0.05) compared to the other dietary groups. The Zn concentration in the kidney of birds fed 100% ITM supplemental level was lower relative to birds fed diet supplemented with CTM. The study showed that trace minerals supplementation improved growth performance of broiler chickens while the chelated form improved the bioavailability of the trace minerals and reduced minerals excreta into the environment.

Key Words: Broiler, chelate, zinc, copper, manganese

P350 Effect of increasing amino acid density on male broiler performance through 33 days Kyle Smith*[1GS], Corey Johnson[1], Rocky Latham[2], Craig Coufal[1], Jason Lee[1] *[1]Texas A&M University; [2]Tyson Foods Inc.*

The objective of the current experiment was to quantify the impact of increasing amino acid density on broiler performance thru 33 days. The experimental design was a one-way ANOVA with 3 levels of amino acid density consisting of a Control, the control +5% and +10% digLys. Each treatment contained 15 replicates with 50 Cobb 500 x Hubbard M99 straight-run broilers per replicate for a total of 2,250 birds placed at d of

hatch for a 33 day assay period. Broilers were provided age appropriate light and supplemental heat and had access to feed and water *ad libitum*. The Control was formulated to digLys levels of 1.18, 1.05 and 0.92% digLys in the starter, grower and finisher phases, respectively. All other essential amino acids were ratioed to digLys and ratios remained constant with increasing digLys levels. Experimental diets were corn-soybean meal based with meat and bone meal included at 5% in the starter and 1% for the remainder of the trial. The starter was fed as a crumble through d12, grower as a pellet through d26, and finisher as a pellet through the conclusion of the study at d33. Body weight (BW), mortality corrected feed conversion ratio (FCR) and feed consumption (FC) were calculated with each dietary phase change. Increasing AA density improved ($P<0.05$) FCR after d12 and elevated BW at d26 which maintained through d33. Regression analysis identified linear relationship between elevated amino acid concentration and improving FCR throughout the study. Feed consumption was increased ($P<0.05$) during the finisher phase with the 10% increase in amino acid density. As expected, linear increases were observed in lysine consumption as amino acid density was elevated which correlated to increased body weight. As anticipated, these data confirm that increasing digLys content improves growth performance, however, this trial quantifies the improvement allowing nutritionists the ability to conduct economic assessments and determine the most profitable digLys content. This research can help broiler nutritionists identify opportunities in feed formulation to target FCR and BW improvements through the use of elevated AA densities.

Key Words: Amino Acids, Broiler, Protein, Performance

P351 Influence of iron source (organic vs inorganic) on broiler performance under a pathogen challenge Jack Garrett*[1], Greg Nunnery[1], James McNaughton[2] *[1]QualiTech, Inc.; [2]AHPharma, Inc.*

Iron is a trace mineral supplemented in broiler diets. Bacterial pathogens, such as E. coli, C. perfringens and E. acervulina have an essential requirement for iron such that they have multiple sequestering mechanisms to obtain iron. This study was designed to evaluate the hypothesis that an organic form of iron (SQM Iron®, QualiTech, Inc.) could limit the availability of iron to such pathogens and reduce their impact on broiler performance. A total of 2496 mixed sex broiler chickens (Ross 708) were randomly assigned to one of 48 pens (12 pens/replicate). Diets (corn-soy based) were formulated to meet industry standards for a Prestarter (0-7d), Grower (8-35d) and Finisher (36-42d) program. Treatments included 1) iron sulfate as the sole supplemental iron source (IONC), 2) SQM Iron instead of iron sulfate (OINC), 3) IONC receiving a pathogen challenge on day 7 (IOPC), and 4) OINC receiving a pathogen challenge on day 7 (OIPC). Broilers received Cocci-Vac day 0. Sub-clinical challenge was provided by obtaining used litter containing E. acervulina (>50,000 oocysts/bird), C. perfringens (>104/bird) and E. coli (>106/bird). Performance was measured every 7 days; lesion scoring, and bacterial counts were done on day 21 and 42. Broiler growth rate and efficiency was significantly ($p<0.085$) impaired by the pathogen challenge every week of the study. Iron source had a significant ($p<0.05$ improvement on weight and efficiency through week 3 with the organic iron source. Week 4-6 had strong trends for organic source improving bird weight and efficiency. Bird mortality showed a significant ($p<0.05$) interaction between source and challenge with those bird receiving the organic source reducing mortality under the challenge conditions. Lesion scores were significantly ($p<0.01$) reduced by feeding the organic iron source (challenged or unchallenged). Iron source had no influence on E. coli counts. Feeding the organic iron source significantly ($p<0.01$) reduced C. perfringens and cocci counts at all times of the study. Results of this study showed that feeding SQM Iron as an organic iron source reduced the growth rate of pathogens. This reduction in growth rate appears to be due to limiting the accessibility of iron for growth of pathogens.

Key Words: iron, broiler, pathogen challenge, organic, SQM

P352 Growth performance and bone characteristics of boiler chicks fed corn-soy diet supplemented with different levels of vitamin premix and different sources of mineral premix Tuoying Ao*, Marquisha Paul, Anthony Pescatore, Lizza Macalintal, Mike Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

Inorganic trace mineral salts in the premix have detrimental effect on the stability of vitamins due to redox reactions. A study was conducted to investigate the effect of dietary supplementation of different levels of vitamin premix (VP) with two different mineral premixes (MP) on the growth performance and bone characteristics of broiler chicks. A 2 x 2 factorial treatment structure included 2 levels of VP (100 or 30% commercial level) and 2 types of MP (inorganic MP at commercial level or Bioplex® MP at 40% commercial level). The premixes with 30% vitamin level and two mineral sources were stored in room temperature for 90 days. One-day old chicks were randomly assigned to four dietary treatments with 12 replicate pens of 22 chicks for 28d. Chicks fed the diet containing Bioplex® MP had higher ($P<0.01$) weight gain and feed intake and lower ($P<0.01$) feed to gain ratio than those fed diet containing inorganic MP. Chicks fed the diet containing 100% VP had higher ($P<0.01$) weight gain and feed intake than those fed diet containing 30% VP. An interactive effect between vitamin levels and mineral sources on performance of chicks was detected ($P<0.01$). Chicks fed the diet containing 100% VP with either source of MP had higher ($P<0.01$) weight gain and feed intake than those fed the diet containing 30% VP with either source of MP. However, the chicks fed the diet containing 30% VP with Bioplex® MP had higher ($P<0.01$) weight gain and feed intake than those fed the diet containing 30% VP with inorganic MP. Chicks fed the diet containing Bioplex® MP had higher ($P<0.01$) breaking strength and ash content of tibia than those fed inorganic MP. Chicks fed the diet containing 100% VP had higher ($P<0.01$) ash% of tibia than those fed the diet containing 30% VP. Data from this trial indicate that supplementation of Bioplex® MP in vitamin mineral premix can positively impact the stability of vitamins.

Key Words: broiler, performance, mineral premix, vitamin premix, vitamin stability

P353 Bioplex® minerals restore the acid-base and electrolyte balance in broiler chicks with nutritionally-induced metabolic acidosis Lizza Macalintal*, Anthony Pescatore, Tuoying Ao, Michael Ford, Karl Dawson *Alltech-University of Kentucky Nutrition Research Alliance*

Acid-base and electrolyte balance in poultry can be influenced by environmental conditions, metabolism, and plane of nutrition including vitamins and minerals. It is crucial in the proper functioning of the metabolic processes otherwise cellular functions will falter. A study was conducted to evaluate growth performance and selected blood gas and electrolytes in broilers provided with diets containing different levels of vitamin premix (VM) and forms of trace minerals. VM was included as either 100 or 30%. Trace minerals were provided as either inorganic (IO, commercial level) or Bioplex® (at 40% commercial level). Cobb male broilers, 1-d of age, were used in this experiment designed as a factorial treatment structure with 4 treatments using 12 replicates pens (22 chicks/pen) each arranged in a randomized complete block design. Growth performance was recorded through d28. Blood samples were collected in lithium heparinized tubes. An i-STAT® handheld point-of-care clinical analyzer loaded with CG8+ & CG4+ cartridges was used for analysis. Providing chicks with 30% VM compared with the standard 100% VM led to a pronounced BW loss at d28, however, the 30% VM + Bioplex®-fed chicks were heavier compared to IO ($P<0.001$). Significant Bioplex® X VM (30%) interactions were observed for HCO_3^- base excess, TCO_2, ionized Ca and hematocrit. Using Bioplex® in the premix but not IO resulted in blood gas and electrolyte values that were comparable with the 100% VM. Birds fed 30% VM (main effect) had lower ($P<0.01$) pO_2, sO_2, pH ($P=0.053$) and K^+ with concomitant increases in ($P<0.03$) lactate, glucose and Na^+. However, Bioplex® tended to reverse the Na^+ effect ($P=0.053$) along with partial CO_2 that tended to be higher. Taken together, these results demonstrated

for the first time the effects of providing a diet with 30% VM or 100% VM with Bioplex or IO minerals on growth performance, blood gas and electrolyte balance. Poor growth index at d28 can be attributed to acid-base and electrolyte perturbation in broilers triggering a nutritionally-induced metabolic acidosis condition. However, the metabolic acidosis brought about by underfeeding VM (30%) can be restored or normalized by using Bioplex® but not IO trace minerals in the vitamin-mineral premix.

Key Words: acid-base balance, acidosis, organic minerals, vitamins, broiler

P354 Effect of different levels of inorganic copper and zinc on growth performance and gut health of broilers challenged with Eimeiria spp. Tatiane Santos*[1], Po-Yun Teng[2], Sudhir Yadav[2], Lorraine Fuller[2], José Roberto Sartori[1], Woo Kim[2] *[1]São Paulo State University (UNESP); [2]University of Georgia*

Coccidiosis is an avian disease caused by *Eimeria* spp. leading to high economic losses in the poultry industry, generating global losses of more than 2.4 billion dollars. Different methods have been used as a preventive measures such as plants and minerals. Copper and zinc are microminerals essential for the immune system of birds with potential benefits to intestinal health. An experiment was conducted to evaluate the effect of different levels of inorganic copper and zinc on growth performance, intestinal permeability and intestinal lesion scores in 20-day-old Cobb 500 broilers challenged with *Eimeria* maxima, *Eimeria* tenella and *Eimeria* acervulina. A total of 360 male chicks were housed in 36 cages in a completely randomized design with six replicates of 10 birds each replicate. There were six corn-soybean meal based dietary treatments: Non-challenge control (NC), Challenge control (C), 100 ppm of copper (C+100Cu), 150 ppm of copper (C+150Cu), 80 ppm of zinc (C+80Zn) and 100 ppm of zinc (C+100Zn). Broilers received the treatment diets for 9 days, and all birds with exception of NC were challenged with *Eimeria* maxima (50,000 oocysts/bird), *Eimeria* tenella (50,000 oocysts/bird), and *Eimeria* acervulina (250,000 oocysts/bird) on day 14. Feed intake, body weight gain, feed conversion ratio, intestinal permeability and lesion score were recorded at d20. The means were subjected to ANOVA and, when significant, were compared by Duncan test ($p<0.05$). The growth performance was not positively influenced by inclusion of inorganic minerals when compared to NC diet. However, the intestinal permeability was improved when the birds received the C+100Zn diet, and the data showed the reduction of intestinal permeability accomplished by the supplementation of Zn. Results from lesion scores showed reduction of this parameter in the duodenum of broilers fed with C+150Cu compared to C diet, and the copper had better influence than zinc on lesion score reduction. Birds challenged with *Eimeria* spp. improved intestinal integrity based on these parameters when the supplementation of inorganic copper and zinc was used, suggesting that Cu and Zn supplementation would be a potential strategy to combat the coccidiosis.

Key Words: growth performance, intestinal permeability, lesion scores, micromineral

P355 Manganese requeriments for broilers fed different mineral sources Bruno Carvalho*[1], Helvio Ferreira Júnior[1], Diego Silva[1], Pedro Arnaut[1], Raully Silva[1], James Pettigrew[2], Melissa Hannas[1], Horacio Rostagno[1] *[1]Universidade Federal de Viçosa; [2]Alltech*

An experiment was conducted aiming to update the manganese requirements for broilers from organic and inorganic sources. For that, 500 male Cobb broilers from 1 to 18 days-old were used. The animals were housed (1-7d) following Cobb guidelines receiving a corn-soybean meal diet with low trace mineral content in the basal diet (13 mg/kg of Mn analyzed) and they were supplemented with 30 mg/kg of Mn (50% of NRC 1994 recommendations) as MnSO4 to avoid high body stores. At 8d-old, birds were weighed and randomly assigned to 10 treatments with 10 replicates of 5 animals each. The treatments consisted of five levels of supplemental manganese (0, 25, 50, 75, and 100 mg/kg) from two sources ($MnSO_4$ with Fe, Cu, and Zn supplemented in as sulfates and Se as selenite, and Bioplex®Mn, Fe, Cu, Zn also supplemented as proteinates and Se-Yeast). The animals were housed in plastic cages equipped with trays and plastic feeders and received demineralized water and feed at libitum. The experimental diets were semi purified (6 mg/kg of basal Mn), with 30% of corn and contained cellulose, phytic acid and phytase to simulate a practical diet. At 17 d-old, the birds and feeds were weighed to record BW, ADG, ADFI and feed efficiency (ADG/ADFI). At 18 d-old, one bird for cage was slaughtered for determination of minerals in tibia, chest and liver. Data were subjected to ANOVA, with the model including source, Mn level, and their interactions, and the requirement was estimate using the quadratic response of each source by orthogonal contrats (if $P<0.05$). Considering 6 mg/kg of Mn in basal diet, the linear results for BW, ADG, ADFI, Mn retention and deposition on tibia suggests that the requirement of Mn is higher than 100 mg/kg for both sources using those parameters, since any interaction between source and levels ($P>0.05$) were verified. However, using the quadratic response for feed efficiency, the Mn requirements is 77.92 mg/kg. Requirements based on Mn concentration in tissues was 92.0 and 68.3mg/kg respectively, for inorganic and organic source in chest and 90.9 and 74.8mg/kg for inorganic and organic source in liver. In conclusion, there are significant differences in the Mn requirements for broilers depending on source and parameters evaluated.

Key Words: Manganese, Requirement, Trace mineral, Broiler

P356 Dietary copper requirements for broilers fed different mineral sources Helvio Ferreira Júnior*[1], Bruno de Carvalho[1], Pedro Arnaut[1], Filipe Monteiro[1], Carolaine Ferreira[1], Diego da Silva[1], James Pettigrew[2], Horacio Rostagno[1], Melissa Hannas[1] *[1]Universidade Federal de Viçosa; [2]Alltech*

Copper (Cu) is responsible for many functions in the animal body, particularly as an enzyme cofactor. Dietary mineral supplementation traditionally uses inorganic sources (such as sulphates). A study was conducted to evaluate the Cu requirements for 17 d-old broilers fed Cu from two different mineral sources. A total of 500 animals were randomly distributed into a 2 x 5 factorial arrangement (2 Cu sources x 5 Cu levels), totalizing 10 treatments, with 10 replicates of 5 birds per experimental unit. Copper sulphate ($CuSO_4.5H_2O$) and copper proteinate (ProCu, Bioplex®Cu), at five supplementation Cu levels (0, 4, 8, 12 and 16 mg Cu / kg) were used to estimate the dietary Cu requirements for broilers. A semi-purified diet was used to obtain low levels of Cu, with 30% of corn, phytic acid and phytase to simulate a practical diet. At 17 d-old, birds and feeders were weighed to record body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (FE). Data were subjected to ANOVA, with the model including source, Cu level, and their interaction. The requirements were estimated by quadratic polynomial model, based on performance responses and Cu concentration in tissues by orthogonal contrasts (at $P<0.05$). Broilers fed ProCu presented greater ($P<0.05$) FE. Increasing levels of Cu showed a linear response ($P<0.05$) for ADFI and quadratic responses ($P<0.05$) for final BW, ADG and FE. No differences ($P>0.05$) were observed between the ProCu and $CuSO_4.5H_2O$ sources for Cu concentration in tissues. Considering the 3 mg Cu/kg of the basal diet, the Cu requirement to maximize final BW, ADG and FE were 9.75, 9.76 and 12.40 mg Cu/kg, respectively. Maximum FE was obtained from Cu levels of 12.83 and 11.75 mg Cu/kg for proteinate and copper sulphate, respectively. Therefore, under the conditions of this experiment, there was a higher requirement for Cu of animals fed ProCu according to the quadratic polynomial model. However, broilers fed ProCu presented higher ($P<0.05$) FE (0.785) compared to those receiving $CuSO_4.5H_2O$ (0.765).

Key Words: Copper, Requirements, Broilers, Feed conversion

P357 Dietary supplementation of an antioxidant (EconomasE™) and two sodium sources on intestinal permeability (gastrointestinal health) and apparent ileal, total tract energy, and nutrient digestibility in 21-day-old broilers challenged with dexamethasone Opeyemi Olojede*[1], Sunday Adedokun[1], Anthony Pescatore[1], Tuoying Ao[2] *[1]University of Kentucky; [2]Alltech Inc.*

When an animal experiences stress factors that mediate the hypothalamic-hypophyseal-adrenal axis, it exhibits a series of changes known collectively as physiological stress. These stress responses are integrally involved with acid-base balance in several species, which can result in physiological, hormonal, and immunological deficits increasing susceptibility to diseases. To test the effect of a commercial algae-based antioxidant, containing Se yeast, EconomasE™ (EcoE) and two sodium sources (NaCl and NaCl+$NaHCO_3$) on growth performance, digestibility, antioxidant and immune status of broilers challenged with dexamethasone (DEX), 336 chicks were assigned to these treatments in a 2 x 2 x 2 completely randomized design with 7 replicate cages and 6 birds/cage. A basal diet containing the supplement and/or either of the sodium sources was fed for 21 days. The stressor, DEX, or deionized water was administered orally at the rate of 1 mg/kg body weight on 3 alternate days (16, 18, 20). On day 21, birds were euthanized to obtain blood and jejunal mucosal samples for antioxidant, and gene expression of mucosal immunity. Dexamethasone reduced body weight gain and feed efficiency ($P<0.0001$) from d 16-21, which ultimately decreased body weight, gain, and feed efficiency ($P<0.0001$) overall (0-21 d). Apparent ileal digestibility of DM, N, and energy (EN) were decreased ($P < 0.05$), and total tract digestibility of DM, N, EN, AME, AMEn were decreased with DEX ($P < 0.05$). Relative weights of liver increased ($P <.0001$) and decreased ($P < 0.0001$) for spleen and bursa of Fabricius with DEX. Inclusion of either sodium source or EcoE in the diets did not affect growth performance and relative weights of the lymphoid organs. However, ileal and total tract DM digestibility increased ($P< 0.05$) with NaCl+$NaHCO_3$. In addition, EcoE increased ileal digestibility of EN, AME, and AMEn. Finally, plasma antioxidant status (SOD, CAT), and jejunal mRNA levels of IL-1β, IL-6, IL-8, IL-10, ZO-1, Occludin 1, Claudin were not affected by DEX, sodium source or dietary supplement. In conclusion, homeostasis was altered with dexamethasone, resulting in low growth performance and nutrient digestibility. While EcoE did not mitigate the performance parameters, it enhanced nutrient digestibility.

Key Words: broilers, dexamethasone, digestibility, sodium source, gut

P358 The Effect of Supplementing broilers with the BASF annual global industry vitamin supplementation survey on performance Frank Parks*, mike coelho, Robert Jones *BASF Corporation*

There is a continued industry focus to decrease FCR (Aviagen, 2018), which means less feed per kg body tissue, and therefore vitamins need to be further concentrated in less and less feed to reach the same vitamin supplementation per kg of body tissue. A study was conducted to determine the response of broilers to different levels of vitamin supplementation based on the 2018 BASF global Industry vitamin supplementation survey (Coelho, 2018). Broilers were stressed to simulate commercial stress conditions. A total of 1350 Cobb 500 male broilers were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (30 birds/pen x 5 vitamin treatments x 9 replications). Broilers were blocked by weight and fed corn/soy diets pelleted at 80C and formulated to meet or exceed NRC (1994) recommendations. Stress was imposed to simulate commercial conditions consisted of 100% old litter, 0.046 m^2/bird, 100,000 oocytes E, acervulina and maxima /bird and 10^8 CFU Clostridium perfringens/bird orally inoculated at day 1. Treatments were NRC, low 25%, AVG, high 25% and high 5% vitamin supplementation based on 2018 industry vitamin survey (Coelho, 2018). At finishing, vitamin supplementation significantly increased body weight up to high 5% vitamin supplementation (1837, 2030, 2285, 2495, and 2650 g, P=0.03, respectively), improved FCR up to Hi 5% (2.15, 2.01, 1.91, 1.86 and 1.78, P=0.03, respectively). Vitamin supplementation decreased finishing mortality up to high 5% vitamin supplementation (7.25, 6.23, 5.21, 4.65 and 4.21%, P=0.02, respectively), improved carcass yield up to Hi 5% (68.0, 70.2, 72.5, 75.5 and 79.1, P=0.03, respectively), breast yield (19.0, 21.1, 23.2, 24.2 and 25.0%, P=0.03, respectively. The profit in $/kg bird and return on extra vitamin supplementation was from low 25% to AVG $0.0062/kg and 41x, respectively; from AVG to Hi25% was $0.0045/kg and 24X; and from Hi 25% to Hi 5% was $0.0041/kg and 7X. In conclusion, due to increased FCR and metabolic stress, vitamin supplementation up to Hi 5% significantly increased bird performance and return on vitamin cost invested.

Key Words: broilrers, vitamin, supplementation, performance, return on investment

P359 Comparison between cholecalciferol vitamin D3 and 25,hydroxychlecalciferol vitamin D3 supplementation on broiler tibia ash, tibia phosphorus and performance Frank Parks*, mike coelho, Robert Jones *BASF Corporation*

Broiler vitamin D3 supplementation increased 89% in the last 18 years (Coelho, 2018) due to an increase in metabolic rate which depressed the neutrophil function requiring more antioxidant/electron transport vitamins (Brea, 2012). A trial was conducted to determine the equivalency between cholecalciferol D3 (D3) and 25,hydroxycholecalciferolvitamin D3 (25,OH D3) by titrating doses of both vitamin D3 and 25,OH D3 and measuring ash %, phosphorus % and body weight at 14 and 28 days. A total of 2500 Cobb 500 male broilers were used in a randomized complete block design with pen as the experimental unit, treatment as the fixed effect, and block as the random effect (25 birds/pen x 10 vitamin treatments x 10 replications). Broilers were blocked by weight and fed corn/soy diets pelleted at 80C and formulated to meet or exceed NRC (1994) recommendations. Litter was used. Treatments were 2000, 3000, 4000, 6000, 8000 and 10,000 IU/kg D3, and D3/25,OH D3 combinations of 1500/62.5, 2300/75.0, 4100/87.5 and 6000/100 IU D3 and mcg/kg 25,OH, respectively. 14 day tibia ash (44.50, 45.32, 46.85, 48.10, 48.89, 49.62, 45.82, 46.95, 48.17 and 48.82 %, P=0.02, respectively; Tibia phosphorus (16.08, 16.88, 17.25, 17.56, 17.73, 18.03, 16.97, 17.34, 17.62 and 17.68 %, P=0.03, respectively; body weight (398.1, 402.0, 437.9, 442.1, 446.4, 452.3, 403.6, 438.3, 442.0 and 445.9 g, P=0.02, respectively. 28 day tibia ash (46.65, 47.98, 50.97, 51.12, 51.25, 51.76, 47.88, 50.83, 51.24 and 51.15 %, P=0.02, respectively; Tibia phosphorus (17.26, 17.46, 17.74, 17.81, 17.89, 17.93, 17.49, 17.65, 17.86 and 17.90 %, P=0.03, respectively; body weight (1210, 1389, 1418, 1473, 1479, 1483, 1380, 1425, 1468 and 1473 g, P=0.02, respectively. Goodness-of-fit tests determined bird performance and mineral retention responses to D3 and 25,HO D3. In conclusion, based on these equations, it was determined that 62.5 mcg and 100 mcg 25,OH D3 are equal to 1600 and 2000 IU D3, respectively in tibia ash, tibia phosphorus and body weight.

Key Words: cholecalciferol, 25,hydroxy vitamin D3, Vitamin D3, Broilers, tibia ash

P360 Evaluation of a novel threonine fermentation product in young male broilers Dalton Dennehy*[1UG], Hunter Walters[1], Keith Haydon[2], Tri Duong[1], Jason Lee[1] *[1]Texas A&M University; [2]CJ Bio America*

In a 28 day experiment a novel threonine fermentation product (≥ 75% threonine) was evaluated in comparison to L-threonine (98.5%) in young male broilers. A total of three experimental treatments were utilized in a randomized complete block design to determine the impact of threonine fermentation product on broiler performance; positive control (PC - included 98.5% L-threonine), negative control (NC - no L-threonine) and a NC + threonine fermentation product to a digestible threonine level equal of the PC fed broilers. Broilers were fed a starter diet from day 1 to 14 and a grower diet from 15 to 28 days of age. One large corn soy based basal diet was manufactured that was deficient in threonine and then L-threonine and cornstarch or threonine fermentation product were added

to generate the three evaluated treatments. Each treatment consisted of 10 replicate pens (28 broilers per replicate pen) for a total placement of 840 Cobb 500 male broilers. Body weight (BW), feed intake (FI), and mortality adjusted feed conversion ratio (FCR) were evaluated on d 14 and 28. Data were analyzed via ANOVA with differences deemed significant at $P \leq 0.05$, and means separated using Duncan's Multiple Range test. As anticipated, the lack of supplemental threonine in the NC negatively ($P < 0.05$) impacted broiler performance as compared to the PC diet with reductions in day 14 and 28 BW and increasing grower and cumulative (d 1 to 28) FCR. Inclusion of the threonine fermentation product to the NC diet increased ($P < 0.05$) BW and reduced FCR on day 14 and 28 compared to the NC fed broilers and restored performance levels similar to the PC fed broilers. These data confirm the importance of adequate threonine supplementation in poultry diets. Furthermore, this novel threonine fermentation product provides similar broiler performance to commercially available L-threonine when included at equivalent digestible threonine levels.

Key Words: threonine, broiler, fermentation product

P361 Effects of including Valine and Isoleucine in poultry diets on pellet quality Kara Dunmire*[1GS], Courtney Truelock[1], Jason Lee[2], Keith Haydon[2], Charles Stark[1], Chad Paulk[1] *[1]Kansas State University; [2]CJ America*

A total of 3 broiler diets were pelleted to determine the effects of diet formulation with crystalline Val and Ile on pellet quality. Dietary treatments consisted of corn and soybean meal (SBM)-based control, the control with crystalline Val, and the control with crystalline Val and Ile. As crystalline amino acids (AA) increased in the diets, corn concentrations increased as SBM and Choice white grease (CWG) were removed to balance for MEn. Diets contained 54.2, 56.4, 57.5% corn, 39.1, 37.1 and 36.2% SBM and 2.5, 2.1 and 1.9% CWG in the control, Val and Val + Ile diets, respectively. Corn was ground to approximately 1,000 µm and used to mix 499 kg of feed per treatment. There were 3 replicates per treatment with time of processing as a blocking factor and treatment order randomized within each block. Diets were pelleted via steam conditioning (25.4 cm × 139.7 cm, Wenger twin staff preconditioner, Model 150) using a pellet mill (CPM Model PM 1012-2 HD) equipped with a 4.8 mm × 31.8 mm pellet die. Target conditioning temperature was 85˚C for 30s at a 15.4 kg/min production rate. Pellet samples were collected and cooled in an experimental counter-flow cooler for 15 min to determine percent fines, Standard Pellet Durability Index (PDI; ASABE S269.4, 2007), Modified PDI (three 19-mm hex nuts) and Holmen NHP100 for 60 s. Hot pellet temperature decreased ($P < 0.01$) in the control diet compared to Val and Val + Ile diets which were 87.3, 87.7, and 87.8 ˚C, respectively. Pellet mill kilowatts (KW) were 9.1, 8.9 and 10.3 for control, Val and Val + Ile diets, respectively. Pellet mill KW increased ($P < 0.05$) in pelleted Val + Ile diets compared to the control and Val diets. Percent fines decreased ($P < 0.01$) as crystalline AA increased in the diet. For the control, Val and Val + Ile diets, PDIs were 66.5, 73.6 and 76.6% for the standard, 37.1, 46.9, and 52.8% for the modified and 53.4, 67.8 and 73.7% for the Holman NHP100 for 60 s methods, respectively. All PDI methods increased ($P < 0.01$) as crystalline AA increased in the diet. In conclusion, diets with increasing crystalline AA, Val and Val + Ile, led to improved pellet quality which can be explained by the 0.4% or 0.6% reduction in added fat with increasing crystalline AA and balancing for MEn in the diet.

Key Words: pellet quality, crystalline amino acids, fat

P362 Digestible lysine requirements during the starter phase for Cobb MVM x Cobb 700 broiler chickens. Leasea Butler*[1,2], Cody Keen[2], Justina Caldas[2], Andy Mauromoustakos[1], Sam Rochell[1], Colin Scanes[1], Michael Kidd[1] *[1]University of Arkansas; [2]Cobb-Vantress, Inc.*

The optimal digestible lysine (dLys) titration level was titrated evaluated forin the Cobb MVM x Cobb 700 broiler chickens during the starter feed phase (0 days to 14 days of age). It was hypothesized that the Cobb MVM x Cobb 700 broiler's requirement of dLys is different than the Cobb 700 Broiler Guide Recommendations of 1.22% dLys and/or the recommendations of the NRC of 1.10% dLys. The broilers were fed isocaloric diets with eight levels of dLys as percentage of the diets (0.90%, 0.98%, 1.06%, 1.14%, 1.22%, 1.30%, 1.38% and 1.46%). Other key essential digestible amino acids were formulated as percentages to dLys ratios. The birds were reared sex separate to determine the response of body weight gain (BW) and feed conversion adjusted for mortality (adjFCR) for males and females. The trial was a completely randomized block design in 96 pens (48 males and 48 females). Data was also combined to estimate an "as-hatched" rearing scenario indicative to broilers reared in the United States. The "Fit Curve" analysis tool in JMP 14 (SAS Institute, Raleigh, North Carolina) was used to determine the proper regression curve for BW and adjFCR. The "Profiling" tool was then used to find the optimal levels for BW, adjFCR and optimization for the interactive responses of BW and adjFCR while trying to reduce feed cost ($FC).

The optimal level of dLys in the starter phase (0 days to 14days of age) for a Cobb MVM x Cobb 700 as-hatched broiler was determined to be 1.25% for BW, 1.20% for adjFCR and 1.24% to optimize BW, adjFCR and reduce $FC . The optimal level of dLys in the starter phase for the male broilers was 1.14% for BW, 1.05 for adjFCR and 1.20% for optimization of BW and adjFCR at the lowest $FC. The optimal level of dLys for the females was 1.34% for BW, 1.25% for adjFCR and 1.27% for optimization of BW and adjFCR at the lowest $FC. The results of the trial would agree support with the hypothesis that the dLys requirements of the Cobb MVM x Cobb 700 broiler is different from the Cobb 700 Broiler Guide and NRC recommendations and suggest a need to revise these recommendations.

Key Words: Lysine, Broiler, Cobb 700, Amino Acid

P363 Impact of methionine source on performance parameters of young male poults Jason Lee*[1], Keith Haydon[1], Roy Brister[2], Terry Olson[2], Nicole Herring[2] *[1]CJ America; [2]Tyson Foods*

The impact of methionine source on performance parameters was investigated in young turkey poults over a 28 day experiment. A total of three experimental treatments were utilized in a randomized complete block design to determine the impact of methionine source on turkey poult performance; L-Methionine, DL-Methionine and dry Methionine Hydroxy Analog-calcium. Poults were fed a prestarter diet from day 1 to 14 and a starter diet from 15 to 28 days of age. Diets were formulated to be equal in digestible methionine level using 100% bioavailabilty for all sources. Each treatment consisted of 10 replicate pens (100 poults per replicate pen) for a total placement of 3000 Nicholas Select male poults. Body weight (BW), feed intake (FI), and mortality adjusted feed conversion ratio (FCR) were evaluated on d 14, 21, and 28. Data were analyzed via ANOVA with differences deemed significant at $P \leq 0.05$, and means separated using Duncan's Multiple Range test. At the conclusion of the prestarter phase (d 14), no differences were observed in any of the evaluated performance parameters including BW, FI, and FCR. Although no differences were observed in BW and FI at the conclusion of the starter phase (d 28), a reduction (P=0.056) was observed in FCR in male poults fed the diet with the L-methionine source as compared to the DL-methionine source (2.6 point increase compared to L-methionine) while the poults fed the dry methionine hydroxy analog-calcium source as intermediate (2.0 point increase compared to L-methionine). These data demonstrate the potential benefit related to performance efficiency when feeding turkey poults L-methionine presumably associated with the elimination of the two step conversion process.

Key Words: Poult, Turkey, Methionine, Performance

P364 Cobb 700 body weight and feed conversion response to increasing digestible lysine by growth phase. Leasea Butler*[1,2], Andy Mauromoustakos[1], Cody Keen[2], Colin Scanes[1], Justina Caldas[2], Michael Kidd[1], Sam Rochell[1], Roy Brister[3], Phillip Smith[3], Steven Bolden[4], Rocky Latham[3], Craig Maynard[1] *[2]Cobb-Vantress, Inc.; [1]University of Arkansas; [3]Tyson Foods; [4]Pilgrims*

The nutritional requirements of Cobb MVM x Cobb 700 are being established. Cobb 700 male broiler's response to digestible Lysine (dLys) was evaluated. Dietary dLys was increased at each growing phase (GP) (GP1=0d to 12d, GP2=12d to 26d, GP3=26d to 35d and GP4=35d to 55d). Male day-old broilers were placed 75 per pen into 48 pens in a completely randomized block design. Data was collected at the end of each GP for body weight (BW) and feed conversion (FCR), and at the end of GP4 processing yield data was collected. The diets during GP1 for treatments (T) 1, 2, 3 and 4 were the control containing 2996 kcal/kg ME and 1.20% dLys. T5 received 3,084 kcal/kg ME and 1.24% dLys and T6 received 3,084 kcal/kg ME and 1.38% dLys. With each GP the diets ME was increased and dLys was decreased for all Ts. Also, with each GP one of the control groups was converted to the same nutritional plane as T5. In GP2 T4 was moved to the T5 diet plane. In GP3 T3 was moved to the T4/T5 diet plane. In GP4 T2 was moved to the T3/T4/T5 diet plane. T6 remained on the highest ME and dLys plane in all GPs. The results in GP1 for BW was higher and FCR was lower for T6 than all other Ts ($P<0.0001$). In GP2 BW was higher for T6 than the other Ts, however FCR was lower for T4, T5 and T6 in comparison to the control T ($P<0.0001$). BW results for GP3 were higher for T6 than T1 and T2, but not different when compared to T3, T4 and T5 ($P<0.0004$). GP3 FCR was highest for T1, T2 and T3, compared to T4, T5 and T6 ($P<0.0001$). GP4 BW was lowest for T1 and highest for T6, with T2, T3, T4 and T5 being similar ($P<0.0001$). FCR in GP4 was high in T1 in comparison to all other Ts ($P<0.0001$). There were no differences in processing yields of carcass, breast fillet, tenders, wings, thighs, legs or abdominal fat as percentages to live BW ($P>0.05$). The results for BW and FCR in all GPs suggest that the dLys requirements are ≥ 1.24% in GP1, 1.19% in GP2, 0.98% in GP3 and 0.90% in GP4 ($P<0.05$), which would suggest that the requirements are different from those recommended in the Cobb 700 Broiler Nutrition Guidelines and that of the NRC recommendations. It is concluded that additional investigations are need within each GP to better define the dLys requirements of the Cobb MVM x Cobb 700 broiler.

Key Words: Cobb 700, Broiler, Amino Acid, Lysine

P365 Moderate reduction in dietary crude protein concentrations enhances amino acid digestibilities and energy utilisation without compromising broiler performance while reducing water intake Peter Chrystal[1], Victor Naranjo[2], Peter Selle[3], Sonia Liu*[3] *[1]Baiada Poultry Pty Limited; [2]Evonik Nutrition and Care; [3]The University of Sydney*

The present study investigated the feasibility of reducing dietary crude protein (CP) levels by the addition of supplemental amino acids (AA). Four iso-energetic diets (AMEn = 3131 kcal/kg) with CP levels ranged from 215 to 171 g/kg in 15 g/kg deductions were offered to 168 Ross 308 off-sex male broilers from 14 to 35 days post-hatch. Diets were corn-soybean meal based and contained 10.9 g/kg SID Lys while meeting the minimum ratios for EAA. Each treatment comprised 7 cages with 6 birds per cage. Reducing CP from 215 to 186 g/kg did not adversely affect weight gain (mean: 1880 g), feed intake (2921 g) or feed conversion efficiency (FCE, 643 g gain/kg feed). However, the further reduction in CP to 171 g/kg significantly depressed FCE by 4.31% (0.622 *vs.* 0.650). Relative abdominal fat pad weights were linearly increased by 69% from 8.64 to 14.62 g/kg ($r = -0.673$, $P < 0.001$) by reductions in dietary CP from 215 to 171 g/kg. Decreasing dietary CP linearly increased the ratio of metabolisable energy to gross energy ($r = -0.770$, $P < 0.001$) and tended to increase AMEn from 2686 to 2849 kcal/kg ($r = -0.371$, $P = 0.052$). From 33-35 days post-hatch, reducing dietary CP linearly decreased water intake from 813 to 628 g/bird ($r = 0.662$, $P < 0.001$) and decreased water to feed intake ratio from 2.19 to 1.83 ($r = 0.756$, $P < 0.001$). Dietary CP did not influence total excreted nitrogen ($P = 0.271$) and total tract nitrogen retention ($P = 0.683$). Reducing dietary CP linearly increased apparent digestibility coefficients of protein (N) from 0.432 to 0.562 ($r = -0.549$, $P = 0.002$) and digestibilities of all 17 AA assessed ($P < 0.05$) in the distal jejunum. Dietary CP did not influence protein digestibility ($P = 0.105$) but reducing CP linearly increased apparent digestibility coefficients of Arg ($r = -0.432$, $P = 0.022$), Ile ($r = -0.506$, $P = 0.006$), Thr ($r = -0.469$, $P = 0.012$), Val ($r = -0.534$; $P = 0.003$), Cys ($r = -0.455$, $P = 0.015$) and Pro ($r = -0.398$, $P = 0.036$) in the distal ileum. The present study indicates that it is possible to reduce dietary CP levels to moderate extents with appropriate additions of supplemental AA without compromising broiler performance and that water intake was reduced and digestibility of AA and protein is enhanced, especially in the distal jejunum.

Key Words: amino acids, broilers, growth, low protein, reduced protein

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