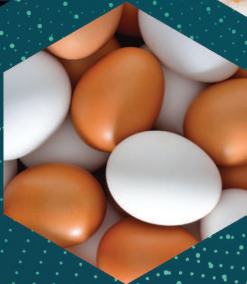


2024

**INTERNATIONAL
POULTRY
SCIENTIFIC
FORUM**

JAN. 29 - 30



ABSTRACTS
2024 International Poultry Scientific Forum
Georgia World Congress Center, Atlanta, Georgia
January 29-30, 2024

Table of Contents

Dendy Keynote Lecture, 1	SCAD II, 51
Teaching, Pedagogy, Extension, 2	Metabolism & Nutrition VIII Feed Additives, 56
Physiology, Endocrinology & Reproduction I Broilers, Turkeys, 4	Metabolism & Nutrition IX Feed Additives, 59
Physiology, Endocrinology and Reproduction II Layers, Breeders, 5	Metabolism and Nutrition X Vitamins and Minerals, 62
Physiology, Endocrinology and Reproduction: Layers, Breeders, 7	Metabolism & Nutrition XI Vitamins and Minerals, 63
Processing & Products, 8	Environment Management V Animal Well-Being, 65
Pathology I, 11	Physiology, Endocrinology and Reproduction: Broilers, Turkeys, 67
Pathology II, 14	Physiology, Endocrinology and Reproduction: Layers, Breeders, 69
SCAD I, 16	Processing and Products, 70
Metabolism and Nutrition I Vitamins & Minerals, 20	Pathology, 74
Metabolism and Nutrition II Amino Acids, 23	SCAD, 79
Metabolism and Nutrition III Feed Additives, 25	Environment, Management: Animal Well-Being (stress, welfare, behavior), 86
Metabolism and Nutrition IV Enzymes, 29	Environment, Management: Environmental Impacts, 91
Metabolism & Nutrition V General Nutrition, 33	Metabolism and Nutrition: Enzymes, 94
Metabolism & Nutrition VI General Nutrition, 35	Metabolism and Nutrition: Amino Acids, 96
Environment Management I Animal Well-being, 38	Metabolism and Nutrition: Vitamins and Minerals, 99
Environment Management II Animal Well-being, 41	Metabolism and Nutrition: General Nutrition, 101
Environment Management III Animal Well-being, 43	Metabolism and Nutrition: Feed Additives, 106
Environment Management IV Environmental Impacts, 44	
Metabolism & Nutrition VII Feed Additives, 47	AUTHOR INDEX, 120

ABSTRACTS

2024 International Poultry Scientific Forum

Georgia World Congress Center, Atlanta, Georgia

January 29-30, 2024

Dendy Keynote Lecture

Safeguarding the future of poultry: Mitigating activist Threats Hannah Thompson-Weeman* *Animal Agriculture Alliance*

With animal rights extremism on the rise, it is increasingly important for everyone in the animal agriculture and food communities to keep security top-of-mind. America's farms, ranches and food processing facilities are the front lines of our food supply. Keeping them safe and secure is essential to animal health and well-being, in addition to ensuring the safety of our milk, meat, poultry, eggs and seafood and protecting the reputation of our community. Additionally, restaurants and food retailers are increasingly dealing with extremist threats, potentially disrupting the flow of the supply chain and the public's ability to access food. The Animal Agriculture Alliance works to safeguard the future of animal agriculture and its value to society by bridging the communication gap between farm and food communities. Part of the Alliance's work includes providing security resources intended to help the entire food chain, from farmers to food brands, protect themselves and their livestock and poultry from threats to the security of their property and livelihoods.

All operations should maintain the priority of keeping their employees safe—nothing is more important. All operations should reevaluate their physical safety and security. All operators should talk to local law enforcement and emergency services to get their best advice. All operators should sit down with their employees to make sure they understand the company's priority on employee safety and facility biosecurity. Large commercial operations should invest in a professional plan to maintain or enhance the security of their facilities and the safety of their employees. Smaller operations can also do good, sound planning and relationship building within their communities. The Alliance recommends that farms, plans and other facilities take basic steps to help maintain security at your facility. These recommendations are not a guarantee of safety, nor are they comprehensive to all farm, ranch or company operations, but they can give a good start to developing an overall security plan to help ensure the safety of your family, your employees and your animals. When in doubt, consult an attorney familiar with your unique situation and local laws. Basic security recommendations include building proactive relationships with law enforcement; carefully vetting job applicants, visitors and anyone seeking information about your facility; limiting access to only verified individuals with a demonstrated purpose for being on a farm or in a plant; and establishing physical security measures such as gates, locks, fences, cameras and lighting.

Key Words: Animal Activists, Threats, Safeguarding poultry

*Author presenting paper

GS Denotes Graduate Student Competition
UG Denotes Undergraduate Presentation

Teaching, Pedagogy, Extension

M1 Sensing and informatics for non-destructive grading of hatching eggs towards next generation hatchery practices Alin Khaliduzzaman*, Md Wadud Ahmed, Mohammed Kamruzzaman
Department of Agricultural and Biological Engineering, University of Illinois Urbana Champaign

Some of the continents and countries might suffer severe food shortage in the next few decades due to climate changes, global unrest, and economic instability. Therefore, we need major technological advancement for egg and poultry production industry to deal with global animal food security and poultry welfare issues. The current hatchery practices, post-hatch grading of day-old chicks, create many consequences of ethical, economic, and environmental issues such as discarding unwanted and abnormal chicks, and keeping the infertile and dead embryos until the hatch which lead higher environmental loads, energy and space consumption. About 10% of eggs are found infertile in hatching eggs, about 3-5% of embryos become dead and 1-3% of chicks become abnormal. Therefore, non-destructive grading of eggs at (pre) incubation using sensing and information technologies might greatly reduce those problems for the future hatchery practices. Spectroscopy (UV-Vis-IR-THz), single wavelength based near-infrared (NIR) optical sensors, multispectral and hyperspectral imaging together with data science can be applied to explore the least explored incubation period. Visual to NIR region have the potentially for grading of eggs based on internal content (e.g., prediction of yolk content, fertility, hatching time), single wavelength based NIR sensor interestingly able to diagnose the live embryo and cardiac abnormality during incubation based on heart rate frequency and pattern. The normal chick embryo showed 200-300 bpm whereas some abnormal embryos with bradycardia showed less than 150 bpm. The imaging technology (diffuse transmissive images and fluorescence images) has also potentially to grade hatching eggs. The terahertz (0.4-2.0 THz) pulse has the potential to explore the eggshell structure, thickness, refractive index thus strength of eggshell. Thus, it can be used to grade eggs suitable for transportation. Therefore, introducing a new course curriculum integrating egg and poultry science, sensing technologies and information science might open a new dimension of teaching and extension materials for the next generation hatchery practices to solve the ethical, economic, environmental issues and to enhance sustainable growth of egg and poultry sector globally.

Key Words: optical sensing, data science, egg grading, education and research, animal welfare

M2 Using Artificial Intelligence Enabled Technologies in Poultry Science Education and Extension Amit Morey* *Auburn University*

Artificial Intelligence (AI) enabled technologies such as ChatGPT have become popular among students but have met with skepticism from faculty and University officials. On one hand, these technologies have empowered us to obtain summarized information on vast number of topics without having to go through multitudes of article collections but on the other hand, it has opened doors to potential plagiarism and other unfair practices. There is a need to evaluate these technologies and use the double-edged sword to our advantage to teach our students and to help our stakeholders. POUL 5110/6110 Poultry Processing and FDSC 5660/6660 Food Microbiology courses were offered in Spring 2023 to graduate and undergraduate students in the Department of Poultry Science at Auburn University. As part of their exam the student groups were required to pro-

vide their inputs and solutions on some real-world, highly complex problems requiring high level of knowledge and cognition. Students were required to provide answers using their knowledge and use two AI engines, copy and paste the AI questions and answers, compare their answers with AI and ultimately do reflective writing. Post-exam surveys demonstrated that the students raised potential concerns about plagiarism and cheating with the technology, however they were satisfied with the comparison and reflection approach. The introduction of easy-to-build GPT platform from ChatGPT is a great feature which allows users to build their own chatbots for a specific topic. A Poultry Processing GPT chatbot was developed using lecture slides and was tested for its functionality. The GPT used the limited knowledge (in bullet points) provided in the slides to comeup with articulated answers to some complex questions but were not detailed enough. Such GPT can be built for stakeholders who can obtain rapid information built on reliable sources and knowledge. Building a GPT also raises several questions about ethics, guardrails, liability, copyrights of materials used for building the GPT, among others. There is no doubt that AI-enabled technologies are here to stay, it is for us to decide and develop it for the benefit our students and stakeholders.

Key Words: Artificial Intelligence, Poultry Science, Extension, Critical Thinking, ChatGPT

M3 Convergence Science and Research: Building New Disciplines to Train Students and Build Future Leaders for the Poultry Industry Amit Morey* *Auburn University*

Convergence Science is the deep integration of knowledge, methods, and expertise from various fields to form new and expanded frameworks. In convergence science, the boundaries between disciplines are blurred or even dissolved. It's not just about collaboration but about integrating and synthesizing disciplines into a coherent whole. Over the past five years, we have practiced convergence science by margining Poultry Science + Supply Chain Management, Poultry Science + Operations Management and Poultry Science + Artificial Intelligence/machine learning. Convergence approach has helped us to cross-train Graduate students in these highly diverse disciplines wherein they take required courses and also 12 credits of courses in their area of convergence. The students then used their knowledge and understanding of these diverse fields to identify and solve some of the vexing industry issues. We have been able to investigate and develop solutions for reducing poultry meat spoilage in supply chain, improving processing efficiencies of further processing plants and developing novel technologies for rapid detection of poultry meat quality and shelf-life. The students who have graduated through this curriculum are poised to solve issues which would be solved by experts in those individual disciplines. For example, the supply chain experts at a company won't have training in food microbiology and vice versa and probably is resulting in significant food safety and spoilage issues leading to economic losses. The second example is where we are actively conducting research on improving efficiency of a further processing plant where the student is using Lean Manufacturing concepts as well as meat chemistry to understand the factors causing loss in productivity and provide recommendations. A Convergence Science student will be able to not only understand the technicality of the supply chain issue but also have a food microbiology vision to solve it effectively. We have graduated one MS and one Ph.D. student while two MS students are currently undergoing Convergence Science training and conducting active research. I implore

all researchers and students to think innovatively to build Convergence Science and Research disciplines.

Key Words: Convergence Science, Poultry Science, Supply Chain, Operations Management, Artificial intelligence

M4 Determining the size and scope of USDA-exempted poultry processing in Pennsylvania Darby Boontarue*^{GS}, Torey Fischer, John Boney *Pennsylvania State University*

Poultry production plays a significant role in Pennsylvania's agricultural income, contributing approximately \$1.7 billion to the state's economy. The USDA has defined six exemption categories that allow small-scale poultry producers, with an annual capacity of 20,000 birds or less, to farm, process, and market their products without continuous inspection. Protecting the commercial poultry industry from disease transmission is of paramount importance. This study aimed to assess the number of birds grown in each USDA exemption category in various poultry-dense Pennsylvania counties, including Lancaster, Lebanon, Berks, Snyder, Juniata, Union, Schuylkill, York, Perry, and Franklin. Data collection involved an anonymous seven-question Qualtrics survey distributed via email to relevant organizations or individuals and in person at farmer's markets and processing facilities. Only 11 out of 69 surveys were completed. Data were analyzed using the FREQ procedure in SAS. The annual bird production ranged from 400 to 4,000 birds, with the following distribution among producers: 36% grew less than 20,000 birds, 27% grew less than 1,000 birds, 18% for personal use, 9% for small enterprises, and 9% did not respond. Many vendors were reluctant to complete surveys, which posed a challenge in collecting data. However, valuable insights were gathered from conversations with poultry producers. Notably, vendors selling processed meat could not accurately quantify their products in terms of whole birds. Additionally, several vendors sourced their poultry products from larger companies that do not fall within the exemption categories, further complicating the assessment of determining the appropriate exemption category for these vendors. In conclusion, it appears that many small-scale poultry producers in Pennsylvania may not be fully aware of the USDA exemption categories and may have selected categories without fully understanding the associated stipulations. State and federal agencies had no previous knowledge of or oversight over small-scale poultry producers. Despite concerns within the commercial poultry industry about the potential risks associated with the small-scale poultry production segment, data gathered does not indicate a significant risk to commercial producers.

Key Words: USDA-exempt processing, extension, small-scale poultry farms

M5 Assessing the need and interest in implementation of an online poultry extension continuing education program Peyton Taylor*^{GS}, Jessica Wells, Kelley Wamsley, Cory Bailey, Ryan Walker *Mississippi State University*

Approximately 110 poultry science degrees are awarded yearly, meaning companies hire nonpoultry degree graduates to meet job demand; these graduates may lack basic poultry knowledge, requiring additional training. Studies show that prior knowledge in an employee's field result in increased job satisfaction and retention. Thus, the current objective was to assess 1) current poultry industry employees' job satisfaction, success, and preparedness; and 2) the need for a poultry extension continuing education (PECe) program to offer additional training for current and future employees. Current personnel (n=128) were surveyed across the industry. Of these participants, 61.7% (n=79) held a management position; and were asked additional questions pertaining to their employees. Data indicated that all participants (100%) were satisfied with their positions and felt successful in their current careers (96%). Additionally, 92.1% of participants noted they felt prepared for their current position in industry. Although most participants reported satisfaction, success, and preparedness in their positions, 85% indicated there is still a need for an online PECe program

prior to entering industry. Additionally, 88% would be interested in this program post-employment, regardless of satisfaction, success, and preparedness and; believed a PECe program would aid in future employee job satisfaction (96%), job retention (97.6%), and job success (99%). Also, those in management positions reported a PECe program would be beneficial for poultry and nonpoultry degree incoming employees (94.9 and 100% respectively). Chi square goodness of fit indicated that all percentages were significantly different ($P < 0.0001$) than the expected 50% of the population. Data suggest industry personnel favor a PECe program despite current job satisfaction, success, and preparedness. It should be noted that 82% of participants who felt prepared for their current position had 6+ years of industry experience. Additionally, due to the small population of new hire (≤ 4 yrs) participants, little can be stated about preparedness of newly hired industry employees. Future research to determine level of preparedness for newly hired populations should be conducted to aid in solidifying the need for a PECe program.

Key Words: job satisfaction, job preparedness, job success, poultry industry employees, continuing education program

M6 Assessing the need and interest in implementation of an online high school dual credit poultry course in the state of Mississippi Peyton Taylor*, Jessica Wells, Kelley Wamsley, Cory Bailey, Ryan Walker *Mississippi State University*

As the poultry industry continues to make more advancements in poultry production, it is critical to keep the industry readily equipped with qualified employees. Researchers have emphasized that there is an ongoing decline in university enrollment; thus, signifying more high school graduates are entering the workforce as opposed to postsecondary education. Career development programs have been implemented in high schools to assist in building career paths for students entering the workforce or postsecondary education. However, these plans are limited and do not showcase the full scope of career opportunities available. With poultry being an opportunity few in high school are familiar with, the objective of this study was to 1) evaluate current application of poultry in high schools and 2) assess the need for an online college dual-credit producing poultry course for current high school students. High school ag teachers across MS were surveyed (n=130) with a return rate of 27% (n=35). Current high school application of poultry, need, and teacher willingness to implement an online dual-credit poultry course were assessed. Of the teacher respondents, 94% ($P < 0.0001$) recognized the importance of teaching poultry; however, only 57.1% ($P = 0.39$) of these teachers cover poultry in their curriculum. It should be noted that 78% ($P = 0.001$) of these teachers stated their students are interested in poultry when offered in the curriculum. Reasons for teachers (42.9%) not covering poultry in their curriculum included: student interest (26.7%), unfamiliar with poultry (33.3%), and time (40%). However, 86% ($P < 0.0001$) of all teachers reported that their students are interested in completing a poultry dual-credit course. Furthermore, 88.6% ($P < 0.0001$) of teachers are willing to implement an online dual-credit poultry course. All data were analyzed using chi square of goodness when compared to 50% of the population. Data suggest a need for an online poultry dual-credit course to expose students to additional careers in poultry. With data illustrating significant differences in students' potential interest in dual-credit and teachers willing to implement dual-credit courses, future research is needed to develop, implement, and evaluate a dual-credit poultry course for MS.

Key Words: dual-credit course, student interest, high school students, poultry

Physiology, Endocrinology & Reproduction I Broilers, Turkeys

M7 Effect of growth rate in the physiology of somatotropic axis in a commercial broiler strain Nabin Neupane*^{GS}, Laura Ellestad, Shailes Bhattra, Prafulla Regmi *University of Georgia*

The growth hormone (GH)/insulin-like growth factor (IGF) system intricately regulates the growth in chickens, wherein IGF action is modulated by IGF-binding proteins (IGFBP). Genetic selection for faster growth in broilers has resulted in changes to the GH/IGF axis, with potential impacts on organ-systems. This study aimed to understand the physiological changes and identify candidate genes in the somatotropic axis of broilers with different growth rates. Four hundred and eighty male Ross-708 chicks were randomly divided into either *ad libitum* (ADLIB) or feed-restricted (RES) groups (8 pens/group; 30 birds/pen) in floor pens. During Wk 1, *ad libitum* feed was provided to all birds whereas from Wk 2 onwards, the RES group received 20% less feed compared to the ADLIB group. Breast muscle (*Pectoralis major*) and liver samples were collected when the ADLIB and RES birds reached target body weights (TW) of 1kg, 2.5kg, and 4kg. Target genes in breast muscle and liver samples were evaluated using RT-qPCR, and results were normalized to beta (β)-actin and glyceraldehyde-3-phosphate dehydrogenase respectively. Data were analyzed using a one-way ANOVA in R-3.6.1 at a significance of $P \leq 0.05$. In the pectoral muscle, the expression of growth hormone receptor (*GHR*), *IGF1*, *IGF2*, and *IGF* receptor 1 (*IGFR1*) genes were greater in the ADLIB birds compared to the weight-matched RES birds at 2.5 and 4 kg TW ($P \leq 0.05$). Hepatic mRNA expression of *IGF1* was not different between the groups; however, *GHR* and *IGF2* were greater in the RES birds than ADLIB birds at 2.5 kg TW ($P \leq 0.05$). Further, mRNA expression of hepatic IGFBP2 was increased in RES birds compared to ADLIB birds at 2.5 and 4 kg TW ($P \leq 0.05$). These results suggest that feed restriction in broilers enhances hepatic GH sensitivity, but this was not reflected in hepatic IGF1 expression. In the RES group, elevated hepatic IGFBP2 could bind to IGF2, preventing its availability and action. Meanwhile, increased *GHR*, *IGF1*, *IGF2*, and IGFBP2 levels in the breast muscle of the ADLIB group potentially result in faster growth, allowing them to reach an equivalent TWs 2, 6, and 10 days before the RES group. In summary, feed restriction modulates the somatotropic axis in a tissue-dependent manner, and this may regulate growth rate in broilers.

Key Words: Broilers, Growth rate, Gene Expression, IGF, IGFBP

M8 Impact of two novel Salmonella bacterin vaccines on broiler chicken immunity and performance Asghar Sedaghat*^{1GS}, Walid Ghazi Al Hakeem¹, Shahna Fathima¹, Syamili Shaji¹, Parimal Sheth², Ramesh Selvaraj¹ *1University of Georgia, 2Endovac Animal Health*

An effective vaccine against *Salmonella* must be able to induce both humoral and cell-mediated immunity. Alum typically induces humoral immunity. Klebsiella antigens can induce cell-mediated immunity. Two vaccines were formulated; Vaccine 1 (ST) as 97% *S. Typhimurium* and 3% Immune Plus® and Vaccine 2 (ST+K) as 77% *S. Typhimurium*, 10% Klebsiella strain KP9580, 10% Klebsiella strain KPZBT01, 3% Immune Plus® to investigate their impact on broiler chicken immunity and performance. A total of 270 eggs were incubated and 0.1 mL of ST and ST+K vaccines were administered *in ovo* on the embryonic day 19. A total of 144 birds were assigned, under the completely randomized design, across three groups: 1) Control; 2) ST; and 3) ST+K. A booster dose was given on d7 of age. ST vaccine increased the antibody titer by 279%, 433%, and 184% on days 14, 21, and 28 of age, respectively. ST+K vaccine increased the antibody titer by 239%, 356%, and 230% on days 14, 21, and 28 of age, respectively. ST+K increased CD4+ T cells in cecal tonsils on day 14 compared to the other two groups ($P < 0.05$). On day 28, ST increased CD8+ T cell percentage ($P < 0.05$), while ST+K decreased the CD4+:CD8+ ratio ($P < 0.05$). ST decreased feed consumption and body weight during the first week ($P < 0.05$) and reduced body weight in the third and fourth weeks of age ($P < 0.05$). However, the treatments did

not affect the feed conversion ratio ($P > 0.05$), and feed intake did not change in the second, third, and fourth weeks of age ($P > 0.05$). In conclusion, both ST and ST+K vaccines enhanced humoral and cell-mediated immune responses.

Key Words: vaccine, broilers, Salmonella, immunity, performance

M9 Evaluating impacts of bacitracin methylene disalicylate supplementation on modern and legacy broilers under heat stress. Colin Barcelo*^{GS}, Laura Ellestad *University of Georgia*

Selective breeding for heavier, faster growing broilers with increased muscle mass has resulted in a diminished capability to tolerate heat stress (HS). Antibiotic growth promoters (AGPs) have been shown to benefit broiler growth performance under HS, but mechanisms are unknown. Rising global temperatures have led to production losses that may be exacerbated by AGP withdrawal. This study aimed to determine effects of bacitracin methylene disalicylate (BMD) at AGP levels on growth performance and blood glucose in modern and legacy broilers exposed to chronic or acute HS. Day (D) old male chicks were randomly assigned into 8 groups ($n=6$ pens/group) with two lines [Ross 708 (ROSS) or Athens-Canadian Random Bred (ACRB)], two diets [antibiotic free (ABF) or BMD-supplemented], and two temperatures [thermoneutral (TN, 78°F) or HS, 95°F for 8h/day]. Birds were fed treatment diets for the entire experiment and subjected to temperature treatments from D32-38. Birds and feed were weighed at D0, D14, D32, and D38. On D32 and D38, whole blood was collected from one bird/pen and analyzed with an i-STAT CG8+. Isolated plasma was analyzed for triiodothyronine (T_3) with a radioimmunoassay. Data were analyzed by three-way ANOVA and Fisher's LSD test where ANOVA indicated significance ($P \leq 0.05$). Final bird body weight had diet-by-line and temperature-by-line interactions. The BMD-ROSS birds were significantly heavier than ABF-ROSS birds ($P \leq 0.05$), and no diet effects on ACRB body weight. The HS-ROSS birds had a lower weight than TN-ROSS birds ($P \leq 0.05$), and no effect of temperature was seen on ACRB body weight. Chronic HS blood glucose levels had a line-by-diet-by-temperature, in which birds of both lines in HS-ABF groups had higher levels relative to TN-ABF groups ($P \leq 0.05$), whereas no significant differences were seen between TN and HS groups on an BMD-supplemented diet. During acute HS, a temperature-by-line interaction was seen for plasma T_3 , where ROSS birds had lower T_3 levels than ACRBs and HS decreased T_3 in both lines ($P \leq 0.05$). Results indicate that selection has altered the broiler physiological response to HS, resulting in suboptimal performance. Notably, AGP addition saw lower glucose levels under HS in both lines and better growth performance in modern broilers.

Key Words: Heat Stress, Broilers, Athens-Canadian Randombred, Glucose, Triiodothyronine

M10 Effect of light spectrum and intensity on female turkey breeder performance and egg quality Clara Ziezold*^{GS}, Gregoy Bedecarrats *Department of Animal Biosciences, University of Guelph*

Turkeys are seasonal breeders that require an increasing photoperiod to activate the reproductive axis. Research in other avian species has shown that red light has the greatest tissue penetration power, resulting in more effective deep-brain photoreceptor stimulation. Thus, housing poultry under red light improves reproductive performance. Based on the results of a pilot study, however, light intensity could be a confounding factor. The aim of this study was to evaluate the effects of white (W) or red (R) spectrum light at low (L; 0.1) or high (H; 0.3 W/m²) intensity on the egg production performance of turkey breeder hens. At 20 weeks of age (woa), 402 female grandparent Hybrid Converter turkeys were randomly allocated to 24 pens within four rooms. Colour was assigned by room and intensity by pen for a split-plot design. Intensity was harmonized to 0.1 W/

m² during the growth phase (20-29 woa). At photostimulation, intensity was increased to 0.3 W/m² in three pens per room and photoperiod was increased as per the breeder's guidelines. Egg production was recorded until 60 woa. Throughout the experiment, body weight and egg quality parameters were measured every two and four weeks, respectively. Data were analyzed with SAS using the GLIMMIX procedure and Tukey's post-hoc test. Cumulative egg production did not differ significantly between treatments, with each hen producing 129, 129, 130 and 132 eggs under WL, WH, RL and RH, respectively. However, eggs produced under L light were heavier ($P < 0.01$) and tended to have higher breaking

strength ($P = 0.0560$). Egg yolk ($P < 0.01$), shell ($P < 0.01$) and albumen ($P < 0.05$) also all increased under L, although relative weights did not differ. This was likely an effect of increased body weight under L ($P < 0.0001$). Uniformity also tended to increase under L ($P = 0.0505$). Overall, these results suggest egg quality can be improved under L without compromising production. Further research should evaluate subsequent impacts on hatchability and poult quality.

Key Words: Lighting, Management, Turkey breeders, Reproduction, Egg quality

Physiology, Endocrinology and Reproduction II Layers, Breeders

M11 Sex differences in intestinal morphology and an increase in diencephalic neuropeptide Y gene expression in Pekin ducks exposed to chronic heat stress. Esther Oluwagbenga*^{GS}, Kolapo Ajuwon, Greg Fraley *Purdue University*

The impact of heat stress (HS) on production performance is intricately linked with feed intake and the integrity and nutrient absorption capacity of the intestines. Therefore, the aim of this study is to investigate the effect of HS on intestinal morphology and the diencephalic appetite-related genes in breeder Pekin ducks. 160 ducks at 35 weeks of age were randomly allocated to two treatment rooms (60 hens and 20 drakes/room). The control room was maintained at a temperature of 22°C and the HS room at 35°C for 3 weeks. On week 3 post HS, 10 hens and 5 drakes were euthanized from each room and jejunum and ileum were collected for histology. Brain samples were collected and snapped frozen for gene expression analysis using qRT-PCR. Data were analyzed with two-way ANOVA and non-normal data were analyzed with Kruskal-Wallis test. A $P < 0.05$ was considered significant and $P > 0.05 < 0.1$ was considered a trend. There was a significant increase in villi width in the ileum ($P = 0.0136$) and jejunum ($P = 0.0019$) of HS hens compared to controls, but no significant differences were observed for drakes. HS drakes showed a higher crypt depth (CD) in the jejunum ($P = 0.0198$) compared to control however no differences were observed for hens. No significant differences were observed in the villi height (VH) and VH:CD ratio. There was an increase in crypt goblet cells (GC) count in the ileum ($P = 0.0169$) of HS drakes compared to HS hens but no differences in the jejunum. There was a trend for higher villi GC count ($P = 0.07$) in the jejunum of HS drakes compared to controls however, no significant differences were observed for hens. There was a significant increase in the crypt GC density ($P = 0.0054$) in the ileum, not jejunum, of HS drakes compared to HS hens. No differences were observed in the villi GC density for both sexes. Further, there was no difference in the proopiomelanocortin gene expression in both sexes but there was a significant increase in the expression of neuropeptide Y (NPY) gene in the diencephalon of HS hens ($P = 0.04$) and drakes ($P = 0.04$) compared to controls. Thus, these data show that there are sex differences in the effect of HS on gut morphology and the upregulation in NPY gene may suggest its central role in mediating response to chronic HS.

Key Words: Orexigenic, Anorexigenic, Heat stress, Pekin ducks

M12 Impact of translucency score and trace mineral supplementation on eggshell quality parameters. Cassidy Morris*^{IGS}, Jonathan Moon¹, Michael Carroll¹, Leticia Orellana², Raquel Burin³, Colwayne Morris³, Austin Jasek³, Duarte Neves³, Ken Macklin¹ ¹Mississippi State University, ²Auburn University, ³Zinpro Corporation

The poultry industry is facing a problem with the fertility of broiler breeder eggs. The use of elevated amounts of trace minerals such as zinc (Zn), manganese (Mn), and copper (Cu) within a broiler breeder diet has been previously linked to an increase in eggshell quality, which in turn may lead to an increased fertility rate. A parameter that has been linked

to an increased eggshell quality is shell translucency. The translucency level of the eggshell can be affected by the mineral levels within the diet. In this study, Zn, Mn, and Cu were added to the water of a commercial broiler breeder house at the level of 40, 40, 7 ppm, respectively. This was conducted on four farms with the second adjacent house not receiving any additional trace mineral supplementation. The objectives of this study were to explore the effects of added amino-acid complexed minerals supplementation on shell translucency scores, coloration lightness (L score), thickness, and breaking strength. In addition, the effect of eggshell translucency score on L score, thickness, and breaking strength was also determined. Translucency was completed with Zinpro® BlueBox™ using a 3-point score system: TS1 = none or few small translucent spots; TS2 = several small translucent spots; TS3 = many large translucent spots. Data was analyzed using PROC GLM procedures in SAS 9.4 with alpha set at 0.05; means were separated by Tukey's Studentized Range Test when appropriate. The results showed that mineral supplementation increased the L score from 74.9 to 75.1 ($P=0.0245$), increased shell thickness from 0.451mm to 0.455mm ($P<0.0402$), increased TS1 eggs by 1.05%, increased TS2 eggs by 5.86%, and decreased TS3 eggs by 6.91% ($P<0.0001$). Significance was found in shell thickness ($P<0.0001$) between all levels of translucency: TS1 eggs (0.462mm), TS2 eggs (0.452mm), and TS3 eggs (0.440mm). Significance was also found for L score ($P<0.0001$) between TS3 eggs (74.2) when compared to TS1 (74.8) and TS2 (75.2) eggs. This study suggests that translucency score is an effective predicting parameter for other eggshell quality characteristics. It also suggests that amino-acid complexed minerals supplementation to broiler breeders can positively impact eggshell quality.

Key Words: translucency scores, trace mineral supplementation, eggshell quality, broiler breeders

M13 Circadian regulation of calcium and phosphorus transport in the shell gland across the laying hen egg production cycle Micaela Sinclair-Black*^{IGS}, Alejandra Garcia¹, Roselina Angel², Bibiana Jaramillo³, Xabier Arbe⁴, David Caver⁴, Laura Ellestad¹ ¹Department of Poultry Science, University of Georgia, ²Animal and Avian Sciences, University of Maryland, ³Iluma Alliance, ⁴H&N International

Layers rely on calcium (Ca) and phosphorus (P) derived from intestinal absorption and bone remodeling to support daily egg formation. As hens age, the physiological systems that control mineral homeostasis become dysregulated, leading to economic and welfare-related issues such as thin eggshells and fragile bones. To identify factors driving these changes, expression of genes involved in Ca and P transport and circulating ionized Ca (iCa) were evaluated from early through extended production stages. Shell gland (SG) tissue and blood were collected from Nick Chick hens (H&N International) during early (25w), mid (43w), and extended (95w) egg production (n=48/age) cycles at times representing bone mineralization (1.5 hours post-oviposition [HPOP]), eggshell calcification (15 HPOP), or the transition between them (6 HPOP and 21 HPOP) (n=12/time point). Levels of mRNA were determined using RT-qPCR and blood was analyzed using an i-STAT (Zoetis) with CG8+ cartridges. Data were

analyzed via ANOVA and means were compared using Fisher's LSD test when ANOVA indicated significance ($P \leq 0.05$). Age-by-HPOP interactive effects were observed for calbindin (*CALBI*) and inorganic P transporter 1 (*P_iT-1*). Both genes increased between 6 and 15 HPOP at all ages, then decreased at 21 HPOP except for 95w, which remained constant ($P \leq 0.05$). No age-by-HPOP interactions were observed for plasma membrane Ca transporter 1 (*PMCA1*) or sodium-calcium exchanger 1 (*NCX1*), though both exhibited main effects of age. Levels of *PMCA1* increased between 25w and 43w but dropped at 95w ($P \leq 0.05$). Expression of *NCX1* remained consistent across 25w and 43w; however, similar to *PMCA1*, also dropped at 95w ($P \leq 0.05$). Blood iCa also showed an age-by-HPOP interaction, in which it steadily decreased from 1.5 through 21 HPOP in younger hens (25,43w); however, iCa exhibited a later decrease at 95w, remaining high at 1.5 and 6 HPOP and not dropping until 15 HPOP ($P \leq 0.05$). In summary, differences observed in both Ca and P transporter expression at 21 HPOP highlight possible dysregulation occurring in the SG of late-production hens. Additionally, the delayed decrease in circulating iCa at 95w suggests that the transitions between bone mineralization and shell calcification may occur less efficiently in aged hens.

Key Words: Layer, Mineral homeostasis, Extended production, Calcium transport, Eggshell formation

M14 Evaluation of supplementation with AlphaD3™ on the physiological regulation of mineral homeostasis and bone integrity in commercial laying hens. R. Alejandra Garcia*^{1GS}, Micaela Sinclair-Black¹, Roselina Angel², Bibiana Jaramillo³, Xabier Arbe⁴, David Caverio⁴, Nabin Neupane¹, Prafulla Regmi¹, Gene Rodrick⁵, Joseph Kindler⁵, Laura Ellestad¹ ¹*Department of Poultry Science, University of Georgia*, ²*Animal and Avian Sciences, University of Maryland*, ³*Illuma Alliance*, ⁴*H&N International*, ⁵*Department of Nutritional Sciences, University of Georgia*

Calcium (Ca) plays a pivotal role in eggshell and bone mineralization, and its homeostasis is influenced by the active form of vitamin D₃ [$1\alpha,25(\text{OH})_2\text{VD}_3$]. As hens age, their ability to convert dietary VD₃ (cholecalciferol) to $1\alpha,25(\text{OH})_2\text{VD}_3$ diminishes, resulting in skeletal health disorders. This study investigated effects of supplementing 1α -cholecalciferol (AlphaD3™, Illuma Alliance) on skeletal parameters and gene expression associated with renal and ileal regulation of Ca homeostasis during early, mid, and extended production. Tissues were collected from Nick Chick hens (H&N International) at 25, 43, and 95 w of age at 1.5, 6, 15, and 21 hours post-oviposition (HPOP), and tibiae were collected at 6 and 21 HPOP. Hens ($n=8/\text{age}/\text{diet}/\text{HPOP}$) were fed a Control (2000 IU/kg VD₃) or AlphaD3-supplemented Control diet (3.5 $\mu\text{g}/\text{kg}$). Levels of mRNA related to VD₃ metabolism and regulation of Ca homeostasis were determined by RT-qPCR. Tibia bone mineral density (BMD) and bone mineral content (BMC) were determined by dual-X-ray absorptiometry, and breaking strength (BS) was evaluated with a 3-point bending test. Data were analyzed by ANOVA and Fisher's LSD test. Renal expression of parathyroid hormone receptor 1 (*PTH1R*) and retinoid-X-receptor gamma (*RXR γ*) showed a 3-way age-x-diet-x-HPOP interaction. At 25 and 43 w, AlphaD3 increased *PTH1R* over Control at 1.5 HPOP ($P < 0.05$). Expression of *RXR γ* at 25 w was higher in Control hens at 15 HPOP, and AlphaD3-fed hens had higher levels at 1.5 HPOP at 43 w ($P < 0.05$). Main diet effects were observed for renal VD₃ 25-hydroxylase and ileal *PTH1R* expression, which both increased with AlphaD3 supplementation ($P < 0.05$). Tibia BMC and BMD had 3-way interactions. At 43 w, AlphaD3-fed hens had higher BMC and BMD at 21 HPOP, while Control fed-hens had higher values at this time at 95 w ($P < 0.05$). Further, tibia BS increased with AlphaD3 supplementation ($P < 0.05$). Results indicate that AlphaD3 may enhance renal and ileal PTH sensitivity, which could improve activation of $1\alpha,25(\text{OH})_2\text{VD}_3$. Thus, greater bone mineralization was observed at peak production for this group during the time of bone depletion for

eggshell formation (21 HPOP), while at later stages it appeared to enhance bone remodeling for this purpose while maintaining bone strength.

Key Words: Vitamin D3, Breaking strength, Bone mineralization, Ileum, Kidney

M15 Leukocyte profiles in blood, spleen, liver, and cecal tonsils following a multi-step commercial Salmonella vaccination program in White Leghorn chicks Chrysta Beck*^{GS}, Jossie Santamaria, Ruvindu Perera, Gisela Erf *University of Arkansas System, Division of Agriculture, Center of Excellence for Poultry Science*

Salmonella vaccine administration in pullet flocks reduces chick morbidity and pathogen load. These vaccines were evaluated for inducing immunological memory, but systemic inflammation associated with these vaccines is unknown. For this study, a commercial *Salmonella* (Sal) vaccination program was administered to Sal-negative, HVT-vaccinated White Leghorn chicks, and leukocyte levels in peripheral blood, spleen, liver, and cecal tonsils (CT) were evaluated. The vaccination program was: V1 oral gavage (OG) with live attenuated Sal-vaccine (LS) or sterile water (C) at day (d) of hatch, V2 OG with LS or C at 6 wk of age, and V3 i.m. injection of killed *S. Typhimurium* (KS) or C at 12 wks. For each vaccination, tissues were collected at 0, 3, 7, and 10 d post-vaccination. Organ single cell suspensions were immunofluorescently stained with chicken leukocyte-specific antibodies, and leukocyte populations analyzed by flow cytometry. Data were expressed as numbers of various leukocytes in the whole organ and as a percentage of total leukocytes present (%). Data were analyzed by two-way ANOVA and reported as different if $P \leq 0.05$. Following V1, leukocyte numbers and proportions increased over time in all organs regardless of treatment. At 7d post-V1, LS-vaccinated chicks had greater % $\gamma\delta$ T cells in spleen and lower ($P=0.07$) % MHCII+ cells in liver compared to C-vaccinated chicks. Following V2, LS-vaccinated chicks had lower % $\gamma\delta$ T cells in spleen, lower % CD4 and CD8 α cells in liver, and greater ($P=0.07$) % B cells in CT compared to C-vaccinated chicks. Heterophils and macrophages (%) increased in liver, spleen, and CT through 7d post-V2 regardless of treatment. Following V3, KS-vaccinated birds had lower % lymphocytes in blood and liver. In both KS and C groups, % heterophils decreased until 3d, 7d and 10d in CT, liver, and spleen, respectively, returning to pre-vaccination levels by 7d in CT and 10d in liver. This study provides novel insight into immune cell changes associated with commercial vaccination programs in poultry. While few changes indicative of an adaptive immune response were observed, the changes in T- and B-cell proportions in CT and spleen were more prominent following LS- than KS-vaccination. Analyses of cytokine- and antibody-profiles are underway.

Key Words: Salmonella, Vaccine, Inflammation, Immunity, Leukocytes

M16 Local and systemic inflammatory responses to Gram- and Gram+ bacterial cell wall components in broiler lines selected for water-efficiency: insights from the dual-window approach. Jossie Santamaria*^{GS}, Chrysta Beck, Sara Orłowski, Maricela Maqueda, Walter Bottje, Gisela Erf *Center of Excellence for Poultry Science, University of Arkansas System, Division of Agriculture*.

The anticipated human population growth will exacerbate water scarcity. For a future with food security and water sustainability, all areas of agriculture must become efficient. For this reason, broiler breeder chickens from a modern random-bred (MRB) population were divergently selected based on low- or high-water conversion ratio (LWCR or HWCR, respectively). To determine the effects of this genetic selection on innate immune function, we evaluated the local tissue and systemic inflammatory responses to intradermal (i.d.) injection of bacterial components, i.e., lipopolysaccharide (LPS) or peptidoglycan (PGN), into the pulp of growing feathers (GF). Ten- to 11-week-old, male, feed-restricted broilers from the MRB, LCWR, and HWCR lines were randomly allocated to three treatment groups, six birds per line and treatment. Treatments consisted of i.d.

GF-pulp injection of 10 μ L of LPS (1 μ g/GF, 12 GF/broiler), PGN (1 μ g/GF, 16 GF/broiler), or endotoxin-free PBS (vehicle injection control). GF and heparinized blood were collected before (0h) and at 6-, 24-, 48- and 72-h post-injection to determine the effects of treatments on leukocyte population profiles in GF-pulp and blood, and on plasma concentrations of α 1-acid glycoprotein (AGP-1). For each treatment, the effects of line, time, and their interactions were analyzed by 2-way ANOVA (pulp) or 2-way repeated measures ANOVA (blood). Multiple means comparisons were conducted as appropriate. Statistical significance was at $P \leq 0.05$. For LPS-injection, there were no line differences in heterophil and macrophage infiltration (% pulp cells) with highest levels ($P \leq 0.001$) at 6- and 24-h, respectively, while lymphocyte levels were higher ($P = 0.035$) in LWCR compared to HWCR, and not different from MRB. PGN resulted primarily in lymphocyte infiltration, reaching peak levels at 48h that were higher in GF-pulps of MRB and LWCR than HWCR ($P \leq 0.001$). There were no line differences in blood leukocyte profiles and plasma AGP-1 for LPS and PGN. The GF and blood dual-window approach provided temporal, quantitative, and qualitative information on local and systemic inflammatory responses, revealing that selection for high water efficiency (LWCR) did not negatively impact innate immune responses to intradermal injection of LPS and PGN.

Key Words: Broiler breeder, Water conversion ratio, Inflammatory response, Lipopolysaccharide, Peptidoglycan

M17 Inclusion of fish meal in broiler breeder rooster diets and the effect on semen quality, egg fertility and hatchability through peak production. Emmillie Boot*^{GS}, Dimitri Malheiros, Kari Harding, Bhavisha Gulabrai, Sophie Chance, Rebecca Wysocky, Ramon Malheiros North Carolina State University

The hatchability of broiler breeder (BB) eggs has decreased significantly over the past decade. Since BB roosters fertilize many eggs a day, improv-

ing or sustaining sperm quality is essential to improving hatchability. The objective of this study was to determine if a 3.2% inclusion of fish meal in the diet of broiler breeder roosters would improve sperm quality parameters and, in response, improve the fertility and hatchability of BB eggs. To evaluate semen quality, 15 BB roosters were assigned to diets with or without fish meal and placed into floor pens. Semen was collected twice from each rooster and analyzed at 34 and 35 weeks of age for a total of 25 samples. Immediately after collection, 100 microliters of each semen sample were diluted into 2mL of semen extender and kept at 40C until testing was completed with the SQA-VtTM Automated Sperm Quality Analyzer. Semen volume, sperm concentration, total sperm count, and sperm motility were measured. To investigate egg fertility and hatchability, six floor pens were used, with 16 hens and 2 roosters in each. All hens were fed a commercial diet according to the breed management guide. Eggs were collected and incubated to assess egg fertility and hatchability three times over the first three months of lay. Egg hatchability and fertility were determined by setting all eggs collected over two days of lay and candling them at 11 days of incubation to remove any eggs that appeared to not be developing. These eggs were then opened and visually inspected to determine the fertility of each egg. At day of hatch, residue was also broken out to confirm fertility in eggs that did not hatch. Data was analyzed via one-way ANOVA in JMP Pro 17, with a significance level of $P \leq 0.05$. All sperm quality parameters were not significantly different between treatments. Egg fertility was also not significantly different; however, hatchability was 10% higher in the diet with fish meal ($P = 0.0410$). Although further research is needed to determine the root cause of the increase in hatchability, this data suggests that a 3.2% inclusion of fish meal in the diets of BB roosters may increase hatchability, providing a potentially valuable diet resource to BB companies.

Key Words: Semen, Fertility, Hatchability, Rooster, Broiler Breeder

Physiology, Endocrinology and Reproduction: Layers, Breeders

M18 The effect of exposure to *Micrococcus luteus* on broiler breeder fertility and egg hatchability Sophie Chance*^{GS}, Ramon Malheiros, Bhavisha Gulabrai, Lin Walker, Aaron Kiess North Carolina State University

Overall hatchability and fertility in broiler breeder flocks have decreased over the past decade and this decrease continues as the flock continues to age. This problem is a compilation of factors including management, genetics, antibiotic removal, and nutrition. Studies are currently working to address problems with low hatchability in the hatchery, however few are looking for ways to improve hatch and fertility in the breeder house. The environmental microbiota in the house is rampant with opportunistic pathogens and symbiotic bacteria that can benefit and harm the hatching egg. In this study, we looked at the impact of a beneficial nest pad bacterium on fertility and hatchability of broiler breeder eggs through peak production. To do this, we added 10⁸ CFU's *Micrococcus luteus* to the nest pads of 8 replicate breeder pens housing 60 hens and 7 roosters each. In addition, 8 pens had their nest pads sprayed with sterile phosphate buffered saline as the control. *M. luteus* was chosen based on its optimum survival conditions, lack of harmful effects on sperm quality, and potential to competitively exclude pathogens. At the start of lay (26-29 wks) 50 eggs per pen were set each week for 4 weeks and the same was repeated at peak lay (33-36 wks). Average hatchability and fertility showed no significant difference between the treated and untreated groups through peak production. Chick weights were also taken and 10 randomly selected chicks from each pen were placed and observed for 7-days to obtain mortality and body weight gain (BWG) data. Data was analyzed via one-way ANOVA in JMP Pro 15, where $\alpha < 0.05$. Results did identify significant differences in average 7-day chick weight ($p = 0.00974$) and

average BWG ($p = 0.00804$) that favored the control group, but no difference in 7 day mortality was detected. Fertility and hatchability results were expected due to hen age and egg quality, however this could change with hen age and reduced shell quality. Overall, this study shows that the introduction of *M. luteus* to the nest pad does not affect egg fertility or hatchability through peak production, but reduces 7-day body weights and BWG. Future research should identify how nest pad bacteria affects fertility, hatchability, and production in late stage breeder production cycles.

Key Words: Broiler Breeder, Hatchability, Fertility, Environment, Nest Box

Processing & Products

M19 The effect of sex, body weight, and parts weight on occurrence of muscle myopathies in broilers Muhammad Ali*^{1GS}, Catherine Fudge¹, Nicolas Mejia-Abauza¹, Jinqun Wang¹, Caitlin Harris², Brian C. Bowker², Chongxiao (Sean) Chen¹ ¹*Department of Poultry Science, University of Georgia*, ²*USDA-Agricultural Research Service*

Breast muscle myopathies in broilers, such as woody breast (WB) and white striping (WS), have not been completely understood. This experiment aims to study the relationship between the sex of birds and meat characteristics on the severity of WB and WS. 384 birds were selected from a flock fed different levels of protein and energy to create a range of different sizes of birds. Sex was identified during the processing, with 198 males and 176 females used for analysis (10 birds with missing tags excluded). Weight of the live bird (BW), hot carcass (HCW), cold carcass (CCW), legs (LW), major (MW), minor (MN), wings (WW), and fat pad (FPW) were recorded. WB and WS were scored from 0-3, with 3 being the most severe myopathies. A T-test was used to compare BW, parts weight, WB, and WS scores between two sexes. Data was grouped by BW or WS scores, and two-way ANOVA was used to analyze interaction of myopathy scores and sex. The regression analysis was used in each sex group that showed interactions. The p-value was accepted at 0.05. Results showed males have higher BW (2957 vs. 2479g/bird), and parts weight (except for FPW) than females (P<0.001). Meanwhile, males also have higher avg WB (1.37 vs. 0.76; P<0.001) and avg WS scores (1.40 vs. 0.98; P<0.001) than females. There is an interaction between WB score and sex in BW (P= 0.031) and HCW (P=0.048). In males, score 3 birds have higher BW than scores 2 and 1, with score 0 being the lowest. However, in females, score 3 birds have the highest BW, but there is no difference in BW between Score 0, 1, and 2. The HCW parameters followed a similar pattern, except female score 2 birds had higher HCW than scores 1 and 0. Furthermore, regression analysis showed male and female responses to WB score differently, with males' BW increased by 148g/score and HCW increased by 124g/score. In females, BW increased by 66g/score, and HCW increased by 53g/score. For main effects, higher WB shows higher CCW and parts weight except for FPW (P<0.001). The main effects of WS show a similar trend (P<0.040). In conclusion, males have higher BW, parts weight (except fat pad), WB, and WS scores than females. Furthermore, males and females respond to WB differently regarding BW and HCW changes, with males having higher coefficients than females.

Key Words: Broiler, Muscle myopathies, Processing, White striping, Woody breast

M20 Cold plasma and plasma-activated antimicrobials: Novel and sustainable technologies to reduce *Listeria monocytogenes* on processing surfaces. Katherine Sierra*^{GS}, Luis Guzman, Laura Garner, Amit Morey *Auburn University*

Listeria monocytogenes (LM) is a fatal foodborne pathogen associated with ready-to-eat (RTE) foods establishes itself in processing environment, food contact and non-contact surfaces making it extremely difficult to eliminate the pathogen using existing sanitation chemicals. We explored (1) cold-plasma (CP), an ionized gas with high energy reactive oxygen and nitrogen species, as a non-chemical antimicrobial intervention to eliminate LM on processing surfaces and (2) plasma-activated chemicals (hydrogen peroxide and quaternary ammonium compounds at lower than recommended concentrations); and assessed their anti-listeria activity on food processing surfaces.

Experiment 1. Three common food processing surfaces (stainless steel, conveyor belt, and rubber) coupons (2.5 cm x 2.5 cm) were inoculated with ~5 logs of LM and placed at 4°C for 30 minutes to allow cell attachment. All samples were exposed (0, 10, and 20 min) to the CP generated using helium gas and an electrical input of 5.75 kW. Experiment 2. Hydrogen peroxide and quaternary ammonium compounds (50% lower than recommended concentration) were activated using CP (5.75 kW) at two

different times (0 and 10min). LM inoculated surfaces were exposed to the activated chemicals for five min. Each sample was diluted in buffer peptone water (BPW) (9mL), and then spread plated on Modified Oxford Agar (MOX) plates and incubated for 24h at 37°C. After 24h, typical *Listeria* colonies were counted and reported as log CFU/sq.cm. The experiment was repeated six times, and the data was analyzed using ANOVA (p<0.05) to find significant differences among the means.

Cold-plasma alone reduced (p<0.05) the population of LM on conveyor belt by 1 and 2 log after 10- and 20-min exposure, respectively. However, rubber and stainless-steel samples did not demonstrate any reductions at 10 min but had a 2-log reduction after 20 min exposure. On the other hand, plasma-activated hydrogen peroxide (3%) and quaternary ammonium compound at (50% lower concentration) were able to eliminate 5 logs of LM on stainless steel after 10min of exposure.

Cold-plasma and plasma-activated sanitizers can have a significant impact on improving LM food safety in RTE processing plants while reducing chemical concentrations.

Key Words: Cold-Plasma, *Listeria monocytogenes*, Contact surfaces, Hydrogen Peroxide, Sustainable alternative

M21 Assessing the Influence of Temperature Fluctuation During Transportation on Chicken Breast Meat Shelf Life through Bacterial Count and Electronic Nose. Vianca Tashiguano*^{GS}, Katherine Sierra, Luis Guzmán, Jaroslav Valenta, Laura Garner, Sungeun Cho, Amit Morey *Auburn University*

Cold-chain disruptions at various stages from the point raw poultry leaves a processing plant, storage distribution until it reaches the consumer can be detrimental to the shelf-life of the product. This study aimed to assess the impact of temperature abuse on fresh chicken breast retail shelf-life. Traditional spoilage studies use standard microbiological techniques; however, the current project uses a novel non-invasive technology called electronic nose which analyzes the volatile profile of raw chicken for estimating spoilage status.

Freshly processed, boneless, skinless chicken breast meat was collected from a commercial poultry processing plant and transported under refrigeration to Auburn University's Department of Poultry Science. The fillets were individually placed in whirl-pak bags and subjected to three temperature regimens; (1) 4 °C for 24h, (2) temperature abuse (TA) for 12 h, and (3) TA for 24h. After temperature abuse, the samples were placed in a walk-in cooler (4C) and samples on days 0, 2, 4, 6, and 8 after treatment. On each sampling day, 3 samples were analyzed for aerobic and anaerobic plate count, and Lactic Acid Bacteria (LAB). Additionally, 3 samples per sampling day were used for volatile analysis using an Electronic nose

Temperature abuse cycles for 12 and 24 h increased the anaerobic and LAB by 1 log while aerobic counts were similar to control samples. The spoilage rate was highest in the 24h TA samples followed by 12h TA and then by control. E-nose analysis indicated the presence of multiple volatiles in control samples on Day 8 while those with temperature abuse contained volatiles such as trimethyl amine, disulfides, formic acid associated with odors such as ammoniacal, rotten cabbage, and pungent, respectively which indicate spoilage.

Key Words: Temperature fluctuation, Cold-chain, Shelf-life, Spoilage, Electronic nose

M22 Detection of Visible and Invisible Fecal Contamination Through Spectral Imaging on Chicken WOGs Micah Black*^{1GS}, Laura Garner¹, Luis Guzman¹, Katherine Sierra¹, Vianca Tashiguan¹, Bet Wu Alvarado¹, Amit Morey¹, Insuck Baik², Kevin Chao², Diane Chan², Moon Kim², Jianwei Qin², Nicholas MacKinnon³, Stanislav Sokolov³, Fartash Vasefi³ ¹Auburn University, ²USDA-ARS, ³SafetySpect Inc.

The USDA-FSIS enforces a zero visible fecal tolerance policy on WOGs to improve *Salmonella* food safety. With the advent of the New Poultry Inspection System, plant workers are trained to detect carcass condemnations including fecal contamination. Research was conducted to evaluate a spectral imaging technology to detect visible fecal matter to improve automation in processing plants. Further, we investigated if the imaging system could detect fecal contamination after the WOGs are reworked (washed) and that invisible (to human eye) fecal can be a source of *Salmonella* contamination.

Processed eviscerated broiler carcasses (8-10lbs live weight) were collected in a processing plant. Fecal contamination from the ceca (~100g) was collected and inoculated with an 18h *Salmonella* Typhimurium culture which was then placed on the breast skin of the chicken carcass for examination. Inoculated carcasses (n=100) were hung by shackle in an ambient light-free box and imaged with a handheld spectral imaging system. Images were taken before inoculation, after inoculation, and after spraying the fecal contaminated area of carcasses with water. Images taken were used for further image analysis using Convolutional Neural Networks (CNN, Microsoft Custom Vision). Carcasses were swabbed after each taken image and incubated for 24 hours in Brain Heart Infusion (BHI) broth for BioMerieux GeneUp *Salmonella* PCR assay testing.

Images determined that fecal contamination can be detected using the spectral camera. Moreover, the image analysis indicated that the remnants of fecal contamination, invisible to human eye, were present on the WOGs. *Salmonella* analysis of all 100 WOGs pre-fecal contamination were negative for the pathogen while those spots were found to be positive for *Salmonella* after reworking the carcasses to remove fecal matter. CNN classification results had 100% precision and 83.3% recall concluding to have a 99.4% accuracy percentage. The research indicates a need to improve reworking and developing improved protocols for removing all traces of fecal contamination to improve food safety.

Key Words: Spectral, fecal contamination

M23 Genomic analysis of Campylobacter jejuni isolated from commercial broiler processing plants. Diksha Pokhrel*^{1GS}, Hudson Thames¹, Mark Arick², Chuan-Yu Hsu², Thu Dinh³, Wes Schilling⁴, Shecoya White⁴, Reshma Ramachandran¹, Anuraj Sukumaran⁵, Kenneth Macklin¹, Li Zhang¹ ¹Department of Poultry Science, Mississippi State University, ²Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University, ³Tyson Foods, ⁴Department of Food Science, Nutrition, and Health Promotion, Mississippi State University, ⁵Freshpet

Campylobacter jejuni is one of the leading causes of bacterial foodborne gastroenteritis in the United States. It is a microaerophilic pathogen and was traditionally considered sensitive to high oxygen in the environment. However, our previous study showed the existence of aero-sensitive (AS), intermediate-aerotolerant (IAT), and hyper-aerotolerant (HAT) isolates among *C. jejuni* isolated from poultry processing plants. There is limited information about the genomic determinants that underlie the aerotolerance phenotypes in *C. jejuni* and their potential linkage to virulence factors (VFs) and antimicrobial resistance (AMR). Therefore, the main objective of this study was to identify the virulence and AMR genes present in three distinct aerotolerant groups of *C. jejuni* isolates using whole-genome sequencing (WGS). Eight *C. jejuni* isolates, 3 AS, 3 IAT, and 2 HAT were used in this study. The WGS was performed in a next-generation sequencing approach including the short-read (PE150) sequencing method via Illumina NovaSeq 6000 platform and the long-read sequencing method via Oxford Nanopore GridION platform. The genome sizes ranged between

1.6 Mbp to 1.8 Mbp. The Resistance Gene Identifier (RGI) with the Comprehensive Antibiotic Resistance Database (CARD) was used to predict the AMR genes. All eight isolates have resistance genes for macrolides, fluoroquinolones, and cephalosporin. Three of the isolates (C-777, C-885, C-901) have genes that encode resistance to aminoglycosides (APH (3')). These isolates were isolated from the same processing plant and are aerotolerant (IAT and HAT). The VFs were analyzed using the VF Database. The VFs involved in adherence (CadF), invasion (CiaB), motility (flaA), and toxin (CdtABC) were present in all isolates. The whole-genome analysis of these *C. jejuni* predicted a diverse range of genetic compositions, AMRs, and VFs. This information will be valuable in understanding the genetic features associated with aerotolerance levels among the isolates. The AMR findings predict the possibility of multidrug-resistant *C. jejuni* in commercial broiler processing facilities possessing public health risks. Further differences in genome among given isolates will be analyzed to understand the specific genes associated with aerotolerance level.

Key Words: Aerotolerance, Campylobacter jejuni, commercial broiler processing, Whole-genome sequencing

M24 Data collection of whole genomes for preparation of groundwork and understanding the role of phages in Salmonella Infantis strains Hailey Fugate*^{1GS}, Yujie Zhang², Li Zhang¹, Yen-te Liao², Vivian Wu² ¹Mississippi State University, ²USDA, Agriculture Research Service, Produce Safety and Microbiology Research Unit

Poultry products have an increasing demand from the constantly growing population. The increase in demand for products has posed a new challenge for the consumers and farmers of the industry. The emergence of Antibiotic-resistant bacteria (AMR) has risen with *Salmonella* strains standing out as potential contributors to the rise of AMR-related illnesses, such as Salmonellosis. A new innovative solution is needed for combating these AMR strains of bacteria. Bacteriophages pose as a promising therapeutic agent due to their bacterial specificity and ability to replicate within hosts. In this study, we collected the genomes of *Salmonella* Infantis and the related phages to understand the role of phages in *Salmonella* strains. We collected and processed 1,891 *Salmonella* phage genomes and 159 *Salmonella* Infantis genomes from the NCBI (National Center for Biological Information) database. Prophage predictions were conducted using PHASTER and PHASTEST tools. The results showed that out of the initial 1,891 phage genomes, 628 complete genomes were identified, with 7 specific to *Salmonella* Infantis. Of the bacterial genomes, 134 were complete. PHASTER predicted 281 intact, 111 questionable, and 588 incomplete genomes, whereas PHASTEST identified 366 intact and 142 questionable genomes, with none classified as incomplete. Descriptive statistical analysis of these data highlighted a high prevalence of intact prophages, underscoring their potential role in bacterial pathogenicity. The study not only elucidates the genetic relationship between *Salmonella* Infantis and its bacteriophages but also contributes to the development of a comprehensive genomic database for training artificial intelligence models to facilitate the development of bacteriophage-based interventions in combating antibiotic-resistant *Salmonella* strains, thereby enhancing food safety and public health.

Key Words: Poultry, Bacteriophage, Salmonella Infantis, Antimicrobial Resistance, Genomes

M25 Use of seeding for Salmonella infantis specific bacteriophage isolation from different poultry associated samples Juan Figueroa*^{1GS}, Steven Kitchens¹, Stuart Price¹, Richard Buhr², Dianna Bourassa¹ ¹Auburn University, ²US National Poultry Research Center

The primary objective of this study was to isolate bacteriophages specific to *Salmonella* Infantis from various samples linked with poultry, obtained from four separate poultry complexes. Samples were obtained from multiple locations within the poultry production and processing system, including housing areas (litter), the processing facility (scalding water, dissolved

air flotation (DAF) water, DAF solids, and carcass/parts rinsing), as well as the broiler gastrointestinal system (ceca). Samples were received and processed by suspending in buffered peptone water and duplicates were prepared for each sample. One treatment was seeded with an inoculation of *S. Infantis*, while the other treatment remained uninoculated. Both sets were subjected to incubation at a temperature of 37°C for a 24 hours. The samples were centrifuged and filtered through 0.45 µm in order to effectively separate the supernatant containing possible bacteriophage from debris and bacteria. The supernatants obtained from each sample were applied in 10 µl droplets onto Luria Bertani magnesium agar plates containing *S. Infantis*, with the aim of detecting potential bacteriophage-induced lysis. The bacteriophage samples exhibiting clearing were classified on a scale from 0 to 4 with 0 indicating no clearance and 4 indicating complete clearance. The evaluation of clearance was conducted based on seeding and sample type. Statistical analysis was conducted using the Kruskal-Wallis Test with a significance level at $P \leq 0.05$ and a trend determined at $P \leq 0.10$. The seeded treatment exhibited a trend of increased clearance compared to the non-seeded samples ($P = 0.0852$). Variations were seen in the types of the samples, with litter (score 1.9) and scalding water (score 2.1) exhibiting the highest degree of clearance in comparison to ceca (score 1.0), DAF solids (score 1.0), DAF water (score 0.8), and rinses (score 0.0) ($P = 0.0047$). These results indicate that sample seeding had the tendency to improve phage clearing during isolation and that litter and scalding water may be preferred poultry sample types for the isolation of bacteriophage.

Key Words: bacteriophage, Salmonella, poultry, litter, scalding water

M26 Evaluating the efficacy of a novel two-sided drop-through photonic decontamination system on Salmonella reduction Abigail McConnell*^{1GS}, Montana Riggs¹, Shijinaraj Manjankattil¹, Sabin Poudel¹, Madalyn Jennings¹, Matthew Hughes¹, Ian Rawson², Richard Buhr³, Dianna Bourassa¹ ¹Auburn University, ²Pulse Forge Inc., ³US National Poultry Research Center, USDA-ARS

This study sought to evaluate a novel two-sided drop-through photonic decontamination system developed by PulseForge Inc. that employs pulsed light technology on whole wings and tenders and its efficacy on reducing *Salmonella* *Infantis*, *Enterobacteriaceae*, and aerobic bacteria counts. Four repetitions for a total of $N = 320$ samples were evaluated with 8 treatments. Treatments included a control (no treatment), pulsed light treatment (PL), 30s water dip, 30s water dip with PL, 30s peracetic acid dip (PAA, 200 ppm), 30s PAA dip with PL, 3 parts simultaneously with PL, and 5 parts simultaneously with PL. 5 wings and 5 tenders were evaluated per treatment per repetition. Parts were inoculated with 0.1 mL of 10^6 *Salmonella* *Infantis* and 1 h was allowed for attachment. Then, each treatment was performed and treatments that included PL were dropped through the machinery and collected in a sterile bag. Samples were evaluated for *Salmonella*, *Enterobacteriaceae* (EB), and aerobic bacteria (APC). Bacterial counts were log transformed and are reported as \log_{10} CFU/mL. Data were analyzed using the general linear models procedure and means were separated by Tukey's HSD with significance at $P \leq 0.05$. Treatment was significant for both wings and tenders ($P < 0.0001$). PL reduced *Salmonella*, EB, and APC by 0.71, 0.66, and 0.62, respectively, on wings, and 0.36, 0.32, and 0.25, respectively, on tenders compared to the control. When water with PL was compared to water alone, *Salmonella*, EB, and APC decreased by 0.64, 0.64, and 0.74 on wings. Only APC was reduced, by 0.29, on tenders when water with PL was compared to water alone. When PAA with PL was compared to PAA, *Salmonella*, EB, and APC decreased by 0.55, 0.30, and 0.35 on wings and 0.75, 0.40, and 0.44 on tenders. Finally, when 3 parts were simultaneously treated with PL, *Salmonella*, EB, and APC were reduced by 0.65, 0.63, and 0.57 on wings, and 0.54, 0.55, and 0.40 on tenders compared to untreated control. Similarly, when 5 parts were treated with PL, *Salmonella*, EB, and APC were reduced by 0.62, 0.64, and 0.64 on wings, and 0.53, 0.53, and 0.43 on tenders. These results demonstrate that the use of PL consistently reduced the levels of *Salmo-*

nella, EB, and APC on single and multiple parts in comparison to parts not treated with PL.

Key Words: pulsed light, salmonella, enterobacteriaceae, poultry parts, intervention

M27 Evaluation of wing damage at different time points during processing Madalyn Jennings*^{GS}, Montana Riggs, Abigail McConnell, Fanny Contreras, Dianna Bourassa Auburn University

Wing damage is a common occurrence found in processing facilities that can negatively affect both the company's efficiency and profitability. There are multiple areas during production and within a processing plant that can result in wing damage including live production, harvest, tipping, shackling, stunning, bleeding, and picking. Our objective was to evaluate the appearance of wing damage at various time points of occurrence within a commercial processing plant. Broilers were evaluated at live haul looking for birds with visually damaged wings. For any bird with a damaged wing, the wings were photographed, the bird was banded, and then placed in the same crates as the birds with undamaged wings. Then birds were sent through processing and carcasses were removed from the line at two different time points, post-bleeding, and post-picking. For the first trial there was a total of 600 birds, with 50 birds tagged at live haul as having various levels of wing damage. In the second trial there was a total of 300 birds, with 15 tagged at live haul. Birds were then stunned and bled and evaluated immediately post-bleed. Wings were defined as damaged or not damaged at each timepoint. Damaged wings were then subcategorized as having bruising, torn skin, bone dislocation, or broken bones. Following picking, damage was further categorized as clean (no blood present) or with blood. Statistical analysis was conducted using the Chi-Square for non-parametric data with a significance level at $P \leq 0.05$. Wing damage increased from 7% at live haul to 22% post-bleed and further increased to 36% post-pick ($P \leq 0.0001$). Within the damaged wings, bruising was higher post-bleed (21%) and post-pick (15%) than at live haul (7%, $P \leq 0.0001$). After picking, dislocations (7%), broken bones (3%), and torn skin (10%) were higher than dislocations, broken bones, and torn skin at live haul (0.1%, 0%, and 0.1%, respectively) and post-bleed (0.8%, 0%, and 0%, respectively, $P \leq 0.0001$). Of all damaged wings post-pick 17% were clean indicating that all damage occurred following blood loss. These data indicate that additional attention is required between live haul and post-pick to reduce the occurrence of wing damage both for animal welfare and final product quality improvements.

Key Words: wing damage, bruising, dislocation, broken, processing

M28 Analysis of metabolic changes in poultry during respiration of carbon dioxide Montana Riggs*^{GS}, Charlene Hanlon, Bethany Baker-Cook, Madalyn Jennings, Abigail McConnell, Juan Figueroa, Matthew Hughes, Dianna Bourassa Auburn University

Controlled atmosphere stunning (CAS) of poultry has begun to grow in popularity due to increased consumer demand for ethically produced meat. However, the effects carbon dioxide has upon metabolic function of poultry has yet to be fully defined. The aim of this study was to evaluate blood metabolites prior to and during the course of carbon dioxide CAS. Broiler chickens were stunned for 5 min (2 min at 25% CO₂, 1 min at 45% CO₂, 1 min at 55% CO₂, 1 min at 65% CO₂). Blood was collected from a total of 63 birds at 0 min (prior to stun), after 1, 2, 3, 4, or 5 min of CAS, and at 6 min (1 min following removal from the CAS chamber). Individual birds were removed from CAS at each evaluated timepoint, the bird was euthanized by cervical dislocation, the neck was cut, and blood was collected from the subsequent flow. For each blood sample, a CG8+ cartridge in an iSTAT Alinity v blood analyzer was used to analyze PCO₂ (mmHg), pH, PO₂ (mmHg), bicarbonate (HCO₃⁻, mEq/L), base excess extracellular fluid (BE_{ecf}, mEq/L), oxygen saturation (sO₂, %), total CO₂ (TCO₂, mEq/L), Na (mEq/L), K (mEq/L), iCa (mmol/L), glucose (mg/dL), hematocrit (%PVC) and hemoglobin (g/dL). Data were analyzed by general linear

regression with means separated by Tukey's HSD. Significance was determined at $P \leq 0.05$. pH values decreased between 0 min and 1 minute (0 min = 7.51, 1 min = 7.31, $P < 0.0001$). $p\text{CO}_2$ increased from 0 min to 2 min (0 min = 32.034, 2 min = 63.934, $P < 0.0001$). HCO_3^- differed over time, but not between timepoints ($P = 0.0345$). BEecf decreased from 0 min to 2 min (0 min = 2.445, 2 min = -4.45, $P < 0.0001$). SO_2 decreased from 0 min to 2 min (0 min = 90.78, 2 min = 74.71, $P = 0.0020$). K increased from 0 min to 6 min (0 min = 6.97, 6 min = 8.57, $P = 0.0029$). iCa increased from 0 min to 4 min, then decreased to base values at 6 min (0 min = 1.27, 4 min = 1.53, 6 min = 1.35, $P = 0.0003$). PO_2 , TCO_2 , Na, hematocrit, and hemoglobin did not change over time ($P = 0.5471$, $P = 0.4180$, $P = 0.7129$, $P = 0.2396$, $P = 0.3249$). Defining these changes will help future research in the understanding of the metabolic changes occurring during controlled atmosphere stunning and how these changes can influence stunning welfare and product quality.

Key Words: Carbon dioxide, broiler, metabolites, controlled atmosphere stunning, blood

M29 Effects of sonication and bird age on egg bacteria levels and egg quality characteristics Matthew Hughes^{*1UG}, Brigid McCrea², Dianna Bourassa¹ ¹*Auburn University*, ²*Alabama Cooperative Extension System*

There is a burgeoning movement among backyard flock owners to clean their shell eggs without the use of chemicals. Sonication uses high-frequency sound waves to agitate water. Studies have shown that sonication can loosen bacteria from a surface, but also results in lysis of bacteria. The objective of this study was to determine if sonication could reduce bacterial load on eggshells without affecting egg quality characteristics.

Eggs were obtained from flocks of three ages, 26, 40, and 57 wk of age. Eggs were divided into three groups: unwashed, water immersion for 8 min, or sonication for 8 min. Following each treatment, eggs were rinsed and rinsates were plated for enumeration on Aerobic Plate Counts (APC) and *E. coli* (EC) petrifilms. Bacterial counts were log transformed and are reported as \log_{10} CFU/mL. Egg quality was evaluated for albumen height, egg weight, Haugh units, shell strength, shell thickness, and yolk color using the Egg Tester UltimateTM. Statistical analysis was completed using the general linear models procedure for APC and egg quality parameters, and Fisher's Exact Test for EC prevalence with significance at $P \leq 0.05$. The main effects of treatment and age and the treatment*age interaction were all significant ($P < 0.0001$) for APC and EC. For the 26 wk flock, unwashed eggs (3.96) had higher APCs than water immersed eggs (3.46), which was higher than sonication (2.41). For the 40 wk flock, sonicated (3.81) and water immersed eggs (3.96) had higher APCs compared to unwashed (3.41). For the 57 wk flock, unwashed eggs (3.71) and water immersed (3.39) had higher APCs than sonication (1.58). For the 26 wk flock, water (5/30) and sonication (0/29) reduced EC prevalence compared to unwashed (16/30). For the 57 wk flock, sonication (0/30) reduced EC prevalence compared to unwashed (6/30) with water immersed eggs (1/29) intermediate. No treatment differences were observed for the 40 wk flock eggs. Cleaning treatments did not affect albumen height, egg weight, Haugh units, shell strength, or yolk color. However, unwashed eggs had thicker shells (0.42 mm) than water immersion (0.39 mm) and sonicator (0.39 mm) treatments. These results show that sonication can enhance the effectiveness of washing eggs with water without a detrimental effect on egg quality.

Key Words: sanitation, table eggs, aerobic bacteria, *E. coli*, egg quality

Pathology I

M30 Evaluation of the pathogenicity of a fowl adenovirus (FAV) group E, 8b field isolate Subhan Ullah^{*GS}, Erich Linnemann, Kathryn McCullough, Holly Sellers *University of Georgia*

Inclusion body hepatitis (IBH), caused by Aviadenoviruses, cause an economically important disease in poultry. Historically, IBH has been considered a secondary pathogen facilitated by immunosuppression. However, in recent years, aviadenoviruses have demonstrated their ability to act as a primary pathogen. Adenoviruses belonging to species D and E are primarily associated with IBH, with FAVE, FAdV8b currently the most prevalent among species D and E. Despite increases in clinical disease and identification of FAVE8b as the primary cause of IBH, evaluation of the pathogenicities of contemporary field isolates has not been performed. In this study, the pathogenicity of an FAVE, 8b field isolate was evaluated in SPF chickens utilizing multiple routes of inoculation, evaluation of clinical signs, body weights, levels of biochemical analytes in plasma, as well as viral shedding and microscopic lesions in the liver. No clinical signs observed in both oral and subcutaneously challenged studies. The subcutaneous challenge study group exhibited higher mortality rate compared to the oral group, and no change in body weights observed. Biochemical analytes results reveal a consistent increase in aspartate aminotransferase, creatine kinase, total protein, globulin and potassium, suggesting pathogenesis in the challenge group from day 6 to day 8 post challenge. However, no change was observed in uric acid, sodium and phosphorous values. Virus shedding was assessed by testing cloacal swabs through PCR, and viral presence detected in both oral and subcutaneous inoculated SPF challenge groups. Gross and microscopic lesions were observed in all challenged birds, followed by a disappearance at day 14 in some challenge birds. Our study on SPF chickens challenge with the pathogenic FAV group E/8b isolate unravels the biochemical and physiological responses, highlighting the impact of FAVE, 8b field isolate on SPF birds. These findings provide a framework for evaluating the pathogenicity of IBH adenoviruses and can be used to determine levels of protection in

progeny from breeders vaccinated with an autogenous vaccine. In addition, this research can be extended to identify potential vaccine candidates for future vaccine development.

M31 Eimeria infection induced hypothermia and modulated genes and proteins related to hypothermia in broiler chickens Janghan Choi^{*1,2}, Jihwan Lee¹, Woo Kim¹ ¹*University of Georgia*, ²*US National Poultry Research Center*

Core body temperature is important for general health, cardiovascular system, immune system, and digestive system in chickens. The purpose of the study was to investigate the effects of *Eimeria* infection on core body temperature and cytoprotective genes and proteins related to hypothermia in broiler chickens. A total of 750 fifteen-day-old broiler chickens were randomly distributed to five experimental groups with 6 replicates of 25 birds per pen. The five experimental groups included 1) non-challenged control (NCC); 2) *Eimeria* challenge 1 (EC1): challenged with 31,250 *Eimeria acervulina*, 6,250 *E. maxima*, 6,250 *E. tenella*; 3) *Eimeria* challenge 2 (EC2): double doses of EC1; 3) *Eimeria* challenge 3 (EC3): double doses of EC2; and 4) *Eimeria* challenge 4 (EC4): double doses of EC3. Orthogonal polynomial contrasts were performed to assess the significance of linear or quadratic effects of different *Eimeria* challenge doses. Core body temperature was linearly reduced by higher doses of *Eimeria* spp. in broiler chickens ($P < 0.05$). The relative mRNA expression of cold-inducible RNA-binding protein (CIRBP) in the liver tended to be increased by higher doses of *Eimeria* spp. ($P = 0.082$). Higher doses of *Eimeria* spp. linearly increased relative mRNA expression of hypoxia-inducible factor 1 alpha (HIF1A) in the liver of broiler chickens ($P < 0.01$). Higher doses of *Eimeria* spp. significantly increased the relative mRNA expression of CIRBP, HIF1A, and heat shock protein 70 (HSP70) ($P < 0.05$) in the duodenum. Higher doses of *Eimeria* spp. linearly (tendency; $P = 0.083$) and quadratically ($P < 0.05$) reduced concentrations of CIRBP in the serum. Therefore, *Eimeria* infection induced hypothermia, which resulted in modulated expression of genes related to hypothermia

and cytoprotective response in broiler chickens. Appropriate temperature management is required to minimize the negative effects of *Eimeria* infection of broiler chickens infected with *Eimeria* spp.

Key Words: broiler chickens, Core body temperature, *Eimeria* infection, hypothermia

M32 Herbal Extract Blend to reduce Coccidiosis and Histomoniasis

Greg Mathis^{*1}, Brett Lumpkins², Lorraine Fuller³, Marc Campbell⁴
¹*Southern Poultry Feed & Research, Inc.*, ²*Southern Poultry Feed & Research, Inc.*, ³*University of Georgia*, ⁴*Infinite Health Labs*

Two of the major parasitic diseases of poultry are Coccidiosis and Histomoniasis. The objective of these studies was to examine the parasitic efficacy of an herbal extract blend (Blend) (Parasitrol, Infinite Health Labs). The Coccidiosis study consisted of 40 cages with 10, day old chicks. The treatments were replicated in 8 randomized blocks. Statistical analysis employed the General Linear Models procedure with a probability level of $P < 0.05$ to determine differences. Treatments were: No Additive, challenged (NMI) and nonchallenged (NMU); Amprolium 125 ppm (feed) (AMP); and Blend 200 ppm, challenged (BI120) and nonchallenged (BU120). In both studies, the Blend was water administered. Birds and feed were weighed by cage on DO, 14, 20, and D28. On D14 challenged birds were orally dosed with *E. acervulina* (200,000 oocyst/ bird), *E. maxima* (100,000 oocyst/ bird), and *E. tenella* (150,000 oocyst/ bird). On D20, 5 birds per cage were coccidia lesion scored (LES) and dropping collected for oocysts per gram (OPG) determination. Comparing NMU to BU200, the Blend did not impact performance. There was a significant statistical separation of treatments by challenge for avg. lesion score and avg. OPGs, respectively: NMI (1.88a / 19760 a), AMP (1.33c / 7854c), BU200 (1.1b / 15249b). FCR D14-28 showed AMP (1.904 bc) and BU200 (2.071b) to be significantly better than NMI (2.563a). The Histomoniasis study consisted of 40 cages with 6 day of hatch poults. The treatments were replicated in 8 blocks, randomized within blocks of 4 cages each. Treatments were NMI, NMU, and Blend 200 ppm, challenged (BI200) and nonchallenged (BU200). Birds and feed were weighed by cage on DO, 14, and D28. On D14 challenged birds were intracloacally dosed with 1 ml Histomonas culture containing 250,000 histomonads. On D28, all birds were humanely euthanized and scored (0-4) for liver and cecal lesions. The Blend did not significantly reduce mortality or cecal lesions. However, birds on the water medication (BI200) did have significantly better FCR (2.427) and WT gain (0.497kg) and lower liver lesions (1.9) compared to NMI (3.376/0.361kg/ 3.4). In these two studies the herbal extract blend showed the potential to reduce some of the negative effects of these two parasitic diseases.

Key Words: Coccidiosis, Histomoniasis, *Eimeria*, Histomonad

M33 Evaluation of an Automated Gel Application System for Delivering Commercial Coccidiosis Vaccine to Broiler Chickens

Matthew Jones^{*1}, Charles Hofacre¹, Brian Jordan², A. Fuller³, Fred Hoerr⁴, Brian Dirks⁵, Eric Aicher⁵, Keith Staggs⁵
¹*Southern Poultry Research Group*, ²*Department of Population Health, College of Veterinary Medicine, University of Georgia*, ³*Department of Poultry Science, College of Agricultural and Environmental Sciences, University of Georgia*, ⁴*Veterinary Diagnostic Pathology*, ⁵*United Animal Health*

Coccidiosis vaccination is an essential part of many coccidia control strategies. While vaccination effectively mitigates consequences of *Eimeria* infection, there are practical limitations to coccidia vaccine application, specifically uniformity of vaccine ingestion. Currently, most coccidia vaccination is administered via coarse spray or a thin gel liquid in the hatchery. The current study evaluated live vaccine cycling when it was applied with a novel automated gel beadlet delivery system (1.0 gram/chick) versus coarse spray vaccination (0.21mL/chick) or oral gavage (0.1mL/chick) in broiler chicks. The coccidia vaccine was diluted so each application method received an equivalent dose. Each treatment was represented

by six replicates of one male Ross broiler chick in an individual cage. Oocyst per gram of excreta was evaluated from each replicate on day 5, 6, 7, 8, 9, and 10. In an adjacent area, a single floor pen (25 chicks) of each vaccine application treatment was placed on fresh pine shavings. Duodenum, jejunum, and ceca samples were collected into formalin on day of test (DOT) 14, 21, and 28 to evaluate progression of the coccidia vaccination histologically. Oocyst data was analyzed by ANOVA and means were subjected to an LSD test at a P -value of 0.05.

No oocysts were detected from any application method on DOT 5 or DOT 6. On DOT 7, at least one bird from each treatment passed *E. acervulina*, *E. maxima*, and *E. tenella* oocysts. On DOT 8, gel application had significantly higher *E. tenella* compared to other groups. There were no significant differences between groups on DOT 9 and DOT 10 as cycling began to decline. Of the placed chicks, 100% (6 of 6) within the gel group passed *Eimeria* oocysts, 67% (4 of 6) of the orally gavaged group passed oocysts, and 50% (3 of 6) of the spray vaccinated group passed oocysts. Histology was observed at later time points in floor pens to monitor vaccine cycling as it would occur in the field. The gel application system had numerically lower cumulative histological coccidia damage on DOT 14, 21, and 28 compared to the other treatments. Greater replication is needed to confirm experimental outcomes, but the initial pilot suggested the novel gel application is a successful method for uniform coccidia vaccine application.

Key Words: Coccidiosis, Vaccination, Gel, *Eimeria*

M34 Anti-coccidial effect of water-soluble extract of rosemary on broilers infected with *Eimeria tenella*

Fang Peng^{*UG}, Zehe Song, Xi He, Haihan Zhang
Hunan Agricultural University

This study was conducted to evaluate the prophylactic and therapeutic activities of water-soluble extract of rosemary (WER) in broilers infected with *Eimeria tenella*. 360 one-day-old Chinese indigenous male yellow-feathered broiler chickens were randomly allocated to six groups: non-infected control (NC) group and positive control (PC) group received a basal diet; DIC group, received a basal diet supplemented with 200 mg/kg diclazuril; WER100, WER200, and WER300 groups received a basal diet containing 100, 200, and 300 mg/kg WER, respectively. At d 21, all birds in the infected groups were orally gavaged with 1 mL PBS of 8×10^4 sporulated oocysts of *E. tenella*, while birds in NC group were administered an aliquot of PBS dilution. The experiment lasted for 29 d, and statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's test using the SPSS 22.0 software.

The results showed that no significant differences were observed in average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of birds among the six groups during d 1 to d 21 ($P > 0.05$). The experimental infestation model was successful by the evidence of the significant reduction in ADG and increase of FCR of birds in the PC group compared to the NC group after challenged with *E. tenella* ($P < 0.05$). However, dietary supplementation of 200 mg/kg WER significantly increased ADG of broilers compared to the PC group after challenged with *E. tenella*. At 8 d post-challenge, dietary supplementation of WER (100, 200, 300 mg/kg) significantly reduced fecal oocyst output of birds as compared to the PC group ($P < 0.001$). Among the three supplementation dose of WER, 200 mg/kg had the minimum fecal oocyst output. Moreover, the anticoccidial index (ACI) of the three groups (WER100, WER200, WER300) are 138.77, 152.88 and 144.97 respectively. And histopathological examinations of cecum exhibited that there were only a few inflammatory cells and coccidium oocysts were found in WER200 group compared with PC group.

Therefore, 200 mg/kg WER possesses the best anti-coccidial effects and dietary supplementation 200 mg/kg WER could improve growth performance of broilers infected with *E. tenella*.

Key Words: *Eimeria tenella*, extract of rosemary, broiler chickens, growth performance, anti-coccidial effect

M35 Comparison of the In Vivo Effects of a Commercial Steroidal Saponin-Based Standardized Premixture and Synthetic Molecules on the Reproduction of Eimeria spp. Bui Hoa^{1,2}, Mohammed el Amine BENARBA^{1,2} ¹LABCOR FEED IN TECH: Université d'Angers, ²NOR FEED SAS

Eimeria spp. completes its life cycle within the host's gastrointestinal tract. Its reproduction has been known to damage enterocytes, leading to decreased growth performance and welfare in chickens. Controlling the reproduction cycle and limiting oocyst excretion could explain the observed efficacy of steroidal saponin-based standardized premixture -Norponin XO- (NPXO) supplementation in chicken. The objective of this study was to evaluate the impact of feed supplementation with NPXO on the reproduction of *Eimeria* spp. in artificially infested chickens and to compare it to the effects of coccidiostats. 70 broiler chickens (Ross 308) were randomly divided into 7 groups. Birds were raised in cages and received a standard diet from D1 to D12. From D13 to the end of the experiment, each group of birds received a supplementation as follows: The untreated control group received feed without any supplementation, while the NPXO group received feed supplemented with steroidal Saponin-Based Standardized Premixture. The other treatment groups were supplemented with coccidiostats (nicarbazin, salinomycin, monensin, and monensin/nicarbazin). Birds were artificially infested at day 14 using a wild-type inoculum. Each bird received 150,000 sporulated oocysts (*E. acervulina*, *E. tenella*, *E. mitis*). Parasite reproduction was monitored using oocyst count in bird droppings during 5 days post-infestation. Starting 96 h post-infestation, daily droppings from each group were collected, weighed, and processed for oocyst count. The Reproductive Index and intensification efficiency were calculated.

Our investigations revealed that NPXO supplements, similar to coccidiostats, decreased the reproductive capability of the parasite. The most notable reduction in Reproductive Index (RI) was observed in birds administered nicarbazin, and those given Norponin® XO2 achieved the second-highest RI reduction. As expected, the birds in the Infected Untreated Control (IUC) group demonstrated the highest RI.

This finding supports the hypothesis that selected and standardized saponins target the extracellular phases of the parasite's development cycle by affecting its membrane. This trial clearly demonstrated that NPXO supplementation could be as effective as many other widely used coccidiostats.

Key Words: Coccidiosis, Broiler chicken, Steroidal saponin

M36 Incidence of Enterococcus faecalis and Escherichia coli coinfection in nonviable embryonated broiler chicken eggs Jodi Delago*, Mueez Ahmad, Enid McKinley, Alexandra Smith *Arm & Hammer Animal and Food Production*

Hatchability rates in the US broiler industry have been declining since 2014. The average weekly hatchability rate in the US in 2023 has been approximately 80.7%, a decrease of more than 4% since 2014. Published studies of nonviable embryos in Canada indicated that *Enterococcus faecalis* and *Escherichia coli* are frequently recovered from nonviable embryos and that infection by one or both bacteria may be contributing to the declining hatchability rates observed. *E. faecalis* and *E. coli* are ubiquitous in the poultry production environment and both are thought to be normal commensals of the poultry gastrointestinal microbiota. Although both bacteria can become opportunistic poultry pathogens, they are frequently recovered in high abundance from the intestinal tract of normal, healthy birds within a day of hatching, resulting in uncertainties around their role in the declining hatchability rates. To better understand this issue and the potential role of co-infection of embryonated eggs with *E. faecalis* and *E. coli* on decreasing hatchability rates in the US, we conducted a survey of nonviable broiler eggs to determine the prevalence of these bacteria in the samples. A total of 405 hatch residue samples were collected from eggs that had evidence of early embryonic mortality from 6 different hatcheries. Samples were collected by opening eggs aseptically and swabbing

the yolk area, followed by standard microbiological methods to determine the presence of *E. faecalis* and *E. coli*. The prevalence of each bacteria recovered alone was compared against the prevalence of co-infection using one-way ANOVA. The prevalence of *E. faecalis* alone was 54 (13%), which was similar to the prevalence of *E. coli* alone at 53 (13%) ($p > 0.5$). However, co-infection with both bacteria was significantly more prevalent than either bacterium alone ($p < 0.0001$), with 174 samples (43%) positive for both bacteria. The remaining 124 samples (31%) were negative for both bacterial species. These results suggest that *E. faecalis* and *E. coli* may contribute to the decline in hatchability rates and that co-infection with *E. faecalis* and *E. coli* may enhance virulence of each bacterium leading to early embryonic mortality.

Key Words: Enterococcus faecalis, E. coli, Hatchability

M37 A pan-genome-wide association study to decipher virulence mechanisms in avian pathogenic Escherichia coli Grayson Walker^{*1GS}, Chalise Brown¹, M Suyemoto¹, Heather Harbottle², Jeff Gilbert², Marilyn Martinez², Steven Foley³, Jing Han³, Madison Johnson³, Luke Borst¹ ¹North Carolina State University College of Veterinary Medicine Department of Population Health and Pathobiology, ²U.S. FDA Center for Veterinary Medicine Office of New Animal Drug Evaluation, ³U.S. FDA National Center for Toxicological Research

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in poultry and is the leading cause of morbidity and mortality in the U.S. poultry industry. Treatment of APEC infections is confounded by widespread antimicrobial resistance, genetic diversity, and numerous overlapping APEC virulence mechanisms. To develop effective interventions, it is crucial to understand the genetic basis of APEC virulence and how these virulence genes may relate to genetic changes associated with antimicrobial resistance. In this investigation, a pan-genome-wide association study was used to correlate APEC genotypes with virulence phenotypes to identify APEC genes associated with embryo lethality. The whole-genome sequences of 97 APEC strains isolated from septic chickens and turkeys were annotated. In the embryo lethality assay, embryos were challenged with APEC strains at 12 days of incubation. Cumulative viability over 5 days was plotted as Kaplan-Meier survival curves and analyzed by the log rank test of significance ($P \leq 0.05$) relative to strains of known virulence. Binary virulence phenotypes (virulent vs avirulent) were assigned to each strain using an established embryo lethality assay with a survival threshold of 50%. Seventy-one of the 97 strains were virulent in embryos. After constructing a pan-genome for the 97 APEC strains, the resulting genotype matrix was aligned with the binary virulence phenotype assignments to complete the pan-genome-wide analysis. Predicted proteins required for exopolysaccharide production including lipopolysaccharide (LPS) and colanic acid exhibited 97% sensitivity in the identification of virulent APEC strains (Odds Ratio = 15.5). Additionally, 180 hypothetical proteins were 100% specific to virulent APEC strains suggesting a number of APEC virulence factors remain uncharacterized. This genetic screen provides insight into APEC virulence, which is important for the development and evaluation of interventions for the treatment, control, and prevention of colibacillosis.

Key Words: Colibacillosis, E. coli, Virulence, Genomics

M38 Capsular deletion mutants in pathogenic Enterococcus cecorum are attenuated in broiler embryo and chick challenge models of infection M Suyemoto*, Grayson Walker, Luke Borst *North Carolina State University College of Veterinary Medicine Department of Population Health and Pathobiology*

Pathogenic *Enterococcus cecorum* (EC) continues to cause losses in the broiler industry due to sepsis and osteomyelitis. Comparative genomic analysis of commensal and pathogenic strains of EC previously identified divergent genomic features in pathogenic strains including alterations in capsular polysaccharides. It was hypothesized that deletion mutants of these putative virulence determinants would be attenuated in an embryo

lethality assay (ELA) and in a chick challenge model of infection. Manipulation of the EC genome has proven difficult; however, deletions in *cpsC* and *cpsO*, genes responsible for capsular biosynthesis, were made with modifications of the homologous recombination method of Thurlow et al. (2009) and transformation protocol of Chatterjee et al. (2019). For the ELA, broiler eggs were inoculated at 12 days of incubation with 10^2 cfu of pathogenic EC wildtype (WT) SA1, SA1 Δ *cpsC*, SA1 Δ *cpsO* or the complemented mutants. Survival in the deletion mutant groups was significantly greater ($P \leq 0.05$) compared to the WT group using the Log-rank Mantel-Cox test. In the chick challenge model, 120 chicks per group were orally gavaged at day of hatch with 10^7 cfu of SA1 or SA1 Δ *cpsC*. Forty chicks were gavaged with PBS and served as negative controls. Sep-

sis was determined by growth of pathogenic EC from spleen cultures collected at 14 and 35 days post inoculation. Free thoracic veterbrae (FTV) were also cultured at day 35. The birds challenged with the WT SA1 parent strain had an overall sepsis prevalence of 55% and FTV infection prevalence of 33%. Neither sepsis nor FTV infection was detected in the SA1 Δ *cpsC* challenged group. Targeted gene deletion mutants in pathogenic EC may lead to a clearer understanding of the pathogenesis of EC and possible interventions for the associated disease.

Key Words: Enterococcus cecorum, gene deletion mutant, challenge model

Pathology II

M39 Impact of Isoquinoline Alkaloids on airsacculitis scores. Cleveson Souza*¹, Arthur Masseur¹, Dimitri de Freitas¹, Elisangela dos Santos², Kelvin da Silva², Nbia Oliveira² ¹Phytophysics, ²Pluma Agroavicola

The objective of this study was to evaluate the effects of Isoquinoline Alkaloids (IQ) on score of airsacculitis in broilers challenged by *E. coli*. Studies with the extract of *Macleaya cordata* composed of IQ have shown positive effects on the performance and intestinal health of broilers, similar the effects of antibiotics. The results demonstrate that IQ decrease intestinal permeability, through the control of intestinal inflammation, consequently, increasing the systemic immunity. A systemic immunity enhanced, may reduce the airsacculitis severity. A total of 120 broilers aged from one to 31 d were distributed in 3 treatments: T1 - Negative control (no challenge); T2 - Positive control (challenge with *E. coli* 1×10^9); T3 -IQ (Sangrovit® ED 100g/ton) with challenge with *E. coli* 1×10^9 , on Mercolab facilities. On day 31 all broilers were euthanized and their air sacs were observed and classified in four score degree: Score 0 = no lesions; Score 1 = light changes; 2 = moderate changes; 3 = severe changes. Statistical analysis was performed with R software. The Pearson chi-squared was performed to test the independence between the variables. There was a statistical difference ($X^2= 33.06$, p-value < 0.001), between treatments. T1 presented 80% of broilers with score 0, and 20% score 1, T2 showed 22% score 0; 65.9% score 1 and 12.1 % score 2, T3 presented 60% of score 0; 40% of score 1, and 0% of score 2. The comparison between T1 and T2 presented ($X^2= 28.209$, p-value < 0.001), the comparison between T2 and T3 presented ($X^2= 14.62$, p-value < 0.001), the comparison T1 vs T3 ($X^2= 2.92$, p-value = 0.218). Therefore, we conclude that IQ (Sangrovit® ED 100g/ton) reduce the frequencies of lesions of airsacculitis of broilers challenged with *E. coli*.

Key Words: Isoquinoline alkaloids, AGP, immunity, airsacculitis

M40 Investigating the pathogenesis and infective dose of Campylobacter hepaticus in white leghorn laying hens. James Wilson*^{GS}, Roel Becerra, Catherine Logue *University of Georgia*

Spotty Liver Disease (SLD), caused by *Campylobacter hepaticus*, is characterized by multifocal 1-2mm white spots on the surface of chickens' livers. *C. hepaticus* SLD infections have not been seen in the US since the 1960s but it has recently re-emerged in free range and cage free rearing layer systems. With increasing potential for SLD to become widespread in egg laying breeds, this study aimed to investigate the pathogenesis of *C. hepaticus*. The objective of this study was to determine the concentration of *C. hepaticus* required to cause clinical disease in white leghorn (WL) laying hens and the pathogenesis of the bacterial infection. For this study, 30 WL layers at 60 weeks of age, were divided into three treatment groups and orally challenged with three different challenge doses of *C. hepaticus*. An unchallenged sentinel bird was added to two groups to monitor for potential transmission. Birds were housed in battery cages in

colony houses and were euthanized weekly beginning four days post challenge. SLD lesion scores were recorded for each bird. Bile, blood, feces, and eggs were collected from euthanized birds and plated on blood agar to assess *C. hepaticus* presence. All samples showing suspect *C. hepaticus* colonies on culture were confirmed by PCR. Statistical analysis using a one way analysis of variance (ANOVA) with multiple comparisons test was used to assess effects of challenge dose on lesion scores and recovery of *C. hepaticus* from bile and blood. No significant differences ($P>0.5$) were observed in the lesion scores recorded for challenges doses although the mean lesion scores were higher for the high challenge dose (mean lesion score 1.2 vs 0.6) compared to the low dose challenge. Similarly, mean lesion scores were not significantly different at 6, 13, 20, 27 or 32 days post challenge ($P > 0.05$). To the author's knowledge, there is currently limited research on the infective dose of *C. hepaticus* required to cause SLD in layers or the bird-to-bird transmission route. This research allows us to examine the level of *C. hepaticus* necessary to cause SLD and its impact on host health.

Key Words: Campylobacter hepaticus, layer, health, challenge, SLD

M41 Combating Necrotic Enteritis: A Breakthrough with L. reuteri Vectored Nanobodies Arvind Kumar*, Dharanesh Gangaiah, Amy McMillan, Desmond Adeniyi, Jacob Shields, Shekar Govindaswamy, Eric Nungester, Olaitan Akintunde, Ali Camara, Emmily Helmes, Nathan Augspurger, Simmi Manuja *BiomEdit*

Necrotic enteritis (NE), caused by *Clostridium perfringens*, has a devastating effect on poultry health and industry economics. The complexity of the disease, aggravated by shifts in gut microbiota and the rise of antibiotic resistance, demands innovative interventions. Addressing this, we introduce a transformative platform technology using engineered probiotic *Limosilactobacillus* (*Lactobacillus*) *reuteri* to deliver nanobodies that target and neutralize NetB and α toxin - key determinants of disease pathology. The pioneering approach presents the first line of poultry - derived *L. reuteri* strains that in addition to inhibiting *C. perfringens* growth also produce a repertoire of bioactive(s), pivotal for maintaining gut health. These strains have shown persistent colonization and protective effects in chicken populations. Notably, a pioneering two-dose administration approach has resulted in significant protection against NE mortality. The efficacy of *L. reuteri* vectored vaccine containing NE01 and NE06 strains was evaluated against mortality in a randomized, partially blinded in a NE challenge study. Birds were housed in battery cages with 11 birds/cage. Equal number of broilers (n=110) received either IVP or distilled water (challenge control) on the day of hatch via coarse spray, and on day 13 via drinking water. The dose of the Investigational Veterinary Product (IVP) per bird was $10E08$ CFU for both vaccinations. All the birds were subjected to NE challenge model consisting of a predisposing dose of *Eimeria maxima* on day 14 followed by a virulent challenge with *C. perfringens* on days 19 and 20. Chickens were observed daily until day 28 and mortality due to NE was evaluated. Mortality due to NE was

the primary outcome of interest, with statistical analysis focused on Prevented Fraction (PF) of each Vaccination Group compared to Challenged Control Group. Mean and 95% confidence interval estimates of PF were derived using Logistic Regression and the Delta Method. These estimates were then used in the context of a two-sided hypothesis test. The IVP significantly reduced the NE mortality in the vaccinates in comparison to the challenge control group with Prevented Fraction of 52%. These findings, in addition to validating the use of *L. reuteri* as an effective live vector for nanobody delivery, also open avenues for combatting complex diseases - showcasing a synergy that could revolutionize disease management in poultry and beyond. The platform with bespoke, genetically tailored probiotics leveraging the natural gut microbiota's dynamics to combat pathogenic bacteria - is a promising scientific approach not only for treating NE but also potentially offers a blueprint for addressing other diseases through similar biological mechanisms. It's a paradigm shift in how we develop dual purpose solutions, with features of a "probiotic and immunoprophylactic" combined as one product, to target both animal health and productivity.

Key Words: Necrotic enteritis, *Clostridium perfringens*, Engineered probiotics, Nanobodies, Innovative biologics

M42 The impact of Salmonella Typhimurium and coccidiosis vaccine on the cecal transcriptome in broiler chickens in the late stage of production Andrea Pietruska^{*1}, Steven Kitchens¹, Rana Tabish², Maria Terra-Long³, Ken Macklin⁴, Stuart Price¹, Rüdiger Hauck^{1,2} ¹Department of Pathobiology, Auburn University, ²Department of Poultry Science, Auburn University, ³Phileo by Lesaffre, ⁴Department of Poultry Science, Mississippi State University

In antibiotic-free and organic poultry production, the use of live vaccines targeting *Salmonella* and coccidia has increased in order to reduce health risks for consumers and to mitigate economic losses. Prior research suggested that in broilers *Salmonella* Typhimurium (ST) infections were more prevalent following coccidiosis vaccination on day 28 of age. Additionally, significant alterations in the expression of tight junction genes were observed when both vaccines were administered. This study investigated the impact of vaccines against ST and coccidiosis on the host transcriptome in the late stage of production. We structured a 2 x 3 experimental design, with groups vaccinated against ST on day 14 or on days 0 and 14. The second factor was the presence or absence of coccidiosis vaccination on day 0. On day 28, all groups were challenged with a ST field strain. Samples of the cecal wall were collected from 6 birds per group on days 35 and 42, and based on mRNA sequencing a transcriptome analysis was performed. The reads were first trimmed with Trimmomatic and then aligned to the NCBI chicken reference genome using Hisat2. The reads were counted using HTSeq. Differential expression and pathway analysis was performed with edgeR and DAVID by fitting a negative binomial regression model. Only the expression of β -spectrin was significantly lower in the group vaccinated against coccidia and ST on day 14 compared to the group vaccinated against coccidia alone (P=0.019). The most substantial difference in gene expression was observed between the group vaccinated against ST on day 14 and the group vaccinated against coccidia only. In this case, there was a significantly lower expression in 1984 genes and a significantly higher expression in 1082 genes. Gene Ontology analysis showed that the most significant pathways in all group comparisons were related to cytosol, protein binding, and ATP binding. No significant changes were observed in pathways related to tight junctions or innate immune response. Our study found that vaccination against ST and coccidia had no significant impact on the transcriptome of broiler chickens compared to the control group. However, vaccination against ST on day 14 alone had a significant influence on the cecal transcriptome.

Key Words: transcriptome, poultry, *Salmonella*, coccidia, vaccine

M43 Binding profiles of H5 influenza A viruses on avian and mammalian respiratory and gastrointestinal tissues Ben Enyetornye^{*GS}, Sarah Carlisle, Brittany Seibert, Lindsey Gay, Carlos Caceres, Chloe Goodwin, Nicole Nemeth, Daniel Perez, Silvia Carnaccini *University of Georgia*

The rapid intercontinental spread of highly pathogenic avian influenza virus (HPAIV) caused by the novel Eurasian-origin H5N1 subtype clade 2.3.4.4b has been devastating for the global poultry industry, wildlife, agriculture and economy. Furthermore, HPAIVs are zoonotic and pose a significant threat to public health. The surface glycoprotein hemagglutinin (HA) plays a vital role in virus binding to the host cell sialic acid receptors, and mediating entry, thus a major determinant of virus host range and tissue tropism. Particularly for recent H5N1 AIV infections, broader host tropism has been observed including species not commonly associated with AIV infections. To this end, we evaluated the binding profiles of different subtypes of influenza A viruses on avian and mammalian respiratory and gastrointestinal tissues. The HA of different H5 AIVs representing recent Eurasian and historical strains were generated over a laboratory-adapted isogenic backbone via reverse genetics. These H5 HAs were incubated with respiratory and gastrointestinal tissues of domestic gallinaceous birds, wild birds, mammals, and human differentiated airway epithelial cells on charged slides overnight. Immunofluorescence assay was then conducted to assess the binding profiles of each H5 HA. Confocal microscopy correlated the binding profiles of the recent H5 HA with historical H5 HA. This was compared to the distribution of α 2,3 and α 2,6 N-linked sialic acid cell receptors. As a control, the binding profiles of the pandemic A/California/04/2009 (pdmH1N1) were compared to the H5 binding profiles within the same tissue types. Each tissue was subjectively scored based on the specific staining on the apical surface of epithelial cells, goblet cells or mucous glands. Criteria utilized for scoring were the percentage of tissue surface positivity and intensity of the immunofluorescence. Generally, we observed an increased trend of binding avidity of the recent Eurasian origin H5 HA to the same tissue types in poultry and non-poultry hosts. These findings suggest an increased trend of the recent AIV towards a broader host range increasing the chances of its adaptation into new host species. This study advance our understanding of the pathogenesis and ecology of AIV in a variety of host species.

Key Words: Virus binding, Avian influenza, sialic acid, Immunofluorescence, mammalian

M44 A delay in reticulin resolution indicates erythrocytes are stressed Paul Cotter^{*} *Framingham State University*

Background. A reticulocyte (Rtc) is a late-stage developmental erythrocyte with retained organelles. Ordinarily, they are detected by vital staining with new methylene blue (NMB) added directly to blood or applied onto the surface of a microscope slide. When used this way NMB will detect network reticulocytes (nRtc) whose remnant reticulin is in a complex 3-D form. In contrast, NMB will not detect remnant reticulin of a smaller punctiform configuration (pRtc). Conversely, theory indicates neither reticulocyte form is detected with Romanowsky stains such as Wright-Giemsa (W-G). The purpose is to demonstrate that some Rtc of duck blood are detected with W-G. The method is a microscopic examination of blood films stained using W-G only. **Results.** Rtc were in blood films from commercial ducks obtained at various ages. Some samples were from nutritional studies; no duck was diseased. Both nRtc and pRtc were found. Some Rtc were irregularly shaped (non-oval), and others were ellipses. Rtc could be faintly stained (hypochromic) or fully hemoglobinized (yellow-orange). **Conclusions.** Reticulocytes detected by W-G are more common than the theory predicts. As they may not be fully hemoglobinized the normal process of reticulin removal was interrupted; an intracellular stress signal. Hypochromic nRtc and pRtc illustrate the independence of hemoglobin synthesis and reticulin removal pathways. They indicate the

importance of evaluating erythroid cells as well as leukocytes in establishing a blood picture.

Key Words: duck blood, reticulin, erythrocyte, hematology

M45 Fowl Adenovirus: Pathogenicity, Epidemiology, and Rapid Detection of Serotype 4 using Insulated Isothermal PCR in Broiler Chickens in the United Arab Emirates. Merghani Adam* *ARABIAN FARMS DEVELOPMENT LLC*

Fowl adenoviruses (FAdVs) are non-enveloped paramyxoviruses with double-stranded DNA molecules and belong to the Avi adenovirus genus. There are also several diseases caused by fowl adenoviruses in broiler chickens, including inclusion body hepatitis, hydropericardium hepatitis syndrome, and adenoviral gizzard erosion caused by fowl adenoviruses. The fowl adenovirus can be divided into five different serotypes (FAdV A, B, C, D, and E). Objectives: This study aims to describe the pathogenicity and epidemiology of Fowl adenovirus disease in the United Arab Emirates and to describe the rapid diagnosis of the disease using Insulated Isothermal PCR as a means of rapid detection. This study was conducted between September 19th and December 2nd, 2022, and was carried out in a closed system at Arabian Farms Development, a sole proprietorship in Al Saad, Al Ain, Abu Dhabi, United Arab Emirates. The duration of this case

report is approximately six weeks. An unsexed Ross 308 hybrid chick was hatched from eggs derived from a 44-week-old Turkish Ross 308 breeder. After hatching, 45,000 chicks per farm were randomly distributed. Additionally, insulated isothermal PCR was conducted along with a necropsy and histopathology. Results It was found that, at 22 days old, the broiler chicken was infected with the disease. Mortality rates ranged from 13, 15.10, and 16.5%, respectively. Clinical symptoms were observed, such as ruffled feathers, diarrhea, anemia, and general weakness. The clinical examination revealed acute necrotic hepatitis, a swollen, yellowish discoloration, an enlarged and friable liver, and an enlarged mottled spleen. Histopathology examination revealed degeneration, necrosis, lymphocytic infiltration, and inclusion bodies of hepatitis in the tissue. Fowl adenovirus was confirmed by histopathology in all samples tested by PCR. The isothermal PCR with specific kits for adenovirus serotype 8b gave accurate and reliable results in the detection of fowl adenovirus in broiler chickens. Additionally, advanced technologies in laboratory diagnosis are being used to diagnose many viral diseases in broiler production, such as the Insulated Isothermal PCR device.

Key Words: Fowl adenovirus, histopathology, serotype 4, broiler, performance

SCAD I

M46 Effect of essential oils, fatty acid and mineral blend product on a live Salmonella vaccine in a Salmonella infantis challenge model Charles Hofacre*¹, Matthew Jones¹, Jennie Baxter¹, Aldo Rossi², Jay Hughes², Roy Berghaus³ *Southern Poultry Research Group, Inc., ²Oil-Dri Corporation, ³The University of Georgia*

Salmonella infantis has become a more prevalent serovar isolated by poultry processing plants and has also been linked to foodborne illness cases by CDC. The live Salmonella vaccines are slightly less effective against serogroup C salmonella such as S.I. Therefore, combining a live vaccine with other interventions may be an effective strategy to control S.I. In this pilot study, there were three treatments: No treatment; Live vaccine alone (MeganVac1ä); Live vaccine with a product that is a blend of essential oils, medium chain FA and a mineral blend (NeutraPathä). The live vaccine was sprayed at one dose per bird at 1 day of age. The study had 300 Aviagen male broilers in six pens (50 birds/pen) with two replicate pens/treatment. Vaccine re-isolation was 4 birds per pen on day 3 of the study. On day 7, all birds were orally gavaged with 5.0×10^7 CFU/chick S.I. Fifteen cloaca swabs per pen were tested on day 15. Then on day 42, 15 ceca were sampled per pen. Salmonella was cultured with tetrathionate/XLT-4; enumeration by micro MPN. Salmonella prevalences were compared between treatments using GEE logistic regression and Salmonella MPN's were compared using linear mixed models. For vaccine re-isolation, there were 100%^a positive in the live vaccine alone and 88%^a vaccine re-isolation in the live vaccine plus NeutraPathä. Results of the S.I. prevalence in cloaca swabs: challenge control 77%^b, vaccine only 57%^{ab} and vaccine plus essential oil/FA/mineral blend 20%^a. Cloaca swab MPN estimated means were 0.57^a log₁₀ MPN/swab for control, 0.88^a for live vaccine only and 0.31^a for vaccine plus essential oil/FA/mineral blend. Ceca prevalence on day 42 were 67%^b for challenge control, 0%^a for live vaccine, and 43%^b for live vaccine plus NeutraPathä. Ceca MPN results were 0.74^a log₁₀ MPN/g for control, N.A. for live vaccine alone, and 0.09^a log₁₀ MPN/g live vaccine plus NeutraPathä. Overall, there were many more individual ceca at less than 1 log of S.I. in the combined treatment than the challenge control. There was a significant reduction in cloaca swab S.I. prevalence with the combined live vaccine and NeutraPathä. There was not a significant effect of the essential oil/FA/mineral blend product on the live Salmonella vaccines ability to colonize broilers.

Key Words: Salmonella infantis, live vaccine, essential oil, fatty acid

M47 Understanding the pathogenesis of Campylobacter hepaticus using different types of layer hen breeds, broiler breeders, and broilers in a well-developed challenge model Roel Becerra*^{GS}, Catherine M. Logue, James Wilson *University of Georgia*

Spotty liver disease (SLD) caused by *C. hepaticus* is an important cause of disease in layers. However, it is not well understood if *C. hepaticus* can cause disease in different chicken breeds, since it is most often seen in brown layer hens with outdoor access. The objective of this study was to determine if different laying hen breeds, broiler breeders and broilers are susceptible to *C. hepaticus*. For this study, 50 chickens; Long Horn white hens, Rhode Island red hens, Rhode Island white hens, broiler breeders, and broilers were divided into 5 groups (n=10) and one negative control group (n=4). A challenge dose of 10¹¹ cfu/ml/hen was used in each bird. An unchallenged sentinel bird was added to each group to monitor potential transmission of *C. hepaticus*. Birds were housed in battery cages in colony houses and were euthanized weekly beginning four days post challenge. Spotty liver disease (SLD) lesion scores were recorded for each bird. Bile, blood, feces, and eggs were collected from euthanized birds and plated to confirm *C. hepaticus* presence by culture and PCR. Challenging at a dose 10¹¹ cfu/mL produced liver lesions in birds, but minimal gross lesions were observed in broilers. Recovery of *C. hepaticus* from sampled tissues was achieved in all birds and histopathology lesions were observed in all bird types. This research showed that *C. hepaticus* can cause disease in layer hens from three different genetic breeder lines and broiler breeders, but minimal disease in broilers.

Key Words: Campylobacter hepaticus, Spotty liver disease, Layers, Broiler breeders, Broiler

M48 Exploring Focal Duodenal Necrosis: A Preliminary Study Investigating Disease Replication Through Challenge Experiments Yu-Yang Tsai*^{GS}, Julia Lima, Klaio Runcharoon, Catherine Lgoue *Department of Population Health, University of Georgia*

Focal duodenal necrosis (FDN), an intestinal disease, ranks among the top five concerns in table egg layers, leading to reduced egg production and significant economic losses to the table egg industry. However, the etiology and pathogenesis are still unclear. This study represents a small-scale

preliminary challenge experiment aimed at replicating the specific lesions associated with FDN in commercial layers.

A total of thirty, 35-week-old laying hens (Hy-line W-36) from a local commercial poultry company were randomly divided into groups, comprising four treatment groups and one control group. All groups received a customized formula feed, considered a potential predisposing factor for FDN. Each designated group were subjected to daily oral challenge using different bacterial cocktails formulated at a concentration of 10^8 - 10^9 cfu/mL. The bacterial cocktails included *E. coli*, *Clostridium perfringens*, *Enterococcus faecalis*, *Gallibacterium anatis*, and *Clostridium colinum*. The control group received PBS as a sham inoculum. The daily oral challenge continued for 14 days, followed by a one-week pause. The egg production rate and egg weight were recorded daily. At seven-day intervals, two layers were selected from each group and euthanized to examine for pathological changes in duodenum.

The identified duodenal lesions included mucosal hyperemia, red foci or patches, and erosions, sometimes extending to the proximal jejunum with hyperemia and red foci associated lesions. Using a lesion scoring system, the highest score among the treatment groups was observed where a combination of *E. coli*, *Clostridium perfringens* and *Clostridium colinum* was used; the lowest score was observed for a combination containing all five strains. Histopathological analysis revealed villous tip necrosis with mucosal exudate mixed with different shaped bacteria. This pilot study offers valuable insight into replicating FDN in layers. Conducting a larger-scale study will be essential to validate the reproducibility of this challenge model.

Key Words: focal duodenal necrosis, challenge experiment, *E. coli*, *Clostridium perfringens*, *Clostridium colinum*

M49 Development of a mobile broiler Salmonella diagnosis system Venkat Umesh Chandra Bodempudi^{*1GS}, Guoming Li¹, Samuel Aggrey¹, Adelumola Oladeinde² ¹University of Georgia, ²USDA Agricultural Research Service

Salmonella is a leading cause of foodborne illnesses, resulting in distressing symptoms such as diarrhea, fever, and stomach cramps in humans. Poultry products are widely recognized as one of the primary sources of salmonellosis. Rapid and accurate detection and characterization methods for *Salmonella* are crucial for timely infection control, enhancing safety, and bolstering biosecurity measures in poultry farms. This study aimed to create a mobile broiler *Salmonella* diagnosis system designed to aid producers in not only swiftly identifying the infection but also in categorizing the severity into low, medium, and high levels during the routine flock inspections. This system featured efficient accuracy, decent processing speed, and cost-effectiveness. A comprehensive broiler feces image classification model was developed using Deep Learning techniques. The training dataset consisted of feces images collected from a broiler *Salmonella* Infantis challenge experiment, and levels of *Salmonella* examined on litter and ceca was used as ground truth for system development. During the development phase, hyperparameters were carefully fine-tuned, maintaining an 80:20 training-to-testing data split and utilizing 100 epochs for model training. Validation loss and accuracy were closely monitored and visualized through histogram graphs, providing real-time insights into model performance optimization and improvement. The developed model was seamlessly integrated into a user-friendly mobile application compatible with a range of mobile devices. The results demonstrated that the mobile application could identify and quantify broiler *Salmonella* infection into low (<2 Log CFU/g), medium (2.5-3.5 Log CFU/g), and high (>3.5 Log CFU/g) levels with over 90% accuracy. The developed tool is useful in enabling timely interventions for infection control within poultry farming, enhancing the overall health and food safety of bird flocks, and contributing to the sustainability of poultry production.

Key Words: poultry, disease, deep learning, mobile application, food safety

M50 Evaluation of a prebiotic mannan-oligosaccharide on Salmonella Enteritidis cecal colonization and immune response of broiler chickens Allison Milby-Blackledge^{*1GS}, Yuhua Farnell¹, Audrey McElroy¹, Christi Swaggerty², Leticia Pachaeco³, Robson Barducci³, Anderson Santos³, Joao Koch³, Thaila Putarov³, Morgan Farnell¹ ¹Department of Poultry Science, Texas A&M AgriLife Research, ²United States Department of Agriculture, Southern Plains Research Center, ³Biorigin Brazil

Salmonella is one of the main foodborne pathogens found in poultry meat and eggs, causing 1.35 million cases of salmonellosis, 26,500 hospitalizations, and nearly 450 deaths in the United States annually. We hypothesized that the administration of ActiveMOS[®] (AM), a prebiotic mannan-oligosaccharide (MOS) derived from *Saccharomyces cerevisiae*, would reduce *Salmonella* Enteritidis (SE) and improve immune function in young broilers. The objectives of this study were to assess the effects of AM on SE cecal colonization (n = 20/treatment), serum cytokines, IgM, and lipopolysaccharide (LPS) concentrations (n = 10/treatment). Day-of-hatch broilers were distributed across eight floor pens where they were randomly assigned into either challenged or unchallenged groups and given one of four diets. The diets were: 0 kg/MT (control), 1.0 kg/MT, 1.5 kg/MT, and 2.0 kg/MT AM. At seven days of age, chicks were orally gavaged with either 0.5 mL of sterile PBS or 0.5 mL of 1.15×10^7 CFU/mL or 9.70×10^6 CFU/mL of SE for replicate 1 and 2, respectively. Cecal colonization, serum *pro-inflammatory cytokines*- interferon gamma (IFN γ), interleukin-2 (IL-2), IL-6, IL-16, IL-21; *anti-inflammatory/regulatory cytokines*- IFN α , IL-10; *chemokines*- regulated on activation, normal T-cell expressed and secreted (RANTES), macrophage inflammatory protein-1 β (MIP-1 β), MIP-3 α ; *colony-stimulating factors*- macrophage colony-stimulating factor (M-CSF); and *growth factors*- vascular endothelial growth factor (VEGF), IgM, and LPS levels were evaluated. Data were analyzed via a one-way ANOVA with an $\alpha = 0.05$. In replicate one, *Salmonella* was significantly reduced by 0.87 and 0.83 log₁₀ CFU/g of cecal contents in the 1.5 kg/MT and 2.0 kg/MT AM inclusions, respectively. In replicate two, a significant 1.26 log₁₀ CFU/g of cecal contents reduction in SE was observed in the 1.5 kg/MT AM diet. Serum MIP-1 β decreased in the SE challenged 1.0 kg/MT and 2.0 kg/MT diets. Higher concentrations of IgM were confirmed in the unchallenged control diet as compared to the 1.5 kg/MT and 2.0 kg/MT AM diets. No significant differences were detected for any other cytokines or for LPS. Overall, these data suggest that AM at the 1.5 kg/MT and 2.0 kg/MT inclusion could be efficacious in reducing SE.

Key Words: cecal colonization, immune response, poultry, prebiotic mannan-oligosaccharide, *Salmonella* Enteritidis

M51 Effect of dietary ginger root extract (GRE) on intestinal Salmonella colonization in broiler chicks. Ikenna Ekenya^{*GS}, Paul Omaliko, Tunde Ogundare, Odinaka Iwuozo, Oluteru Orimaye, Oluwabunmi Apalowo, Yewande Fasina ^{North Carolina Agricultural and Technical State University}

Salmonella spp. is the predominant bacteria causing foodborne gastroenteritis in humans. Ginger root extract (GRE) could be an alternative to antibiotic use and has antibacterial and immunomodulatory properties. A 3-week isolator cage trial was conducted to determine the efficacy of dietary GRE in reducing intestinal colonization by *Salmonella enteritidis* (SE) in broiler chicks. Day-old Ross 708 male broiler chicks were obtained from a commercial hatchery, and randomly assigned to 6 treatments in a 2 by 3 factorial design. The CON treatment consisted of chicks given corn-soybean meal (SBM) basal without SE challenge. Treatments GRE1 and GRE2 consisted of chicks given a corn-SBM basal diet containing 0.75% and 1.5% GRE respectively, without SE challenge. The CON-SE, GRE1-SE, and GRE2-SE consisted of chicks that were fed diets like those given to CON, GRE1, and GRE2 treatments respectively, and challenged with SE (2.25×10^8 CFU SE/mL) at 2 days of age. Each treatments had 4 replicate pens, with each pen housing 10 chicks. On d 2, 5 and 12 post-challenge (PC), ceca SE was enumerated on xylose lysine tergitol-4

(XLT4) agar supplemented with Nalidixic acid. Differential leukocyte counts (DLC) were measured on d 12PC. Growth performance was also monitored throughout the experiment. Data were analyzed using SAS at a 5% level of significance. Results showed that chicks in CON-SE, GRE1-SE, and GRE2-SE had cecal SE concentrations ranging from 0.57 to 5.94 Log₁₀ CFU/g ceca milieu throughout this study, thereby indicating that SE infection was established in these chicks. Similarly, liver invasion at d5PC showed positive results for all challenge treatment, and negative for unchallenged treatment, describing the virulence of SE. By d 12PC, chicks in GRE2-SE had lower SE count ($P < 0.05$) compared to GRE1-SE. There were no differences ($P > 0.05$) in growth performance parameters among treatments. DLC by d12PC revealed that GRE treatments exhibited innate immunity, while CON and CON-SE treatments exhibited adaptive immunity. It was concluded that GRE at 1.5% level of the diet reduced intestinal SE colonization and enhanced innate immunity in broiler chicks without negatively impacting growth performance.

Key Words: Ginger root extract, Salmonella enteritidis, Ceca colonization, Growth performance, Differential leukocyte count

M52 Assessment of the effects of feed sanitizer Finio® on the horizontal transmission of Salmonella Infantis, performance, and carcass yield in broiler chickens for a period of 35 days Juan Latorre*¹, Raul Rosalino-Marcon¹, Roberto Señas-Cuesta¹, Makenly Coles¹, Guillermo Tellez-Isaias¹, Billy Hargis¹, Callie Selby², Enrique Montiel²
¹University of Arkansas, ²Anitox Corporation

The present study investigates the potential impact of the feed sanitizer Finio® on the horizontal transmission of *Salmonella* Infantis (SI), carcass yield, and performance over a 35-day period in broiler chickens. International trade of poultry and poultry products may require feed to go through extra measures to maintain Salmonella-free status as a food safety measure. In the present study, the feed sanitizer Finio®, a synergistic blend of phytochemicals and carboxylic acids, was included in broiler starter, grower, and finisher diets at an inclusion of 5 lbs/ton. 720-day-old male Cobb-500 broiler chickens were randomly allocated in floor pens (8 replicates/group and 30 chickens/replicate) into one of three groups: 1) Negative control, non-treated feed, and no challenge; 2) Positive control group, non-treated feed, and challenge with SI; 3) Experimental group, feed treated with Finio® and challenge with SI. On the day of hatch, seeder chicks ($n=8$) were orally gavaged with 10⁶ CFU/chick of SI. Horizontal transmission to unchallenged chicks ($n=22$), broiler performance, and processing yield were evaluated at d35 of age. Data from SI (Log CFU/g), performance, and carcass yield were analyzed using ANOVA with SAS in a randomized design. Statistical differences between means were considered at a $P < 0.05$. The incidence of SI was compared using the chi-squared test of independence. Birds fed a Finio® treated diet exhibited statistically ($P < 0.05$) lower CFU and incidence (%) of SI in the ceca on days 14, 28, and 35 of age in comparison to the positive control. Statistical differences in performance and mortality between treatments were not observed. At processing, similar results between treatments were obtained in total meat or breast yield. These data suggest that feed sanitizers can prevent feed source pathogens as well as the horizontal transmission of SI in broiler chickens from d0-35 without affecting overall performance and breast yield. To make chicken production more sustainable and safer, more research is needed to confirm these findings and explore Finio®'s additional applications.

Key Words: Broiler, Feed Sanitizer, Salmonella Infantis, Performance, Carcass yield

M53 Characterizing pathogenicity traits of emerging serogroups of Avian Pathogenic E. coli (APEC) using in vitro and in vivo assays Klao Runcharoon*^{GS}, Julia Lima, Yu Yang Tsai, Catherine Logue
Department of Population Health, College of Veterinary Medicine, University of Georgia

Colibacillosis caused by Avian pathogenic *Escherichia coli* (APEC), is a disease of poultry that results in severe morbidity and mortality leading to significant economic loss worldwide. Currently, there are approximately 188 O groups linked with bacterial antigenicity and pathogenicity. Among APEC, serogroups O1, O2, and O78 are most often implicated in disease. However, our APEC collection from avians diagnosed with colibacillosis in Georgia poultry populations has identified several new emerging serogroups. The aim of this study was to evaluate the pathogenicity level of these emerging serogroups using the serum-resistance assay and embryo lethality assay (ELA) and hypothesizing there would be strains that exhibited higher virulence than others. We selected 10 novel APEC strains from our collection for our analysis including: O15, O25, O45, O62/O68, O86, O88, O91, O115, O152, and O155. The serum resistance assay were performed using 96-well plates and the selected bacterial strains were inoculated into chicken serum. The number of colony-forming units (CFU) was determined at 0 h, 4 h and after 24 hrs incubation at 40°C. The results showed that selected APEC strains exhibited serum resistance with higher concentrations evident after 4 hours of incubation ($p < 0.05$) except for O25. For the ELA, each challenge group consisted of 10, 12-day-old SPF embryonic eggs that were inoculated with the different APEC strains (300-500 CFU/0.1 ml) via the allantoic fluid. Negative control (PBS) and positive control (APEC WT O18) were included. Eggs were candled daily, and deaths were recorded for 6 consecutive days. Isolates causing embryo death of >29% and <10% were considered as virulent, and avirulent. The results showed that all APEC strains were virulent with the highest mortality (100%) caused by serogroups O152 and O145 while the lowest was found for O88 with 50% mortality. Collectively, our 10 APEC novel strains exhibited virulence traits both in serum resistance and ELA and support further investigation of their pathogenicity in birds in order to establish new protection plans against these emerging serogroups.

Key Words: E.coli, poultry, colibacillosis, Embryo lethality assay, serum resistant

M54 Genomic insights into Type VI Secretion System in Avian Pathogenic Escherichia coli Julia Ienes-Lima*^{GS}, Klao Runcharoon, Cierra Dunham, Catherine Logue
University of Georgia

Avian pathogenic *Escherichia coli* (APEC) is one of the most common bacterial pathogens of poultry and causes the disease colibacillosis, leading to morbidity and mortality in avian species. Currently, APEC is considered a significant threat to global poultry production and food security. The Type 6 Secretion System (T6SS) plays an essential role in the pathogenesis of several bacterial species. Specific T6SS genes, including *icmF* and *hcp*, are implicated in APEC adherence and invasion of epithelial cells, as well as its survival within macrophages. Three distinguishable and conserved T6SS loci are characterized in APEC strains, and recent studies suggest they are also involved in APEC virulence. This study aims to analyze the frequency of these three T6SS loci in a collection of APEC genomes of strains isolated from production birds of Georgia. Nineteen APEC genomes were analyzed, and the sequence type (ST) and serotype of each isolate were identified using MLST and SeroTypeFinder, respectively. The T6SS loci were identified through BLAST analysis, using the T6SS sequences from *E. coli* strain ED205 as a reference. To establish a positive presence of specific loci, a query cover and percentage identity threshold of 60% and 70% were set, respectively. Among the APEC genomes analyzed, 15 different O groups, 10 different H groups, and 15 different STs were identified. At least one T6SS locus was observed in eleven (58%) genomes. The prevalence of T6SS1, T6SS2, and T6SS3 loci was 26% ($n=5$), 32% ($n=6$), and 0% ($n=0$), respectively. Analyses are ongoing to identify the prevalence of T6SS genes among T6SS-positive genomes and explore the relationship between T6SS, *E. coli* sequence type (ST),

and serotype. The presence of T6SS loci, particularly T6SS1 and T6SS2, suggests their potential involvement in APEC virulence. In summary, this study contributes to our understanding of the molecular epidemiology of APEC, especially on the prevalence of T6SS loci and genetic diversity among APEC strains. Further investigations will enhance our ability to develop targeted strategies for the prevention and control of colibacillosis in poultry populations.

Key Words: *E. coli*, APEC, T6SS, poultry, virulence

M55 Updates on current understanding of penguin-stance myotendinopathy in broiler breeder males Jason Sousa*^{1GS}, Robin Gilbert², Jenny Nicholds¹, Fred Hoerr³ ¹*Poultry Diagnostic and Research Center, University of Georgia*, ²*Wayne-Sanderson Farms*, ³*Veterinary Diagnostic Pathology*

Penguin-stance myotendinopathy in broiler breeder males has been reported as an emerging issue within the poultry industry, but there is no published description of the condition. In this report we summarize current knowledge of this condition, including cases not yet reported, and discuss current theories regarding the etiology and pathogenesis.

The condition primarily affects broiler breeder males resulting in impaired mobility characterized by a penguin-like posture. Affected breeders are typically 12-22 weeks of age with male mortality in a range from 0.01-6% per flock. Often culled due to lameness, affected birds tend to be easiest to identify during handling for vaccination. Characteristic gross lesions include bilateral hemorrhage associated with tearing of the iliotibialis muscles and fascia, including the aponeurosis. Histopathology reveals muscle fiber necrosis, edema, fibroplasia, and dissociation of tendon collagen in the iliotibialis aponeurosis and possibly other flexor tendons. The lesions are suggestive of biomechanical failure of tendonous tissue and muscle insertion sites. Features of infectious and nutritional causes have not been evident based on clinical presentation and diagnostic testing completed to date.

The condition is observed infrequently in pullets and similar posture changes and gross lesions have been observed in one-day-of-age male chicks. Chicks observed with this posture have gross lesions including subcutaneous edema and hemorrhage and tearing of the iliotibialis muscles and fascia. Histopathologic lesions at the myotendon junction consist of acute myxomatous tendon degeneration; edema, hemorrhage, and granulation tissue formation; and acute degeneration of skeletal muscle.

Gross lesions are typically bilateral, but unilateral lesions have been identified in the leg contralateral to a leg with vaccine reaction. This supports the current theory that biomechanical stress plays a role in the pathogenesis of this condition, with greater stress placed on the leg without vaccine reaction. Within these flocks, birds with bilateral lesions can be found suggestive that the condition is pre-existing and exacerbated by vaccine reaction.

Future work aims to determine the scope of this problem and factors predisposing birds to this condition.

Key Words: bilateral muscle tear, broiler breeders, lameness, myopathy, penguin walking

M56 Meta-analysis of the normal chicken cecal microbiota Matheus Santini*^{1,2UG}, Andrea Pietruska³, Zubair Khalid³, Ruediger Hauck^{1,3} ¹*Department of Poultry Science, Auburn University, Auburn, AL 36849*, ²*Faculty of Veterinary, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil*, ³*Department of Pathobiology, Auburn University, Auburn, AL 36849*

The study of intestinal microbiota of birds is crucial for understanding their impact on animal health and production. To properly assess microbiota variations in chicken populations, it would be useful to establish a representative baseline for what can be considered a "normal" microbiota.

This meta-analysis focused on the cecal microbiota in control groups of chickens, aiming to create this reference. The goal was to enable the identification of significant differences and help the interpretation of results in future experiments.

The criteria for experiments to be included in this meta-analysis were (1) having an untreated control group, (2) Illumina next generation sequencing of the 16S rRNA gene to characterize the microbiota of the ceca, and (3) availability of raw sequence data. For a pilot study, 17 experiments met the requirements. Raw data of control groups was downloaded from the NCBI Sequence Read Archive. The datasets were imported individually into QIIME 2 and processed with standard methods. Merged relative frequency tables, taxonomies and metadata were imported into R and analyzed using the phyloseq package. Investigated parameters were alpha and beta diversity, and characterization of the core microbiota.

Differences in alpha diversity were tested by Kruskal-Wallis test, revealing significant differences between each study, impacting Observed richness, Shannon index, Simpson index, and Pielou's evenness ($P < 0.001$). Significant differences in Pielou's evenness were observed along geographic location ($P = 0.037$) and housing ($P = 0.009$). Spearman correlation test showed a positive correlation of age with all alpha diversity metrics ($P < 0.01$). However, bird type did not significantly influence alpha diversity. PERMANOVA showed significant differences in the beta diversity between experiments, but no significant influence of any of the other tested factors. Analysis of the core taxa revealed that 18 genera, almost all in the class Clostridia, were present in more than 50% of samples in more than 50% of the experiments.

In conclusion, this study provides valuable information about the influence of age, geographic location and housing and their correlation on alpha and beta diversity of the cecal microbiota in chickens.

Key Words: Meta-analysis, Intestinal microbiota, Illumina 16S rRNA, Alpha diversity, Beta diversity

M57 MinION amplicon sequencing rapidly and accurately resolves mixed ARV populations in diagnostic samples Olatunde Oluwayinka*^{1GS}, Erich Linnemann¹, Kelsey Young², James Stanton², Holly Sellers¹ ¹*Department of Population Health, University of Georgia*, ²*Department of Pathology, University of Georgia*

Molecular characterization of avian reovirus (ARV) field isolates relies on PCR amplification of the viral Sigma C (SC) encoding region of the S1 segment followed by Sanger sequencing. However, approximately 20% of all field isolates contain mixed populations of reoviruses. Currently, resolution of mixed populations is based on cloning of the SC amplicons, followed by sequencing of 5 clones. While useful, this process is time-consuming and may not identify all isolates in the mixed population given that only 5 clones are sequenced. In this study, we evaluate a MinION-based amplicon sequencing method (MinION Ampseq) for genotyping mixed populations of ARV isolates from clinical cases. Ten field isolates were evaluated in this study. All isolates were previously characterized by RT-PCR and sequencing. Six out of the ten samples were identified as mixed populations and were cloned and sequenced. Total RNA was extracted from cell culture isolates followed by reverse transcription and PCR amplification using modified sigma C specific primers. PCR products were barcoded for multiplexing, processed into a 1D sequencing library and sequenced using the MinION Mk1b device. The bioinformatics pipeline included reads classification, mapping ARV classified reads to a custom library of SC sequences and consensus building.

MinION Ampseq method identified the same reovirus subpopulations as the cloning-based approach in all 10 cases. In four cases, it detected an additional 5 strains. Pairwise analysis of counterpart consensus nucleotide sequences revealed 95-100% similarity between the results of both methods. Genetic cluster classification for isolates in each case remained consistent regardless of protocol. MiniON-Ampseq-derived consensus

sequences for five strains not detected by cloning had low read numbers (123 to 1048). This suggests that the limitation of the cloning method may be due to sequencing of a small number of clones which diminishes the probability of detecting low-abundance strains. In conclusion, our results

show that the MinION Ampseq protocol was capable of identifying individual reovirus sequences in mixed populations with a sample-to-result turnaround time of 10hrs per 10 cases.

Key Words: MinION, sequencing, reovirus, mixed, populations

Metabolism and Nutrition | Vitamins & Minerals

M58 Effect of organic Zn sources on performance, tibia parameters and gut permeability of broiler chickens to an Eimeria challenge Mohammad Pilevar*, Oluyinka Olukosi *Department of Poultry Science, University of Georgia*

The risk of antibiotic resistance for chicken consumers has pressured the industry to reduce or eliminate chemotherapeutic agents as growth promoters and use alternatives. Coccidiosis is an enteric disease of poultry that hinders growth performance and impairs gut health. In our previous study, ZL increased apparent digestibility and bioavailability compared to inorganic Zn sources. The current experiment investigated the effect of four Zn sources on broiler performance and gut permeability under a coccidiosis challenge. A total of 350 one-day-old chicks (Cobb 500) were randomly allocated to treatments in a 5x2 factorial arrangement of 7 replicates each. Factors were 5 diets: 1) control without supplemental Zn (C); 2) C plus 80 mg/kg Zn sulfate (ZS); 3) C plus 80 mg/kg hydroxychloride Zn (ZH); 4) C plus 80 mg/kg Zn glycine (ZG); 5) C plus 80 mg/kg Zn lysine-glutamate (ZL) with or without challenge. On day 17, half of the birds were orally gavaged with a mixed *Eimeria* solution containing 12,500 *E. maxima*; 12,500 *E. tenella*; 62,500 *E. acervulina* in 1 ml distilled water, and the other half gavaged with 1 ml distilled water. Performance parameters were evaluated during the starter phase (0-10 days) and grower phase (11-23 days). One bird per cage was gavaged with fluorescein isothiocyanate dextran solution (FITC) on day 22 (5dpi), 120 minutes before collection of blood, to evaluate the permeability of the intestinal barrier. On day 23 (6 dpi), one bird per cage was euthanized for tibia parameters. No significant Zn sources x *Eimeria* challenge were observed for performance, tibia ash or weight, or gut permeability. The *Eimeria* challenge reduced the overall weight gain, tibia weight, and ash weight but tended to increase the percentage of bone ash ($P = 0.059$). Of all the Zn sources studied, ZL increased ($P < 0.5$) weight gain compared to ZS in the grower phase. Moreover, Zn sources did not have any effect on gut permeability. In conclusion, the *Eimeria* challenge decreased the proportion of the organic content of the tibia, and supplementation of ZL partly alleviated the negative effects of a mixed *Eimeria* infection on weight gain via mechanisms different from enhancement of gut integrity during the challenge.

Key Words: Zinc, inorganic, organic, Eimeria, performance

M59 Performance and tibia mineralization response of YPM x Ross 708 male broilers subjected to a necrotic enteritis challenge and fed diets containing varying limestone particle sizes and calcium concentrations from 1 to 35 days of age Joseph P. Gulizia*^{1GS}, Rana W. Tabish¹, Jose I. Vargas¹, Jose R. Hernandez¹, Cristina T. Simões¹, Eva G. Guzman¹, Samuel J. Rochell¹, Ruediger Hauck^{1,2}, Matthew A. Bailey¹, William A. Dozier, III¹, Klint W. McCafferty³, Wilmer J. Pacheco¹ *¹Department of Poultry Science, Auburn University, ²Department of Pathobiology, Auburn University, ³USDA-ARS Poultry Research Unit, Mississippi State*

This study evaluated the effect of limestone particle size (LPS) and calcium (Ca) concentrations on broiler performance and tibia mineralization from 1 to 35 d of age. In total, 2,100-d-old male broilers (YPM x Ross 708) were distributed into 70 floor pens. Each pen was assigned to 1 of 7 treatments (10 replicates/treatment). This experiment was a 2 x 3 + 1 factorial arrangement including 2 LPS (200 and 910 μm) and 3 Ca concentrations (adequate, reduced, low). For each growth phase, dietary Ca was a two-step, 10-point reduction from the adequate Ca concentration (Starter: 0.95%; Grower: 0.85%; Finisher: 0.75%). The 6 factorial treatments were

subjected to a necrotic enteritis (NE) challenge, while the + 1 treatment served as an unchallenged control with the diet containing 200 μm LPS and adequate Ca at each feeding phase. Feed intake, BW, and FCR were determined at 17, 21, 26, and 35 d of age. Left tibias (4 birds/pen) were excised (d 21 and 35) to determine weight and shear strength. Data were analyzed as a 2 x 3 (LPS x Ca concentration) factorial arrangement using the GLIMMIX procedure of SAS. Means were separated using PDIFF with significance considered at $P \leq 0.05$. The NE challenge reduced BW and increased FCR but did not impact mortality ($P < 0.05$). Performance and tibia mineralization were not altered by LPS ($P > 0.05$). However, broilers fed diets with adequate and reduced Ca had higher cumulative BW compared to broilers fed diets with low Ca ($P < 0.05$). Day 1 to 35 FCR was 3 points lower when broilers were fed a diet with adequate and reduced Ca compared to low Ca ($P < 0.05$). There was a LPS x Ca concentration interaction for 1 to 35 d FCR ($P < 0.05$). Increasing LPS from 200 to 910 μm decreased 1 to 35 d FCR in the adequate Ca group, whereas FCR increased in the low Ca group as LPS increased from 200 to 910 μm . Broilers fed a diet with low Ca had a lower d 21 and 35 tibia weight and shear strength compared to broilers fed adequate and reduced Ca diets ($P < 0.05$). This study demonstrated that reducing dietary Ca concentrations by 0.10% from the adequate Ca can maintain broiler performance and tibia mineralization during a NE challenge. However, this outcome will be influenced by LPS and needs to be further considered when formulating broiler diets.

Key Words: limestone, particle size, calcium, necrotic enteritis, broiler

M60 Influence of different calcium levels and limestone particle sizes on the intestinal microbiome in broilers upon subclinical necrotic enteritis challenge Rana Waqar Tabish*^{1GS}, Joseph Gulizia¹, Jose Vargas¹, Jose Hernandez¹, Cristina Simões², Eva Guzman¹, Wilmer Pacheco¹, Samuel Rochell¹, Matthew Bailey¹, William Dozier¹, Klint McCafferty³, Ruediger Hauck^{1,4} *¹Department of Poultry Science, Auburn University, ²Federal University of Santa Maria, ³USDA-ARS Poultry Research Unit, ⁴Department of Pathobiology, Auburn University*

High levels of readily soluble calcium in poultry diets can increase the incidence of necrotic enteritis (NE) by favoring the growth of *Clostridium perfringens*. The aim of this study was to assess the impact of different dietary calcium concentrations and limestone particle sizes on the intestinal microbiome of broilers during a subclinical NE challenge. The study followed a 3 x 2 + 1 factorial design with 6 treatments subjected to a subclinical NE challenge and an unchallenged control. Each treatment had 10 replicates with 30 birds per pen. Main effects included calcium concentrations (Starter: 0.95, 0.85, and 0.75%; Grower: 0.85, 0.75, and 0.65%; Finisher: 0.75, 0.65, and 0.55%; standard, reduced, and low) and limestone particle size (910 and 200 μm ; coarse and fine). The unchallenged control was fed a diet containing standard calcium concentrations and 200 μm limestone particle size. The challenged treatments were gavaged with a tenfold dose of a live coccidia vaccine on day 14 and 10⁸ CFU *Clostridium perfringens* on day 18. Jejunal content was sampled from 10 birds per treatment on day 21. The intestinal microbiome was investigated by 16S rRNA next-generation amplicon sequencing and analyzed using the Qiime2 pipeline. Faith phylogenetic diversity (PD) was higher ($P < 0.05$, Kruskal Wallis test) in the group receiving standard calcium concentrations compared to groups receiving reduced or low calcium concentrations. Additionally, the unchallenged control had a higher ($P < 0.05$) Faith PD than the group re-

ceiving reduced calcium concentration with coarse limestone particles. In terms of beta-diversity, as indicated by Bray-Curtis and UniFrac distances, the groups receiving low or reduced calcium with fine limestone particles diverged ($P < 0.05$, PERMANOVA) from the unchallenged control group. Moreover, the group receiving standard calcium with coarse particle size displayed a significant distinction from the unchallenged control. These findings were consistently supported by clustering patterns observed in Principal Coordinate Analysis-generated emperor plots. Conclusively, different calcium concentrations and limestone particle sizes had more pronounced effects on alpha- and beta-diversity than the NE challenge itself.

Key Words: calcium, limestone, particle size, *Clostridium perfringens*, broilers

M61 The use of PolyTransport technology to reduce the bioavailability of iron to enteric pathogens Jessica Brown^{*IGS}, Ashley Tarcin¹, Rachel Ganske¹, Joshua Jendza², Eric Neeno-Eckwall³, Jessica Hite³, Steven Ricke¹ ¹*Meat Science and Animal Biologics Discovery, Department of Animal and Dairy Sciences, University of Wisconsin-Madison*, ²*Qualitech Inc.*, ³*School of Veterinary Medicine, Department of Pathobiological Sciences, University of Wisconsin-Madison*

Dietary supplementation with iron is routine in the poultry industry. However, supplemental iron not only supports the host but also the microbial community within the gut. This can lead to an internal competition between host and gut pathogens for bioavailable iron sources. Iron also plays a role in virulence regulation in intestinal pathogens, magnifying the significance of this competition. The objective of this study was to evaluate the ability of the PolyTransport technology in SQM iron, a metal polysaccharide complex, to reduce the bioavailability of iron to *Salmonella* and *E. coli* in an *in vitro* system. Nine *Salmonella* and four *E. coli* were grown as individual overnight cultures. A 96-well plate was prepared with the following treatments: (1) 2, (2) 5, (3) 10ppm SQM w/o Fe, (4) 2, (5) 5, (6) 10ppm SQM, (7) 2, (8) 5, (9) 10ppm FeSO₄, (10) -CTRL, (11) +CTRL. Treatments were diluted in sterile water and combined (1:1) with 2× LB broth, chelated (1-11) with 100ppm 2,2-dipyridyl or unchelated (12). A pin replicator was used to inoculate the treatment plate before being incubated for 24 h at 37°C in a TECAN Infinite 200 Pro with OD measurements taken at 600nm every 10 min. Growth rates were calculated in R Studio using growthrates linear fit function. Three independent replicates were performed for each strain and data was analyzed in a linear mixed effect model with means separated by Sidak ($P \leq 0.05$). In four of nine *Salmonella* strains, the 10ppm SQM treatment reduced the rate of growth compared to the 10ppm FeSO₄ treatment (0.46-0.57 and 0.57-0.67 lnOD/h; $P < 0.05$). While significant effects were not observed in all *Salmonella* strains, numerically, the growth rates observed for the SQM treatments were consistently lower than those in the FeSO₄ equivalent. *E. coli* was less impacted by the PolyTransport technology, as three of the four strains did not exhibit growth rate differences between SQM and FeSO₄ treatment ($P > 0.05$). The SQM matrix itself did not appear to have any innate antimicrobial properties beyond the restriction of iron. It can be concluded that the PolyTransport technology can restrict iron bioavailability to pathogenic bacteria within an *in vitro* system; however, growth rates and efficacy may vary due to iron dependency and sequestration systems.

Key Words: Salmonella, E. coli, Iron, Nutrient Bioavailability

M62 Starter diet non-phytate phosphorus concentration and research facility do not influence regression-derived ileal phosphorus digestibility of soybean meal for broiler chickens Vitor Santos Haetinger^{*IGS}, Jung Sung¹, Sunday Adedokun², William Dozier³, Carl Parsons⁴, Markus Rodehutsord⁵, Olayiwola Adeola¹ ¹*Purdue University*, ²*University of Kentucky*, ³*Auburn University*, ⁴*University of Illinois at Urbana-Champaign*, ⁵*University of Hohenheim*

A study was conducted across 4 institutions to evaluate the effects of starter diet non-phytate phosphorus (nPP) concentration on subsequent

phosphorus digestibility, and the consistency of a protocol for the determination of ileal phosphorus (P) digestibility of soybean meal (SBM) using the regression analysis. Ross 308 broiler chickens received a starter diet with 3.5 or 4.5 g nPP/kg (96 cages per diet, 8 broilers per cage) for 18 days. Subsequently, birds from each starter diet were allocated to 3 semi-purified diets in which dietary total Ca: total P was maintained at 1.3:1.0 and formulated with 400, 510, or 620 g SBM/kg (32 cages per diet, 6 broilers per cage) until ileal digesta collection on day 21. Statistical analysis was conducted as a randomized complete block design in split plot analysis with whole plot for 2 starter diets and subplot for the 3 grower diets. In addition, regression analysis of digestible P with P concentration in diet was conducted for institution and starter diet. Institution affected ($P < 0.05$) all growth performance criteria in the starter and grower phases; and birds that received the lower nPP starter diet tended ($P < 0.10$) to have lower body weight gain and feed intake in the starter period and, presented lower ($P < 0.05$) body weight gain and feed intake in grower period. The regression-derived ileal P digestibility of SBM was similar regardless of starter diet nPP or institution, ranging from 44 to 49% in the 3.5 g nPP/kg starter, and from 40 to 44% in the 4.5 g nPP/kg starter (SE = 3.07), with pooled digestibility values of 46.0 and 42.0% for 3.5 and 4.5 g/kg nPP groups (SE = 2.94), respectively. In conclusion, the regression-derived P digestibility of SBM determined for the different nPP concentration starter diets or experimental institutions were not different. Because the protocol employed resulted in regression-derived digestibility of P that is not different across institutions, the protocol is recommended for studies to determine digestible P content of feed ingredients.

Key Words: digestibility, phosphorus, phytate, regression, soybean meal

M63 Standardized ileal digestibility of calcium and phosphorus in inorganic sources for broilers Chae Won Lee^{*GS}, Su Hyun An, June Hyeok Yoon, Inho Cho, Yun Ji Jeon, Changsu Kong *Kyungpook National University*

This study aimed to determine the standardized ileal digestibility (SID) of calcium (Ca) and phosphorus (P) in various feed ingredients using the direct method and to assess the potential influence of sex on SID for Ca and P. To estimate basal endogenous losses (BEL) of Ca and P, a Ca-P-free diet was formulated. Three other semi-purified diets were prepared, each containing monocalcium phosphate (MCP), dicalcium phosphate (DCP), or monosodium phosphate (MSP), with limestone as the sole source of Ca, P, or both Ca and P. These diets provided 4.21 g/kg of non-phytate P from MCP, DCP, or MSP, and the MSP with limestone diet included 7.5 g/kg of Ca. All diets included 5 g/kg of chromic oxide as an indigestible index. On day 18, 256 male and female broilers were individually weighed and randomly assigned to four treatments, each with four replicates for each sex (8 birds per cage). This allocation followed a randomized complete block design based on body weight. On day 21, the birds were euthanized using carbon dioxide, and ileal digesta samples were collected from the distal two-thirds section of the ileum. The SID of Ca and P were calculated by adjusting apparent ileal digestibility for BEL. Data analysis utilized the SAS MIXED procedure, with experimental diets and sex treated as fixed variables and the block considered as a random variable. The cage served as the experimental unit, and statistical significance was accepted at $P < 0.05$. Means were differentiated using Tukey's test. No significant interaction between experimental diets and sex regarding the SID of Ca or P was found, and no effect of sex on SID was observed. BEL values for Ca and P were determined to be 58.98 and 66.37 mg/kg of dry matter intake, respectively. The standardized ileal Ca digestibility of MCP, DCP, and limestone was found to be 84.8%, 70.1%, and 52.6%, respectively, while the standardized ileal P digestibility of MCP, DCP, and MSP was determined to be 91.7%, 76.8%, and 94.4%, respectively. In conclusion, this study demonstrates that sex does not affect the SID of Ca or P. Given the variation in digestibility of Ca and P among different feed ingredients,

consideration of the specific type of feed ingredients used in formulation is crucial.

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

M64 Availability of iron from commercial limestone and dicalcium phosphate for broilers Julmar Feijo^{*IGS}, Sergio Vieira¹, Douglas Maria¹, Raquel Horn¹, Walter Altevogt¹, Samantha Silveira¹, Brenda Nicola¹, André Favero² ¹Universidade Federal do Rio Grande do Sul, ²Santa Livia Farm

Iron (Fe) is routinely supplemented in broiler diets aiming to prevent dietary deficiencies. Limestone and dicalcium phosphates are the richest sources of Fe in poultry feeds; however, its availability from these ingredients is unknown. The present research was conducted with the objective of evaluating the contribution of limestone and dicalcium phosphate as sources of Fe for broilers. A total of 576 one-day-old male Cobb x Cobb 500 were placed into battery cages (0.9 x 0.4 m²) and allocated in 6 treatments with 12 replicates of 8 chicks each. Chicks were fed a corn-soy Fe depletion diet (58.2 ± 2.4 mg/kg Fe) from placement to 7 d. At d 8, birds were randomly distributed into dietary treatments having gradual increases of Fe (obtained by replacing lab grade calcium carbonate and phosphoric acid by commercial type limestone and dicalcium phosphate). Feeds did not have animal by-products and contained nutrient and energy as usual in broiler integrations. Treatments with gradual increases of Fe had 55.5, 88.5, 121.5, 154.5, 187.5, 220.5 mg/kg Fe formulated, corresponding to 57.6 ± 2.1, 92.0 ± 2.3, 124.1 ± 2.7, 159.3 ± 3.1, 187.2 ± 3.2, 223.7 ± 3.6 mg/kg analyzed). No effects were found for dietary Fe on live performance, hematocrit and hemoglobin at d 28 (P > 0.05). Increasing dietary Fe from limestone and dicalcium phosphate at expense of calcium carbonate and phosphoric acid led to a linear reduction in the percent ileal digestible (Y = 13.0788 - 0.0461x, R² = 0.8140, P < 0.001), however, as total dietary Fe increased by adding limestone and dicalcium phosphate, an increase in Fe retention per bird was observed (Y = 15.1779 - 0.0503x, R² = 0.7293, P < 0.001). Serum ferritin and Fe content in livers indicated some utilization from Fe originated from the two rock based feed ingredients. It is concluded that Fe from limestone and dicalcium phosphate can be partially utilized by birds. It is estimated that each increase of 100 mg/kg from limestone and dicalcium phosphate leads to increments in retention and serum ferritin were of 0.5 mg/bird and 7.0 ng/ml, respectively.

Key Words: broilers, iron, limestone, dicalcium phosphates, retention

M65 The effects of a natural source of vitamin D (Trisetum flavences) on broiler breeder production and offspring performance Raquel Horn^{*IGS}, Sergio Vieira¹, Dimitri Freitas², Douglas Maria¹, Julmar Feijó¹, Walter Altevogt¹, Samantha Silveira¹, Brenda Nicola¹, André Favero³ ¹Universidade Federal do Rio Grande do Sul, ²Phytobiotics Brazil, ³Santa Livia Farm

A natural source of vitamin D has been marketed in several countries throughout Latin America (Active D[®], produced from *Trisetum flavences*). The present research was conducted using a natural source of vitamin D (*Trisetum flavences*) as a dietary supplement for broiler breeders. Evaluation was done on breeder performance. Three hundred and twenty Cobb 500 breeder hens and 32 Cobb broiler breeder males, both 41 wk of age, were randomly allocated in 16 pens (2.0 x 2.5 m). Hens were initially fed pre-starter layer feed from 41 to 42 wks of age and then fed the experimental diets from 43 to 62 wks (both formulated with corn, soybean meal and wheat bran). Treatments were two with 8 replications of 20 hens and two males each, which consisted of a commercial type breeder hen diet (total vitamin D from cholecalciferol 2,000 UI) or the same diet but supplemented with 200 g/ton of *Trisetum flavences*. Eggs were daily collected, and all hatchable eggs laid to 62 wks were weighed previously to incubation. Hatching male chicks were allocated into 16 floor pens (1.65 x 1.65m) in two groups corresponding to the feeds provided to the breeders. Replications per treatment were 8 totaling one hundred ninety-two

birds. Live performance was evaluated weekly until day 21. Data were submitted to an ANOVA using the PROC MIXED and PROC GLM of SAS (2013). The *Trisetum flavences* source vitamin D supplement diet fed to breeders did not affect egg production, settable and cracked eggs (P > 0.05). However, it demonstrated superior outcomes (P ≤ 0.05) in terms of fertility, overall hatchability, and lower numbers of contaminated eggs (97.8%, 82.1% and 0.99%, respectively). For the offspring performance, no differences in FCR were found between the dietary treatments (P > 0.05), however, BWG was improved when chicks originated from hens that were fed the supplementation with 200 g/ton of *Trisetum flavences* source vitamin D at 7 days and 14 days (P ≤ 0.05). It is concluded that *Trisetum flavences* is an effective alternative natural source of vitamin D for broiler breeders.”

Key Words: Natural source, *Trisetum flavences*, Broiler breeder, Offspring, Performance

M66 Evaluation of aragonite as a calcium source in laying hen diets Alyssa Lyons^{*IGS}, Steve Dowd², Jon Hart², M Persia¹ ¹Virginia Tech, ²Ag Source LLC

Experiments were conducted to determine the bioavailability of fine ground aragonite (FGA) using 0-14 d old broilers and to determine the use of FGA on performance, eggshell quality, and tibia ash (TA) of laying hens from 28-56 wks. In experiment 1, 450 as hatched Ross 708 broilers were fed a basal diet without either LS or FGA containing 0.35% Ca and LS and FGA additions resulting in 0.43, 0.51, 0.59, and 0.67% Ca. Body weight gain, feed intake (FI) and feed efficiency (FE) were measured 0 to 14 d after which all remaining chicks were euthanized for TA. Slope ratio analysis was conducted and the relative bioavailability of FGA to LS using TA was 179%. In experiment 2, 180 Hy-Line W-36 laying hens were fed diets containing LS or FGA at 2.46, 3.28, or 4.10% Ca, corresponding to 60, 80 and 100% of breeder recommendations from 28-56 wks. The experiment was set up as a 2 x 3 factorial with Ca source and concentration, resulting in 6 treatments of 10 cages of 3 laying hens. Hen-day egg production (HDEP), egg weight (EW), egg mass (EM), FI, and FE were calculated over two wk periods. Body weights were measured and eggs collected every four wks to determine shell thickness, relative weight, breaking force, and egg specific gravity. Data were analyzed as a factorial with repeated measures in SAS (P ≤ 0.05) and means were separated using Tukey's HSD test and TA was analyzed in JMP Pro 16.0 (P ≤ 0.10). Interactions occurred for HDEP, EW, EM, and FE between Ca concentration and source. In general, 100% LS resulted in the lowest performance among the treatments. Hens fed 100% LS (568 g/kg) had the lowest FE compared to 80 and 100% FGA (583 and 584 g/kg; P ≤ 0.01), with other treatments being intermediate. There was an interaction between Ca concentration and source for TA where all FGA treatments maximized TA, however, TA increased in a dose dependent manner for LS (P = 0.07) suggesting that the lower concentrations of limestone were insufficient to maintain egg production and TA over time. Limestone increased shell quality for all parameters measured (P ≤ 0.05). These data indicate that FGA may be used as a higher available alternative Ca source in laying hens based on performance, but more understanding of how FGA is used for eggshell formation is needed.

Key Words: limestone, aragonite, bioavailability, laying hen

Metabolism and Nutrition II Amino Acids

M67 Assessing Ross 708 broiler responses to practical adjustments of dietary valine and isoleucine in diets moderately increased in leucine Benjamin Angel^{*IGS}, Kenneth Nelson¹, Savannah Wells-Crafton¹, Mathews Costa¹, Mitchell Vaught¹, Chance Williams², Jason Lee³, Michael Kidd¹ ¹University of Arkansas, ²Wayne-Sanderson, ³CJ America-Bio

An experiment was conducted to assess the need of Val and Ile formulation increases in diets with increased Leu via high-protein dried distillers grains in late feed phases. Male Ross 708 broiler chicks (1,728) were placed in floor pens, 12 birds/pen, measuring 3' x 4' ft with fresh pine wood shavings. Birds were raised in a commercial designed facility with solid walls and tunnel ventilation. Common diets were fed *ad libitum* to birds from 0-14 d (crumble form) and 14 to 28 d (pellet form) of age. Two treatment diets (1.40 and 1.60 Leu:Lys) in two feed phases (28 to 38 and 39 to 49 d of age) were created to blend three levels of both Val and Ile. From 28 to 38 d of age Val:Lys and Ile:Lys were 0.77, 0.80, and 0.83 and 0.66, 0.69, and 0.72, respectively. From 39 to 49 d of age, Val:Lys and Ile:Lys were 0.78, 0.81, and 0.84 and 0.67, 0.70, and 0.73, respectively. The resultant pelleted 18 treatments (2 x 3 x 3 factorial) were replicated 7 times and two additional treatments (one treatment with increased Leu to 1.60 via L-Leu and one treatment with increased Arg:Lys (1.08 to 1.28) via L-Arg in the high Leu:Lys) were replicated 9 times. Pen density was reduced to 10 birds per pen at d 28. Live performance was assessed from 28 to 49 d of age and processing was assessed on 576 birds thereafter. Data was analyzed using JMP Pro 17 with statistical significance set to $P \leq 0.05$. Significant interactions or main effects did not occur. Assessing Leu in late finishing Ross broilers can be accomplished with corn-based dried distillers grains of L-Leu. Increasing the dietary Arg:Lys ratio from 1.08 to 1.28 did not improve performance or yields. Corn and corn co-products fed to finishing Ross 708 broilers resulting in a 20 point increase in the Leu:Lys (up to 1.60) does not appear to warrant formulation adjustments in Val or Ile.

Key Words: Nutrition, Isoleucine, Valine, Leucine, Broiler

M68 Dietary arginine responses of Ross 708 broilers reared under cyclic elevated temperatures. Annalise Anderson^{*IGS}, Jason Lee², Roshan Adhikari², Samuel Rochell¹ ¹Department of Poultry Science, Auburn University, ²CJ America, Inc.

The objective of this study was to evaluate the effects of dietary arginine level on performance and processing characteristics of broilers subjected to a cyclic heat stress (HS) challenge model. A total of 1,200 male Ross 708 broiler chicks were fed common starter (0-17 d) and grower (17-27 d) diets before being fed 1 of 6 target dietary digestible Arg:Lys ratios (80, 92, 104, 116, 128, and 140) across both finisher 1 (27-38 d) and finisher 2 (38-46 d) phases. Birds were reared in floor pens and treatments were replicated by 8 pens of 25 birds. At placement, barn temperature was set to 32°C and decreased to maintain bird comfort until initiation of the HS challenge at d 28. From 28-47 d, barn temperature was maintained at 32°C for 12 h daily (7:30 h to 19:30 h) and reduced to 24°C each night. At 32, 39, and 46 d, cloacal temperatures were measured in 2 birds per pen at approximately 06:30 (thermoneutral) and 2:30 (HS). At 48 d, 8 birds per pen were processed and deboned to determine carcass and parts weights and yields. Data were analyzed by one-way ANOVA with pen location as a random blocking variable. Linear and quadratic contrasts of Arg:Lys ratio were also performed. Statistical significance was determined at $P \leq 0.05$. Feed conversion ratio improved linearly with increasing Arg:Lys ratio from both 27-38 d ($P = 0.001$) and 27-46 d ($P = 0.012$), but no effects were observed for body weight gain or feed intake ($P > 0.05$). Cloacal temperatures were approximately 1.6°C higher during HS than during the thermoneutral period at each time point, and were influenced by dietary Arg. At 32 and 39 d, cloacal temperatures during HS decreased quadratically ($P = 0.022$ and 0.021 , respectively), while 46 d temperatures decreased linearly ($P = 0.011$) as Arg:Lys ratio increased. For processing

characteristics, there were linear increases ($P < 0.05$) in chilled carcass and tender weights and yields with increasing Arg, with quadratic responses ($P < 0.05$) observed for breast fillet and total breast weights and yields. Overall, the results of this study indicate that in addition to improving feed efficiency and 48 d processing yields, dietary Arg may also influence core body temperature in a dose dependent manner in Ross broilers subjected to a cyclic HS challenge.

Key Words: Arginine, Cyclic heat stress, Broiler, Processing characteristics, Cloacal temperature

M69 Influence on the hypothalamic expression of feed intake control genes and jejunal morphology in broiler chickens receiving increasing levels of digestible lysine and branched-chain amino acids. Adeleye Ajao^{*GS}, Oluyinka Olukosi *University of Georgia*

Lysine and BCAA (branched-chain amino acids) are essential for poultry nutrition, with comparable roles in animal physiology. In parallel, leptin and adiponectin regulate satiety, while ghrelin influences appetite, collectively orchestrating a complex interplay in the regulation of poultry feeding behavior and metabolism. The hypothesis of this study was that Lys and BCAA would influence these feed intake control centers in a similar way, thus affecting weight gain. The objective was to compare the impact of increased digestible levels of Lys and BCAA ratio on the expression of amino acid (AA)-mediated neuropeptide hormones controlling satiety and appetite, nutrient transporters, and histomorphology indices in broiler chickens. A total of 450 Cobb 500 male broiler chicks (off-sex) were distributed into 30-floor pens using a randomized complete block design, with 15 birds per pen on day 0. All chicks received the same starter diet (220g/kg, 3000 kcal/kg AME) during the initial 8 days. Subsequently, for the grower phase (day 8 to 28), they were provided with five experimental diets (D). D1 met the recommended AA levels. D2 and D3 were similar to D1 but had Lys levels increased by 15% and 30% above the requirement, respectively. D4 and D5 were similar to D1 but had BCAA added at 15% and 30% above the requirement, respectively. On day 21, the jejunum and hypothalamus were collected from one bird per cage and snap-frozen in liquid nitrogen prior to RNA extraction and quantitative RT-PCR assays. The villus height (VH) and crypt depth (CD) were measured in jejunal tissues. There were no significant treatment effects on VH, CD, or VH:CD. Additionally, there were no significant treatment effects on the expression of neuropeptide hormones such as adiponectin, ghrelin, and leptin. The mRNA expression of peptide and AA transporters measured showed no significant differences ($P > 0.05$) among the dietary groups. Overall, increased levels of digestible Lys or BCAA produced similar feeding patterns, as observed in the feed intake measurements and enhanced nutrient utilization, indicating a comparable proteinogenic value for both amino acids under the condition of the study.

Key Words: LYSINE, BCAA, GHRELIN, LEPTIN, HISTOMORPHOLOGY

M70 Effects of the different ratios of branched chain amino acids (BCAAs) on growth performance, meat quality, and intestinal health in broilers fed with low and high crude protein diet. Doyun Goo^{*IGS}, Amit Singh¹, Janghan Choi², Milan Sharma¹, Deependra Paneru¹, Jihwan Lee¹, Woo Kim¹ ¹Department of Poultry Science, University of Georgia, ²United States National Poultry Research Center, USDA-ARS

The branched-chain amino acids (BCAA), including leucine (Leu), valine (Val), and isoleucine (Ile), are indispensable amino acids with similar structures, and impact poultry growth, intestinal health, and immunity. In general, poultry feed contains more Leu than Val and Ile. However, when chickens are fed low crude protein (CP) diets, there can be an imbalance in BCAAs, negatively affecting growth performance. Therefore, we aimed to investigate how different BCAA ratios in both low and high CP diets in-

fluence growth performance, meat quality, and intestinal health in broilers. The study was conducted using a completely randomized design, employing a 4×2 factorial arrangement. A total of 1,280 0-d-old male Cobb 500 broilers were allotted to 8 groups, each with 8 replicates, and raised until 42 days. The four different BCAA ratios were as follows: 1) Low Leu + high Val/Ile; 2) Normal BCAAs with soybean meal (SBM) without wheat middling (WM); 3) Normal BCAAs with WM; and 4) High Leu + Low Val/Ile. The two different dietary CP groups were: 1) Low CP (Starter, 20.5%; Grower, 18.5%; and Finisher, 17.5%); 2) High CP (Starter, 22.5%; Grower, 20.5%; and Finisher, 19.5%). All data were subjected to statistical analysis using 2-way ANOVA, and Tukey's HSD test or Student's t-test was applied if the $P < 0.05$. The Low CP group significantly increased body weight gain (BWG), feed intake (FI), carcass weight, and relative pectoralis major weight when compared to the High CP group ($P < 0.001$). The High Leu + Low Val/Ile group significantly decreased BWG, FI, feed efficiency, carcass weight, and relative pectoralis major/minor weight when compared to the other BCAA ratio groups ($P < 0.001$). The Normal BCAAs with SBM group significantly increased BWG and feed efficiency when compared to the Normal BCAAs with WM group ($P < 0.001$). No significant differences were observed in intestinal morphology and microbiota analysis among all the treatments. In conclusion, maintaining a well-balanced BCAAs ratio based on SBM without WM has a positive effect on chicken growth, and a marginally-low CP diet can also help improve growth performance with the current BCAA ratio. Further studies are necessary to determine optimal BCAA balance when the chickens are fed high SBM/protein diets.

Key Words: BCAA, Broiler, Crude protein level, Growth performance, Soybean meal

M71 Effects of varying branched-chain amino acid ratio using corn gluten meal, L-isoleucine, and L-valine on 0-21d turkey poul performance. Emily Estanich^{*1GS}, Kristina Bowen¹, Lucas Knarr¹, Elizabeth Lynch¹, Sally Noll², Arturo Garcia Morales³, Joseph Moritz¹ ¹West Virginia University, ²University of Minnesota, ³Butterball, LLC

Providing amino acids (AA) in concentrations that optimize production performance is critical to turkey production. The branched-chain amino acids (BCAA) leucine (Leu), isoleucine (Ile), and valine (Val) are essential AA needed for protein synthesis and energy production, in addition to performing anabolic signaling functions via activation of the mechanistic target of rapamycin (mTOR) pathway. Due to the similarity of chemical structure among the three BCAA, imbalanced dietary ratios can lead to metabolic competition and enzymatic degradation of limiting BCAA, particularly in diets with excess Leu. Concentrated corn protein ingredients such as corn gluten meal (CG) contain high levels of Leu and thus can alter BCAA requirement; however, L-Ile and L-Val may be added to balance BCAA ratios. The objective of this study was to evaluate the effects of manipulating BCAA ratios in diets using CG, L-Ile, and L-Val on live performance of Nicholas Select turkey hens from 0-21d of age. Using a 38.4% corn and 49.8% soybean meal-based diet as the Control, five treatments were created to model the impact of BCAA ratio: Low CG (5.5%), High CG (11%), High CG + Val, High CG + Ile, and High CG + Val + Ile. Formulated Leu:Lys ratios were 1.30, 1.43, 1.60, 1.58, 1.60, and 1.58, respectively. Diets were fed to 15 replicate cages of 8 poults from 0-3d and 6 poults from 3-21d, utilizing a randomized complete block design. Performance was measured on day 14 and 21 and analyzed using a one-way ANOVA followed by Fisher's LSD test where appropriate ($\alpha=0.05$). Performance data at day 14 and 21 indicated that High CG decreased feed intake, bird weight, live weight gain, and increased feed conversion ratio relative to the Control ($P<0.05$). Additions of L-Ile and L-Val produced performance equivalent to the Control ($P<0.05$) at d14 and decreased feed conversion ratio relative to the Control ($P<0.05$) at d21. These results suggest that starter diets containing high Leu from a concentrated corn protein ingredient can decrease hen performance but may be restored with additions of L-Iso and L-Val. However, in practical settings, nutritionists

should assess the costs associated with BCAA supplementation versus diets with high inclusions of soybean meal.

Key Words: branched-chain amino acids, turkey production, corn gluten meal

M72 Understanding the branched-chain amino acid interactions in Hy-Line W-36 layer pullets during 6-12 weeks of age Charis Waters^{*1GS}, Roshan Adhikari², Jason Lee², Ruben Kriseldi³, Michael Elliot⁴, Kelley Wamsley¹, Pratima Adhikari¹ ¹Mississippi State University, ²CJ Bio America, ³Aviagen, ⁴A&E Nutrition Services, LLC

The objective of this study was to investigate the relationship between branched-chain amino acids (BCAA) on the performance and bone quality of Hy-Line W-36 layer pullets during the grower phase, 6-12 weeks of age. A total of 2,160 6-week-old pullets were randomly placed into 120 brooder pens according to a 2^3 full factorial central composite design (CCD) with 20 treatments (14 treatments and 6 center points). Each treatment consisted of varying digestible Val:Lys (65 to 95), Ile:Lys (58 to 88), and Leu:Lys (120 to 220) ratios; digestible Lys was formulated at 0.88% per the Hy-Line management guide. Feed was weighed weekly to determine feed intake (FI) and birds were weighed at 6 and 12 weeks of age to determine body weight gain (BWG), average daily gain (ADG), and feed conversion ratio (FCR). At 12 weeks of age, one bird per pen (6 birds/trt), was randomly selected and euthanized, and the right tibia and femur were removed to evaluate the bone quality. Data were analyzed as a CCD using the surface response option of JMP v. 15. Body weight gain (546.72 g; $P=0.0582$; $R^2=0.72$) and ADG (13.01 g; $P=0.0584$; $R^2=0.72$) were optimized at the highest Val:Lys (95), Ile:Lys (88), and Leu:Lys (220) ratios. However, when Val:Lys was reduced at the highest Ile:Lys and Leu:Lys, BWG and ADG decreased. Feed intake during week 6 of the study (49.41 g; $P=0.0687$; $R^2=0.71$) was optimized at the lowest Val:Lys (65) ratio. Moreover, a trend toward significance was observed for FI during week 3 of the study (46.49 g; $P=0.1120$; $R^2=0.67$) in which the lowest Val:Lys (65) ratio showed the most improvement. A trend toward significance was also observed for FCR (0.49; $P=0.1587$; $R^2=0.64$) which was optimized at the lowest Ile:Lys (58) ratio and the highest ratios of Val:Lys (95) and Leu:Lys (220). Dietary BCAA had little effect on altering bone ash and bone-breaking strength ($P > 0.10$). Results indicate that performance metrics such as BWG, ADG, and FCR can be maximized in diets with high Leu:Lys (220) and Val:Lys (95) ratios. Adjusting Leu levels could influence the Val and Ile needed to achieve max BWG and ADG. In addition, low Val levels increased FI, this could be due to deficient levels causing birds to increase FI to meet their requirement.

Key Words: branched-chain amino acid, pullets, surface response, performance, bone quality

M73 Precision nutrition: The value of considering the energy, amino acid content, and digestibility values of soybean meals in feed formulation. Thiago H. Yabuta, Edgar Oviedo-Rondón* *Prestage Department of Poultry Science, North Carolina State University*

Precision nutrition depends on using accurate nutritional information in feed formulation. Soybean meals (SBM), the primary amino acid (AA) source vary by country of origin influencing diet precision. Results from NIRS may be beneficial in describing the nutrient value of SBM sources. This study evaluated the differential feed cost, and relative value of SBM from three origins: Argentina (ARG), Brazil (BRA), and the United States (USA). The global database from NIRS Precision Nutrition Evaluation (PNE) from Adisseo[®] was used to assess differences in proximate composition, AMEn, AA content and digestibility. The PNE use direct calibration with *in vivo* of experiments to estimate AMEn and AA digestibility. Information from two harvest (2020 and 2021) were used for least-cost feed formulation (Concept 5.0[®]). Starter, grower, and finisher broiler diets were formulated following Aviagen (2022) nutrient recommendations. Diets for two production phases of Brown and White W-36 layers were

formulated following Hy-Line recommendations (2022). Average market prices (2023) were applied to all ingredients except for corn and SBM. High, medium and low prices of SBM (590.0, 471.5, 353.0 \$/MT) and corn (323.0, 236.22, 198.82 \$/MT) were used in a factorial combination to obtain 378 diets. The relative SBM economic value (\$/MT) was estimated using the following formula: Relative value of SBM (\$/MT) = Base SBM (USA) price (\$/MT) – $[(TDC_{\text{test}} - TDC_{\text{Base}}) / SBM_{\text{test}} \times 1000]$; where “TDC” is the total diet cost (\$/MT), assuming the inclusion of the specified SBM, and “SBM” is the amount of SBM included in the diet (kg/MT). Results indicated significant differences ($P < 0.001$) among SBM country of origin for nutrient, energy, and AA digestibility. Despite ingredient price and nutrient per year variability ($P < 0.01$), diets formulated with USA SBM were cheaper ($P < 0.01$) for broilers (2.76–9.84 \$/MT) and layers (0.41–13.45 \$/ton). Exceptions were noted for ARG SBM in Brown phase 2 and White layers with high or medium SBM with high corn prices. The USA SBM had a premium value that ranged from 7.94–29.22 \$/MT for broilers, and 2.08–46.50 \$/MT for layers. In summary, these analyses demonstrated economic benefits of considering accurate SBM nutrient and energy value description in feed formulation.

Key Words: Feed formulation, precision nutrition, soybean meal, amino acid digestibility, feed costs

M74 Evaluation of 24 commercial lots of soybean meal of different origins indicates that both KOH protein solubility and trypsin inhibitor analyses are needed to determine soybean meal quality Nelson Ruiz*¹, Carl Parsons², Benjamin Parsons³, Keshun Liu⁴ ¹Nelson Ruiz Nutrition, LLC, ²Department of Animal Sciences, University of Illinois, ³Department of Poultry Science, University of Arkansas, ⁴Grain Chemistry and Utilization Laboratory, National Small Grains and Potato Germplasm Research Unit, USDA

A set of 24 solvent extracted commercial soybean meal (CSBM) samples, which corresponded to the same lots of CSBM used in the field, were

evaluated. The CSBM were from different origins (Argentina, Bolivia, Paraguay, Trinidad & Tobago, U.S.). The objective herein was to evaluate the relationship between KOH protein solubility (KOHPS, a measurement of overprocessing) and trypsin inhibitor activity (TIA, a measurement of underprocessing and overprocessing) and to determine if their ranges of adequacy overlap. The 24 CSBM samples were analyzed for: 1) KOHPS (Ruiz et al. 2022 IPSF Abstract M105), 2) TIA by the new official AOCS enzymatic method (Ba 12a-2020) and a NIR method (Evonik AminoN-ir®), and 3) in vivo amino acid (AA) digestibility determined utilizing the precision-fed cecectomized rooster bioassay as described by Corray et al. (2018, Poult. Sci. 97:3987-3991). Correlations were conducted using PROC GLM in SAS (2013) and the significance value was determined at $P < 0.05$. The KOHPS values ranged from 88 to 52%, while TIA expressed as TUI/mg ranged from 12.8 to 1.0 and TIA expressed as mg trypsin inhibited (TId)/g ranged from 4.9 to 0.6 (Evonik NIR method). There was a positive correlation ($P < 0.05$) between the AOCS enzymatic method and the Evonik NIR method. Lysine digestibility coefficients (dLYS) ranged from 0.9602 to 0.7018 and were positively correlated with KOHPS ($P < 0.05$). Ruiz et al. (2022 IPSF Abstract M106) previously reported that CSBM that was adequately processed (i.e. not overprocessed) exhibited 80 to 85% KOHPS, which correlated with 0.88 dLYS coefficient or greater. Samples that exhibited 80 to 85% KOHPS herein had variable TIA. Some of these samples contained 3.5 to 5.3 TUI/mg (2.5 to 3.0 mg TId/g) and the field assessment was satisfactory; however, some of these samples contained 6.4 to 12.8 TUI/mg (3.4 to 4.9 mg TId/g), which correlated with the observation in the field of rapid feed passage and poor zootechnical performance in broilers internationally. In conclusion, KOHPS and TIA adequacy ranges overlap and consequently both measurements are necessary to adequately determine the quality of CSBM of different origins.

Key Words: Soybean meal, Trypsin inhibitor activity, Amino acid digestibility, Rapid feed passage, Overprocessing

Metabolism and Nutrition III Feed Additives

M75 Dietary oleuropein supplementation affects lipolysis in broilers. Usman Sulaiman*^{1,IGS}, Reagan Vaughan¹, Paul Siegel¹, Dongmin Liu², Elizabeth Gilbert¹, Mark Cline³ ¹School of Animal Sciences, Virginia Polytechnic Institute and State University, ²Department of Human Nutrition, Foods and Exercise, Virginia Polytechnic Institute and State University, ³School of Neuroscience, Virginia Polytechnic Institute and State University

Purpose: The aim of this research was to investigate the effect of dietary oleuropein supplementation on broiler adipose tissue physiology during the first 10 days post-hatch. Oleuropein is a phenolic compound found in olives that may have anti-obesity properties in mammals. Excessive adipose tissue in broilers is a significant concern in poultry production due to the impact on meat quality, economic viability, and metabolic health in breeders.

Experimental Design: 68 day-of-hatch Cobb-Hubbard broilers were divided into dietary treatment groups receiving different amounts of oleuropein (0, 125, 250, or 500 mg/kg of commercial starter diet). Diets were fed ad-lib and on day 10 post-hatch, blood and adipose tissue samples (abdominal and subcutaneous) were collected from 12 birds in each group. Plasma was harvested from the blood to measure non-esterified fatty acids (NEFA). The mRNA levels of key regulators of adipogenesis and lipolysis (C/EBP α , C/EBP β , DGAT2, LPL, PPAR γ , SREBP1, HSL, ATGL, MGLL, NPY) were assessed by real-time PCR. Data were analyzed by ANOVA, with the model including effects of diet, depot, and interaction. Post-hoc means were separated using Tukey's test, with an alpha level of significance of 0.05.

Results: At day 10 post-hatch, body weights were reduced in the 125-dose group relative to other groups. In both adipose tissue depots, lipolytic enzyme (HSL, ATGL, MGLL) mRNAs were more significant in the 125-dose group compared to other groups. In contrast, the mRNA of DGAT2, an enzyme involved in triglyceride synthesis, was reduced in the 125-dose group relative to other groups. The PPAR γ (master regulator of adipogenesis) mRNA was reduced in the 125-dose group compared to other groups. The C/EBP β (regulator of adipogenesis) mRNA was more significant in the 125-dose group compared to other groups. Plasma NEFAs were more significant in the 125-dose group compared to other groups.

Conclusion: Results suggest that oleuropein has pro-lipolytic and anti-adipogenic properties in broilers. Effects were observed only in response to the lowest dietary inclusion level of oleuropein. This may provide a means to reduce excessive adipose tissue accumulation in broilers.

Key Words: oleuropein, broilers, adipose tissue, gene expression, dietary supplement

M76 Role of Solergy as an alternative energy source in low and high-fat diets fed to broilers Catherine Fudge*^{1,IGS}, Octavi Colom², Ekaitz Maguregui², Houssem Kadarar², Chongxiao Chen¹ ¹University of Georgia, ²Igusol Advance S.A.

This study was conducted to evaluate the efficacy of an energetic metabolism enhancer (Solergy) to replace soybean oil in formulation on growth performance in broilers. A total of 960-day-old Cobb 500 male chicks were randomly distributed to 6 treatments with 8 replicates of each. Treatments consisted of 2 basal diets containing low fat (LF: starter 1.5%, grower 2%

and finisher 2.5% soybean oil) or high fat (HF: starter 3%, grower 3.5% and finisher 4% soybean oil), in which soybean oil was replaced by Solergy at a 1:10 ratio at different levels (No replacement (CON), low Solergy (LS), 50%, 40%, and 30% of replacement for each phase, or high Solergy (HS), 60%, 50%, and 40% of replacement for each phase) to make six treatments: LF-CON (low fat control diet), LF-LS (low fat, low Solergy), LF-HS (low fat, high Solergy), HF-CON (high fat control diet), HF-LS (high fat, low Solergy), and HF-HS (high fat, high Solergy). Body weight (BW), feed intake (FI), and mortality corrected feed conversion ratio (FCR) were calculated at the end of starter (D14), grower (D28) and finisher (D42). Data were analyzed using one-way ANOVA in SAS 9.4, and means were separated using Tukey HSD. Results were considered significant at $P \leq 0.05$. In starter, low- and high-Solergy replacements reduced 30, and 36 Kcal/kg metabolic energy (ME) in LF and 60 and 72 Kcal/kg ME in HF diet, but BW, BWG, and FI were not negatively impacted. Whereas FCR was increased in LF-LS, LF-HS ($P=0.0054$) and HF-HS ($P=0.0216$) compared to control. There were no impacts on growth performances for all Solergy treatments during the grower (32 and 40 Kcal/kg ME reduction in LF, and 40 and 70 Kcal/kg reduction in HF) and finisher phase (30 and 40 Kcal/kg ME reduction in LF; 48 and 64 Kcal/kg reduction in HF), except the HF-LS, and HF-HS increased FI ($P=0.0162$). For the overall period (0-6wks), Solergy replacements in both LF and HF diets did not impact the overall growth performance. The results suggest that Solergy can be used as a potential alternative to fat in diet formulations, allowing for a reduction in energy content without compromising growth performance. Further research could focus on optimizing the Solergy dosage to maximize the benefits while minimizing any potential negative effects on feed efficiency.

Key Words: Energy, Solergy, Alternative, Fat

M77 Effect of low protein soybean meal with soy hull or wheat bran inclusion to corn-based diets on ileal digestibility, jejunum histomorphology, and expression of nutrient transporters under mild coccidiosis Shravani Veluri*^{1GS}, Oluyinka Olukosi *Department of Poultry Science, University of Georgia*

We previously reported that a low-protein soybean meal, LPSBM vs. high-protein SBM (HPSBM), produced greater body weight in the starter phase of broiler chickens and marginally higher body weight on d42 in cocci-challenged broilers. This study investigates possible mechanisms accounting for the differences in performance we reported. A 42-day floor pen trial with 10 treatments and 6 replicates/treatment with 15 birds/replicate was used to study the objective. Treatments were arranged in a 5×2 factorial with factors as diets (5 diets) and cocci (challenge; CH vs. no challenge; NCH). Diets were: 1. LPSBM, 43% CP; LPSBM was prepared by mixing soy hull (SH) with HPSBM, 48% CP; 2. corn-HPSBM diet; 3. corn-LPSBM + xylanase, 4. HPSBM+ 5% wheat bran (WB) and 5. HPSBM+ xylo-oligosaccharides. All the diets were isonitrogenous and isocaloric with the same level of digestible AA. Birds in CH group were challenged with a mixture of *Eimeria* species on d15, while NCH group received tap water. At day 21, ileal digesta were collected to determine ileal amino acid digestibility (IAD). Jejunum tissue was collected for histology and mRNA expression of nutrient transporters and tight junction genes, and breast tissue for protein synthesis (mTOR, EEF2, 4EBP1) and degradation genes (Myog, MYF5, FBX09). Cocci challenge decreased ($P < 0.05$) IAD of all the AA. Threonine digestibility was greater ($P < 0.05$) for LPSBM and LPSBM+ xylanase than for other diets. The villi were shorter, and crypts were deeper ($P < 0.05$) in the CH group. Cocci challenge increased $P < 0.05$ claudin and GLUT1 mRNA expression but decreased ($P < 0.05$) expression of Bo⁺AT. There was higher mTOR and MYF5 ($P < 0.01$) expression in CH. There was significant diet \times cocci for expression of EEF2, 4EBP1, Myog, and FBX09 ($P < 0.05$). There was a decrease in mRNA of EEF2, 4EBP1, and FBX09 in NCH group. However, in the CH group, LPSBM + xylanase tended to decrease the mRNA expression of the genes. In conclusion, mild cocci infection negatively

affected ileal histomorphology and decreased AA digestibility, whereas LPSBM had variable positive effects, which partly explained the marginal increase in weight gain previously observed for birds receiving diets with LPSBM compared to HPSBM.

Key Words: soyhull, wheat bran, coccidiosis, prebiotic, xylanase

M78 Evaluating the effects of a dietary monoglyceride supplement on intestinal integrity and host defenses during a broiler coccidiosis infection Laney Froebel*^{1GS}, Julianna Jespersen¹, Kaitlyn Sommer¹, Cameron White¹, Adriana Barri², Adebayo Sokale², Ryan Dilger¹
¹University of Illinois, ²BASF SE

Coccidiosis is a disease caused by the *Eimeria* protozoan and costs the poultry industry billions annually in production losses. As the usage of antimicrobials continues to decline, it is essential to investigate alternative strategies to combat this costly disease. In this study, the combined effects of butyric, caprylic, and capric monoglycerides (MG) were evaluated in promoting intestinal integrity and host defenses during a coccidiosis challenge. A total of 480 male Ross 308 chicks at 2 d post-hatch were randomly assigned to 1 of 8 treatment groups with 6 birds allotted to each of 10 replicate cages. The study was conducted as a 2×4 factorial arrangement with factors including: 1) coccidiosis challenge status (2 levels; non-challenged or challenged) and 2) MG supplementation (4 levels; 0, 500, 1000, or 1500 g/metric tonne). After a 14-d acclimation period, birds were orally gavaged with 1 mL of either water (non-challenged) or a $5 \times$ dose of commercial vaccine (challenged). Day of inoculation was designated as 0 d post-inoculation (DPI). Growth performance data were analyzed by a 2-way ANOVA using SAS and effects were considered significant when $P < 0.05$. Histopathological outcomes were scored with discrete and ordinal values (0-5), and cumulative probabilities were obtained using the PLM procedure of SAS via a Tukey-Kramer test. In the pre-inoculation period (study d 0-14), birds did not differ in body weight gain, feed intake, or FCR ($P > 0.05$). However, in the infection period (study d 14-28) and overall study (study d 0-28), a main effect of challenge status was observed, where infected birds had impaired growth performance ($P < 0.05$) compared with uninfected birds. No interaction effect of MG supplementation and challenge status was observed for growth performance during the study. On DPI 7, challenged birds supplemented with 500 g/metric tonne of MG had lower ($P < 0.05$) cumulative scores of coccidia and small intestine histopathology compared with other challenged treatments. Overall, we conclude that MG supplementation at 500 g/metric tonne may help maintain intestinal integrity during a coccidiosis infection in broilers.

Key Words: coccidiosis, monoglycerides, broiler, nutrition

M79 Evaluating the effect of bacitracin methylene disalicylate on cecal microbiota of broilers during a subclinical necrotic enteritis condition Shailes Bhattarai*^{1GS}, Monika Proszkowiec-Weglarz², Laura Ellestad¹
¹University of Georgia, ²United States Department of Agriculture, Agriculture Research Service, Animal Biosciences and Biotechnology Laboratory

The ban on antibiotic growth promoters (AGPs) has led to a rise in sub-clinical necrotic enteritis (SNE) that could impact broiler health by altering the intestinal microbiome. Although AGP regulation of the intestinal microbiome potentially promotes broiler growth efficiency, further study is needed to understand their impact on the microbiome in broilers facing a SNE challenge. This study examined the effects of feeding bacitracin methylene disalicylate (BMD) at AGP levels on the cecal microbiota in SNE-challenged broilers. Day (D)-old male Ross chicks were divided into three groups ($n=5$ pens/group): unchallenged control (Control), challenged without BMD supplementation (SNE), and challenged with BMD supplementation (SNE+BMD). Challenged birds were orally gavaged with *Eimeria maxima* oocysts on D14, followed by *Clostridium perfringens* oral gavage from D19-D21. Cecal contents were collected from one

bird/pen at 0 (D21), 24 (D22), 72 (D24), and 168 (D28) hours post final infection (HPFI) for bacterial DNA isolation. The V3-V4 hypervariable region of the 16S ribosomal RNA was sequenced using Illumina's MiSeq platform, and sequences were processed with Quantitative Insight into Microbial Ecology 2 software. Data were analyzed with non-parametric tests and differences were considered significant at $P \leq 0.05$. Analysis of α -diversity by the Shannon index showed higher species diversity in both the SNE and SNE+BMD groups compared to the Control group at 24 HPFI ($P \leq 0.05$). The unweighted UniFrac distance metric, a measure of β -diversity, revealed that the microbial communities in both the SNE and SNE+BMD groups were more dissimilar from those in the Control group at 24 HPFI ($P \leq 0.05$). By 72 HPFI, the SNE group had a higher relative abundance of Firmicutes than the Control group ($P \leq 0.05$). Linear discriminant analysis (LDA cutoff > 2) effect size identified distinctive taxa, primarily from Firmicutes, that differentiated Control and SNE groups and SNE and SNE+BMD groups at all HPFI ($P \leq 0.05$). The number of distinct bacterial taxa increased between 0 and 72 HPFI. Regardless of BMD supplementation, SNE challenge led to alterations in the cecal microbiota that were closely associated with pronounced shifts in bacterial species within Firmicutes during early infection periods.

Key Words: 16S ribosomal RNA, Firmicutes, Subclinical necrotic enteritis, alpha-diversity, beta-diversity

M80 Effect of dietary postbiotics supplementation on growth performance and immune response in broilers challenged with necrotic enteritis Hanseo Ko^{*GS}, Doyun Goo, Jihwan Lee, Woo Kyun Kim *University of Georgia*

This study aimed to evaluate the effect of dietary postbiotic supplementation (Lumensa LFM, Verdesian Life Sciences) on growth performance, gut integrity, and immune response in broilers under necrotic enteritis (NE) challenge conditions. A total of 216 broilers (as hatched, male Cobb 500) were allocated into three treatments with six replicate cages (12 birds per cage) for 28 d. Three treatments comprised non-challenged control, NE challenged control (CC), and CC with dietary 500 ppm of postbiotic supplementation group. All birds in NE-challenged groups were orally challenged with *Eimeria maxima* (10,000 oocysts per bird) at d 14 and *Clostridium perfringens* (CP, 10^8 CFU/mL per bird) at d 18 for inducing NE. Growth performance, daily feed intake (FI), and mortality were measured daily or weekly. The CP load in small intestinal digesta and total ceca were evaluated at d 21 and d 28. Gut permeability test and NE lesion scoring were conducted at d 20, d 21, and d 28. Gene expression in the jejunal tissue was measured at d 21. One-way ANOVA with Tukey's post hoc test was used for all data analyses. During the starter phase (0 - 14 d) before the NE challenge, dietary postbiotic supplementation significantly improved ($P < 0.05$) body weight gain (BWG) and FI compared to the non-supplementation group. During the NE-challenged period (14 - 28 d), the dietary postbiotic group had significantly improved FI by 9.7%, BWG by 6%, and FCR by 0.07 points compared to the NE-challenged control ($P < 0.05$). The dietary postbiotic supplementation significantly reduced CP counts in cecal content and upregulated ($P < 0.05$) the expression of IL-10 in the jejunum compared to the NE-challenged control. Moreover, the dietary postbiotic supplementation significantly reduced fecal oocyst shedding and fecal moisture content compared to the NE-challenged control and numerically reduced gut permeability and lesion score compared to the NE-challenged control. In conclusion, the results of this study indicated that dietary postbiotic supplementation would be a promising antibiotic alternative to alleviate the negative effect of growth performance and gut health in broilers challenged with NE.

Key Words: Postbiotics, *Eimeria maxima*, *Clostridium perfringens*, Necrotic enteritis, Broiler

M81 Probiotein® effect on metabolite dysregulation of 14-day poultry cecal contents. Elena Olson^{*IGS}, Alysson Freedman¹, Lindsey Wythe², Chamia Chatman¹, Erica Majumder¹, Abe Sheaffer³, Steven Ricke¹ ¹*University of Wisconsin-Madison*, ²*Texas A&M University*, ³*SweetPro*

This study aimed to assess the impact of ProBiotein®, a yeast fermentate (PB), in broiler diets on cecal function. Each diet was supplemented with PB, ranging from 0% to 0.75%. The metabolomic profiles of cecal contents from d-14 of the 42-d trial were analyzed. The metabolite extracts (N = 38, k = 4) were analyzed using ultra-high-performance liquid orbitrap chromatography-mass spectrometry (UHPLC-MS), employing a data-dependent acquisition for the tandem MS workflow. MS1 spectra were matched to spectra and compounds in the Human Metabolome Database using MetaboAnalyst 5.0. Statistical analysis was also carried out using MetaboAnalyst 5.0. Pairwise comparisons were conducted via a t-test ($P < 0.05$) to investigate potential differences in metabolite features between treatment groups. The functional analysis was performed using Mummichog 2.0 (P-value < 0.00001). The highest dysregulated metabolites were associated with the 0% and 0.5% PB groups. These metabolites were related to plant and fatty acids compounds and were downregulated in the 0.5% PB. Based on the *Gallus gallus* model, functional analysis revealed significant down-regulation of metabolites in 16 pathways in the 0.5% PB group compared to the control. Meanwhile, the functional analysis based on the *Escherichia coli* model, representing the overall cecal community, showed six significantly down-regulated metabolites between the 0% and 0.5% PB groups. These pathways were associated with the host and the cecal microbiome, with linoleic acid metabolism, primary bile acid biosynthesis linked to the host, and methionine and biotin metabolism associated with the cecal microbiome. Purine and arachidonic acid metabolisms were associated with both the host and the cecal microbiome. Isogingerenone B has been identified as a promising biomarker for evaluating the utilization of Probiotein® due to its consistent increase in response to varying treatment concentrations. Ultimately, 0.5% PB treatment appeared to have a more favorable impact on nutrient utilization by both the host and cecal systems compared to the 0%, 0.2%, and 0.75% treatments. This finding suggests that PB at 0.5% concentration improved both host and bacterial functions within the poultry cecal system by the d-14 of the trial.

Key Words: poultry, yeast fermentate, metabolomics, ceca

M82 Supplementation of novel probiotics improves layer performance and modulates cecal microbial populations Ragini Reddyvari^{*GS}, Venkata Praveen Raja Kosuri, Sulthana Humayoon Muttathukonam, Yuying Ren, Eswari Kanike, Maiuri Gao, Mary Anne Amalaradjou *University of Connecticut*

Antibiotics used to be employed to enhance layer performance and egg production. However, there is a pressing demand for alternative approaches due to the development of antimicrobial resistance in humans and animals. Probiotics are one of the many viable alternatives that can potentially replace antibiotic growth promoters. However, research on the use of probiotics as an alternative is still very limited in obtaining consistent and impactful production. Hence, we evaluated the efficacy of novel probiotics to improve feed efficiency, performance, and bird health. Two novel probiotics, namely *Lactobacillus rhamnosus* NRRL-B-442 (LR) and *Lactobacillus paracasei* DUP 13076 (LP), recently identified to promote pullet growth, were employed in this study. The experiment was set out as a completely randomized design. A total of 300 19-week-old Lohmann lite laying hens were randomly allocated into 4 treatments with 5 replicates of 15 birds each for 48 weeks of age. The experimental treatments included a corn-soybean meal-based diet without probiotics (Control) and the feed supplemented with $9 \log$ CFU/kg of LP, LR, and a probiotic cocktail of LP and LR (PR). Data were analyzed using GraphPad (Version 9.3.1), and $P < 0.05$ was considered significant. The results showed that the feed conversion ratio (FCR) was significantly improved ($P < 0.05$) in the LP group (2.94) when compared to control (3.22), LR (3.20), and PR (3.47). Specifically, the feed intake was significantly lower in the LP group (117g/

bird) when compared to the control (128g/bird), LR (128g/bird) and PR (140g/bird). In terms of egg production, the PR and LP groups significantly increased production by 5 and 3%, respectively, when compared to the control. Further, we also observed higher cecal *Lactobacilli* counts and reduced *E. coli* counts in LP, LR, and PR when compared to control. Overall, supplementing probiotics LP and LR in layer diets improved layer performance while modulating cecal microbial populations. Hence, these novel probiotics can be employed as feed supplements to improve layer performance. Additional studies are underway to evaluate the probiotic effect on layer health and their application as a pre-harvest intervention to improve egg safety.

Key Words: probiotics, in-feed supplementation, layer performance, feed conversion ratio, cecal microflora

M83 Effects of enteric released or pure benzoic acid on Ross 708 male broilers raised on used pine shaving litter Cooper Fritzen^{*IGS}, Zhigang Tan², Wenzhen Chen², Haijun Liu², Xianfeng Peng², Zonghua Qin², Michael Persia¹ ¹Virginia Tech, ²Guangzhou Insighter Biotechnology Co., Ltd

An experiment was conducted to evaluate the effects of pure benzoic acid (PBA) or enteric-released benzoic acid (EBA) on performance, intestinal histology and permeability, apparent ileal nutrient digestibility (AID), cecal bacterial enumeration, and litter dry matter (DM) of broilers raised on used litter. Day-old male Ross 708 broiler chicks were housed in floor pens (0.08 m²) and assigned to: negative control (NC), without feed additives; positive control (PC), NC + 50 ppm bacitracin methylene disalicylate; PBA, 1000 ppm of PBA; EBA, 330 ppm of EBA. Each trt was replicated with 12 pens of 37 birds. Body weight gain (BWG) and mortality-corrected feed conversion ratio (FCRm) were calculated over the 0-14, 0-28, and 0-42 d periods. Pooled ileal contents were collected from 6 birds per pen on d 28 for AID. On d 28 and 42, duodenum and jejunum samples from one bird per pen were collected for histology. Cecal contents were collected from one bird per pen on d 28 and 42. On d 29, serum FITC-dextran was completed to determine intestinal permeability. Data were analyzed using ANOVA, and if $P \leq 0.05$, means were separated using Student's t test (JMP Pro 16). From 0-14 d, NC resulted in improved FCRm in comparison to PC but was similar to all other treatments. However, from d 0-28 onwards, PC outperformed NC in either BWG or FCRm ($P \leq 0.05$). From 0-28 d, EBA and PBA significantly improved BWG (1,165 and 1,155g v. 1104g) and FCRm (1.316 and 1.338 v. 1.381) compared to NC ($P \leq 0.05$). However, from d0-42 EBA alone improved BWG (2,642 vs. 2,520 g) and FCRm (1.450 vs. 1.496) in comparison to NC ($P \leq 0.05$). The PBA was similar to NC for BWG and FCRm from d0-42. The supplementation EBA and PBA altered duodenum and jejunum histology inconsistently. There were no differences in AID and intestinal permeability ($P > 0.05$). There were no differences in coliform counts ($P > 0.05$). However, PBA significantly reduced enterococcus counts at 42 d compared to NC (4.11 v. 4.59 log₁₀ cfu/g). These results suggest that 1000 ppm of pure benzoic acid or 330ppm of enteric-released benzoic acid can both impact broiler performance and intestinal health, with enteric-released benzoic acid showing better growth performance, but the mode of action of BA remains unclear.

Key Words: performance, benzoic acid, intestinal histology, bacterial enumeration, broiler

M84 Effects of saponin blend on the performance and oocyst shedding of young laying hens exposed to coccidial vaccine challenge and residual manure access. Delaney Groves^{*IUG}, Emily Kimminau², T Karnezos², M Persia¹ ¹Virginia Tech, ²Land O'Lakes

An experiment was conducted to evaluate the various concentrations of a saponin blend supplemented to young laying hens exposed to coccidial vaccine from 21 to 39 weeks of age. In total, 168 Hy-Line W36 laying hens were housed 3 hens to a cage (72 sq in/bird) with 14 replicate cages

for each of the 4 treatments: a non-supplemented and non-vaccinated positive control (PC); a non-supplemented and vaccinated negative control (NC); and two treatments (300 Sap and 600 Sap) vaccinated and supplemented with 300 or 600 g/ton of Saponin (SarSap3TM, Land O' Lakes, Arden Hills, MN). Coccidial vaccination occurred two days after hens were transferred to experiment diets and consisted of approximately a 50x dose of Coccivac[®] B52 (Merck Animal Health, Rahway, NJ) diluted into 1 ml of tap water and orally gavaged to each hen. Hens were control fed 97g of feed daily and eggs produced (EP) and mortality were collected and weighed daily. Feed intake, EP and feed conversion ratio were calculated in 3-wk periods. Egg yolk color score (YCS) and oocyst shedding (OS) were determined weekly over the first 6 wk. Hens were weighed every 6 wk. Performance data were analyzed using repeated measurements, if significance was detected, Tukey's HSD test was used to separate means ($P \leq 0.05$). Yolk color score and oocyst shedding were analyzed by ANOVA over individual wk and if significance was detected, means were separated via LSD test ($P \leq 0.05$). Coccidia decreased EP between PC (90.9%) and NC (87.5%; $P \leq 0.05$) and the 300 Sap and 600 Sap were intermediate (88.6 and 89.5%), respectively. In the second wk, the YCS was reduced with coccidia between the PC (7.9) and the NC (6.7) with the 600 Sap (7.2) higher than the NC, but still reduced compared to the PC ($P \leq 0.05$). This response is likely related to *E. acervulina* altering fat digestion in the duodenum. As expected, the NC shed the highest number of oocysts and the non-vaccinated PC the least with the two Sap treatments intermediate, although the response was not consistent over time. Overall, coccidia reduced laying hen performance, decreased YCS and increased OS and at least the 600 mg/ton saponin blend was able to partially ameliorate the negative effects of the coccidia.

Key Words: Coccidiosis, Saponin, egg production, yolk color score, oocyst shedding

M85 The effects of Magni-Phi on the performance response of commercial white layers during pullet rearing and through a single laying cycle Dimitri Malheiros^{*GS}, Kenneth Anderson *North Carolina State University*

One of the most prevalent poultry diseases is caused by *Eimeria* protozoan parasite resulting in an enteric disease infection called coccidiosis. In this current experiment, saponins were sourced from Quillaja Saponaria to reduce coccidia oocyst production. In this grow/lay experiment, 988 W-36 pullets were used in a 2X4 factorial design. The grow period consisted of two feeding programs, a Control (CON) and Magni-Phi (MP) treatments. In the lay cycle, the grow treatments were divided to compose the four lay treatments. The experimental lay treatments were allocated in the feed as C-C, C-MP, MP-C, and MP-MP. Water and feed were supplied *ad libitum*, and vaccine schedule and lighting program followed W-36 management guide. The hens remained unchallenged in this trial, with only exposure to the research station's facilities natural biologicals which did not include coccidia. Feed consumption, FCR (g egg/g feed), egg production, Hen-Housed, Hen-Day, USDA Grading distribution, and egg incomes were also calculated on a per-period basis. Egg quality parameters like Haugh unit, shell color, yolk color, egg weights, shell and vitelline strength and elasticity were analyzed every other period from 17-69 weeks. Data was analyzed using JMP Pro 17 running a two-way ANOVA with $\alpha < 0.05$ using Tukey's HSD to make comparisons and determine differences. During the grow period birds in the control group (C) consumed more feed than birds fed the Magni-Phi (MP) diet, but no differences in body weights were observed. Egg production, feed consumed, hen day, and hen housed production showed significant results in the lay periods, with C-C treatment groups showing statistically significant higher results when compared to MP-MP treatments. Albumen height and Haugh Unit showed significance, with MP-MP treatment showing higher values when compared to C-C treatment. Overall, our study explores the potential supplementation of Magni-Phi in the poultry industry as it offers a possible reduction of rearing feed costs with comparable pullet development, while

in the laying phase maintaining comparable egg yields compared to the control, with an improvement in physical quality indicating an economically viable utilization even in healthy laying hens during production.

Key Words: Magni-Phi, Saponin, Quillaja tree, Eimeria, Coccidia

M86 Evaluating the efficacy of humic acids in alleviating aflatoxin B1 toxicity in turkey poults Jesus Maguey-Gonzalez*¹, Maria de Jesus Nava², Juan Latorre¹, Ileana Loeza¹, Roberto Senas¹, Sergio Gomez³, Abraham Albores², Xiangwei Du⁴, Guillermo Tellez¹, Billy Hargis¹ ¹*Department of Poultry Science, University of Arkansas, ²UIM, L5: LEDEFAR, FES-C, UNAM, ³CENID-INIFAP, ⁴Veterinary Diagnostic Laboratory, Iowa State University*

Humic acid (HA) derived from worm compost was evaluated as an adsorbent for aflatoxin B₁ (AFB₁) in turkey poults. The experiment involved

the inclusion of 0.25% (w/w) HA in the turkey poults consuming feed contaminated (250 ng AFB₁/g). Three hundred fifty one-day-old female Nicholas-700 turkey poults were randomly assigned to five groups: negative control (basal diet), positive control (basal diet + 250 ng AFB₁/g), HA (basal diet + 0.25% HA), HA + AFB₁ (basal diet + HA + 250 ng AFB₁/g), and zeolite + AFB₁ (basal diet + 0.25% zeolite + 250 ng AFB₁/g). Each group had seven replicates with 10 poults (n=70). HA supplementation improved body weight (BW) (p<0.05), body weight gain (BWG) (p<0.05), and feed conversion rate (FCR) (p<0.05). HA also mitigated the adverse effects of AFB₁ on most serum biochemical parameters (p<0.05) and histological findings (p<0.05). These findings suggest that HA can effectively counteract the toxic effects of AFB₁ in turkey poults and remove AFB₁ from contaminated diets.

Key Words: Humic acids, Aflatoxin B1, Adsorbents, Turkey poults

Metabolism and Nutrition IV Enzymes

M87 Effect of dietary supplementation of a multi-carbohydrase enzyme complex to broiler diets with or without energy reduction on performance, carcass traits and intestinal morphology Carlos Soto*¹, Hector Leyva¹, Brian Dirks¹, Absaar Mahmood², Ehsaan Khan², Asif Rana³, Nadim Amarin¹ ¹*United Animal Health, ²University of Veterinary and Animal Sciences, ³Hivet Animal Health Business*

The present study was conducted to evaluate the effect of dietary supplementation of a multi-carbohydrase enzyme complex (MCE; Enspira®+, United Animal Health, US) to broiler diets with or without Metabolizable Energy (ME) reduction on performance, carcass traits and intestinal morphology. A complete randomized design with a 2x2 factorial arrangement (0 or -70 ME kcal/kg reduction, with or without MCE) was employed to distribute 4 dietary treatments (trt) consisting of PC, no ME reduction, no MCE; NC, ME reduction, no MCE; PCE, no energy reduction + MCE at 100 ppm; and NCE, ME reduction + MCE at 100 ppm. A total of 1,000 day-old straight-run Ross 308 broiler chickens were distributed into 40 floor pens with 25 birds each. The feeding program consisted of 3 dietary phases (starter 0-10 d; grower 10-24 d; finisher 24-35 d). Feed intake (FI), body weight gain (BWG), FCR (corrected for mortality), and European production efficiency index (EPEI). were determined. At 35 d, 18 birds/trt were processed for the evaluation of carcass yield (CY), leg quarter weight (LQW), and breast weight (BTW). Additionally, 2 birds/pen, were selected to evaluate villus height, crypt depth, and villus height/crypt depth ratio (VH:CD). Data were subjected to two-way ANOVA and means were separated using Fisher's LSD test (P<0.05) when appropriate. Cumulatively (0-35 d), an interaction (P<0.05) was observed for FI, BWG, FCR, EEI, CY, LQW, and VH:CD. The NC had higher (P<0.05) FI compared to all other trt. NCE had higher (P<0.05) BWG compared to PCE. Additionally, both PCE and NCE had higher (P<0.05) BWG compared to PC and NC. FCR was improved by 14% (P<0.05) in the NCE trt compared to NC. EEI and CY were higher (P<0.05) in NCE compared to NC and PC. NCE had a higher (P<0.05) LQW and BTW compared to PCE. Additionally, both PCE and NCE had higher (P<0.05) LQW and BTW compared to PC and NC. VH:CD was improved (P<0.05) in both MCE supplemented trt compared to PC and NC. In conclusion, the results suggest that MCE inclusion in boiler diets with or without ME reduction-maintained performance and improved carcass traits and intestinal morphology. Moreover, a higher response to MCE dietary supplementation was observed in the ME reduced treatment.

Key Words: Carbohydrase, Gut Morphology, Carcass Traits

M88 Validation of the inclusion rate of a β-xylanase and β-glucanase enzyme preparation (Natugrain® TS) in corn-soy diets for broiler chickens. A. Sokale*¹, G. Pasquali², A. Amerah³ ¹*BASF Corporation, ²BASF SE, ³Cargill Animal Nutrition*

This study aimed to investigate the effects of different inclusion rates (0, 50, 100, 150 and 200 g/MT feed) of a β-xylanase and β-glucanase enzyme preparation (Natugrain® TS, BASF) in corn-soy-based diets on the apparent metabolizable energy (AME) and nutrient digestibility in broiler chickens. A total of 300 Ross 308 male broilers (5 birds/cage × 12 cages/treatment) were randomly allotted to the 5 dietary treatments in a randomized complete block design. A corn-soy-DDGS-based diet containing phytase (Natuphos E®, BASF) at 1000 FTU/kg was formulated and fed from day 1 to 24. Feed was pelleted at 75°C and TiO₂ was used as a marker. Excreta samples and ileal digesta were collected on days 23 and 24, respectively, and analyzed for gross energy and nitrogen (N). Ileal digesta samples were also analyzed for starch and fat. Quadratic polynomial (QP) model was used to fit the titrated levels for the treatments LSMeans for each response variable. The levels which maximized response variable from the QP model are reported. Additionally, One-Way ANOVA was used to analyze data when there is no significant Goodness of fit (P<0.05) for the response variable. There was a quadratic effect (P<0.05) of Natugrain TS supplementation on the AME of the diet, indicating that the supplementation of Natugrain TS maximized AME of diet at 100-120 g/MT. One-Way ANOVA showed significant differences among treatments for nitrogen retention (P<0.05), with 100 g/MT showing 6.9% improvement over the control group. A tendency for a quadratic response (P=0.077) was observed on the coefficient of ileal nitrogen digestibility, which has been maximized when Natugrain TS was added at 100-120 g/MT feed. No significant effect (P>0.05) of Natugrain TS inclusion rate on the coefficients of ileal energy, starch, and fat digestibility were observed. This trial showed that addition of Natugrain TS on top of a phytase-supplemented (Natuphos E at 1000 FTU/kg) corn-based diet improved AME and nitrogen retention at an inclusion rate dependent manner. Inclusion of Natugrain TS at 100 g/MT feed improved diet AME by 101 kcal/kg and nitrogen retention by 6.9%. In conclusion, under the trial conditions, the optimum inclusion rate of Natugrain TS in corn-soy-DDGS-based diets for broilers is 100 g/MT feed.

Key Words: broilers, digestibility, NSP-enzymes, β-xylanase, β-glucanase

M89 Effects of high dose of phytase with a β -xylanase and β -glucanase enzyme on plasma myo-inositol and growth performance of broiler chickens. A. Sokale^{*1}, A. House², D. Wicker², C. Middlebrooks², D. Golz¹, K. Riley³, N. Tillman⁴ ¹BASF Corporation, ²Fieldale Farms Corporation, ³Cargill Animal Nutrition, ⁴Nutritional Statistics LLC

This field study aimed to investigate the effects of high dose of a bacterial 6-phytase and a β -xylanase and β -glucanase enzyme combination on growth performance and plasma myo-inositol concentration. A total of 864 broilers were randomly allotted to 2 experimental treatments: xylanase and phytase at 1500 FTU/kg feed (1500) and a xylanase-glucanase and phytase at 2500 FTU/kg feed (2500). Birds were allocated to 48 pens in a randomized complete block design (each treatment comprised of 12 male pens and 12 female pens with 18 birds/pen). Diets reduced in P, metabolizable energy, calcium, and sodium across 4 feeding phases from 0-45 d were formulated. All feeds were pelleted and fed ad libitum. On d25, blood samples were collected from 1 male and 1 female bird per pen (n=12) for evaluation of plasma myo-inositol. Feed intake and individual body weight (BW) were measured at d17, 28, 38, and 45. Average daily gain (ADG), feed intake, and feed conversion ratio (FCR) were calculated. Data were analyzed using Mixed model of JMP PRO 17 according to a 2x2 factorial design with phytase level and sex as main effects. At 0-17 d, there was a significant phytase level \times sex interaction for FCR ($P=0.028$). At 0-28 d, there were significant phytase level \times sex interactions for BW ($P=0.014$), ADG ($P=0.015$), and FCR ($P=0.007$). Significant improvement for BW ($P=0.009$) and ADG ($P=0.009$) at 0-17 d as well as for BW ($P=0.035$), ADG ($P=0.034$), and FCR ($P=0.049$) at 0-28 d were observed with higher phytase level. At 0-38 d, FCR was significantly improved ($P=0.028$) while from 0-45 d, FCR was numerically improved ($P=0.064$) in higher phytase level. There were significant differences in performance variables between males and females at each phase, with males having the most improvement. Plasma myo-inositol concentration was significantly higher ($P=0.035$) in the 2500 group (0.284 mmol/l) compared to the 1500 group (0.244 mmol/l). Additionally, there was a significant difference ($P=0.029$) in plasma myo-inositol between males (0.243 mmol/l) and females (0.286 mmol/l). In conclusion, this study demonstrated that supplementation of phytase at 2500 FTU/kg with xylanase-glucanase enzyme improved the growth performance of the birds, with differential expression of plasma myo-inositol.

Key Words: broilers, myo-inositol, NSP-enzymes, performance, phytase

M90 Equivalence of metabolic energy release of a mannanase with and without the addition of xylanase and glucanase in broilers fed a corn-soy diet Rodrigo Messias^{*1}, Rita Vieira², Anna Fickler³, Michele Bernadino², Rosana Maia¹, Edney da Silva² ¹Basf SA, ²Unesp, ³BASF SE

A metabolism trial divided into two periods was conducted to evaluate the energy release of a commercial β -mannanase when used with and without the addition of xylanase and glucanase based on equivalence. The trial was carried out at Unesp-Jaboticabal, Brazil. For both periods, experimental diets were formulated using corn and soybean meal. In all diets, 500 FTU/kg of phytase was added, following the complete matrix recommended by the manufacturer. The treatments were the same for both periods: T1: Diets meeting all of Rostagno et al. (2017). T2: A reduction of 50 kcal/kg compared to T1 (T1 - 50 kcal/kg) by reducing the oil content. T3: A reduction of 100 kcal/kg compared to T1 (T1 - 100 kcal/kg). T4: A reduction of 150 kcal/kg compared to T1 (T1 - 150 kcal/kg). T5: T4 + Mannanase (800 TMU/kg feed). T6: T4 + Mannanase (800 TMU/kg feed) + xylanase+glucanase (560 TXU/kg and 250 TGU/kg feed). In period one, 300 broiler chicks were divided into 10 replicates with 6 chicks per cage. In period 2, 10 replicates were kept but with 4 birds per cage. Period 1 took place from day 7 to day 17 with 5 days of adaptation and 5 days of total excreta collection. Period 2 from day 28 to day 36 day with 5 days adaptation and 3 for excreta collection, following a completely randomized design. In both trials, statistical analysis was performed with linear regression considering response Metabolic Energy (ME) kcal/bird/

day, and Energy intake kcal/bird per day as predictors. For T5 and T6, energy intake was used to estimate the energy equivalence for ME. In the first period, there was a statistically significant linear effect for ME and ME corrected by nitrogen ($P=0.0003$ and $P=0.0004$, respectively). The energy equivalence for mannanase was 52 kcal/kg, and for mannanase + xylanase and glucanase it was 89 kcal/kg. In the second trial, a statistically significant linear effect for ME and ME corrected by nitrogen ($P=0.0049$ and $P=0.0058$, respectively) was observed. The energy equivalence for mannanase was 60 kcal/kg, and for mannanase + xylanase and glucanase it was 108 kcal/kg. In conclusion, enzymes demonstrated an additive effect in terms of energy release. The average energy release was 59 kcal/kg for mannanase and 98 kcal/kg for mannanase + xylanase and glucanase.

Key Words: mannanase, Enzymes, Energy, metabolism, equivalence

M91 Impact of a multi-activity non-starch polysaccharide degrading enzyme on the performance, nutrient digestibility and nitrogen utilisation of broilers Leni Kuterna^{*1}, Sebastian Kaczmarek², Joan Edwards¹ ¹Palital Feed Additives B.V., ²Poznan University of Life Sciences

Non-starch polysaccharide degrading enzymes (NSPases) improve the zootechnical performance and nutrient use efficiency (NUE) of poultry consuming fiber-rich feed. This has benefits for the sustainability of poultry production systems, as well as potentially their environmental impact in terms of nitrogen (N) emissions. However, the concept of NSPases as a tool to decrease N emissions has not been explored to date. In order to investigate the merit of this concept, a trial was conducted to assess the impact of a multi-activity NSPase (containing both xylanase and glucanase) on the performance, total tract digestibility and N efficiency of broilers.

Ross 308 birds were randomly allocated into two groups: control and NSPase. For each group, birds were divided into 10 pens of 10 birds. Each group had the same basal diet in each of the feeding phases: starter (1-14 d), grower (15-35 d) and finisher (36-42 d). In the NSPase group basal diets were supplemented with 100 ppm of the multi-activity NSPase AveMix® XG 10. At d 14, 35, and 42 the following parameters were assessed: bodyweight gain (BWG), feed intake (FI), and feed conversion ratio (FCR). Fat total tract digestibility and dry matter (DM) and N retention were assessed on day 36 using TiO₂ as a marker. N retention and N efficiency were also calculated based on average BWG per bird during the trial (i.e., 1-42 d). Data were analyzed using a T-test with the pen as the experimental unit. Statistically significant ($P < 0.05$) differences relative to the control are stated.

NSPase increased BWG at the end of the grower (1720 vs 1504 g) and finisher (2909 vs 2586 g) phases but not the starter. No effect on FI occurred. FCR was improved by NSPase in both the grower (1.615 vs 1.823) and finisher (1.600 vs 1.748) phases but not the starter. NSPase increased the retention of DM (66.1 vs 60.1%) and N (57.3 vs 49.0 %), as well as fat total tract digestibility (69.7 vs 58.8%). Overall, NSPase resulted in decreased N excretion (67.5 vs 73.9 g N/bird), and increased N retention (84.4 vs 75.0 g N/bird) and N efficiency (56 vs 50 %).

In conclusion, as well as improving performance and NUE the use of a multi-activity NSPase, AveMix® XG 10, has merit as a tool to potentially decrease N emissions from poultry production systems.

Key Words: Poultry, Feed additive, Growth performance, NSP, Nitrogen balance

M92 Efficacy of xylanase supplementation in lower energy laying hens diet Lucio Araujo*¹, Rasha Qudsieh², Sudhir Yadav², Sandra Rodrigues², Yun-mei Lin², Douglas Faria¹, Giovana Longhini¹, Mario Lopes¹, Cristiane Araujo^{1,3} ¹University of Sao Paulo - Faculty of Animal Science and Food Engineering, ²BioResource International, ³University of Sao Paulo, School of Veterinary Medicine and Animal Science

The use of enzymes in poultry production has become a necessary tool for nutritionists to reduce production costs and improve the performance of birds. An experiment was carried out to evaluate the use of a xylanase (Xylamax[®] - XM) in the feeding of commercial layers from 20 to 40 weeks of age. A total of 280 white Lohmann LSL layers were used, distributed in 5 treatments (T1 - positive control diet; T2 - Positive control - 100 kcal (NC); T3 - NC + 50g/MT XM; T4 - NC + 100 g/MT XM; T5 - NC + 150 g/MT XM) in a completely randomized design with 7 replicate cages/treatment, and 8 hens/cage. Birds were fed on a maize and soybean meal-based diet. Performance data (egg production, feed consumption (g/day), feed conversion - kg/dz and kg/egg mass, and egg mass) and egg quality (egg weight, shell strength, shell thickness, albumen height, and Haugh unit) were evaluated. One-way ANOVA was performed using SAS and means were separated by Tukey's test with significance set at $P < 0.05$. Supplementation with 50 and 100 g/TM XM increased egg production by 5% and 6%, respectively, compared to the NC ($P < 0.0001$), egg mass was increased by 4g due to the supplementation of either 50 or 100 g XM/MT, compared to NC ($P < 0.0001$). The dirty eggs tended to decrease by 1% due to supplementing 100 g/MT XM ($P = 0.0951$). There were no significant differences between treatments for egg weight, feed consumption, and feed conversion ratio (kg/dz and kg/egg mass). Regarding egg quality, there was a reduction in albumen height with the use of 50 and 150 g/MT XM ($P = 0.0027$) and in Haugh unit with the supplementation of 50 g/MT XM ($P = 0.0020$) compared to eggs from the PC treatment. However, shell breaking strength was higher ($P = 0.0003$) in eggs from birds fed diets containing 50 g (4.30 kgf) and 100 g (4.46 kgf) /MT XM compared to eggs from NC treatment (4.01 kgf). Shells were thicker ($P = 0.0405$) in eggs from birds fed diets containing 100 g/MT XM compared to eggs from birds in the NC treatment (0.40 vs 0.38 mm). Based on these results, it can be concluded that supplementation with 100g/MT XM provides the best improvement in performance and eggshell quality in commercial laying hen while lowering the energy content of the diet by 100 kcal/kg which can lower the feed cost and reduce production costs.

Key Words: Carbohydrase, Egg quality, Exogenous enzyme, Performance, Production costs

M93 Effects of superdoses of phytase on peaking Hy-Line W-36 laying hen egg production and egg quality Sophia Bianchi*^{1GS}, Brooke Bodle², Courtney Poholsky¹, Darby Boontarue¹, John Boney¹ ¹Pennsylvania State University, ²DSM Nutritional Products

New generations of feed enzymes are included to enhance nutrient utilization. This study was designed to evaluate the efficacy of a new generation phytase, HiPhorius (HP), and protease, ProAct 360, when fed to peaking hens. Dietary treatments included Positive Control (PC), Negative Control 1 (NC1) at 0.15% nPP, NC1+600 FYT/kg HP, NC1+900 FYT/kg HP, Negative Control 2 (NC2) with a 2% crude protein (CP) reduction, NC2+ProAct360, and NC1+600 FYT/kg HP+ProAct 360. A total of 378 Hy-Line W-36 hens were randomly allocated to nine replicate cages per treatment with six hens per cage. Peaking 1 and Peaking 2 diets were provided throughout the 17-wk study. Phytase activity was analyzed after feeding and were higher than expected. Phytase activity ranged from 1,391-1,508 FYT/kg in Peaking 1 and ranged from 1,112-1,365 FYT/kg in Peaking 2 diets. Considering the elevated phytase activity, the new objectives were to determine how superdoses of HP affected egg production and egg quality when fed to hens from 18-35 wk. The GLM procedure of SAS was used to analyze weekly performance in an ANOVA and then repeated measures ANOVA were conducted for 4-week periods (Pd; Pd1 = 18-21wk, Pd2 = 22-26wk, Pd3 = 27-30wk, Pd4 = 31-34wk). The MIXED

procedure was used to conduct preplanned contrasts to compare PC vs NC1, NC1 vs diets containing HP, and PC vs diets containing HP. When considering the ANOVA for individual wk and the repeated measures ANOVA for each Pd, neither egg production (EP%) nor egg quality were affected by dietary treatment ($P > 0.05$). However, when contrasts were utilized, EP% differences were apparent. In Pd2 and Pd4, hens provided the PC had higher EP% than those hens provided the NC1 ($p = 0.013$ and $p = 0.0082$, respectively). As hens approached peak production in P2, those provided superdoses of HP tended to have higher EP% than those provided the NC1 ($p = 0.059$). While dietary treatment did not affect EP% or egg quality in Pd3 ($P > 0.05$), hens provided diets containing HP had improved EP% compared to those provided NC1 ($p = 0.0072$) in Pd4. In Pd1-P4, the EP% of hens provided treatments with superdoses of HP was similar to hens provided the PC ($P > 0.05$). These data support the inclusion of superdoses of HiPhorius in peaking diets with reduced nPP.

Key Words: phytase, protease, laying hens, phosphorus, crude protein

M94 Evaluation of almond hulls as an alternate feed ingredient on performance, egg quality and digestibility in Lohmann LSL-Lite layer hens Nelly Cribillero*^{1GS}, Craig Wyatt², Kelley Wamsley¹, Timothy Boltz¹, Li Zhang¹, Klint McCafferty³, Pratima Adhikari¹ ¹Poultry Science Department, Mississippi State University, ²AB Vista Feed Ingredients, ³USDA-ARS-Poultry Research Unit

This study evaluated the effects of almond hulls (AH) and cellulase-xylanase enzyme (E) on the performance, egg quality, and nutrient digestibility of laying hens from 22–41 wks. A total of 504 Lohmann LSL-Lite hens were randomly assigned to cages (117.5 sq in/hen) with 4 hens/cage and 72 hens/treatment in a 3x2 factorial arrangement of AH concentrations (5%, 10%, and 15%) and E (yes or no) in a randomized complete block design. Diets were formulated to be isocaloric and isonitrogenous and were as follows: T1 (standard control diet based on corn-SBM), T2 (5% AH), T3 (5% AH+E), T4 (10% AH), T5 (10% AH+E), T6 (15% AH), and T7 (15% AH+E). Hens were fed 100 g/day. Egg quality was measured every four weeks, and ileal digesta was collected at the end of the study. A two-way ANOVA using PROC GLM SAS 9.4 was used to analyze data and significance was considered at $P < 0.05$. Orthogonal contrasts were used to separate treatment means. An interaction between AH and E was observed on hen-day egg production (HDEP) ($P < 0.05$). Hens fed T3 had a higher HDEP than those fed T2 and T4, however it was lower than those fed T1. A main effect of AH ($P < 0.05$) and E ($P < 0.05$) was observed to affect feed intake (FI). Hens fed diets with 5% AH had a higher FI than those fed diets with 10% and 15% AH, similarly, FI was lower with E supplementation compared with those without. A main effect of AH affected feed conversion ratio (FCR). Hens fed 15% AH had an improved FCR based on egg mass ($P < 0.05$) and feed per dozen of eggs produced ($P < 0.05$) than those fed diets with 5% and 10% AH. Likewise, AH and E interacted to affect egg weight ($P < 0.05$) and eggshell weight ($P < 0.05$), with those fed T5 and T6 exhibiting the highest egg weight compared with all other treatments, whereas hens fed T2 exhibited a higher eggshell weight than those fed T5 and T6. Digestibility of CP was affected by AH ($P < 0.05$) and E ($P < 0.05$) with those fed 5% AH and no E exhibiting higher CP digestibility than those fed other treatments. In conclusion, the supplementation of 5% AH in laying hen diets for the entire peaking phase indicates that it does not produce detrimental effects on FI and egg production.

Key Words: almond hull, enzyme, performance, egg, hen

M95 Litter consumption does not have an effect on the phytase response of Hubbard × Ross 708 broilers reared in floor pens Kristina Bowen*^{1GS}, Lucas Knarr¹, Mark Jackson², Joseph Moritz¹ ¹West Virginia University, ²Huvepharma

Phytase research has traditionally been conducted by rearing broilers in raised wire cages, whereas commercially grown broilers are reared on built-up litter. Cage-based studies may not accurately represent commer-

cial bird performance response to phytase. Birds with access to litter consume it, possibly increasing phosphorus intake and giving the impression that lower phytase concentrations are required. The authors hypothesized that broilers reared in cages would express a greater response to phytase relative to birds reared in floor pens because they are not consuming soluble phosphorus via litter. The objective of this experiment was to determine the effect of different concentrations of Optiphos Plus on d0-21 live performance and tibia mineralization of male Ross 708 chicks reared in raised-wire cages and floor pens. A nutritionally adequate positive control (PC; 0.90% calcium and 0.45% nonphytate phosphorus [nPP]) and a negative control (NC; 0.65% calcium and 0.20% nPP) diet were conditioned for 30 sec at 82°C, pelleted, and analyzed within a multiple comparison. Optiphos Plus was added to the NC diet at 250, 500, 1,000, 1,500, 2,000, and 2,500 FTU/kg. A two-way ANOVA was employed to analyze the 6 (Phytase level) × 2 (Housing system) factorial arrangement of treatments. Diets were fed to 8 replicate pens of 22 chicks and 8 replicate cages of 7 chicks. On d21, live performance and tibia ash content were measured. In both housing systems, at least 1,500 FTU/kg increased weight and live weight gain (LWG) relative to the NC diet and to similar levels as the PC diet ($P < 0.05$). Tibia ash percent was restored to PC levels with the addition of 1,500 FTU/kg or greater in both systems ($P < 0.0001$). Bird weights and LWG decreased and feed conversion ratio increased for birds reared in pens relative to cages ($P < 0.0001$). There was a housing system by phytase level interaction where tibia ash mg/chick did not differ between systems at 250 FTU/kg, but above this the cages expressed improved bone mineralization ($P < 0.05$). The phytase response between the housing systems was similar, indicating that the potential consumption of litter likely does not have a significant impact on broiler performance. The phytase demonstrated optimal efficacy at an inclusion of 1,500 FTU/kg.

Key Words: phytase, floor pens, cages, bone mineralization, live performance

M96 Response of broilers to varying carbohydrase supplementation within reduced nutrient corn and soybean meal-based diets on 0 to 41 d performance and processing Emily Myers^{*1GS}, Shelby Corray², Kelley Wamsley¹ ¹Mississippi State University, ²DSM Nutritional Products

Supplementation of exogenous enzymes into commercial broiler diets is a common practice to combat inherent antinutritional factors (i.e., carbohydrases targeting non-starch polysaccharides). An experiment was conducted to optimize the inclusion of a novel carbohydrase (CAR) on 42d broiler performance and processing, using three inclusions (CAR1=250 AMNU/kg, CAR2=500 AMNU/kg, CAR3=1000 AMNU/kg), in reduced nutrient corn-soybean meal diets. This was a randomized complete block design consisting of male Ross 708 × Ross YP broilers reared in 96 pens (0.080 m²/bird). Data were analyzed using PROC GLM in SAS 9.4. Six dietary treatments (TRT) were tested, including a positive control (PC), negative control (NC), NC+CAR1, NC+CAR2, or NC+CAR3, and NC + commercial carbohydrase (NC+CCAR). All diets were formulated to meet 95% of breeder recommendations for digestible AA, with the AME reduced by 100 kcal/kg for the PC and 200 kcal/kg for the NC, for each phase. Starter (d0-14) data demonstrated that birds fed PC had the lowest d14 BW, d0-14 BW gain (BWG), and Avg. feed intake/bird (AFI) compared to all other TRT ($P < 0.05$); though no difference was detected for FCR ($P > 0.05$). For the grower phase (d14-28), birds fed PC had the lowest FCR, followed by NC+CAR1, which were similar to NC and NC+CAR2; birds fed NC+CAR3 and NC+CCAR had the highest FCR ($P < 0.0001$). Only a trend was detected during the finisher phase (d28-41), wherein birds fed NC+CCAR had the highest AFI vs. all other TRT, with PC birds having the lowest ($P = 0.0823$). Cumulatively (d0-41), birds fed PC had the lowest FCR as compared to all other TRT ($P = 0.0003$), followed by NC+CAR1. Birds fed NC and NC+CCAR had the highest FCR, though similar to all other TRT except PC and NC+CAR1. On d42, four birds ±100 g of Avg. BW/pen were processed. While no significance was found for carcass or total breast weight ($P > 0.05$), total breast yield

was significantly lower for PC fed birds, which were only similar to birds fed NC+CAR2 ($P = 0.0286$). Overall (d0-41) data reveals benefit for novel CAR supplementation, though further research is needed to optimize enzyme-substrate interaction and economic return, depending upon feed substrate and phase of growth when male broilers are fed reduced nutrient corn-soybean meal diets.

Key Words: broiler, carbohydrase, exogenous enzyme, performance, processing

M97 Effect of a multi-activity non-starch polysaccharide degrading enzyme on nitrogen balance and growth performance in broilers: a meta-analysis of trial data. Alexia Godbout^{*1}, Joan Edwards², Leni Kuterna², Marie-Pierre Létourneau-Montminy¹ ¹Laval University, ²Palital Feed Additives

With fiber-rich feeds, a multi-activity enzyme (glucanase and xylanase) can be used to break down non-starch polysaccharides (NSP) and enhance nutrient availability. A meta-analysis was performed to quantify the effect of a multi-activity enzyme supplement on the growth performance and nitrogen balance of broilers. The database was made up of 18 non-published trials conducted between 2003 and 2021 with broilers aged 0-42 d. Trial basal diets were mainly wheat-based with some corn-based. Treatment groups were control (basal diets without enzyme) or enzyme (basal diets with 25 ppm to 100 ppm of AveMix[®] XG 10). Within each trial, all other nutrients were similar between treatment groups. Two sub-databases were created to also study the impact of feeding phase: starter and grower/finisher. Trial feeding phases starting before 11 d of age were classified as starter, and other feeding phases classified as grower/finisher. N balance was calculated using the previously published value of 29 g N retention/kg of body weight gain. To assess enzyme effects within each feeding phase, Minitab software (version 21.4.0) was used with linear general models and trial as a fixed effect. During the starter phase, enzyme supplementation increased ADG (27.3 vs 26.5 g/d; $P < 0.001$) whereas ADFI and FCR were not affected. N retained (g/d) was increased by 3.4 % ($P < 0.001$) with enzyme supplementation. In contrast, N intake (g), N excreted (g) and N efficiency (%) were not affected. During the grower/finisher phase, enzyme supplementation increased broiler ADG by 4.59 g/d ($P < 0.001$), and decreased FCR by 6.4 % ($P < 0.001$). Enzyme supplementation affected N balance during the grower/finisher phase: N retained (40.2 vs 38.1 g $P < 0.001$) and N efficiency (52.8 vs 49.6 %; $P < 0.001$) increased, whilst N excreted (35.8 vs 38.2 g $P < 0.001$) decreased. The impact of enzyme supplementation was higher during the grower/finisher phase. It is speculated that this may be due to the longer duration and/or higher feed intake during this phase. In conclusion, a multi-activity enzyme supplement can improve the growth performance and N utilization of broilers.

Key Words: Broilers, Feed additive, Growth performance, NSP, Nitrogen balance

Metabolism & Nutrition V General Nutrition

M98 A horizontal mixer and a batch-to-horizontal mixer system increased the mix uniformity of free methionine in complete diets relative to a vertical mixer Lucas Knarr*^{GS}, Kristina Bowen, Emily Estanich, Joseph Moritz *West Virginia University*

Mix uniformity (MU) is affected by mixer type (MTY) and mix time (MTI). The coefficient of variation (CV) of a selected nutrient/ingredient marker is typically utilized to measure MU. The CV of a batch of mixed feed can be calculated based on the analysis of ten representative samples, and an industry-acceptable CV is < 10%. This study aimed to determine the effect of a vertical mixer, horizontal mixer, and batch-to-mix transfer system on mix uniformity while using adequate or inadequate mixing times for two different diet formulations. Both a corn-salt mixture and a complete diet were mixed in a 2 (MTI) x 3 (MTY) factorial arrangement of three replicates. Chloride ions (Cl⁻) were used in experiment one as a marker for the CV in the corn-salt mixture, and crystalline DL-Methionine (Free Met) and crystalline L-Lysine-HCl (Free Lys) were used in experiment two as markers for CV in the complete diet. Increasing MTI decreased Cl⁻ CV by 3.91% (P = 0.017). An interaction between MTY and MTI affected Free Met CV (P = 0.005). The horizontal mixer and transfer mixing system did not demonstrate changes in the Free Met CV compared to the vertical mixer by manipulating MTI. The authors hypothesize that the horizontal mixer is a superior type that influenced the MU of the treatments using the horizontal and transfer MTY. The transfer system resulted in the lowest Free Lys CV (P = 0.017); however, no treatment provided an industry-acceptable MU. In conclusion, Cl⁻ and Free Met may be appropriate markers for MU in corn-salt mixtures and complete diets, respectively, and the transfer mixing system may contribute to an acceptable MU.

Key Words: Mix Uniformity, Mixer Type, Mix Time

M99 Effects of grinding different sorghum grain varieties with a hammermill on subsequent particle size and flowability Walter Friesen*^{GS}, Haley Ottott, Charles Stark, Chad Paulk *Kansas State University*

Sorghum grain is a common commodity used as an energy source in poultry diets due to high concentrations of starch. Different sources of sorghum can be classified as waxy or non-waxy varieties. Non-waxy sorghum is defined by having a ratio of 25% amylose to 75% amylopectin, while waxy sorghum is characterized by having 90% or greater amylopectin. The objective of this experiment was to evaluate the effects of grinding waxy and non-waxy varieties of sorghum with a hammermill on subsequent particle size and flowability. Yellow dent corn, red non-waxy, red waxy, and white waxy sorghum (20 kg each) were ground with a laboratory-scale 1.5 HP Bliss Hammermill (Model 6K630B) through a #10 screen (3.97 mm). Each variety was ground at three separate time points to provide three replications per treatment. For each replication, samples were collected and analyzed for particle size geometric mean diameter (d_{gw}), standard deviation (S_{gw}), and angle of repose (AoR). There was an overall treatment effect ($P < 0.001$) for d_{gw} , S_{gw} and AoR. When grinding different sorghum varieties with consistent hammermill settings, red waxy (664 μ m) and white waxy (688 μ m) varieties of sorghum had greater ($P < 0.05$) d_{gw} when compared to the red non-waxy sorghum (567 μ m) which was also greater than the yellow dent corn (438 μ m). Red waxy (2.39) and white waxy (2.39) varieties of sorghum had decreased ($P < 0.0001$) S_{gw} when compared to red non-waxy sorghum (2.84), which was also less than yellow dent corn (3.34). Red waxy (43.4) and white waxy (43.3) sorghum varieties had improved ($P < 0.0001$) flowability properties when compared to that of red traditional sorghum (45.6) which was also improved compared to yellow dent corn (53.1). In conclusion, waxy sorghum varieties ground with consistent hammermill settings had greater geometric mean diameter particle size, reduced variation, and improved flowability properties compared to that of red non-waxy sorghum and yellow dent corn. Future research will utilize ground grain in this experiment

to determine the effects of sorghum grain variety on the pelleting process and will be presented with the grinding experiment.

Key Words: Sorghum, Variety, Processing

M100 Feed bin fill level impacts on-farm feed particle and nutrient distribution and subsequent broiler performance over time Courtney Poholsky*^{GS}, John Boney *The Pennsylvania State University*

Recent studies showed that pellets, fines, and nutrients can segregate in poultry houses. There is a need to investigate if bird performance is impacted by feed particle and nutrient segregation. Therefore, a field study was conducted to assess feed and nutrient distribution in feed lines over time and examine the relationship among feed bin dynamics, feed and nutrient flow, and broiler performance. Feed (65% pellets: 35% fines) was provided to broilers as part of a standard grow-out. The 152 m long commercial house was equipped with two feed lines and two migration fences, creating three distinct locations in the house. Hanging scales were present in each of the three locations to capture bird weights throughout each day. Feed samples were collected at 25, 75, and 125 m along each feed line. Before sampling, designated feed pans were emptied. Feed was then augered throughout the feed lines to fill the empty pans. Sample collection occurred over five days. Feed samples were sifted using a No. 6 ASTM sieve for pellet-to-fine ratio determination. Amino acids and mineral concentrations of the feed were assessed at commercial laboratories. Feed bin inventory and average bird weights were recorded daily using an intelia FarmHub system. Feed analysis data were analyzed in a repeated measures ANOVA using PROC MIXED in SAS. Pearson's correlations were performed on all data using PROC CORR in SAS. Percent pellets varied throughout the house, increasing as feed was augered away from the hopper ($P < 0.001$). Daily variations in percent pellets were also apparent ($P < 0.001$). Bin fill correlated with percent pellets ($r = -0.400$; $P = 0.027$), indicating more pellets in the feed pans as the feed bin emptied. Average concentrations of minerals and amino acids in the house varied day by day ($P < 0.05$) with the highest and lowest concentrations observed on days 1 and 5, respectively. Average bird weights correlated with location in the house ($r = 0.553$; $P = 0.002$) and percent pellets ($r = 0.600$; $P = 0.001$), suggesting that birds placed at the end of the house (100-150 m) consumed more pellets and gained more weight compared to other birds in the house. In conclusion, feed bin dynamics contributed to variations in percent pellets and nutrients, ultimately impacting broiler performance.

Key Words: feed bin, broiler, amino acids, minerals, nutrient segregation

M101 Stochastic feed formulation: examining the cost of uncertainty in soybean nutrient composition. Caleb Marshall*^{IGS}, Thiago Yabuta¹, Manoel Garcia Neto², Edgar Oviedo-Rondon¹ *¹Prestige Department of Poultry Science, North Carolina State University, ²Faculdade de Medicina Veterinária, Universidade Estadual Paulista*

Currently, poultry feed is formulated using least-cost linear programming (LP) to produce nutritionally balanced diets while minimizing feed cost. The LP assumes the nutrient composition of feedstuffs are fixed and ignores nutrient variability. To address this issue, nutritionists apply a margin of safety (MOS); thereby, ensuring minimum nutrient requirements are met. An alternative approach is stochastic programming (SP), a non-linear method which considers ingredient variability. This study aimed to evaluate the impact of utilizing two MOS methods compared with LP and SP on diet cost by considering AMEn, CP, and digestible amino acid (AA) variability of SBM. The energy, nutrient mean, and SD of SBM sources (Argentina; ARG; Brazil, BRA; and United States; USA) were obtained from a global database (NIRS Precision Nutrition Evaluation, PNE, from Adisseo®) for two years (2020 and 2021). Significant variability in AMEn, CP and AA concentration and digestibility were observed between origins ($P < 0.001$). Practical starter and grower diets were formulated following

Aviagen (2022) recommendations for ME, dLys, dTSA, and dThr, using the following methods: LP, 5% MOS to requirements (MOS-5%), MOS with 69% probability adjustment using SBM nutrient's SD (MOS-SD), and SP with a 69% probability to meet nutrient requirement. As expected, MOS-5% was over formulated, resulting in higher nutrients and average cost for 2020 (starter, 33.78; grower, 38.10 \$/MT) and 2021 SBM (starter, 33.76; grower, 38.07 \$/MT). While MOS-SD and SP achieved the same nutrient levels as LP, MOS-SD formulas had consistently higher cost compared with SP in the starter for 2020 SBM (2.98, 4.21, and 2.15, \$/MT; USA, BRA, and ARG, respectively) and 2021 SBM (3.06, 5.73, and 2.15, \$/MT; USA, BRA, and ARG, respectively). Similar, results were obtained in the grower formulations for 2020 SBM (2.62, 1.30, and 1.57, \$/MT; USA, BRA, and ARG, respectively) and 2021 SBM (0.38, 4.37, and 1.10, \$/MT; USA, BRA, and ARG, respectively). Notably, increased SBM SD resulted in higher utilization of SBM, DDGS, and poultry fat to ensure the minimum nutrient requirement was met. Overall, SP formulations achieved cheaper diet cost at a higher confidence to meet nutrient specifications compared with MOS-5% and MOS-SD.

Key Words: feed formulation, stochastic programming, linear programming, nutrient variability, margin of safety

M102 Precision Nutrition: The value of soybean meals by origin in poultry and swine feed formulation. Thiago Yabuta*^{UG}, Joaquín Cabanas-Ojeda, Edgar Oviedo-Rondón *Prestage Department of Poultry Science, North Carolina State University*

Soybean meal (SBM) is the main source of amino acids (AA) for poultry and swine and accounts for 25% of the metabolizable energy. Variability due to SBM origin affects cost and diet precision to provide nutrients. The present study evaluated the difference between using the average nutrient values of SBM without considering their origin, and the inclusion of SBM from five origins: North Carolina (NC), Eastern (EAST) and Western (WEST) US Corn Belt, Argentina (ARG) and Brazil (BRA) analyzed with NIRS AMINONIR® (Evonik) from 2020 and 2021. These sources had significant differences ($P < 0.001$) in AA and energy content. Diets were formulated in a least-cost formulation software (Concept 5.0®). Starter, grower, and finisher broiler diets were formulated following the Ross 708 (2019) nutrient specifications. Grower brown and white layer diets for phases 1 and 2 were formulated following the Hy-Line (2021) nutritional guide. For swine, diets for growing boars and gilts, phases 2 and 3 were formulated based on PIC (2021) guide. High market prices were used for all ingredients except for SBM. Four SBM prices (590, 511, 432, and 353 \$/MT) were evaluated in a sensitivity analysis formulating 440 diets in a factorial arrangement. The relative SBM economic value (\$/MT) was estimated as $\text{Base SBM price (\$/MT)} - [\text{TDC}_{\text{test}} - \text{TDC}_{\text{Base}}] / \text{SBM}_{\text{test}} \times 1000$; where "TDC" is the total diet cost (\$/MT), assuming the inclusion of the specified SBM, and "SBM" is the amount of SBM included in the diet (kg/MT). The Base SBM was the NC SBM. Results indicated no interactions ($P > 0.05$) and diets including NC SBM were cheaper ($P < 0.001$) for broilers (1.40–34.32 \$/MT), layers (0.27–27.24 \$/MT), and swine (0.18–15.90 \$/MT). Based on relative SBM value, the NC SBM had a premium value (\$/MT) over the other sources ranging from 8.17 to 98.73 for broilers, 10.57 to 99.74 for layers, and 0.80 to 106.36 for swine. When comparing diet nutrient composition, SBM as a commodity with average nutrient values disregarding their specific nutrient content by origin can cause deficient diets when EAST and WEST SBM are included, and nutrient excess in diets with NC and BRA SBM. In conclusion, feed formulation should consider the origin, nutrient and energy content of each SBM source to reduce cost and nutrient waste.

Key Words: precision nutrition, soybean meal, country of origin, diet cost, nutrient waste

M103 Determination of nitrogen corrected true metabolizable energy values of poultry feed ingredients by near infrared reflectance spectroscopy Reed Dillard*^{GS}, Adam Davis *University of Georgia*

Near infrared reflectance spectroscopy (NIRS) ingredient analysis has the potential to allow nutritionists to obtain real-time nutrient specifications of their ingredients which will aid in the formulation of diets that more precisely meet dietary requirements. In the current research, NIRS technology was assessed for its potential to rapidly predict the nitrogen corrected true metabolizable energy (TME_N) values of poultry feed ingredients. The Leghorn rooster bioassay was utilized to determine the TME_N value of 701 feed ingredients. A sample of each of these feed ingredients was finely ground and scanned across the near infrared spectrum using a Bruker MPA: FT-NIR Spectrometer equipped with OPUS software. From these scans an all-encompassing poultry feed ingredient NIRS calibration curve to predict TME_N was developed with 150 samples and then validated with another 551 samples. The calibration and validation feed ingredient samples were distributed across more than 75 different ingredient types. For the validation samples, the relationship between the predicted values obtained from the NIRS calibration curve versus the rooster bioassay determined values had an R^2 value of 0.95 and a residual predictive deviation value of 4.43. But more importantly, 97% of the validation samples had predicted values that deviated less than plus or minus 10% from their bioassay determined TME_N values, with the remaining 3% of samples having predicted values deviating less than 15% from their bioassay determined value. These results indicate that this TME_N calibration curve would be acceptable for an initial assessment of the TME_N of an ingredient for which a more accurate ingredient specific TME_N NIRS calibration curve did not exist. However, for poultry diet formulation, ingredient specific TME_N NIRS calibration curves would be necessary as these curves predict TME_N values with much greater accuracy.

Key Words: Feedstuff evaluation, Digestibility, Feed formulation

M104 Utilization of near-infrared reflectance spectroscopy to predict the digestible lysine, methionine, threonine and nitrogen-corrected true metabolize energy content of mechanically and solvent-extracted soybean meals Thomas Jones*^{GS}, Reed Dillard, Coleman Hatmaker, Chongxiao Chen, Adam Davis *University of Georgia*

Soybean meal (SBM) is a widespread constituent of poultry diets internationally. However, variations in agronomic conditions, cultivar variety, and processing techniques can alter digestible lysine, methionine, threonine, and nitrogen-corrected true metabolize energy content (TME_N) of SBM. To detect these deviances in nutritional value, broiler or rooster bioassays with subsequent laboratory analyses can be completed to determine digestible amino acid and TME_N content. But, these bioassay determinations are expensive and time-consuming. In contrast, near-infrared reflectance spectroscopy (NIRS) has the potential to enable the rapid prediction of nutritional component values of feed ingredients upon delivery and prior to their incorporation into poultry diets. The goal of the current research was to design NIRS calibration curves that accurately predicted the TME_N and digestible lysine, methionine, and threonine content of solvent-extracted and mechanically processed SBM. The TME_N and digestible amino acid content for over 100 SBM samples was determined by the intact and cecectomized rooster bioassays, respectively. Each SBM sample was uniformly ground prior to obtaining a complete near-infrared spectral analysis using a Bruker MPA: FT-NIR Spectrometer equipped with OPUS software. Roughly half of the samples were used to construct each calibration curve, while the remaining half were used to validate each calibration curve. For the validation samples, the relationship between the predicted values obtained from the NIRS calibration curve versus the rooster bioassay determined values had correlation coefficients (R^2) values ranging from 0.83 to 0.90 for digestible lysine, methionine, and threonine content and 0.98 for TME_N . However, for all three amino acids and TME_N , the validation samples had predicted values that deviated less than plus or minus 2.5% of their bioassayed determined value, except

for two samples in the methionine validation and one sample in the threonine validation, which had NIRS predicted values that deviated more than 2.5%, but less than 5% of their bioassayed values. This research indicates

that NIRS can be used to accurately predict TME_N and digestible lysine, methionine, threonine content of the SBM samples for poultry diets.

Key Words: Poultry, Feed formulation, Ingredient quality

Metabolism & Nutrition VI General Nutrition

M105 Comparison of ingredient markers and near-infrared spectroscopy (NIRS) to assess mix uniformity Courtney Poholsky*¹, Sophia Bianchi¹, Darby Boontarue¹, Caitlin Rvans², Craig Wyatt², John Boney¹ ¹Penn State University, ²AB Vista

Mix uniformity (MU) is a quality control parameter used to evaluate diet homogeneity. Different ingredient or nutrient markers are used throughout the industry to measure the coefficient of variation (CV) in a batch of feed. However, lab analysis of traditional markers is costly and time-consuming. Two experiments (EXP) were conducted to compare different ingredient markers and near-infrared spectroscopy (NIRS) to assess MU and evaluate the impact of sample preparation (unground vs ground) on MU measured using NIR spectral scans. All feed was mixed using a 454 kg Scott paddle mixer for 3 different mix times (60, 180, and 330 sec). In EXP 1, a broiler starter diet was used. Treatment mix times were arranged in a completely randomized design with 3 replicate batches of feed. In EXP 2, 3 unique diets (broiler starter, finisher, and withdrawal) were mixed at each mix time and diet type served as a blocking variable. After mixing, 10 mash samples were collected from the top opening of the mixer using a grain probe. Samples were analyzed to calculate CV for: L-Lys-HCl, sodium, chloride ion, manganese, Microtracers (Red #40 and Blue #40), spectral scans (NIRS), crude protein (CP; NIRS), crude fiber (CF; NIRS), and crude fat (CFat; NIRS). Regarding NIRS analysis, samples were scanned as mash feed collected directly from the mixer (unground) and as ground feed that was passed through a coffee grinder to reduce the particle size. Data were analyzed in a one-way ANOVA using the GLM procedure of SAS. Pearson's correlations were performed for each EXP to explore relationships between markers. Overall, all markers showed a reduction in CV as mix time increased from 60 to 330 sec, except for CF and CFat. In EXP 1, L-Lys-HCl positively correlated with sodium ($r=0.982$) and chloride ion ($r=0.931$) as markers for MU ($P<0.01$). In EXP 2, MU using NIRS spectral scans correlated with markers L-Lysine-HCl ($r=0.684$) and manganese ($r=0.700$; $P<0.05$). Scanning unground samples using NIRS resulted in higher standard deviations compared to the ground samples. These data indicate that MU results are influenced by the selected marker and total mix time. In addition, NIRS spectral scans may be a rapid, cost-effective tool to measure MU, provided that the sample is ground prior to scanning.

Key Words: mix uniformity, coefficient of variation, NIRS, correlations, Microtracers

M106 Effect of dietary inclusion of corn samples sourced from different origin on broiler performance, processing yield, and nutrient digestibility from 1 to 35 days of age Jose Vargas*^{GS}, Joseph Gulizia, Jose Hernandez, Eva Guzman, Cristina Simoes, Leticia Orellana, Wilmer Pacheco *Department of Poultry Science, Auburn University*

The nutritional value of corn is influenced by origin since it is determined by genetics, agronomic practices, and post-harvest processing. Therefore, understanding of the influence of corn origin on broiler production is important for nutritionists who use corn from different sources. This experiment studied the effect of corn source (USA local (USA-L), USA reimported (USA-R), Argentina (ARG), and Brazil (BRA)) on broiler performance, processing yield, and nutrient digestibility from 1 to 35 d of age. A total of 1,200-day old YPM × Ross 708 male broilers were randomly sorted in 48 floor pens, each containing 25 chicks and subjected to four experimental diets, resulting in 12 replications per treatment. Nutritional content of the corn samples was analyzed by near-infrared spectroscopy

and used to calculate their inclusion in diets formulated to meet nutritional requirements on a least cost basis for YPM × Ross 708 male broilers. Data were analyzed as a one-way ANOVA and means were separated using Tukey's HSD test, with statistical significance considered at $P \leq 0.05$. No differences ($P > 0.05$) were found in BW from 1 to 35 d of age among the treatments. Similarly, no differences ($P > 0.05$) were found in FI and FCR from 1 to 21 d of age. Nonetheless, broilers fed diets formulated with corn from ARG and BRA had a higher FI compared to broilers fed diets formulated with corn from USA-L from 1 to 35 d of age (3,470 and 3,463 vs 3,372; $P = 0.012$). Lastly, broilers fed diets with USA-L and USA-R corn had lower FCR than broilers fed diets with BRA corn from 1 to 35 d of age (1.376 and 1.385 vs 1.401; $P < 0.0001$). Broilers fed diets with corn from ARG had a higher chilled carcass weight compared with broilers fed diets with inclusion of USA-R corn (1,971 vs 1,911; $P = 0.032$). Corn origin did not influence ($P > 0.05$) crude protein, fat, calcium, and potassium digestibility. Nonetheless, broilers fed diets with inclusion of corn from ARG had a higher phosphorus digestibility in comparison with broilers fed diets with inclusion of corn from USA-L (79.92 vs 75.13; $P = 0.0066$). In conclusion, corn origin influenced FCR, chilled carcass weight, and phosphorus digestibility of broilers from 1 to 35 d of age.

Key Words: corn origin, broilers, growth performance, processing yield, nutrient digestibility

M107 Impact of low-quality soybean meal and live coccidiosis vaccination on performance, relative organ weights, and heterophil:lymphocyte ratio in broiler chickens Makenly Coles*^{IGS}, Aaron Forga¹, Kyle Teague¹, Ross Wolfenden², Jundi Liu², Juan Latorre¹, Guillermo Tellez-Isaias¹, Billy Hargis¹, B. Graham¹ ¹University of Arkansas, ²Animal Nutrition BU, Eastman Chemical Company

Dietary soybean meal (SBM) quality can negatively impact intestinal health and performance in broiler chickens. The purpose of the present study was to develop a model to compare the effects of dietary SBM quality and live coccidiosis vaccine cycling on organ weights and performance in broiler chickens. At day of hatch (DOH), chicks were randomly assigned to one of two groups ($n=12$ pens/group): 1) high-quality (HQ) SBM or 2) low-quality (LQ) SBM. Group 1 received HQ SBM diet formulated to meet or exceed requirements. Group 2 received LQ SBM diet designed to induce inflammation resulting in lower body weight (BW) and decreased feed conversion ratio (FCR). The LQ SBM was produced using a 1:1:1 mixture of uncooked SBM, normal SBM, and overprocessed SBM (autoclaved ~125C for 60 minutes). Chicks were orally gavaged with a 1X dose of a commercial live coccidiosis vaccine containing *E. acervulina*, *E. tenella* and *E. maxima* at DOH. Pen weights were recorded at DOH and weekly thereafter to determine average BW and BW gain. Feed was weighed weekly to calculate feed conversion ratio (FCR) and feed intake (FI). On D15, D28, and D42, individual organ weights were recorded ($n=24$ chickens/treatment) to calculate relative organ weights (%). Blood was also collected ($n=10$ chickens/treatment) to assess heterophil:lymphocyte ratio (H:L ratio). Data were analyzed using Analysis of Variance with significantly ($P \leq 0.05$) different means separated using Student's T-test. Average BW (g) was significantly higher in the HQ group than the LQ group at D7, D21 and D28. At D42, the HQ group had an average BW significantly higher than the LQ group. Both average BWG (g) and FCR were significantly improved from D0-D42 for the HQ group compared to the LQ. Additionally, the LQ group had significantly higher relative whole gut and pancreas weight (%) than the HQ group on D15,

D28 and D42, whereas the HQ group had markedly ($P \leq 0.05$) higher liver and spleen weights (%) on D15. There were no statistical differences between the two groups at any time point evaluated for H:L ratio. These results indicate that the LQ SBM diet reduced overall performance and impacted organ development in broiler chickens. The impact of these dietary treatments on the gut microbiome is currently being evaluated.

Key Words: soybean meal, broiler chickens, performance, organ weights

M108 Effect of Sub-Clinical Mycotoxin Exposure on Growth Performance and Immune Response in Eimeria-Challenged Pullets Deependra Paneru^{*1GS}, Milan Sharma¹, Hanyi Shi¹, Hamidreza Naeini¹, Venkata Reddy Choppa¹, Doyun Goo¹, Revathi Shanmugasundaram², Woo Kim¹ ¹Department of Poultry Science, University of Georgia, ²Toxicology and Mycotoxin Research Unit, USDA-ARS

An experiment was conducted to investigate the effects of subclinical mycotoxin exposure on the growth performance and immune cell dynamics in pullets exposed to coccidiosis. Using a 2x2 factorial design, a total of 288 four-week-old Hy-Line W36 pullets were randomly allocated to four treatment groups, each containing six replicated cages with 12 birds. The experimental groups were exposed to either non-contaminated or mycotoxin-contaminated diets and subjected to either a non-challenge or a challenge with *Eimeria* spp. Mycotoxin-exposed group was fed a diet with naturally contaminated corn distillers dried grains with solubles included at the level of 20% in the feed, providing an average of 1.3 mg deoxynivalenol, 56 µg zearalenone, and 0.06 mg fumonisins per kg of feed. *Eimeria*-challenged group was orally inoculated with sporulated oocysts of 50,000 *E. acervulina*, 10,000 *E. maxima*, and 10,000 *E. tenella* per dose on the second week of the experiment. Growth performance, intestinal permeability, intestinal lesions, and immune cell dynamics were measured at 6 and 14 days *Eimeria* post-inoculation (DPI). Data were subjected to two-way ANOVA for the main and interaction effects, and the significance level was set at 0.05. *Eimeria* infection significantly reduced feed intake (FI) by 23% and body weight (BW) by 14% from 1 to 6 DPI, while increasing the intestinal permeability at 5 DPI, intestinal lesions, and the ratio of CD4⁺ to CD8⁺ T cells in the cecal tonsils at 6 DPI ($P < 0.05$). Mycotoxin exposure further decreased the FI by 3.8% and BW by 1%, increasing the intestinal lesions and the CD4⁺:CD8⁺ T cells ($P < 0.05$). From 7 DPI, FI started to recover in the *Eimeria*-challenged group and completely recovered in 14 DPI. However, recovery was less effective with mycotoxin exposure. At 14 DPI, the CD4⁺ to CD8⁺ T cell ratios were similar in the non-challenged and *Eimeria*-challenged group. However, the pullets exposed to mycotoxins had a higher CD4⁺ to CD8⁺ ratio, which was further increased with *Eimeria* infection. It can be concluded that the presence of mycotoxins in the feed can negatively affect the growth performance and impair the recovery of the pullets infected with coccidiosis.

Key Words: Subclinical mycotoxin exposure, Growth performance, Immune response, Pullets, Coccidiosis

M109 Effect of metabolizable energy levels and feed form on broiler performance and feed wastage from 1 to 21 days of age Jose Hernandez^{*GS}, Joseph Gulizia, Jose Vargas, Eva Guzman, Isadora Pegoraro, Beatriz Onishi, Wilmer Pacheco Department of Poultry Science, Auburn University

This study evaluated the main effects and interactions of two metabolizable energy levels and three feed forms on broiler performance and feed wastage from 1 to 21 d of age. A total of 648 YPM x Ross 708 male broilers were randomly distributed in 72 battery cages (9 birds/cage) and assigned to six treatments (12 replicates/treatment). Starter diets were formulated to contain two metabolizable energy levels (standard energy (SE) = 2,979 kcal/kg and reduced energy (RE) = 2,875 kcal/kg). Both diets were fed as mash, crumbles conditioned at 85°C, and crumbles conditioned at 90°C. Body weight gain (BWG) and feed intake (FI) were determined at 10 and

21 d of age and FCR was adjusted for mortality. On d 10, 15, and 18 feed wastage (FW) was collected from the excreta trays under every battery cage to adjust FI results and to calculate wastage as g/kg. Data were analyzed as a 2 x 3 factorial arrangement (metabolizable energy level x feed form) using the GLIMMIX procedure of SAS to evaluate main effects and interactions. Tukey's HSD test was used to separate means and statistical significance was considered at $P \leq 0.05$. Broilers fed SE diets had higher ($P < 0.05$) BWG (868 vs. 809 g) and lower ($P < 0.05$) FCR (1.28 vs. 1.36 g:g) from 1-21 d than broilers fed RE diets. Broilers fed mash diets had lower ($P < 0.05$) BWG (734 vs. 896 and 886 g) and FI (1049 vs. 1223 and 1215 g) from 1-21 d compared to broilers fed crumbles conditioned to either 85 or 90°C. In addition, d 1-21 FCR (1.34 vs. 1.30 and 1.32 g:g) was higher ($P < 0.05$) when broilers were fed mash compared to broilers fed crumbles. Furthermore, broilers fed mash diets had higher ($P < 0.05$) FW (28.6 vs. 2.3 and 3.0 g/kg) compared to broilers fed crumbles conditioned to either 85 or 90°C. Overall, broilers responded both to energy level and feed form with the best performance achieved when broilers were fed SE crumbled diets, irrespective of conditioning temperature.

Key Words: energy, feed form, broilers, growth performance, feed wastage

M110 Influence of energy and stocking density on body composition & egg production in broiler breeders Cole Umberson Umberson^{*GS}, Diego Martinez, Pramir Maharjan, Nawin Suesuttajit, Jordan Weil, Craig Coon University of Arkansas

Energy in egg production is crucial for the onset and persistency of egg production in broiler breeders. Hatching egg production creates an energy supply demand in the body that is supplied via the diet (protein, carbohydrates, and fat) and body energy utilizing a combination of protein and lipid turnover. 3,400 Cobb 500 FF broiler breeder chicks were randomly distributed into 48 pens at two stocking densities during pullet rearing (HS, 1.72 sq. ft. /bird; LS, 1.32 sq. ft. /bird) and in production (HS, 1.85sq. ft. /bird; LS, 2.3 sq. ft. /bird). From 16 to 20 wks. of age pullet feed allocation was increased to increase body fat (BW: +40%). At 16 wks., pens were switched to different energy diets (HE, Higher energy, 2900 Kcal; LE, Lower energy, 2800 Kcal) and source of energy (LE, standard diet; HEC, higher energy calorie diet; HEO, higher energy oil diet). Body composition (Dual X-ray absorptiometry; DEXA) and egg production were measured. Birds were infused with 15N-phenylalanine and deuterium oxide and samples were collected for the determination of protein and lipid turnover. Increased pre-peak egg production was observed in birds with higher percent body fat ($P = 0.0001$). However, post-peak egg production was increased in birds with lower percent body fat and ash ($P = 0.0046, 0.0057$, respectively). Egg production was increased in both pre- and post-peak production with lowered stocking density and higher energy level diets (P -value < 0.05). In conclusion, egg production was increased by lowering the stocking density, feeding higher energy level diets, and increasing the percent body fat of broiler breeder females.

Key Words: Body composition, Energy, Stocking density, Fat, Broiler breeders

M111 A 51-week egg production assessment of White Leghorn hens fed on diets having a 400 kcal ME/kg range Douglas Maria^{*1GS}, Sergio Vieira¹, Raquel Horn¹, Walter Altevogt¹, Julmar Feijó¹, Samantha Silveira¹, Brenda Nicola¹, Raissa Menezes¹, André Favero² ¹Universidade Federal do Rio Grande do Sul, ²Santa Livia Farm

The study aimed to evaluate the performance and egg characteristics of White leghorn type laying hen chickens fed on diets with graded increases in metabolizable energy (ME) from 25 to 76 weeks. Hens were placed in wire cages (0.33 m length, 0.46 m width, 0.40 m height), two per cage. Each cage had one stainless steel nipple drinker and one trough plastic feeder. Hens were completely randomized into 4 treatments with 12 replicates each, in a total of 96 hens and 48 cages. Hens were *ad libitum*

fed a common diet (2,750 kcal ME, 17.2 CP, 4.3 Ca and 0.48 Av.P) from 18 to 24 wks. Experimental feeds had 2,550, 2,650, 2,750 and 2,850 kcal ME/kg, with all other nutrients formulated to be equal and were provided from 25 to 76 wks, divided into 13 periods of 28 d. Eggs were daily collected. Data were analyzed by PROC MIXED using periods as repeated measures, whereas total eggs produced were analyzed by PROC GLM using SAS 9.3. Means were compared using Tukey ($P < 0.05$). Body weight, and specific gravity of eggs at 76 wks were not affected by the treatments ($P > 0.05$). Feed intake decreased linearly at a rate of 2.2 g per 100 kcal ME increase in the feeds ($P < 0.05$) whereas egg weight and egg mass increased linearly at 0.7 g and 0.8 g for each 100 kcal ME increment ($P < 0.05$). Quadratic results pointed to the highest egg total production 350 eggs/hen and FCR 0.032 g/dozen eggs occurring at 2,729 kcal and 2,860 kcal, respectively ($P < 0.05$). Yolk, albumen, eggshell weight, and eggshell thickness linearly increased at the rate of 0.2, 0.5, 0.05 g and 1.82 μm for each 100 kcal ME feed increment ($P < 0.05$). Haugh units were quadratically adjusted with the maximum response at 88.1 units at 2,707 kcal ($P < 0.05$). At the current market feed costs in Brazil the lower production egg cost was obtained from hens fed at 2,750 kcal ME, R\$ 0.18 (US\$ 0.036) per egg unit. Outcomes from the present study can be used to predict the impact of dietary energy on the final egg production costs.

Key Words: Bovans white, Eggs, EMAn, Performance

M112 Determining optimum dietary balanced protein levels in white and brown egg-type pullets: outcomes on body weight, feed intake, and sexual maturation. Jo Ann Chew*^{1GS}, Laura Star², Martin Zuidhof¹
¹Department of Agricultural, Food and Nutritional Science, University of Alberta, ²Schothorst Feed Research

The objective of this study was to determine the amino acid requirements of two egg-type pullet strains prior to sexual maturity. A dose-response study was conducted during the pullet rearing phase using six isocaloric diets containing 60, 70, 80, 90, 100 (control), and 110% of recommended dietary balanced amino acid profiles based on the ideal protein concept. Two additional choice treatments enabled birds to select from either 60, 80, and 100%, or 70, 90, and 110% diets. Sixty-four Bovans Brown (Brown) and sixty-four Babcock White (White; $n=128$; 8 birds per strain \times dose level) pullets were assigned to either of two floor pens from 0 to 30 weeks of age. All birds were fed with multi-feeder feeding stations that allocated one of four diets to each individual bird. As such, each bird served as an experimental unit. The birds were photostimulated at 17 weeks of age, and all treatments received the same commercial pre-lay and layer diets after 18 weeks of age. Body weight, feed intake, and age at first egg response data were collected, and cumulative digestible lysine intake was the independent x-axis variable. Data were analyzed using non-linear (broken line) regressions, as well as ANOVA, where Tukey's multiple range test was used to separate means. Based on the broken line regression, the cumulative digestible lysine intake requirements (after which no further body weight increase was observed) were calculated as 3.74 g for Brown and 3.31 g for White strain pullets at 5 weeks of age (35 d), and 16.28 g for Brown and 12.90 g for White strain pullets at 10 weeks of age (70 d). By 17 weeks of age (119 d) BW was similar for all treatment groups. Birds on the 60% diet had greater daily feed intake than other treatments at 17 weeks of age. White birds achieved sexual maturity earlier than Brown birds (147 versus 149 d, respectively; $P < 0.001$). No differences were observed between amino acid levels on the age at first egg. Overall, the results indicated that a diet ranging from 60% to 110% of amino acids from the ideal protein concept resulted in no difference on age at sexual maturation. Depending on subsequent egg production efficiency, these somewhat surprising results may have substantial impact on pullet nutrition strategies and pullet rearing costs.

Key Words: dietary balanced protein, precision feeding, laying hens, sexual maturation

M113 Effect of temperature on chicken egg quality available at grocery stores Usman Bin Saud*¹, Muhammad Zeeshan Alam Khan², Usman Elahi³, Nadir Shaban¹, Sara Malik¹, Naseem Saud Ahmad²
¹Food Sciences & Technology, Allied Health Sciences, Superior University, ²Department of Pharmacology, Azra Naheed Medical College, Superior University, ³Faculty of Agriculture & Veterinary Sciences, Superior University

Objective: The objective of this work was to assess the effect of temperature on different quality parameters of poultry eggs.

Methods: A total of 50 unfertilized eggs were purchased from poultry farms and grocery stores in winter and 50 in summer from the same farm and stores. Geometric parameters (such as weight W, length L, and breadth B) of individual eggs were measured. Formulae derived from geometry of ovoid were applied to measure volume (V), surface area (SA), shape index (SI) and geometric mean diameter (GMD) of eggs. The eggs were broken on a flat surface. Albumen height (AH), albumen length (AL), albumen width (AW), yolk height (YH), diameter (YD) and pH of egg pulp were measured. Egg shells were dried and their weight was measured. Different formulae were applied to calculate pulp ratio, albumen index (AI), yolk index (YI) and Haugh unit (HU).

Results: Independent sample *t* test was used to compare the geometric and internal egg quality parameters of summers and winters. There was an insignificant difference in size, weight, and length of eggs albumen height, yolk height, breadth, pulp ratio as well as pH of egg pulp ($p > 0.05$).

The calculated parameters during summer e.g. SI, SA, V, and GMD were 77.22 ± 1.93 , 73.03 ± 7.22 , 59.05 ± 8.6 and 4.82 ± 0.25 respectively. The SI, SA, V and GMD during winter were 73.66 ± 1.74 , 70.02 ± 2.86 , 55.09 ± 3.36 and 4.70 ± 0.10 respectively. There was no significant effect of seasonal variation on geometric parameters. Internal egg quality parameters like AI, YI and HU during winter were $5.02\% \pm 1.8$, $61.57\% \pm 14.9$ and 68.51 ± 11.50 respectively whereas during summer AI, YI, HU were $4.08\% \pm 2.09$, $57.87\% \pm 10.17$ and 67.91 ± 17.35 respectively. The pH of egg pulp was 7.54 in winter and 7.73 in summer.

Conclusion: Geometric parameters showed insignificant differences in weight and size, but internal egg qualities were within the normal range.

Key Words: Temperature, egg albumen, geometric parameters, egg quality, haugh score

M114 Productive energy (Arkansas Net Energy) is more sensitive than classic net energy determination to performance and actual net energy fractions for gain and maintenance Diego Martinez*, Nawin Suesuttajit, Cole Umberson, Craig Coon
University of Arkansas

An energy system is needed to optimize feed formulation and influence economics and sustainability. This study assessed the sensitivity of productive energy (PE) and classic net energy (CNE) to BW gain (BWG), feed conversion ratio (FCR), and net energy for gain (NEg) and maintenance (NEm), and developed models to predict BWG, FCR, and protein accretion (PAC). 1920 chicks in 96 pens were assigned to one of 8 blocks and 12 experimental diets, which varied in total digestible amino acids (TDAA; T1-T3) or digestible (dig.) starch and TDAA (T4-T6), or increasing (oil, TDAA; T7-T9) or reducing (soy hulls; T10-T12) densities. Blocks received a standard diet before transitioning to successive one-week treatment intervals, starting after the previous block. Birds were control-fed to control energy intake. In each block, BWG and FCR were assessed, body protein, fat, and energy gain (NEg) were determined with dual-energy X-ray absorptiometry, and heat production (NEm, fasting one) in calorimetry chambers. Diet N-corrected apparent metabolizable energy (AMEn), non-starch polysaccharides and dig. fat, dig. starch, dig. crude protein (dCP), and TDAA were determined. CNE was calculated as AMEn - heat increment and PE as NEg + NEm. A Completely Randomized Block Design with 12 treatments, 8 blocks, and 1 replication per block was used. ANOVA and Tukey tests were run. The sensitivity of the

energy systems was determined by comparing Tukey outcomes for PE and CNE with those of BWG, FCR, NEg, and NEm. Linear mixed modeling used JMP. BWG, FCR, PAC, NEg, and PE were positively influenced ($P < 0.05$) by TDAA or diet density ($P < 0.05$) and negatively by diet dilution. The PE (not AMEn or CNE) efficiency to produce BWG and PAC was stable ($P > 0.05$) across treatments. PE was 2.3, 1.8, 1.8, and 1.8 times more sensitive to BWG, FCR, NEg, and NEm, respectively, than CNE. Models to predict BWG, FCR, and PAC based on digestible nutrients were validated ($\text{adjR}^2 > 0.95$). Models to predict BWG ($\text{adjR}^2 = 0.99$), FCR ($\text{adjR}^2 = 0.86$), and PAC ($\text{adjR}^2 = 0.98$) based on PE were validated. The one for FCR also included dCP. In conclusion, PE is 90% more sensitive than CNE to changes in performance and actual net energy constituents (NEg, NEm) than CNE, and models to predict BWG, FCR, and PAC based on PE were developed.

Key Words: Productive energy, Arkansas Net Energy, net energy, predicting performance, broilers

M115 Predicting processing weights and broiler economics with productive energy (Arkansas Net Energy) Diego Martinez, Nawin Suesuttajit, Cole Umberson, Craig Coon* *University of Arkansas*

Nutritionists need an energy system to enhance feed formulation and impact broiler sustainability and economics. This study developed models for the growth rate of processing weights (carcass, breast fillet, tenderloins, wings, leg quarters), carcass market value gain (MVG), and gross profit gain (GPG) of broilers. 96 floor pens (20 chicks each) were assigned to one of 12 treatment diets (within 8 blocks), which consisted of changing levels of total digestible amino acids (TDAA; 1-3) or TDAA and digestible starch (4-6), or increasing (7-9) or reducing (10-12) diet density. Blocks initially received a standard diet, followed by consecutive weekly treatment periods, one block at a time. Feed was restricted to ensure similar energy intake. Feed intake was recorded. Body energy gain (NEg) and weights of each processing part were determined with dual-energy X-ray absorptiometry, and heat production (NEm, fasting) in calorimetry chambers. Diet N-corrected apparent metabolizable energy (AMEn) was determined, and CNE (AMEn – heat increment) and PE (NEg + NEm) were calculated. MVG was calculated with the market price of each part, and GPG was the difference between MVG and daily feeding cost. One-way ANOVA (12 treatments, 8 blocks, and 1 replication per block) and Tukey tests, along with linear mixed modeling, were used to analyze the data in JMP. MVG and GPG data was Ln-transformed. The growth rate of all processing parts, MVG, and GPG were positively influenced by TDAA ($P < 0.05$). PE (not AMEn or CNE) efficiency to produce carcass or breast gains showed no change among treatments ($P > 0.05$). AMEn and CNE showed no influence on the growth rate of processing parts ($P > 0.40$ and 0.06 , respectively), MVG ($P = 0.66$ and 0.13 , respectively), or GPG ($P = 0.10$ and 0.13 , respectively). Simple linear models were validated to predict the growth rate of processing parts ($\text{adjR}^2 > 0.97$), MVG ($\text{adjR}^2 > 0.99$), and GPG ($\text{adjR}^2 > 0.98$). The PE model for MVG indicated that per each continuous +100 kcal/kg diet PE, each 56-d-old bird gained +3.53

cents of market value (\$35,000 per million birds produced). In conclusion, PE is a strong predictor of processing weights, carcass market value, and gross profit. Models were developed to predict these traits based on PE.

Key Words: Productive energy, Arkansas Net Energy, net energy, processing, broiler economics

M116 Wheat bran particle size influenced jejunum expression of nutrient transporter and ceca short chain fatty acid profile in broiler chickens receiving corn-soybean meal-based diets supplemented or not with a stimbiotic Shravani Veluri*¹, Gemma Gonzalez-ortiz², Mike Bedford², Oluyinka Olukosi¹ ¹*Department of Poultry Science, University of Georgia*, ²*AB Vista and Feed Ingredients*

Wheat bran (WB) inclusion and stimbiotic (STB) supplementation are hypothesized to increase fiber fermentation in the ceca. There are indications that WB particle size significantly affects gastrointestinal tract development and ceca fermentation capacity, whereas coarse WB enhances the gizzard development, fine particles can enter the ceca easily and increase fermentation. A total of 760 Cobb 500 broilers were allocated to 48-floor pens with six treatments and 8 replicates per treatment to study the influence of WB and STB supplementation on jejunum expression of nutrient transporters and ceca short-chain fatty acid (SCFA) profile. Treatments were arranged in a 3×2 factorial with WB (0% WB, 5% coarse WB, 5% fine WB) and STB (0 or 100 g/ton of feed). Jejunum tissue and ceca contents were collected on d 18 and 42 for mRNA expression of nutrient transporters, tight junction genes, and SCFA profile, respectively. There was neither significant interaction nor significant main effect of WB particle size or STB on the expression of glucose transporters (GLUT1, GLUT2, GLUT5, SGLT1, and SGLT4), protein/amino acid transporters (PepT1, rBAT, SLC7A7, SCL7A2, SLC7A9), and tight junction gene claudin 1 both at d 18 and 42. There was no significant interaction nor significant main effect for all the SCFAs measured at d 18 except for total SCFA production determined at d 42. Fine WB inclusion decreased total SCFA content in ceca compared to coarse WB. However, when supplemented with STB, there was no difference in total SCFA production between coarse and fine WB. There was neither significant interaction nor main effect for caecal acetate, propionate, butyrate, and valerate at day 42, however, diets with either coarse or fine WB decreased ($P < 0.05$) ceecal BCFA compared to diets without WB. Stimbiotic tended to decrease cecca isobutyrate ($P = 0.086$) and isovalerate (0.081) concentrations. In conclusion, the increase in nutrient digestibility with fine WB inclusion reported earlier for this study are not reflected in the expression of nutrient transporters. However, a decrease in BCFA with stimbiotic supplementation or wheat bran inclusion indicates preferential fermentation of fiber rather than protein, with positive implications on the gut health of the broiler chickens.

Key Words: Stimbiotic, wheat bran, SCFA, nutrient transporters, particle size

Environment Management | Animal Well-being

M117 Deep learning algorithms for tracking individual chicken for locomotion analysis Xiao Yang*^{GS}, Ramesh Bist, Bidur Paneru, Lilong Chai *University of Georgia*

Poultry locomotion is an important indicator in evaluating animal health, welfare and farm productivity. Traditional methodologies, i.e., manual observation or the use of wearable devices, encounter significant challenges, including potential stress induction and behavioral alteration in animals. This research introduced an innovative approach that employs an enhanced track anything model (TAM) to track chickens in various experimental settings for locomotion analysis. Utilizing a dataset comprising

both dyed and undyed broilers and layers, the TAM model was adapted and rigorously evaluated for its capability in non-intrusively tracking and analyzing poultry movement by intersection over union (mIoU) and the root mean square error (RMSE). The findings underscore TAM's superior segmentation and tracking capabilities, particularly its exemplary performance against other state-of-the-art models, such as YOLO (You only look once) models of YOLOv5 and YOLOv8, and its high mIoU values (93.12%) across diverse chicken categories. Moreover, the model demonstrated notable accuracy in speed detection, as evidenced by an RMSE value of 0.02 m/s, offering a technologically advanced, consistent, and non-intrusive method for tracking and estimating the speed of chickens.

This research not only substantiates TAM as a potent tool for detailed poultry behavior analysis and monitoring but also illuminates its potential applicability in broader livestock monitoring scenarios, thereby contributing to the enhancement of animal welfare and management in poultry farming through automated, non-intrusive monitoring and analysis.

Key Words: Poultry locomotion, deep learning, track anything model, animal welfare, non-intrusive tracking

M118 Probiotic and diet treatment improves growth while reducing feed intake in breeder pullets housed in floor pens. Nikolas Faust^{*1GS}, Mazette Croom¹, Marvin Vides¹, Rosemary Walzem^{1,2} ^{1Texas A&M University- Department of Poultry Science, 2Texas A&M University-Graduate Faculty of Nutrition}

Breeder pullets begin feed restriction (FR) ~ 3 weeks post-hatch. A welfare issue, FR effectively increases hen livability and chick yield. Prior battery studies that administered a modified diet and anorexigenic probiotic (P) decreased feed intake (FI) as much as 29%. This experiment's goal was to determine whether appetite suppressive effect would persist with birds raised in floor pens.

200, day-old breeder pullets from each of two commercial strains were placed in 3' X 3' floor pens for 21 days. Chicks were randomly assigned by breed to one of 4 groups (n=50) with similar starting bodyweights (BW) by breed (Breed 1 (B1): 38 ± 3g Breed 2 (B2): 45 ± 4g). Treatments were combinations of usual (US) or high oleate (OL) diets +/- P.

P dose was 1x10⁸ CFU *E. coli* Nissle 1917/mL/bird/day. OL was oleic acid rich compared to US. All diets met breeder recommendations. We report 21-day BW (n=50 birds/treatment/breed), day 14-21 FI (n=10 pens/treatment/breed), 21-day liver (LPW) and intra-abdominal adipose (APW) percent of BW, and percent hepatic fatty acids (HFA). Two-way ANOVA tested effects of diet and breed; Tukey-Kramer was used for post-hoc means testing. Mean values ± SEM were different (SIG) at p≤0.05. Standard 21 d BW: B1, 308g; B2 420g. BW(g) of B1 (US, OL, US+P, OL+P), were 313±51^C, 236±43^D, 418±67^B, 287±73^C; & B2 437±63^B, 415±70^B, 522±90^A, 442±74^B, respectively. FI (g/b/d) of B1 (US, OL, US+P, OL+P) were 30±6^F, 28±5^F, 38±6^E, 28±5^F; & B2 42±5^{DE}, 38±6^E, 53±5^B, and 46±5^{CD} respectively.

Across all diets and breeds, probiotic treatment increased BW by 27% (p < 0.034). Despite a general BW increase, P treatment reduced FI by 26% in B1-OL and 13% in B2-OL fed compared to their US+P fed counterparts. A SIG breed effect was found with B1 being more responsive to diet and P than B2. LPW was 3.4 -4.0% of BW in all groups. HFA distributions reflected diet fed and overall APW was similar within breed, with APW of B2 groups averaging 1.4%, SIG higher than APW of B1 which averaged 1.0%. Breeds differed in appetite regulation. Outcomes suggest that probiotics aimed at altering appetite operate within the complex biological controls of growth and feeding behavior. Future studies are needed to maximize effect and return.

Key Words: Broiler Breeder, Welfare, Endocannabinoid, Probiotic, n-Acylethanolamines

M119 What's in a quack? Effects of Pekin duck vocalizations on conspecific physiology. Jenna Schober^{*GS}, Serena Wesley, Isabella Taylor, Collette Pluec, Melanie Bergam, Jeffrey Lucas, Gregory Fraley *Purdue University*

Identification of stressors has primarily been reactive rather than proactive, leading to a reduction in health and productivity. We hypothesize that a bird's vocalizations reflect ducks' current wellbeing. If we understand the meaning behind individual quacks, calls could be used to reduce stress levels. We tested 17 Pekin ducks (5 hens and 12 drakes) with playbacks of 5 different vocalizations, no noise stimulus, and white noise. We tested 5 distinct vocalizations produced by just hens (pips, harmonics and egg laying calls), and ones made by both sexes (AMs, and honks). Trials

consisted of an initial recording in a quiet condition with 5 consecutive measurements of heart rate, blood pressure and respiratory rate. A specific vocalization was then played on repeat while 8 more measurements of blood pressure and heart rate and a recording of respiratory rate. Finally, 5 measurements of heart rate and blood pressure were taken post playback and a final recording of respiratory rate. Blood pressure measurements and heart + respiratory rates were each aggregated using principal component analyses. The first principal component loaded strongly and positively (PRIN 1, systolic, diastolic, and MAP: lambda=2.48) and the second principal component loaded strongly and positively (PRIN 2, l=1.09). Repeated measures ANOVA indicated that the AM long call, honk, harm call, egg lay squiggle, and white noise significantly increased blood pressure (PRIN 1) when compared to the no noise control (treatment effect: F_{6,108}=2.56, P=0.013). The AM long call in particular caused the ducks' blood pressure to rise significantly (t₁₀₈=2.80, P=0.006). In addition, the hens' blood pressure was significantly higher than the drakes' blood pressure (sex effect: F_{1,108}=10.74, P=0.0014). There was no significant treatment effect (F_{6,108}=0.17, P=0.98) or sex effect (F_{1,108}=0.01, P=0.93) on PRIN 2. In practice these results could be used to detect early stages of stress in ducks before they impact production. Since the AM long vocalization had the most significant physiological response across the ducks, poultry producers could electronically monitor their flocks' vocalizations and receive alerts as to when a stressor is detected.

Key Words: vocalizations, Pekin duck, welfare, physiology

M120 Changes in central dopamine and serotonin activity associated with age but not with preening cup use in grow-out Pekin ducks Melanie Bergman^{*1GS}, Jenna Schober¹, Alexandra Grief¹, Richard Novak¹, Collette Pluec¹, Drew Frey², Heidi Parnin², Gregory Fraley¹ ^{1Animal Sciences, Purdue University, 2Culver Duck Farms, Inc.}

Preening cups (PCs) offer Pekin ducks an enrichment that is a semi-open water source to improve welfare. We used mass spectrometry and qRT-PCR to measure brain neurotransmitter levels and gene expression for serotonin (5-HT) and dopamine (DA) synthesis and metabolism to understand ducks' affective states with PCs. We assessed 4 commercial duck barns (~2000 ducks/PC) to evaluate affective state in ducks housed with PCs. Brain samples were collected from ducks actively using a PC and ducks not using a PC (CON). Brain collection occurred prior to PC placement (day 21), then one week after PC placement (day 28), and one day prior to processing (day 35; N = 6 per age/state). Welfare transects were performed in the barns by 3 separate individuals before brain collection as previously described by our lab. Brains were microdissected to collect three brain areas: caudal mesencephalon (CM), rostral mesencephalon (RM), and diencephalon (DI). The right hemisphere brain areas were used for mass spectrometry to calculate neurotransmitter turnover. Welfare transect data were analyzed by Chi-square (N = 4 barns/age). All barns showed excellent welfare scores, but no significant differences were observed by age before or after PC placement. There were no significant differences in 5-HT turnover among ages or with PC use. Significant decreases were observed in DA turnover in the CM (p<0.05), RM (p = 0.07) and DI (p<0.001) over time, but not between PC and CON. The left hemisphere brain areas were used for qRT-PCR to measure the synthetic enzymes for 5-HT and DA, tryptophan (TPH1 & TPH2) and tyrosine hydroxylase (TH), respectively. These data were analyzed using a pairwise Wilcoxon rank sum test. The CM and DI brain areas showed no significant differences across age or PC use for relative mRNA levels. Within the RM, there was a significant (p=0.04) increase in relative TPH2 mRNA levels for the CON (day 35) when compared to CON and PC (day 28). Our data suggest that DA, but not 5HT, activity increases as ducks age over a production period that may or may not be related to PC placement. However, our data suggest that PC use may not impact affective state in ducks, as determined by brain biogenic amine activity.

Key Words: Pekin duck, commercial, open water, enrichment, affective state

M121 The effects of dietary oregano oil on production performance, plasma chemistry, and egg quality traits of laying hens during early-lay phase. Alexa Johnson*^{GS}, Gracie Anderson, Mireille Arguelles-Ramos, Ahmed Ali *Clemson University*

Essential oils are one alternative feed additive that can be provided to poultry in lieu of antibiotics as growth promoters. Oregano oil has been used previously in many poultry studies with positive effects. The objective of this study was to explore the effects of the essential oil of oregano (EOO) on health, performance, and egg quality in Hy-Line Brown laying hens. At 24 weeks old, 270 hens were randomly divided into a corn-soybean basal diet or treatment diet with EOO (Ecodiar®, 0.275 g/kg basal) across 10 pens (27 birds/pen). Production measures, egg quality, and blood samples were taken at 24, 30, 36, and 40 weeks. Blood samples were taken from 3 birds per pen. Differences within measured parameters across time points and groups were assessed using GLMM with Tukey's Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. The feed conversion ratio was improved in EOO birds at 30 and 40 weeks compared to CON ($p \leq 0.05$), with no differences in average daily feed intake and egg production. Triglycerides were lower at 24 ($p=0.025$) and 36 ($p=0.031$) weeks, and cholesterol levels were lower at 24 weeks (0.036) in EOO hens compared to CON hens. High-density lipoprotein levels were higher at 24 ($p=0.033$) and 36 ($p=0.029$) weeks in EOO birds. Concentrations of total antioxidant capacity (TAC), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were significantly higher at all timepoints in EOO birds compared to CON ($p \leq 0.05$). Levels of malondialdehyde (MDA) and alanine transferase (ALT) were lower at all timepoints in EOO group ($p \leq 0.05$), while Aspartate transferase (AST) was lower in EOO birds at 24 weeks ($p \leq 0.05$). Relative albumen weight and Haugh units were higher in EOO group ($p \leq 0.05$), with no differences in relative eggshell weight, relative yolk weight, yolk color, eggshell thickness, and eggshell strength across all weeks ($p \geq 0.05$). Egg MDA was lower in EOO eggs compared to CON eggs at all timepoints ($p \leq 0.05$). Within the CON treatment, MDA was significantly lower at 24 weeks compared to all other weeks, with a marked increase each consecutive week ($p \geq 0.05$). In conclusion, oregano essential oil benefits laying hens' performance, blood lipids, antioxidants, and egg quality, making it a cost-effective poultry health enhancer.

Key Words: laying hens, oregano oil, production performance, plasma chemistry, egg quality

M122 Influence of perch provision during rearing on musculoskeletal health of pullets Mallory Anderson*^{GS}, Alexa Johnson, Cerano Harrison, Jeryl Jones, Ahmed Ali *Clemson University*

Providing opportunities for exercise during pullet rearing represents a proactive strategy for mitigating the risk of bone fractures during the subsequent lay phase by promoting increased bone mass. This study aimed to assess whether the introduction of multi-tier perches could effectively prepare pullets for the lay phase by enhancing their musculoskeletal health. Pullets ($n=810$) were housed with multi-tier perches (P, $n=15$ pens) or without (NP, $n=15$ pens) from 0-17 weeks of age. Over the course of the study, the activity levels of 90 birds per week were individually tracked for three consecutive days at 5, 11, and 17 weeks of age. At weeks 11 and 17, 60 birds were euthanized and subjected to computed tomography scans to determine tibial bone mineral density and bone cross-sectional area. Further analysis included assessments of muscle deposition, tibial breaking strength, tibial ash percentage, and serum concentrations of bone-specific alkaline phosphatase (BALP) and procollagen type 1 N-terminal propeptide (PINP) as markers of bone mineralization ($n=90$ birds/week). Differences within measured parameters across time points and groups were assessed using GLMM with Tukey's Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. Results revealed that P pullets exhibited increased vertical activity levels ($P < 0.05$), although overall activity levels during weeks 5, 11, and 17 did not significantly differ from NP pullets ($P > 0.05$). Notably, the tibiae of P pullets exhibited greater total and cortical bone mineral density at week 11 and larger cortical bone cross-

sectional areas, along with higher total and cortical bone mineral densities at week 17, compared to NP pullets ($P < 0.05$). At week 11, P pullets displayed larger leg muscles, including triceps, pectoralis major and minor, and leg muscles, and this difference persisted at week 17 ($P < 0.05$). In both weeks, P pullets showed increased tibial breaking strengths, higher ash percentages, and higher concentrations of BALP and PINP compared to NP pullets ($P < 0.05$). Our findings indicate that the provision of multi-tier perches improved musculoskeletal health via increased load-bearing exercise and could be used as a tool to increase bone mass prior to the start of the lay phase.

Key Words: pullet, perch provision, musculoskeletal health

M123 Effects of interrupting scotophase period on hen performance, welfare, and egg quality Alexis Clark*^{GS}, Ari Bragg, Ahmed Ali, Mieille Argüelles-Ramos *Clemson University*

Laying hens in commercial systems are typically given 16 h of continuous light and 8 h of continuous dark (scotophase) during laying period. Most eggshell formation happens during the scotophase when hens are not eating and do not have calcium available in the digestive tract, which impacts the eggshell quality and bone health. During late lay, it is not uncommon to see eggs with poor eggshell strength. This trial aimed to explore the effects of interrupting the scotophase period on hen performance, welfare, and egg quality. 240 Hy-line W36, 17-week-old pullets were housed in 12 pens (20 hens/pen). Hens in the control (C) group (6 pens) were subjected to the routine lighting schedule (16 h of artificial light and 8 h of darkness daily), while the test group (W1) was subjected to an interrupted scotophase light schedule: 15 h of artificial light, 4 h of darkness, 1 h of light and then 4 h of darkness. Egg production and feed intake were monitored, and eggs were collected for internal and external egg quality parameters for 3 days during week 50 of age. Further analysis included assessing physical measures of welfare using a 0-2 scale. Differences within measured parameters across groups were assessed using GLMM with Tukey's Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. Average daily feed intake/bird (gm) and %hen-day were consistent across treatments (C:115.6, 93.6, W1:119.8, 94.9). No significant differences were detected between groups in average egg weight (C:63.25, W1:64.17 g), albumen weight (C: 61.07, W1: 61.85 %), Haugh unit (C: 86.97, W1: 85.89), and yolk color (C:7.12, W1:7.15). However, eggshells of W1 eggs had significantly higher breaking strength (33.6N) compared to C eggs (27.22N; $p=0.003$). Moreover, eggshell thickness (mm) and weight (g) in W1 eggs (0.39, 6.3) were higher compared to C eggs (0.35, 5.71; $p=0.021$, 0.019, respectively). No significant differences were observed in physical measures of welfare (average score of keel bone damage (C:0.31, and W1:0.28), footpad dermatitis (C:0.25, W1:0.17), feather damage (C:0.69, W1:0.28), and comb and skin lesions (C:0.28, W1:0.11)). In conclusion, interrupting the scotophase period improved eggshell thickness and weight without impacting bird performance and welfare.

Key Words: scotophase, egg quality, laying hen, keel bone, calcium

M124 Assessing the relationship between stress and production in four strains of laying hens housed in a cage-free environment Bhavisha Gulabrai*^{GS}, Kenneth Anderson, Sophie Chance, Allison Pullin, Aaron Kiess *North Carolina State University*

In order to satisfy consumer demands and potentially enhance laying hen well-being, the egg industry is shifting to cage-free production systems. The objective of this study was to determine if genetic strain influenced stress and egg production parameters in a cage-free environment. It was hypothesized that stress levels would be higher in white laying hens compared to brown laying hens but there would not be a difference in production attributes between strains. Four laying hen strains (W36 White, H&N White, Hyline Brown, and Bovan Brown; $N=300$ hens) were divided amongst twelve 3.2m x 1.2m floor pens (25 hens/pen; $n=3$ pens/strain). Each pen contained nest boxes, perches, feeders, and bell drinkers. Egg

production was recorded daily, feed weigh back and body weight data were collected every period (28 days), and egg quality was performed every other period. Stress physiology parameters (i.e., plasma corticosterone (CORT), heterophil lymphocyte ratios, and heat shock protein (HSP) gene expression) were collected at three different time points of the laying hen cycle: the pullet stage (17 weeks of age (WOA)), start of lay (22 WOA), and peak lay (32 WOA). All data were analyzed using JMP Pro 17 through a two-way ANOVA where $\alpha < 0.05$, and Tukey's HSD was utilized to identify significant differences. The Bovan Brown produced more eggs per hen, had superior hen-day production, and the highest yolk color DSM ($p < 0.0001$) when compared to all other strains. Hyline Browns had higher body weights and produced more XL eggs ($p < 0.0001$) but laid more floor eggs ($p = 0.0001$) than all other strains. Although no differences in CORT were observed, there were differences between time points in HSP 70, 90a, and 90b gene expression. A trend ($p = 0.055$) was found associated with strains expressing varying levels of HSP 70, with H&N Whites having the highest and Bovan Brown the lowest. Our hypothesis was partially incorrect, such that strains differed in both stress and production metrics in cage-free housing. These findings provide valuable information for table egg integrators aiding them in making more informed decisions in selecting laying hen strains that may be best for their particular cage-free production system.

Key Words: laying hen, cage-free, production, stress

M125 Evaluating the effects of depopulation treatments on corticosterone in laying hens Kari Harding^{*1GS}, Sanjay Shah², Ramon Malheiros¹, Kenneth Anderson¹ ¹*Prestage Department of Poultry Science, North Carolina State University*, ²*Biological and Agricultural Engineering, North Carolina State University*

In 2015, 2022 and starting 2023 the egg industry has been hit by the worst highly pathogenic avian influenza outbreaks in U.S. poultry history affecting more than 90 million birds. The results from these outbreaks have

shown that decisive measures to end the suffering of diseased poultry as well as preventing the spread of infection to the healthy poultry flocks are effective and become increasingly important. Three methods used in this study were ventilation shutdown plus heat (VSDH), ventilation shutdown plus heat and humidity (VSDHRh), and ventilation shutdown plus CO₂ (VSDCO₂). The study's objective was to evaluate corticosterone levels in laying hens as they underwent these three depopulation methods. The hypothesis for this study was that the corticosterone levels would be similar between treatments. Three different phases were completed with 1 and 2 conducted in environmental chambers, and phase 3 scaled to match industry conditions. Phase 1 determined average time of death (TOD); Phase 2, monitored the progression of the bird's corticosterone levels from onset to TOD. Phase 3 was scaled up to compare more to what could be seen in an industry setting. Blood was collected prior to the birds undergoing treatment, and immediately after death. Corticosterone was extracted and evaluated using the Caymen Chemical Corticosterone Kit. Statistical analysis was performed using JMP Pro 15 with a one-way ANOVA and a significance value of $\alpha < 0.05$ and a Tukey's HSD to evaluate differences. There were no significant differences between treatments in Phase 1, however there was a trend toward significance ($P = 0.0710$). Only the VSDH sequence was significantly different with greater corticosterone levels before the birds entered the chamber than any other time points. VSDHRh method did trend ($P = 0.07$) toward being significant. There was a significant increase in corticosterone levels with VSDHRh in Phase 3. This significance could be due to the humidity altering how the hen physiologically dealt with dissipation of core body heat, as opposed to seeing no significance in the chambers. Under ideal conditions the depopulation methods were equivalent however, the influence of environmental factors will always affect the physiological response of the birds as seen in the scale up.

Key Words: Layer, Corticosterone, Depopulation, Ventilation Shutdown Plus, Humidity

Environment Management II Animal Well-being

M126 Water consumption profiles of commercial free-range layers William Strickland^{*GS}, Michael Czarick, Brian Fairchild *University of Georgia*

Water consumption is a critical metric in layer management reflecting both bird health and performance. Daily water usage has long been used as an indicator of performance by layer producers. Sudden decreases in water usage can indicate problems with the water supply system, the potential onset of a health issue, or since water consumption is affected by feed intake, missed feedings. The problem is these issues are not found until the daily totals are reviewed the following day. The advent of ultrasonic water meters now allows for highly accurate minute-by-minute water usage monitoring, which allows the opportunity to explore how layer water usage patterns change over the day so potential problems may be identified more quickly.

Two commercial free-range layer houses in Northeast Georgia were fitted with ultrasonic water meters and water usage was collected every five minutes for five months with a remote data collection system. At the beginning of the study, each house had between 10,500-12,000 hens that were 46-51 weeks old. Models for each house were created to evaluate the consistency of the hourly bird water usage profile of each day. This was a linear mixed-effect model with random day effects, fixed time-period effects, an error covariance structure that accounts for heteroscedasticity, and an ARMA correlation structure.

The day-to-day SD for house one was estimated to be 4.79 and 1.19 for house two. Error SD was estimated to be 6.95 and 0.48 respectively. Water usage increased linearly by 336% through the day and peaked three hours

before the dark period. Variability also increased later in the day. The average hourly usage of the first four hours was between 1.1-1.2 GPH/1000 birds with SD between 3.84-5.47, whereas the last four hours usage was between 4.8-5.1 GPH/1000 birds and SD of 6.88-10.37.

Monitoring daily water usage patterns offers a potential advantage, allowing deviations to be seen in periods of the day as opposed to the next day. The model structure used here may form an outline for how models can be created to analyze water usage patterns. Further research on the water usage pattern can enhance our understanding of influencing factors and improve the speed and accuracy of abnormality detection.

Key Words: Management, Water Consumption, Modeling, Layer

M127 Zero-shot image segmentation for monitoring thermal conditions of individual laying hens Mahtab Saeidifar^{*1GS}, Guoming Li², Lilong Chai², Ramesh Bist² ¹*Institute for Artificial Intelligence, Franklin College of Arts and Sciences, University of Georgia*, ²*Department of Poultry Science, College of Agricultural and Environmental Sciences, University of Georgia*

Body temperature is a critical indicator for health and productivity of egg-laying chickens and other domesticated animals. Recent advancements in thermography allow for precise surface temperature measurement without physical contact with the animals, reducing animal stress from human handling. Gold standard temperature analysis via thermography requires manual selection of limited points for object of interest, which could be time-consuming and inadequate for representing the comprehensive thermal profile of a chicken's body. The objective of this study is to leverage a

zero-shot artificial intelligence technology for the automatic segmentation of individual laying hens within thermal images, providing insights into their overall thermal conditions. A zero-shot image segmentation model (Segment Anything, "SAM") was modified by replacing manual mouse clicks of target points with automatic selection of the highest temperature point in each thermal image. The model was also incorporated with pre- and post-processing techniques to improve segmentation accuracy. Three versions of SAM models (i.e., SAM, FastSAM, and MobileSAM) and two segmentation algorithms (i.e., Yolov8 and MaskRCNN) were comparatively evaluated to determine the optimal one for bird segmentation from thermal images. A total of 1,917 thermal images were collected from cage-free laying hens (Hy-Line W36) at 77-80 weeks of age. The image dataset exhibited considerable variations such as feathers, bird movement, body gestures, and the specific conditions of cage-free facilities. The experimental results demonstrate that SAM consistently outperformed the rest of the four models regarding segmentation success rate (84.4%), Intersection of Union (85.53%), precision (93.61%), recall (91.01%), and F1 score (92.05%). The optimal model, SAM, was pipelined to extract the averages ($^{\circ}\text{C}$) of mean (27.03, 27.04, 28.53, 26.68), median (26.27, 26.84, 28.28, 26.78), 25th percentile (25.33, 25.61, 27.26, 25.53), and 75th percentile (28.04, 27.95, 29.22, 27.55) of surface body temperature of individual laying hens from weeks 77-80, respectively. The developed pipeline is a useful tool for automatically evaluating poultry thermal conditions.

Key Words: poultry, thermography, artificial general intelligence, image analysis

M128 Automatic analysis of group-level activity of broilers with heat stress operations Oluwadamilola Oso*^{GS}, Nicolas Mejia-Abauza, Chongxiao Chen, Sammy Aggrey, Guoming Li *University of Georgia*

Heat stress (HS) negatively influences chickens' welfare and performance, causing economic losses. Group-level activity indicators (e.g., Broiler Activity Index, BAI) have been developed to quantify animal behavior and welfare but not been used to understand broiler well-being under HS. Higher BAI presumably indicates higher levels of group activity. The objective of this research was to assess the BAI of broilers influenced by HS operations. Three pens (control groups) of Cobb 500 broilers (22 birds for each) were fed with commercial standard diet, while another three pens (variation groups) with 0.05% 25-hydroxyvitamin D₃ created additional variations to verify the BAI performance under HS operations. Broilers in both groups were raised in the standard environment and exposed to cyclical heat stress (8 am-6pm) starting from 28d. Temperatures ($^{\circ}\text{C}$) before (25-26d), during (28-29d, 8 am-6 pm), and after (28-29d, 6 pm-8 am) HS operations were 27.73 ± 1.91 , 29.82 ± 1.58 , and 25.69 ± 1.67 , respectively. A camera was installed above each pen to capture top-view videos at a sampling rate of 15 frames per second. A pen area in each frame was cropped, and the BAI was computed by comparing pixel differences between consecutive frames across videos and then summing up the differences. The one-way ANOVA statistical test was conducted to compare the behavior differences with the significance level being 0.05. Analysis of both groups revealed that BAI was higher ($P \leq 0.0001$) before HS compared to after HS while BAI during HS was lower ($P \leq 0.0001$) relative to before HS. In the variation groups, broilers before HS operations had higher BAI than during HS operations ($P = 0.042$); but the BAI differences before, during, and after HS operations were not significant in control groups ($P = 0.296$). In the 24-hours activity trend (during and after), broilers in variation group had higher BAI than those in control ($P \leq 0.0001$). The automated and non-invasive BAI could be a potential indicator to predict birds experiencing HS, but the effectiveness could be influenced with various diets as differences in diets may lead to differences in endogenous heat load.

Key Words: Computer vision, housing management, image processing, behavior, poultry

M129 Reviewing the trait definition of mortality in broilers Jennifer Richter*^{IGS}, Fernando Bussiman¹, Jorge Hidalgo¹, Vivian Breen², Ignacy Misztal¹, Daniela Lourenco¹ ¹*University of Georgia*, ²*Cobb-Vantress, Inc.*

As meat chickens have been bred to grow larger and faster, mortality rates have shown an increase at the phenotypic level, thus making this a trait of importance for both producers and consumers. Since animal welfare has gained more attention, one of its aspects, both financially detrimental as well as a contributor to societal worries, is mortality. Broiler survival has genetic factors, as there is a clear difference between individuals as to their ability to live. Birds that die do not pass on their own genes due to natural selection, but there is still genetic variability within those birds that do survive. Selection against mortality is currently being implemented in most breeding programs as a binary outcome that does not consider differing genetic factors that might be influencing the phenotype at differing stages of the bird's life. The purpose of this study was to investigate alternative trait definitions that might improve evaluations against broiler mortality at the genetic level, improving one aspect of welfare over generations. A dataset was provided by Cobb-Vantress, Inc. with two mortality traits: a binary mortality (OM) and a categorical weekly mortality (WM). These two traits were used to develop five additional trait definitions: broiler mortality (BM), early and late mortality (EM & LM), and two repeatability models with either a binary or cumulative approach (CM & RM). Variance components estimation and prediction of genomic breeding values were performed for each trait definition using the BLUPF90 software suite. Heritability estimates ranged from 0.01 to 0.15, indicating there is genetic variability within the population across these trait definitions. The genetic correlation between EM and LM is 0.81, indicating that these traits are different traits with differing genetic backgrounds. Linear regression (LR) validation was performed to compare trait definitions. The LR accuracies showed that EM and LM used together gave the same accuracy (0.38 & 0.43) as the baseline mortality (0.43) while giving a more precise picture of mortality. Therefore, the use of EM and LM together may enhance the genetic evaluation of mortality in broiler breeder populations compared to the baseline mortality.

Key Words: Chicken mortality, Heritability, Accuracy, Genetic correlations

M130 Chronic stress in fast- and slower-growing broilers raised in high- or low-complexity environments Alexandra Ulans*^{GS}, Kathryn Walsh, Leonie Jacobs *Virginia Tech*

Fast-growing broiler chickens (FG) can experience poor welfare associated with rapid weight gain. The use of slower-growing strains (SG) or increasing environmental complexity may mitigate this. The objective was to assess the impact of environmental complexity on chronic stress in FG and SG by measuring feather corticosterone concentrations (fCORT).

Six-hundred FG (Ross 708) and 600 SG (Hubbard Redbro) were raised in 24 pens with standard (low complexity=LC) or complex (permanent and temporary enrichments; high complexity=HC) environments, resulting in 6 replicates per treatment. Primary wing feathers were collected from 6 broilers at 4, 5, and 6 weeks of age and 1, 2, and 3 kg of body weight, with each bird sampled once (n=576). Feathers were weighed, fCORT extracted and assayed using a commercial ELISA kit. Data were analyzed in JMP Pro using a mixed model with strain, complexity, and age or weight as predictors, and pen as random factor. Post-hoc comparisons were corrected using Tukey's HSD. LSmeans \pm SEM (pg fCORT/mg feather) are presented.

fCORT concentrations differed as birds aged ($F = 6.89$, $p = 0.001$), with 4-week-old broilers (6.96pg/mg) showing higher concentrations than 5- and 6-week-old broilers (5: 5.50pg/mg; 6: 4.96pg/mg; SEM=0.04, $p < 0.03$). Strain, complexity, and their interaction did not impact fCORT at 4, 5, or 6 weeks of age.

fCORT concentrations differed between strains depending on body weight ($F = 6.46$, $p = 0.002$), with 1-kg FG showing higher levels compared

to FG and SG at all other sampled weights (all $p < 0.001$). In addition, 1-kg SG (7.04pg/mg) had higher fCORT levels than 3-kg FG (4.40pg/mg, SEM=0.05, $p=0.008$).

FCORT levels in FG in LC tended to be higher than in SG regardless of weight (FG 6.77pg/mg vs SG 5.33pg/mg; SEM=0.4, $F=3.19$, $p=0.089$), but did not differ in HC.

Early life may be more stressful than later life regardless of strain, stabilizing in week 5. FG experienced more chronic stress at 1 kg, which decreased as they gained weight. SG broilers consistently experienced less chronic stress than FG, except when comparing 1-kg SG with 3-kg FG broilers. When raised without enrichments, FG broilers experienced somewhat more chronic stress than SG broilers. The use of slow-growing strains could reduce chronic stress in commercial production.

Key Words: animal welfare, broiler chicken, chronic stress, corticosterone

M131 Using quantitative ultrasound machine as a noninvasive bone health assessment tool in broilers Nicolas Mejia-Abauza*^{1GS}, Catherine Fudge¹, Muhammad Ali¹, Jinqian Wang¹, Austin Jasek², Colwayne Morris², Raquel Burin², Duarte Neves², Chongxiao Chen¹ ¹University of Georgia, ²Zinpro Corporation

Lameness is one of the major causes of mortality in broilers and results in economic losses and animal welfare concerns. Establishing a noninvasive, on-farm tool to assess bone health in broiler chickens is beneficial and could improve flock management. Quantitative ultrasound is used in humans to characterize bone structure using attenuation and speed of sound (SOS) measurements. However, this method is not well studied in broilers. The current study aimed to explore using SOS measured with an ultrasound machine as an indicator of bone mineralization in broilers. Forty-eight randomly selected birds from a 42-day-old broiler flock were examined with an ultrasound device (HMD-A3) to obtain SOS in the shank (un-feathered). The shank length (L) and diameters (posterior to anterior (W1) and medial to lateral (W2)) were measured using a caliper. The shank volume (VOL) is approximately calculated by $L \times W1 \times W2$. Furthermore, whole body and shank bone mineral density (BMD) and bone mineral content (BMC) were determined by Dual-energy X-ray absorptiometry (DEXA) scanner. The correlation was obtained using a linear regression model in JMP 17.0. Significance level was considered at $P < 0.05$. The results showed a significant positive linear relationship between SOS and shank BMD ($P = 0.0346$), as well as between SOS x VOL and shank BMC ($P = 0.0186$). The current results indicate that SOS could reflect the bone mineral density in the shank. No significant correlation was found in shank BMD and whole-body BMD ($P = 0.1875$). However, shank BMC is correlated to both whole-body BMD ($P < 0.001$) and BMC ($P < 0.001$). Ultimately, SOS x VOL is positively correlated with whole-

body BMC ($P = 0.0264$) in relation to SOS x VOL. In conclusion, SOS could be used as an indicator of shank BMD, and the product of SOS with volumetric approximate parameter could be used as an indicator of shank BMC and, ultimately, to evaluate whole-body BMD and BMC in 42 day-old broilers. The current study shows the possibility of using quantitative ultrasound as a noninvasive bone mineralization assessment tool in broilers. However, a dataset with more observations at different ages is necessary to establish an accurate prediction of BMD and BMC in broilers.

Key Words: Speed of sound, DEXA, BMD, BMC, bone quality

M132 Influence of age and dietary fats on welfare response in broiler chickens. Paul Omaliko*^{1GS}, Yewande Fasina¹, Hayley Sutherland², Peter Ferket³, Marisa Erasmus² ¹North Carolina Agricultural and Technical State University, ²Purdue University, ³North Carolina State University

Diet can play a variety of roles in the welfare of broiler chickens. Dietary fat types and quantities can modify gut microbiota, which could influence fear response and welfare parameters. This study investigated the influence of dietary fat types and age on welfare parameters (Feather cleanliness (FC), Hock burn (HB), Footpad dermatitis (FPD), Gait score (GS), Breast blisters, Novel object test (NOT), Stationary person test (SPT). Day-old Ross 708 male broiler chicks (560) were obtained from a commercial hatchery, and randomly assigned to seven dietary treatments. Treatments consisted of basal corn-soybean meal (SBM), into which poultry fat (CON), olive oil (OLIV), fish oil (FISH), canola oil (CANO), lard (LARD), coconut oil (COCO), or flaxseed oil (FLAX) was incorporated at 3% level. Each treatment consisted of 5 replicate pens, with each pen housing 16 chicks. Welfare measures were assessed on 27, 36, 43, and 50 d and analyzed using PROC LOGISTIC in SAS 9.4 at a 5% level of significance. Fear levels were assessed through two fear tests: The SPT and NOT and were analyzed using PROC LOGISTIC in R studio. Age influenced the outcome of the welfare and fear tests. The outcome of the NOT at the different ages indicated that fear levels decreased with increasing gait scores at 43 and 50 d, whereas the fear levels increased with increasing gait scores at 27 and 36 d of age. In comparison, the outcome of the SPT suggested that the fear response increased with decreasing and increasing gait scores at d 27 and 50 respectively. CANO, COCO, and FISH treatments displayed higher odds ($P < 0.05$) of having better gait scores. CANO and COCO recorded superior ($P < 0.05$) hock scores while FISH had a higher odd of cleaner feathers at d 50 ($P < 0.05$). Footpad was recorded at d 50 and there was no evidence of breast blisters. It was concluded that fear response and welfare in broiler chickens were influenced by age, type of test used and to an extent dietary fat type.

Key Words: Broiler, Dietary fat type, Fear response, Gait score, Footpad dermatitis

Environment Management III Animal Well-being

M133 Automatic Gait Score Evaluation for Individual Broilers in Group Settings Using Deep Learning and Machine Learning Models Mustafa Jaihuni*¹, yang zhao¹, Hao Gan², Tom Tabler¹, Hairong Qi³, Maria Prado¹ ¹Department of Animal Science, University of Tennessee Knoxville, ²Department of Biosystems Engineering, University of Tennessee Knoxville, ³Department of Electrical Engineering and Computer Science, University of Tennessee Knoxville

Gait score (GS) assessment is essential in broiler welfare management. It relies on manual assessments by experts, which can be time-consuming, resource-intensive, and prone to inter-observer bias, hindering timely interventions. Deep learning (DL) models, on the other hand, provide cost-effective methods to evaluate GS via automated detection of broilers in real-time. This study was conducted to 1) develop a vision-based system

using YOLOv8 DL model to automatically determine individual broilers' mobility indicators, including daily walking distances, average speeds, and walking time; and 2) utilize Machine Learning (ML) models to regress their GS level using the mobility indicators. Consequently, 10 broilers were distinctively color-coded, and video recorded for 15 minutes per hour via a top-view standard camera daily for 41 days. An expert evaluated broilers' GS two times per week, yielding a total of eight manual assessments per broiler. Thus, the manual and automatic mobility data were correlated through four ML models. The middle sized YOLOv8m model was trained, validated, and tested with 600, 150, and 50 images from the recordings yielding 91%, 89%, and 87% accuracies in identifying individual broilers with different color codes, respectively. The model estimated the average total daily distance traveled by all the birds to be 540.8 ± 378.5 cm. It was found that the average mobilities of the birds did follow the

deteriorating trends of the manual GS results throughout the eight weeks. Additionally, out of four ML models, the Random Forest (RF) model, outperforming other counterparts, was able to predict GS with a generalized 0.64 R² and 78% classification success rates.

Key Words: gait score, YOLOv8, Random Forest, broiler welfare

M134 An evaluation of egg quality parameters across two multi-tiered aviary designs Kathryn Baugh*¹, Cara Robison¹, Janice Siegford¹, Ahmed Ali² ¹Michigan State University, ²Clemson University

The laying hen industry is transitioning to cage-free systems; balancing egg production and profitability with bird welfare will ensure long-term sustainability. Commercially available multi-tiered aviaries offer easily accessible resources for hens along with automated management processes for the producer, with a variety of different configurations on the market. To evaluate if a difference in aviary layout influences egg quality, this study evaluated and compared eggs produced in Big Dutchman's NATURA 60 and NATURA Step systems. NATURA 60 aviaries provide an enclosable tiered system, nests on the top level, and maintenance aisles, while NATURA Step systems have an open design and full floor utilization, maximizing available space for hen resources. At 16 weeks of age (WOA), floor-reared Hy-Line Brown pullets were moved into 7 aviary rooms, 4 with NATURA 60 systems and 3 with NATURA Step. Each room was further divided into 4 sections. NATURA 60 rooms were stocked with 144 birds/section and NATURA Step rooms were stocked with 164 birds/section to allow for a minimum of 929 cm² of floor space per bird. At 22, 27, 31, 35 WOA, 18 nest eggs per section were collected, weighed, and measured for shell compression strength with a texture analyzer. Component weights and percentages, shell thickness, and albumen height were evaluated for 12 eggs from each section. Due to identical genetics and nutrition, no significant differences in egg quality between systems were expected. Data were analyzed with PROC MIXED (SAS 9.4) with fixed effects of system, age, and their interaction and random effect of room. Data are presented as lmeans ± SEM. Between 22 and 35 WOA, egg weight, percent yolk, shell weight and shell thickness increased, while percent albumen and Haugh units decreased (P<0.001 for all). NATURA

Step eggshells were lighter in weight (5.91±0.02 g) than NATURA 60 shells (5.98±0.02 g; P=0.042). No significant differences were found in shell compression strength or shell percentage by age or system. Overall, egg quality was functionally consistent across the two studied aviary designs. Most age-related changes (e.g., egg weight, shell thickness and Haugh units) were consistent with previous findings. Data collection will continue every 4 weeks through 60 WOA.

Key Words: poultry, layer, egg, cage-free, aviary

M135 Evaluating carcass characteristics of three Naked Neck genotypes reared under different production systems Arif Hameed*¹, Shahid Mehmood¹, Sohail Ahmad¹, Arshad Javid² ¹Department of Poultry Production, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan, ²Department of Wildlife and Ecology, Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences, Lahore, Pakistan

Present study evaluated the carcass traits of three Naked Neck genotypes reared under different production systems. For this, 90 cockerels (16-week-old) were selected from three Naked Neck genotype i.e., Full Feathered (FF), Partial Feathered (PF), and Naked Neck (NN) that were reared under three production systems i.e., aviary, backyard, and open sided (30 birds from each production system and 10 birds from each genotype). A completely randomized experimental design was applied according to 3 × 3 factorial arrangement of treatments. Data were evaluated regarding pre-slaughter weight, shank, head, empty intestine, gizzard, liver, heart, neck, carcass, leg quarter, thigh, drumstick, and breast weight and carcass yield. Significant interactions between production system and chicken genotype were noted in pre-slaughter, neck, carcass, leg quarter, and breast weight; however, rest of the traits did not differ among the treatment groups. It was concluded that variations exist in carcass traits of Naked Neck genotypes when reared under different production systems.

Key Words: Naked Neck genotypes, Production systems, Carcass weight, Giblet weight

Environment Management IV Environmental Impacts

M136 Validation of a rail mounted robot in a commercial broiler house Tanner Thornton*^{GS}, Yang Zhao, Shawn Hawkins, Robert Burns, Tom Tabler *University of Tennessee-Knoxville*

The poultry industry plays a pivotal role in addressing global food demands. However, it faces challenges related to operational efficiency, animal welfare, and labor costs in commercial broiler houses. In response, this paper presents a comprehensive validation of the application of a rail-mounted robot system in a commercial broiler house environment. The aim of this study was to evaluate the feasibility, benefits, and potential drawbacks of implementing a rail-mounted robot in this specific context. The experimental setup involved the deployment of a rail-mounted robot equipped with various sensors and tools within a typical commercial broiler house. Data collected by the robot allowed for an in-depth analysis of the robot's performance in tasks such as mortality detection, environmental monitoring, and animal welfare assessment. The results revealed that the rail-mounted robot successfully addressed several critical issues in commercial broiler house management. The robot's ability to measure ammonia, airspeed, temperature, and humidity were all examined at various heights. Readings obtained from the Scout sensors were found to be in strong agreement with our reference sensors, as evidenced by an R² value of 0.92 for temperature measurement and 0.90 for relative humidity (RH) measurement. The accuracy of the Scout temperature and humidity sensors was determined to be +/-1.5°C and +/-10.6%, respectively. Furthermore, the system offered real-time mortality monitoring, which enhanced

overall operational control and animal welfare management. Mortalities were validated by marking the actual locations of mortalities, then comparing them against the robot's mapping system. The mortality numbers were also validated. Though the robot's ability to map mortalities was demonstrated, the accuracy of mortality detection was low (R²= 11%). The findings suggest that the implementation of poultry robots may revolutionize broiler house management and contribute to the sustainability of the poultry industry, yet further improvements are still required to enhance the accuracy of the robot on specific parameters.

Key Words: Animal welfare, Robot, Real-time mortality detection, rail-mounted

M137 Increase broiler movement effect on woody breast occurrence in a fast and slower growing strain Cirenio Hisasaga*^{1,2GS}, Maja M. Makagon¹ ¹Center for Animal Welfare, Department of Animal Science, University of California, ²Animal Biology Graduate Group, University of California

Woody breast (WB) presents as hardening and paleness of the *Pectoralis major* muscle and has been reported to affect up to 96% of broilers. Fast growth, high breast yield, and inactivity have been suggested to contribute to WB prevalence. We hypothesized that increased movement would lead to a lower prevalence of WB, with a greater effect in a faster growing strain. We further hypothesized that increased movement and strain would

impact the bone quality of the tibia. A total of 188 Ross 708 (faster growing) and 213 Ranger Gold (slower growing) broilers were raised to their target ages of 42 and 56 days. The birds were reared in 10x10 ft pens in groups of 23-24 (Ross 708) and 26-27 (Ranger Gold; 8 pens/strain). Four pens/strain were assigned to an exercise treatment. The other four served as controls. The exercise regimen was applied over 6hrs during each weekday with the goal of increasing frequencies of standing and walking. WB severity was determined for each individual at target age. Treatment effects were analyzed using a one-tailed Fisher's Exact Test. Tibia BMC (g) and BMD (g/cm²) were measured for each individual using a dual-energy x-ray absorptiometry. These data were analyzed using linear mixed models. Increased movement reduced the overall prevalence of WB in both strains (Ross 708: 77.5 vs 90.5% control, p=0.013; Ranger Gold: 57.9 vs 76.4% control, p=0.005). The prevalence of severe WB was also reduced (Ross 708: 12.9 vs 24.2% control, p=0.02; Ranger Gold: 4.7 vs 0.02% control, p=0.01). Tibial BMC and BMD were not impacted by treatment or the interaction between treatment and strain (all p>0.25). Strain affected BMC (p=0.003) and BMD (p=0.03), with Ross 708 broilers having higher values. However, on a per unit of final body weight, Ranger Gold birds had a significantly higher BMC (p=0.006) and BMD (p=0.01) compared to Ross 708 birds. The results of this proof-of-concept study suggest that a reduction of WB prevalence can be achieved by increasing broiler movement.

Key Words: broiler, slower-growing strain, woody breast, activity

M138 Individual and combined effects of the addition of organic acids in water on jejunum and ileum histomorphometry and cecal microbiome of broilers with or without Campylobacter inoculation Luis Munoz*^{1GS}, Li Zhang², Linan Jia², Haley Nabors², Matthew Bailey¹, Cesar Escobar¹, Dianna Bourassa¹, K Macklin² ¹Auburn University, ²Mississippi State University

The use of organic acids (OA) supports gut health in poultry, leading to improved nutrient utilization and to an environment less favorable for the proliferation of foodborne pathogens, including *C. jejuni* (CJ). The objective of this study was to evaluate the effects of OA, a CJ inoculation, and their combination on broiler intestinal health and physiology. The experimental design consisted of a 4x2 factorial using a randomized complete block with pen location being the blocking factor. A total of 2,240-day-old Ross 708 males were randomly distributed in 64 pens with 8 replicate pens/trt and 35 birds/pen. Each pen was assigned to 1 of 4 water treatment groups: negative control (water), intermittent OAA (2 mL/L; d 1 to 5 then 1 d per week), continuous OAA (2 mL/L), and continuous OAB (2.6 mL/L) and to 1 of 2 inoculations: PBS or CJ at 10⁴ CFU/mL administered via oral gavage on day 21. Duodenum, jejunum, and ileum (DJI) samples were collected from 3 birds/pen on d 42 to assess villus length, crypt depth, and their ratio (V:C). Cecal content samples were collected from 2 birds/pen on d 42 to assess cecal microbiome diversity. The cecal microbiome was investigated by 16S rRNA sequencing and data was analyzed using the EMU software (v3.2.0). Data were subjected to ANOVA using GLIMMIX (SAS v 9.4) and means were separated by Tukey's HSD. There were no interaction effects (P>0.05) of water trt and inoculation on histomorphometry of the DJI. However, the birds that were inoculated with CJ had increased crypt depth in the jejunum (P=0.0018) and had a reduced villus height and V:C in the ileum (P=0.0134 and P=0.0003) than birds inoculated with PBS. Similarly, no interaction effects (P>0.05) of water trt and inoculation on microbial alpha- or beta-diversity were observed. However, the inoculation with CJ significantly reduced the Chao1 and Shannon indexes (P<0.001 and P=0.007) and the beta-diversity in the bacterial community (P=0.0001) compared to the PBS inoculated birds. Overall, under the conditions of this study, broiler intestinal health and physiology was not altered by the addition of organic acids in water. How-

ever, the inoculation with CJ reduced the richness and evenness of the bacterial species in the ceca and the beta-diversity of the community.

Key Words: organic acid, water treatment, Campylobacter jejuni, intestinal histomorphology, cecal microbiome

M139 Characterizing the microbiome of litter and water lines of broiler farms with varying water quality. Tolulope Tiwa Ogunjipe*^{1GS}, Li Zhang², Tomi Obe¹ ¹University of Arkansas, ²Mississippi State University

Preharvest control strategies to reduce *Salmonella* contamination are increasingly becoming the focus of the poultry industry. Environmental sources such as litter and water lines are potential harborage sites for pathogenic microbes; and microbial profiling of these sites, especially in water lines where they are likely to form biofilm, could help integrators identify contributing factors to *Salmonella* colonization in poultry. Hence, this study evaluated the microbial community of the litter and water lines within ten commercial broiler farms. Given the history of the water quality from the integrator, these farms were divided into two groups of five farms each having normal and high sulfur-iron content (NSI & HSI). On each farm, three random houses were visited for a total of 30 houses. Two litter and four water line (biofilm) samples were collected in each house using boot and gauze swabs, respectively. All samples were analyzed for *Salmonella* and microbial ecology determined by 16S rRNA sequencing. The data was analyzed using ANOVA in JMP, and R-Studio. The overall *Salmonella* prevalence in litter was 91% while that of the biofilm was 4% (obtained from HSI group). The HSI farms had a higher average *Salmonella* load in litter and biofilm (4.58 and 0.50 Log₁₀ CFU/sample) than the NSI farms (3.64 Log₁₀ CFU/sample). Alpha diversity differed significantly between the litter and biofilm of NSI farms (p < 0.05) but not in HSI farms (p > 0.05). Irrespective of farm group, the litter samples exhibited higher Shannon diversity indices compared to the biofilm samples. Beta diversity revealed significant differences in the microbial diversity of the NSI & HSI farms (p < 0.05). At the species level, the microbial communities of the litter of NSI farms were dominated by *Aerococcus urinaeequi* and *Bacillus licheniformis* while HSI farms were *A. urinaeequi*. Moreover, the biofilm of NSI farms were mainly *B. licheniformis* (a probiotic) whereas the HSI farms were *B. cereus* (a notable biofilm pathogen). This data suggests that water quality can influence the microbial composition within poultry house environments, particularly water line biofilm. Effectively removing these biofilms is vital for reducing *Salmonella* at pre-harvest, which is critical to ensuring food safety.

Key Words: microbiome, Salmonella, poultry, biofilm, water quality

M140 Impact of sodium bisulfate application to litter, feed, and water on broiler performance and litter quality during a 42 day grow-out Jorge Urrutia*^{1GS}, Craig Coufal², Kelley Wamsley¹ ¹Mississippi State University, ²Jones Hamilton Company

Sodium bisulfate (SB) is commonly used as a poultry litter treatment (PLT®) due to its acidic characteristics to improve litter quality. It has also been used as an animal feed grade (AFG®) additive and pH water treatment (PWT®). The optimal application strategy to utilize all three of these products has yet to be established, thus leading to the objective of this study to maximize broiler performance and litter quality during a 42-day grow-out using varied strategies of SB applications. From 0-35 d, there were 6 treatments (TRT): TRT 1-Basal diet, TRT 2- Basal+PLT, TRT 3-Basal+High (3.6 kg/tonne) AFG, TRT 4-Basal+High AFG+PLT, TRT 5-Basal+Medium (2.7 kg/tonne) AFG+PLT, and TRT 6-Basal+Low (1.8 kg/tonne) AFG+PLT. From 35-42 d, TRT 5 and 6 received PWT (water pH at 3.5), as well as a portion of TRT 3 and 4, creating two more TRT (TRT 7-Basal+High AFG+PWT and TRT 8-Basal+High AFG+PLT+PWT). All TRT were distributed as a randomized complete block design to pens containing male Ross 708 x YP broiler chicks and reused litter (0.05 m²/bird; 10 reps/TRT at d 42). For PLT TRT, application occurred at d -1 (56.7 kg/100 m²) and at d 18 (34 kg/100 m²). Live performance (FCR,

BW; 0-42 d) and litter quality variables (pH and % moisture; d -1, 0, 18, and 19) were measured and analyzed using SAS 9.4. For d 0, 18, and 19, PLT-treated litter had a lower pH vs. no PLT ($P < 0.05$). At d 42, litter pH was significantly impacted by TRT ($P < 0.0001$). For bird performance from 0-35 d, birds fed TRT 3 (High AFG), as well as TRT 4 and 5 (High and Medium AFG, respectively, + PLT) had improved FCR vs. birds fed TRT 2 ($P = 0.0362$). For d 35 BW, no significant difference was found ($P = 0.8038$). From 0-42 d, birds fed TRT 3 and 4 had a reduced FCR vs. TRT 2 birds; all other TRT were similar to TRT 1 ($P = 0.0369$). These data indicate that PWT did not negatively impact litter quality or bird performance. Overall, litter pH and FCR improved with the addition of AFG in the feed as well as the combination of AFG in the feed and PLT on the litter; however, drier litter may have impacted the magnitude of response. Future research should focus on varying PLT and AFG inclusions to optimize litter quality and bird performance, as well as investigate SB product influence on gut health parameters.

Key Words: Sodium bisulfate, litter quality, feed additive, broiler, water treatment

M141 Effect of two commercial broiler house window configurations on light intensity and uniformity Joshua Etherton^{*1GS}, John Linhoss¹, Jeremiah Davis², Jessica Starkey¹, Joseph Purswell³ ¹Auburn University, ²Auburn University, National Poultry Technology Center & Biosystems Engineering, ³USDA ARS Poultry Research Unit, Mississippi State

Light is a crucial factor in the successful management of broilers, influencing both the physiological and behavioral aspects of broilers in a commercial poultry house. Recent changes in consumers' desire for alternative rearing programs have prompted integrators to adopt varying lighting strategies, including provision of natural light via windows. The objectives of this study were to compare light intensity, spatial distribution, and uniformity in two 18.2 × 182.9 m commercial broiler houses with different window configurations during brooding and tunnel conditions. House 1 had 23 translucent windows (16.5 ft²) that were all located on the north wall. House 2 had 58 translucent windows (6.1 ft²) located on both the north and south sidewalls and two additional windows of the same size on the west end wall (brooding end). Data acquisition systems were constructed to collect data at 750 locations per replicate in both houses. Two replicates were collected for tunnel and brooding conditions in each house at solar noon ± 2 h. Three house sections (fan, mid, and pad) were statistically compared as well as whole house data for both tunnel and brood programs. Average house illuminance was assessed in house 1 and house 2 treatments during tunnel and brood treatment testing through the utilization of PROC MIXED. For tunnel conditions, whole house light intensities were not different, however, light intensities were significantly higher ($P < 0.05$) in the mid and fan sections of house 2. Mean light intensity values were 1.8 times as high in the mid section and were also 6.5 times higher in the pad section in house 2. Light intensities for their brood program was not statistically different for the houses' whole brood sections, however, the light intensity was significantly higher ($P < 0.05$) in house 1 in the mid house section. This study showed that differences in house design, in regard to window size and placement, influence light intensity and decrease spatial uniformity within the houses.

Key Words: Broiler management, light intensity, window configurations, spatial distribution, commercial poultry houses

M142 Commingled or sex separate rearing with standard or increased feed intake on body composition and reproductive performance of broiler breeder pullets Hunter Mason^{*GS}, Luis Avila, Venkata Choppa, Woo Kim, Jeanna Wilson *University of Georgia*

Due to differences in body size and growth potential, it is encouraged to raise broiler breeder pullets and cockerels separately (SS), instead of traditional commingling (CO), where pullets and cockerels are grown together. The objectives of this study were to compare the reproductive

performance and body composition of pullets reared SS or CO. In addition, half of the SS and CO pens were fed a standard pullet allowance (STD) as recommended by primary breeders with the remaining pens fed a higher (HI) industry allowance (average 24% more feed). The 4 rearing treatments (TRT) were: STD-SS, STD-CO, HI-SS, and HI-CO. Day old, Ross 708 (Aviagen) pullet chicks ($n = 1856$) were randomly distributed between eight pens, having two pens/TRT. SS pullets ($n = 253$ birds/pen) had no contact with Aviagen YP males, while CO pullets were reared at a 5:1 pullet-to-male ratio ($n = 211$ pullets and 42 males/pen). All birds were fed the same diets and feed was adjusted weekly based on body weight. At 21 wk, pullets were moved to hen pens ($n = 36$ total pens at 9/TRT; $n = 41$ birds/pen) with 4 YP males. Each wk, 33% of the pens for each TRT were weighed to adjust feed allowance. At 25 ($n = 5$ birds/TRT) and 60 wk ($n = 9$ birds/TRT), breasts and ovaries were excised and weighed from sampled hens. From 25 to 60 wk, egg production, shell quality, and hatchery performance were recorded. Data were analyzed by SAS v. 9.4, using GLM procedure and means were separated by LSMEANS at a significance of $P \leq 0.05$, and tendencies declared when $0.05 \leq P \leq 0.10$. From wk 21 to 60, pullets reared SS were heavier than CO ($P < 0.01$). Rearing method did not impact uniformity. However, STD pullets were more uniform than HI ($P < 0.01$) during late stages of lay (wk 46-60). At 25 wk, HI pullets had greater meat deposition as compared to STD ($P = 0.029$). Ovary weights were unaffected by TRT at all ages. Egg production of STD-CO pullets during late lay was greater than all other TRT ($P < 0.01$). Egg-shell quality was not impacted regardless of TRT. Compared to CO, SS rearing tended to increase fertility (94.9 vs 93.5; $P = 0.08$) and hatchability (85.9 vs 84.3; $P = 0.08$) in late lay. Overall, SS rearing had no negative impact upon pullet BW and uniformity while having a positive effect on fertility and hatchability late in lay.

Key Words: broiler breeder pullet, commingled, feed allowance, reproductive performance

M143 Assessing the risk of antimicrobial resistant enterococcal infections in humans due to bacitracin usage in poultry Randall Singer^{*}, Timothy Johnson *University of Minnesota*

Bacitracin is a cyclic polypeptide antimicrobial commonly used in the feed or water of poultry in the U.S. Resistance to bacitracin is not of primary concern in human health. However, if the use of bacitracin were to select for resistance to other important antimicrobials due to co-selection, also referred to as co-resistance, then the use of bacitracin could present a human health risk. The objective of the present study was to perform a quantitative risk assessment (QRA) to estimate the potential risk in the U.S. of human infection with antimicrobial resistant bacteria, namely *Enterococcus faecalis* and *Enterococcus faecium* derived from chicken and turkey products, as a result of bacitracin usage in U.S. poultry. The modeling approach begins with the annual number of healthcare-associated enterococcal infections in the U.S. and then estimates the number of these infections that would be resistant to antimicrobial therapy and that would be derived from poultry sources because of bacitracin use in poultry. Attribution estimates were generated by analyzing whole genome sequencing data and then comparing the resistance gene overlap among host species with random forest classification models. While approximately 60% of *E. faecalis* and *E. faecium* derived from poultry were predicted to possess bacitracin resistance based on the presence of the *bcrABDR* gene locus, very few human-derived isolates possessed this trait. Furthermore, no vancomycin or linezolid resistant strains of *E. faecalis* or *E. faecium* were detected in poultry sources between the years 2002 and 2019. The model estimated the number of antimicrobial resistant *E. faecalis* and *E. faecium* cases per year that might resist therapy due to bacitracin use in poultry as 0.23 and 0.18, respectively. This translates to an annual risk estimate of less than 1 in 1 billion for members of the U.S. population for each bacterial species, representing a negligible risk. Even with the use of risk maximizing assumptions and parameter estimates, the results indicate

that there is a high probability that the use of bacitracin according to label instructions in U.S. poultry presents a negligible risk to human health.

Key Words: Bacitracin, Enterococcus, Quantitative risk assessment

M144 Effect of a calcium and magnesium source on organ physiology, blood mineral profile and general performance of White Laying hens.

Fausto Solis*, Rajasekhar Kasula., Christopher Olinger *Wenger Nutrient Science and Innovation Center (WNSIC), The Wenger Group,*

Calcium (Ca⁺) is a macro-mineral that animals need for proper bone metabolism, and general metabolism. Similarly, dietary magnesium (Mg⁺) has shown to improve egg production, eggshell strength, egg weight and reduce stress in laying hens. The purpose of the present study was to evaluate an intermediate source of Ca⁺ (30%), Mg⁺ (4.5%) termed CalMag-Carb compared to a typical calcium carbonate (38% Ca⁺ and 0.5% Mg⁺) or dolomite (30% Ca⁺ and 10% Mg⁺). The importance of testing CarMagCarb is to determine its feasibility and safety in laying hen production. The study parameters included the content of Ca⁺, Mg⁺ and P in the blood, bone strength, eggshell strength, and general performance of laying hens. CarMagCarb was supplemented as a replacer of the current calcium carbonate in the layers from week 36 to 47. A total of 120,000 hens were

randomly distributed in three treatments connected to two rows inside of a commercial caged layer facility. The treatments were: 1. control: calcium carbonate, 2. CarMagCarb50 substituting 50% of the Calcium Carbonate, and 3. CarMagCarb100 substituting 100% of the calcium carbonate. The data were analyzed with the General Linear Model (GLM) of the Statistical Analytical Software (SAS) with a probability of error of 5%. There was not consistent effect of CarMagCarb on the calcium and phosphorus content of the blood; however, at week 5 of the trial, the level of Mg⁺ was increased from 3.72 in the control to 4.13 in the 100% replacement of calcium carbonate. Similar results were observed at week 9 when the Mg⁺ content was increased from 4.0 in the control to 4.88 in the CarMagCarb 100. The eggshell strength improved from 3861 g in the control group to 3951 g in the CarMagCarb100. Egg production was not significantly affected by the supplementation of CarMagCarb as a substitute of the calcium carbonate. The egg production was 94.42% in the control group, 94.98% in the CarMagCarb50 and 94.57% in the CarMagCarb100. In conclusion, the results of the present study suggest that CarMagCarb can be safely supplemented in laying hens without negative impact on physiology, egg quality and performance parameters.

Key Words: calcium, magnesium, CarMagCarb, layers, eggs

Metabolism & Nutrition VII Feed Additives

T144 Effect of a slow vs a fast soy protein source in starter diets on broiler performance Megan Bible*¹, Simone Rasmussen², Alfred Blanch² ¹Hamlet Protein Inc, ²Hamlet Protein A/S

Previous research has shown differences in various soy-based proteins based on in-vitro protein hydrolysis (Bible et al., 2023). But, the impact of an ingredient's hydrolysis rate on broiler performance has yet to be established. The effect of two soy-based protein sources characterized by different rates of protein hydrolysis (slow vs fast) in starter diets on broiler performance was evaluated. 1,560 Ross 308 one-day old male broilers with initial weight of 39.5 ± 1.9 g were randomly allocated to one of two dietary treatments (30 pens/treatment; 26 birds/pen). Treatments were: corn-wheat-based starter diets containing 28.95% soybean meal (SBM) and 4.68% of a soy protein concentrate (SPC; slow protein) or 28.83% SBM and 5.00% of an enzyme-treated soy protein (ESPI; fast protein). All birds received the same corn-wheat-SBM grower and finisher diets. Diets were formulated to meet breed nutrient recommendations. The starter diets were formulated to be isocaloric and isonitrogenous with the inclusion of SPC and ESPI balanced on protein contribution. Birds were weighed on d 10, 28 and 42 and overall unadjusted (FCR) and mortality adjusted feed conversion ratios (mFCR) were calculated. Protein hydrolysis rate was determined for the two starter diets in-vitro using pH-stat method as described by Bible et al., 2023. Performance data were analyzed using GLM in the statistical software R (v.4.0.2) with means separated using Tukey's test. pH-stat data were analyzed using a pairwise t-test. Significance was declared at P<0.05. The speed of protein hydrolysis (k-value) was greater with ESPI in the diet compared to SPC (111 vs 85 μL/mole·s; p=0.04). Birds fed ESPI weighed significantly more on d 10 (271 vs 248 g), 28 (1.58 vs 1.48 kg) and 42 (2.99 vs 2.89 kg) compared with the SPC-supplemented birds (p<0.05). Overall FCR and mFCR did not differ significantly (p>0.05). In conclusion, the protein source affected the in-vitro protein hydrolysis rate of a complete feed. This result carried over into birds where the fast protein source (ESPI) resulted in higher weight at end of the starter and market phases than the slow protein source (SPC). Results indicate that dietary protein hydrolysis rate in the starter phase does affect the overall broiler performance at market age.

Key Words: Protein kinetics, Soy protein, Broiler performance

T145 Impact of Black Soldier Fly Larvae Frass (EnviroFeed) on Broiler Growth Performance Daniel Adams*¹, Brett Lumpkins², Greg Mathis², Elizabeth Koutsos¹ ¹EnviroFlight LLC, ²Southern Poultry Feed & Research

The poultry industry is always in need of alternative feed ingredients that can provide a source of animal protein, fat, and other dietary components. Insect-based ingredients are currently considered one of these alternatives and are present in many animal feed and pet food diets worldwide. Insect agriculture is of interest since it often requires fewer land and water resources than other ingredients such as soybean meal or corn. The by-product produced from rearing insects is known as Frass, and contains chitin-rich exoskeletons, and digested feedstocks. Frass is commonly used in fertilizer applications but is also gaining traction as a feed ingredient for livestock species. However, frass composition and value may vary due to the starting feedstock and manufacturing variables, so it is imperative to test each frass source to understand its value as a feed ingredient. Two experiments were conducted to determine the nutritional value of a commercially manufactured BSFL Frass in broiler diets. In both studies, male Cobb 500 chicks were randomly assigned to one of two treatments fed a basal diet or a diet containing EnviroFeed (frass from EnviroFlight commercial operations) and raised to 42 days. EnviroFeed was incorporated into the diet at 2.5% during the starter phase (0-14d), 5.0% during the grower phase (14-28d), and 10% during the finisher phase (28-42d). In both studies, there were 10 pens per treatment, study 1 had 20 chicks per pen, for a total of 200 chicks per treatment, while study 2 contained 45 chicks per pen, for a total of 450 chicks per treatment. Pen was the experimental unit in both studies. Group weights and feeder weights were measured weekly throughout the study to determine weight gain, feed consumption, and feed conversion ratio. During study 1, blood was also collected on day 42 for serum chemistry analysis. Data were analyzed using a one-way ANOVA in JMP 17. Means were adjusted using Tukey's post hoc test with significance determined at P<0.05. Frass, in the form of EnviroFeed, produced broilers similar in BW and FCR compared to a Control diet. These results indicate that EnviroFeed is a safe and effective ingredient for inclusion in broiler diets. Future research is needed to optimize the inclusion rate of frass in broiler diets.

Key Words: Black Soldier Fly, Broiler, Growth Performance, Insect Frass

T146 Comparative efficacy of a *Bacillus*-based direct fed microbial and an antibiotic on performance and intestinal morphology of broiler chickens Hector Leyva-Jimenez*, Yemi Burden, Katherine McCormick, Derek Haag, Adrienne Woodward, Brian Dirks *United Animal Health Inc.*

Boiler chickens from commercial rearing facilities are subjected to a variety of stressors that increase disease susceptibility and reduce growth. Direct-fed microbials (DFM) can be employed to introduce beneficial bacteria to the digestive tract of poultry and through diverse mechanisms boost performance. The present study was conducted to evaluate the effect of supplementing broiler diets with DFM or bacitracin methylene disalicylate (BMD) on performance and intestinal morphology using a complete randomized design of 3 treatments including negative control (NC, basal diet), positive control (PC, basal diet + BMD at 55 ppm), and the basal diet + Novela® ECL DFM (United Animal Health, US) at 3.68×10^5 cfu/g of feed (NECL). Each treatment included 10 replicate floor pens (used litter) with 40 male broilers per pen. All experimental diets were formulated based on corn and soybean meal. The diets were pelleted and offered ad libitum. The feeding program consisted of 3 dietary phases (starter 0-14 d; grower 14-28 d; finisher 28-42 d). Feed intake, body weight (BW), body weight gain (BWG), and FCR (corrected for mortality) were determined at the end of each feeding phase. Five birds per treatment were randomly selected and ileum samples were taken to evaluate villus height (VH), crypt depth (CD), and villus height/crypt depth ratio (VH:CD) at 28 days of the trial. Data were subjected to one-way ANOVA and means were separated using Fisher's LSD ($P < 0.05$). Cumulatively at the end of the grower phase (0-28 d) both PC and NECL had increased ($P = 0.003$) BW and BWG compared to NC. FCR improved ($P < 0.001$) by 4.5% and 3.7% for PC and NECL respectively, compared to NC. Although not statistical, numerical improvements were observed in both VH ($P = 0.131$) and CD ($P = 0.103$) for PC and NECL compared to NC, leading to a numerical improvement in VH:CD ($P = 0.792$) for NECL compared to both the NC and PC. Cumulatively after 42 d, FCR was adjusted to a common BW of 2.94 kg (C-FCR). C-FCR was improved ($P < 0.001$) by 3.9% and 2.7% for PC and NECL respectively, compared to NC, and were not different ($P > 0.05$) from each other. In conclusion, the dietary supplementation of DFM to broiler diets improved cumulative growth performance and yielded similar results to the antibiotic BMD.

Key Words: *Bacillus*, broiler, antibiotic, direct-fed microbial, performance

T147 From microbe to bird: In-depth evaluation of a DFM application during intestinal dysbiosis Sasha van der Klein*, Kirsty Gibbs *Danisco Animal Nutrition & Health (IFF)*

The impact of strategies to mitigate intestinal dysbiosis can be unclear owing to the nonspecific manifestation of the condition. This study elucidated the effect of a water applied direct fed microbial (DFM) at pen, host, and microbiome level. Treatments were an unchallenged control (UC), a dysbiosis challenged control (CC), and a CC with daily applied dual DFM administered via the waterline containing *Lactobacillus acidophilus* AG01 and *Bifidobacterium animalis* subsp. *Lactis* AG02. The challenge included a predisposition of *Escherichia coli* at d 7 of 1×10^8 CFU/bird, an *Eimeria* challenge at d 15 of 4000 oocysts/bird each of *E. tenella*, *E. maxima* and *E. acervulina*, and 1×10^9 CFU/bird of *Clostridium perfringens* (CP; netB-positive strain) at d 18-20. Three-phase corn/soy diets were fed. The 12 pens per treatment had clean wood shavings with 25 Ross 308 birds each. Performance was recorded up to d 42 and at d 24 and d 42, ileal and cecal content samples were used for enumeration of *Lactobacillus*, CP, and *E. coli*. Serum samples were analyzed for the acute phase protein alpha-1-acid-glycoprotein (AAGP), and ileal mucosal scrapings were analyzed for sIgA. Cecal samples were analyzed for volatile fatty acid (VFA) composition at d 24. Statistical analysis used ANOVA with treatment as a fixed effect and Tukey's adjustment for means separation. At d 24, ileal *Lactobacilli* were increased from 6.48 to 6.99 \log_{10} CFU/g ($P = 0.041$), cecal *E. coli* was decreased numerically from 7.05 to 6.68 \log_{10} CFU/g (P

$= 0.27$), and microbial lactic acid production was increased by 280% with the dual DFM compared to the CC. Immunological parameters showed a host-level response, as circulatory AAGP increased by 34% (d 24, $P = 0.005$) and 27% (d 42, $P < 0.001$) between the UC and CC and the dual DFM reduced AAGP with 17% (d 24, $P = 0.068$) and 33% (d 42, $P < 0.001$) compared to the CC. sIgA increased by 16% with the DFM compared to the CC at d 42 ($P = 0.001$), indicating intestinal immunological defense was enhanced. The dual DFM improved BW by 3% and FCR by 2% compared to the CC. The current holistic analysis of microbial, host, and pen level responses to challenge and the dual DFM indicate that microbial composition and VFA production may aid in restoring intestinal balance during periods of dysbiosis.

Key Words: Microbiome, coccidiosis, DFM, probiotic, dysbiosis

T148 Reducing the burden of necrotic enteritis: a cross study analysis on water applied DFMs in broilers Sasha van der Klein*, Kirsty Gibbs *Danisco Animal Nutrition & Health (IFF)*

Necrotic enteritis (NE) is a complex and impactful disease in current poultry production. Challenge models studying NE solutions often fail to show repeatable results, creating a perception that the effects are not consistent. This research aimed to study the overall efficacy of a water-line applied direct fed microbial (DFM) as a NE solution by combining the analysis of three US-based experiments performed between 2019 and 2023. The dual strain DFM comprised of *Lactobacillus acidophilus* AG01 and *Bifidobacterium animalis* subsp. *Lactis* AG02. Performance parameters and NE lesion scores were analyzed in all studies. Treatments consisted of an unchallenged control (UC), a challenged control (CC), and CC supplemented with a daily applied DFM at 1×10^8 CFU/bird/day. Application occurred when lights were turned on. The challenge consisted of COCCIVAC®-B52 (Merck Animal Health) 10x overdose at d 14 to predispose NE and 1×10^8 CFU/ml *Clostridium perfringens* (*netB*⁺) at d 16-20 for induction. In two trials Ross 308 birds were used, in one trial Cobb 500. Each trial had 12 pens per treatment with 40 birds each pen. Birds were placed on reused litter and fed a three phased corn/soy diet. Live performance was recorded up to d 42 and NE lesion scoring (0-4) was measured at d 21 and d 28 in 5 birds/pen. Combined data was analyzed with ANOVA using a mixed model with experiment as a random effect and treatment as a fixed effect, using Tukey adjustment for multiple comparisons. Compared to the CC, the dual DFM improved bodyweight by 8% ($P = 0.001$), FCR by 3% ($P = 0.004$), and reduced NE scores of those animals positive for NE (score > 1) from 1.49 to 1.09 ($P < 0.001$). Induction level of NE (percentage of birds in a pen with NE score > 1) at d 28 was 64.6% in the CC compared to 9.44% in the UC. The DFM treatment reduced the induction of NE to 31.5% ($P < 0.001$). Mortality was low in the UC (1.7%) and mildly increased in the CC (3.1%), where the dual DFM reduced mortality to 1.5% ($P < 0.018$) compared to the CC. The dual DFM improved broiler performance and reduced the effects of the NE challenge, suggesting DFM supplementation may reduce the development of NE when birds are challenged. This dual DFM can serve as a valuable tool in the search of antibiotic alternatives.

Key Words: poultry, DFM, necrotic enteritis, *clostridium perfringens*, probiotic

T149 The effect of an all-natural blend of yeast complex carbohydrates and yeast culture metabolites on broiler growth performance, breast meat yield, and Salmonella prevalence Theresia Lavergne*, Carl Jones, Charlie Elrod *Natural Biologics, Inc.*

Cascade (Natural Biologics, Newfield, NY) is an all-natural blend of yeast complex carbohydrates and concentrated yeast culture derived from *Saccharomyces cerevisiae*. This study was conducted to determine the effect of Cascade on broiler growth performance and *Salmonella* prevalence. A total of 1,200 male Ross 708 broilers were allotted to dietary treatments: 1. Control, 2. Cascade (100 g/ton), 3. yeast culture (YC; 750 g/ton), or 4.

hydrolyzed yeast+yeast culture (HY/YC; 100 g/ton). This was a randomized complete block design with 15 pens per treatment and 20 birds per pen. Broilers were reared on used litter and were not administered a disease or pathogen challenge. Broilers were fed a standard starter, grower, and finisher diet from day 0 to 14, day 14 to 28, and day 28 to 42, respectively. Birds and feed were weighed on days 0, 14, 28, and 42 to calculate body weight gain (gain), feed intake (FI), and feed:gain (FG). On day 41, cloacal swabs were collected from five birds per pen for determination of *Salmonella* prevalence and load. At the termination of the study, three birds per pen were processed to determine percent carcass and breast yield. From day 0 to 14, chicks fed Cascade had higher ($P<0.01$) gain than chicks fed the control, YC, or HY/YC, and better ($P<0.01$) FG than chicks fed the control or HY/YC. Broilers fed Cascade tended to have higher ($P=0.13$) gain than broilers fed HY/YC from day 14 to 28. From day 28 to 42, broilers fed HY/YC had the lowest ($P<0.05$) gain and FI, as well as numerically higher ($P=0.114$) FG than broilers fed Cascade. For the overall trial, gain and FI were higher ($P<0.01$) for broilers fed Cascade or YC than for broilers fed HY/YC. Although not statistically significant, broilers fed Cascade had three points better FG than broilers fed either YC or HY/YC from day 0 to 42. Percentage carcass yield and percentage total breast meat yield were lowest ($P<0.01$) for broilers fed HY/YC, and similar for broilers fed Cascade or YC. *Salmonella* prevalence was numerically highest and *Salmonella* load (CFU) was highest ($P<0.05$) for broilers fed HY/YC. Cascade can be utilized to improve growth performance, carcass yield, and breast yield of broilers. Additionally, Cascade can reduce *Salmonella* prevalence and load in live broiler production.

Key Words: prebiotics, complex carbohydrates, Saccharomyces cerevisiae, Salmonella, broilers

T150 A microencapsulated blend of botanicals can enhance growth performance and improve gut health of broiler chickens Benedetta Tugnoli^{*1}, Martina Felici², Andrea Piva^{1,2}, Ester Grilli^{2,3} ¹*Vetagro S.p.A.*, ²*University of Bologna DIMEVET*, ³*Vetagro, Inc*

Botanicals are widely used in poultry nutrition for their various biological properties (antimicrobial, anti-oxidant, anti-inflammatory) that can help to support growth and health of animals. The aim of the study was to evaluate the effects of a botanicals-based feed additive, microencapsulated in a lipid matrix, on growth performance and gut health of broilers vaccinated for coccidiosis.

A total of 924 day-old male chicks (Ross 308) were vaccinated for coccidiosis (Livacox®), divided in floor pens (22 birds/pen) and assigned to 2 groups (21 pens/group): control group (CON), fed a basal diet; treated group (TRT), fed a basal diet supplemented with a microencapsulated blend of botanicals at 200 g/MT of feed. Oocysts per gram (OPG) of feces were determined weekly per group while at day 28 some birds (8 birds/group) were sacrificed to collect intestinal samples (duodenum and ileum) for gene expression analysis of gut health markers by qPCR (tight junctions components and nutrients transporters). Overall the study lasted 42 days with growth performance recorded throughout the study. Data were analyzed with T test and differences considered significant at $P<0.05$. For both groups *Eimeria* shedding showed a first peak at day 7 and the highest peak at day 21, with OPG values numerically lower in TRT than in CON group at each time-point ($P>0.05$). TRT group showed improved growth performance compared to CON group: already in the first phase (0-14 days) the treatment significantly increased BW (+35 g) and reduced FCR (-0.06) compared to control ($P<0.0001$). BW was significantly higher in TRT than in CON group also at day 28 (+79 g), day 35 (+80 g) and overall at day 42 (+70 g), ($P<0.01$ each).

Gene expression analysis showed a modulation of many markers for TRT compared to CON group: the treatment increased tight junctions markers (ZO1, Claudin-1, JAM2) in both duodenum and ileum, while increased the nutrient transporter for peptides (Pept1) in duodenum and for glucose (GLUT2) in ileum ($P<0.05$ each).

To conclude, the microencapsulated blend of botanicals used in this study has the potential to enhance the growth performance of broilers vaccinated for coccidiosis, while improving the gut health.

Key Words: botanicals, microencapsulation, cocci vaccination, broilers, gut health

T151 Reduction in aminoglycoside and macrolide antibiotic resistance genes in E. coli isolates recovered from broilers fed a feed additive over 15 months. Lindsay Leonard^{*}, Enid McKinley, Jodi Delago, Alexandra Smith *Specialty Products Division, Arm and Hammer Animal and Food Production, Church and Dwight Co., Inc*

Antibiotic resistant (ABR) bacteria are an issue that is of concern for both human and animal health. In 2017 the FDA began limiting the use of antibiotics in agricultural settings. As an alternative, feed additives have been shown to be valuable at managing levels of pathogens. Some studies have found that the use of additives can decrease the number of ABR genes detected in pathogens, while some studies found no impact. The objective of this study was to assess the prevalence of 7 ABR genes in *E. coli* isolates collected over a 15-month period from a broiler complex that implemented an Arm & Hammer (A&H) feed additive program with refined functional carbohydrates and *Bacillus* probiotics to aid in the transition to a No Antibiotics Ever (NAE) diet. Seven months into the NAE program bacitracin methylene disalicylate (BMD) was implemented in the diet. Gastrointestinal tracts were collected from broilers every 2-3 months from a total of 339 birds. Mucosal associated bacteria were grown on selective media to isolate a total of 1583 *E. coli* isolates. DNA was isolated then screened using a PCR with specific primer pairs targeting 7 ABR genes: trimethoprim (*drfA14*), tetracycline (*tetA*), aminoglycoside (*aac3-Vla* and *aph3'-la*), beta-lactam (*blaTEM-1*), macrolide (*mphA*), and sulfonamide (*sul1*). The NCBI Pathogen Detection database was used to investigate the prevalence of these 7 ABR genes in *E. coli* and *Shigella* genomes from US isolates reported during the same 15-month period. The results showed that when birds were on the A&H additive there was a significant decrease, over time, in the levels of the *aac3-Vla* (high of 33% to low of 2%), *aph3'-la* (high of 15% to low of 1%), and *mphA* genes (high of 5% to low of 0%) ($p<0.05$, Logistic Regression Likelihood Ratio Test). The other 4 genes (*blaTEM-1*, *tetA*, *sul1*, and *drfA14*) remained unchanged over time. All 7 genes were found to be increasing over time during the same period in US environmental and clinical isolates from all species ($p<0.05$, χ^2 test for trend). In conclusion, trends in the prevalence of ABR genes observed in *E. coli* isolates from broilers fed an A&H additive were improved compared to trends observed in US NCBI isolates, supporting the hypothesis that feed additives can impact ABR gene prevalence.

Key Words: Feed additive, E. coli, Antibiotic Resistance

T152 Reducing the bioavailability of a mix of mycotoxins in broiler chickens with a clay- and algae-based detoxifier Marie Gallissot^{*1}, Sofie Rutjens², Maria Rodriguez¹, Mathias Devreese² ¹*Olmix Group*, ²*Ghent University, Faculty of Veterinary Medicine*

Poultry feed is often contaminated with mycotoxins that can impair animal health and performance, even at levels below official guidance, with a cost to the U.S. poultry industry estimated at M\$900 annually. The use of mycotoxin detoxifiers in the feed can limit these adverse effects. The objective of this study was to test, in a validated toxicokinetic model, the ability of an algae-clay-based detoxifier to reduce the exposure of broilers to mycotoxin polycontamination (deoxynivalenol, DON, ochratoxin A – OTA and aflatoxin B1 – AFB1). The study included 16 male broiler chickens (Ross 308) of 1.6 kg BW. After an acclimatization period of one week, the birds were fasted for 12 h and then administered a single oral bolus with either the mycotoxins ($n = 8$) or the mycotoxins in combination with the detoxifier ($n = 8$) (0.5 mg DON/kg BW, 0.25 mg OTA/kg BW, 2.0 mg AFB1/kg BW, 2.5 g detoxifier/kg feed), administered in a parallel study design. Blood samples of the 16 broiler chickens were taken at dif-

ferent time points between 0 h (before administration) and 24 h (post administration, p.a.). The analysis of deoxynivalenol-3-sulphate (DON-3S, DON main metabolite), OTA and AFB1 in plasma was performed using UHPLC-MS/MS. The area under the curve from time zero to the last point above the LOQ ($AUC_{0 \rightarrow t}$), maximal plasma concentration (C_{max}) and time at maximal plasma concentration (T_{max}) were calculated. Results showed that the mycotoxin detoxifier significantly altered the $AUC_{0 \rightarrow 12h}$ of DON-3S in broiler chicken ($P=0.012$). The detoxifier also decreased the absorption of OTA as indicated by a significantly decreased $AUC_{0 \rightarrow 24h}$ ($P<0.001$). Lastly, the detoxifier affected the absorption of AFB1 with a significantly decreased $AUC_{0 \rightarrow 8h}$ ($P<0.001$), C_{max} ($P<0.001$) and a significantly delayed T_{max} ($P=0.025$). Consequently, the relative oral bioavailability ((average $AUC_{0 \rightarrow t}$ mycotoxin + detoxifier / average $AUC_{0 \rightarrow t}$ mycotoxin) * 100) of DON-3S, OTA and AFB1 when administered with the detoxifier were, respectively 60.1%, 55.7% and 35.9%. Therefore, it can be concluded that the tested algae-clay based mycotoxin detoxifier reduces the oral absorption of multiple mycotoxins orally administered to broiler chickens in a single oral bolus model and can be of interest for the poultry industry.

Key Words: Toxicokinetic, Mycotoxins, Broiler chickens, Algae, Clay

T153 Impact of Isoquinoline Alkaloids and Essential Oils addition in broiler feed on intestinal health. Kelvin da Silva¹, Nbia Oliveira¹, Elisangela dos Santos¹, Dimitri de Freitas², Arthur Masseur², Cleverson Souza*² ¹Pluma Agroavicola, ²Phytobiotics

The objective of this study was to evaluate the effects of Isoquinoline Alkaloids (IQ) + essential oils (EO) on broilers' histological intestinal inflammatory parameters and Fluorescein isothiocyanate-dextran (FITC-d) levels, compared to a conventional additive program. A total of 400 broilers aged from 1 to 28d were housed in pens (1,2m x 1,5m) in the experimental farm from Pluma Agroavicola. The experiment followed a randomized design, with 10 replicates of 20 birds for each of the 2 treatments: T1 - Control diet with Enramycin 10 ppm; and T2 - Control diet with IQ (Sangrovit® ED 100g/ton) + Blend of EO (carvacrol e cinnamaldehyde 100g/ton). On day 28, 10 birds of each treatment were euthanized by cervical dislocation and samples of ileum were collected for histological analysis. The I See Inside (ISI) methodology was used, and it is based on a numeric score of alteration, and FITC-d measurement's by fluorimetry. The parameters evaluated were: Lamina Propria Thickness, Epithelial Thickness, Proliferation of Enterocytes, Inflammatory Cell Infiltration on Epithelium, Inflammatory Cell Infiltration in the Lamina Propria, Increase of Goblet Cells, Congestion and Presence of Oocysts. Data were analyzed by the Shapiro-Wilk normality test. The non-parametric data were submitted to the Kruskal-Wallis test ($P<0.05$). There was a statistical difference ($P<0.05$) between the means of the treatments in most parameters evaluated, except for Congestion and Presence of Oocysts ($P>0.05$). The Total ISI Score was statistically ($P<0.05$) lower for the T2 group (T1=6.41 and T2=3.24), the T2 group had statistically ($P<0.05$) lower scores than T1 group for Lamina Propria Thickness (1.2 and 2.22, respectively), Epithelial Thickness (0.28 and 0.49, respectively), Proliferation of Enterocytes (0.23 and 0.36, respectively), Inflammatory Cell Infiltration on Epithelium (0.21 and 0.38, respectively), Inflammatory Cell Infiltration in the Lamina Propria (0.86 and 2.22, respectively) and Increase of Goblet Cells (0.44 and 0.74, respectively). To FITC-d, was a statistical difference ($P<0.001$), T1(0.278 $\mu\text{g/mL}$) and T2 (0.212 $\mu\text{g/mL}$). Therefore, we conclude that IQ + EO control intestinal inflammation, and reduce gut permeability, improving broilers' intestinal health.

Key Words: Isoquinoline alkaloids, AGP, gut health, gut immunity

T154 Yeast cell wall with essential oil improves performance in broilers challenged with coccidiosis Fernando Souza¹, Melina Bonato*¹, Ricardo Barbalho¹, Lcio Arajo², Aaron Kiess³, Claudia Castañeda⁴ ¹ICC Brazil, ²Sao Paulo University, ³North Carolina State University, ⁴Engrain

As antibiotic-resistant bacteria associated with animal agriculture continue to be identified globally, research exploring antibiotic alternatives is ongoing. These concerns must be addressed, and reliable antibiotic alternatives must be identified. This study aimed to evaluate the effects of essential oils with yeast cell walls on broiler performance and health while those broilers experienced stress/disease from coccidiosis. The trial was conducted at Mississippi State University. It used 3,024 male Ross708 distributed in 4 treatments in a factorial arrangement that considered two doses of essential oil (EO - 300 g/MT and 600 g/MT) composed of a blend of carvacrol and thymol and the presence or absence of yeast cell wall (YCW - zero or 500 g/MT) composed by 20% of mannan oligosaccharides and 35% of β -glucans. The levels of the diets were the same, and no antibiotics or anticoccidials were used. A coccidiosis challenge was administered on day 14 of the grow-out through oral gavage with a 20X vaccine dosage. On days 1, 14, 21, 28, and 41, the performance, weight of the organs, and coccidiosis score were measured. It was observed an interaction between essential oil and yeast cell wall; the treatments with 600 g/MT of EO and 500 g/MT of YCW showed higher weight gain and final weight at all ages than other treatments; without YCW, there weren't differences between the dosages of EO. For the accumulated feed intake, there was a difference between the main effect of EO, which showed better results with the 600 g/MT doses, and the final feed conversion rate was better for the interaction of 600 g/MT of EO and 500 g/MT of YCW. It was evaluated the bursa, duodenum, jejunum, ileum, spleen, and ceca for the morphometry analysis. An increase in the weight of the Bursa at 13 days old in the YCW treatment was observed, and, at 28, a decrease in the weight of the bursa and jejunum at the same treatment. For the score lesions in the intestine, lower values for scores 2 and 3 were observed for the association of EO and YCW. In conclusion, the treatment with 600 g/MT of essential oil and 500 g/MT of yeast cell wall improved performance and lesion score of coccidiosis in the intestine of the broilers.

Key Words: essential oil, yeast, β -glucans, coccidiosis

T155 Multiple-trial analysis shows improved broiler performance for a blend of natural essential oils Ellen Hambrecht¹, Melchior de Bruin*², Marcos Rostagno³ ¹Selko, ²Nutreco Exploration, ³Selko USA

The gastrointestinal tract has been recognised as a complex sensory organ capable of detecting and processing a wide range of signals from its environment through receptors located on enterocytes. Targeting these receptors through selected plant metabolites may trigger local and systemic physiological responses that in turn affect metabolism, immunity, hormonal secretion, inflammation, and ultimately, animal performance. Fytera Perform (FP) is a micro-encapsulated blend of three natural essential oils from clove, cinnamon and oregano. The purpose of the present multiple-trial analysis is to evaluate the potential of FP as a natural performance enhancer in commercial broilers. Six studies were included, all conducted at independent contract research organizations. FP was added at 25 ppm to standard commercial diets without antibiotics and fed throughout the cycle of 42 days. Three studies were conducted under high copper (Cu) levels, one under nutritional Cu levels and two used a 2x2 factorial design with FP and Cu supplementation levels as main factors. In the high Cu treatments, IntelliBond Cu was added at 125 ppm to starter and grower diets, whereas nutritional Cu levels corresponded to 13 ppm. In five studies, reused litter was used to simulate mildly challenging commercial conditions. Data were analysed using one-way ANOVA with trial considered as a random variable. Differences were considered statistically significant at $p<0.05$. The feed conversion ratio (FCR) was adjusted for mortality and body weight and averaged 1.67 across all trials. In all but one comparison to the control diet, broilers that received FP had an improved FCR by an average of 5.1 points ($p<0.01$). Final body weight aver-

aged 2433 g and was 44 g higher ($p < 0.05$) for broilers receiving FP. There was no treatment effect ($p > 0.05$) on feed intake, which averaged 3988 g. Overall, there was not a statistically significant interaction between Cu level and FP ($p > 0.05$); effects of FP were similar under both nutritional and high levels of Cu. In conclusion, this multiple trial analysis shows that the phyto-actives in Fytera Perform synergistically support nutrient utilisation and energy partitioning to enhance broiler performance.

Key Words: Feed conversion ratio, Essential oils, Broiler performance, Body weight gain, natural performance enhancement

T156 Reduction of mortality in broiler chickens as a result of feeding a stimbiotic Gemma González-Ortiz*, Michael Bedford *AB Vista*

A holistic exercise was conducted to evaluate the effectiveness of supplementing a stimbiotic (Signis®, AB Vista) into broiler diets on bird mortality. A total of 53 studies evaluating a stimbiotic were conducted by AB Vista between 2017 and 2022. The database for all studies included factors such as the project code, year, dietary phases, diet composition, dietary nutrients, feeding plan, breed, sex, bird body weight gain, feed intake, feed conversion ratio and mortality. The effects of stimbiotic on broiler chicken mortality was determined by comparison with the relevant control treatment, the difference being termed the delta (δ) in mortality. Because in some studies there was more than one control diet, it yielded multiple

comparisons from one study, thus resulting in a total of 85 total observations. Data was visualized and checked for outliers before analysis with JMP 17 (SAS). Principal component analysis, decision trees and regression models were applied to identify the factors with the most influence on prediction of δ Mortality. Models were then cross-validated. The Ross 308 strain was the most prevalent strain (79%) used, diets were pelleted in most studies (69%) and all feed was available *ad libitum*. The majority of trials contained corn as the sole cereal source (46%), or corn combined with wheat (27%). The average trial length was 39 days (range from 10 to 42). Data showed average mortality of the control treatments was 4.57% (ranging from 0% to 18.13%). Based on the regression model, control bird mortality was found to be the only significant variable influencing δ Mortality in a quadratic relationship explaining 64% of the variation ($P < 0.05$). The quadratic regression model was validated twice by randomly splitting out 60% of the observations for training and using the remaining 40% of the observations for validation. Such a process increases the reliability and robustness of the prediction model given the limited sample size. This holistic approach allows an objective interpretation without bias on the efficacy of a stimbiotic on mortality reduction highlighting its association with gut function and health.

Key Words: stimbiotic, broiler chickens, mortality, holo-analysis, gut health

SCAD II

T157 Gene expression in Harderian glands and tracheas with maternal antibodies 24 and 48 hours after vaccination with LaSota Newcastle Disease Virus Taina Lopes*, Jannis Nankemann¹, Andrea Pietruska¹, Raimundo Marquez¹, Cassandra Kitchens¹, Camila Guevara¹, Haroldo Toro¹, Ruediger Hauck^{1,2} *¹Department of Pathobiology, Auburn University, ²Department of Poultry Science, Auburn University*

Newcastle disease (ND) affects poultry, causing significant economic losses. Current vaccines do not prevent infection and virus shedding. Identifying immune-related genes as markers for effective vaccination will help with the testing of new vaccines. This study examined immune gene expression in chicken Harderian glands (hg) and tracheas (tc) after ND vaccination. Fifty-six one-day-old commercial layer chicks with maternal antibodies against NDV were vaccinated either on day 1 or 14 of age with NDV LaSota strain via the oculo-nasal route (vacc), while control birds were mock-vaccinated with PBS. Hg and tc were collected 24 h and 48 h post-vaccination (h_{pv}), and total RNA was extracted. Transcriptome analysis was performed by Illumina sequencing. Reads were trimmed and aligned to the NCBI chicken reference genome using Hisat2. Reads were counted using HTSeq. Differentially expressed genes (DEGs) and differentially regulated pathways related to the innate immune system were identified using edgeR, limma and amiGO. Pairwise comparisons were made between the vacc and control birds at each age for each organ and each time point. In hg of 1d old birds around 1000 DEGs were found 24 and 48 h_{pv}. The JAK-STAT cytokines receptor and hormone activity pathways were upregulated 24 h_{pv}, and neuropeptides, apoptosis, and protein binding were upregulated 48 h_{pv}. Downregulation of neuropeptides, interleukin, and production of immune cells-related pathways were detected 24 h_{pv}, when JAK-STAT cytokines receptor, DNA and nitrogen catabolism, and peptides fragmentation pathways were downregulated 48 h_{pv}. Fewer DEGs were detected in tc. In birds vaccinated at 14d of age, less than 500 DEGs were found in hg and tc. Upregulated pathways 24 h_{pv} included miRNA metabolism, cytokine signaling, and interferon production, meanwhile miRNA metabolism, innate immune response paths and peptide hormones were upregulated 48 h_{pv}. Downregulation was marked for the cell death, DNA and nitrogen catabolism, cytokine receptors 24 h_{pv} while defense cell apoptosis, B and T cell homeostasis and immune cells production were downregulated 48 h_{pv}. In conclusion, this

study identified marked differences in gene expression after ND vaccination varying within organ, age and time point after vaccination.

Key Words: Immunity, Transcriptome analysis, Gene Ontology, KEGG analysis, Bioinformatics

T158 Rapid access to on-farm biosecurity expedites the U.S. poultry industry's response to and recovery from endemic and emerging diseases. Jason Galvis*, Rocio Crespo, Lilian Hutchens, Kelsey Mills, Gustavo Machado *Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University*

The well-established U.S. National Program Improvement Plan (NPIP) provides a guideline for enhancing the biosecurity features of poultry farms to improve preparedness for outbreaks of several diseases, including high pathogenic avian influenza (HPAI). Participation in the NPIP is voluntary. However, due to the need to monitor procedure data during outbreaks, the U.S. Department of Agriculture highly recommends participation. Understanding the strengths and weaknesses of biosecurity measures in place at individual farms is necessary to prevent the spread of disease. Nevertheless, due to the absence of standardized data input and lack of evaluation tools, the U.S. Poultry industry needs help in analyzing and comparing the quality of the biosecurity plans. Members of the poultry industry, government officials, and academic scholars assembled the Rapid Access Biosecurity (RAB) app (RABapp™) consortium to 1) reduce ambiguity regarding the construction of on-farm biosecurity plans, 2) provide rapid access to standardized data input to biosecurity plans at a national level, and 3) track movements between farms allows for the ability to efficiently develop and utilize disease spread transmission model and transform these models into decision support tools for poultry health official and poultry industry. As a result, RABapp™ allows users to monitor farms in real-time through updated and standardized biosecurity data and assess infection risk using disease spread models. It also facilitates the creation of control zones and expedites the distribution of movement permits within them, strengthening surveillance strategies. The adoption of RABapp™ by the swine industry in the U.S. was a success, with 33 companies across 21 states joining the consortium. This has resulted in the addition of nearly 10,000 biosecurity plans to the app, a success that can

be attributed to the valuable tools that enable effective farm monitoring. We anticipate replicating this success in the poultry industry. In summary, the RABapp™ tool can help producers, veterinarians, and government officials to more easily manage flocks during the HPAI epidemic to prevent the spread of the disease.

Key Words: Rapid Access Biosecurity, on-farm biosecurity, movement data, disease transmission

T159 Characterization of *Castellaniella* spp.: a new bacterial pathogen associated with mortality in broiler breeders Yi-Chen Luo*^{GS}, Tiffani Allen, Jenny Nicholds, Grazieli Maboni *university of georgia*

Since 2018, the Poultry Diagnostic and Research Center (PDRC) at the University of Georgia has identified unusual bacterial infections in broiler breeders presenting increased mortality, lameness, and swollen wattles. To date, cases were gathered from four integrators with farms spanning three states – Georgia, Texas, California, and North Carolina. A total of 20 isolates were obtained from blood agar, forming white mucoid colonies within 24–48 hours at 37°C. There was no growth on MacConkey, but Gram stain revealed pure Gram-negative short rods. Even though clinical presentation resembles *Pasteurella multocida*, analysis of the 16S rRNA gene sequencing revealed 99% homology with *Castellaniella* species. The minimum inhibitory concentration (MIC) of antimicrobials suggested resistance to penicillin (95%, n=19, MIC ≥8), ceftiofur (95%, n=19, MIC ≥4) and susceptibility to tetracycline (100%, n=20, MIC ≤4), spectinomycin (90%, n=18, MIC ≤16), enrofloxacin (95%, n=19, MIC ≤0.25) and florfenicol (95%, n=19, MIC ≤1). *Castellaniella* spp. has been isolated from the environment knowing for its capability for biodegradation of high nitrogen waste and antibiotics, but in animals it was also reported to be associated to the cause of death of small lagomorphs presenting suppurative inflammation in internal organs. To our knowledge, *Castellaniella* spp. has not been acknowledged as a pathogen in poultry, and there are no documented instances of its presence in avian species. Our future work will provide a comprehensive genomic characterization based on whole genome sequences and will set a model to examine the *in vivo* pathogenesis of *Castellaniella* spp. in birds.

Key Words: Castellaniella, broiler breeders, phenotypic characterization, antimicrobial susceptibility

T160 Supplementation of a sulfate polysaccharide extracted from marine algae positively influences performance and gut integrity of broilers during a necrotic enteritis challenge Candice Blue*^{IGS}, Maria Garcia-Suarez², Elise Nacer-Khodja², Rami Dalloul¹ *University of Georgia, ²Olmix SA*

Necrotic enteritis (NE) is a complex poultry disease caused by the Gram-positive, spore-forming, opportunistic pathogen *Clostridium perfringens*. In poultry production, losses can be attributed to reduced performance and higher mortality rates. This study employed an experimental NE model involving co-infection with *Eimeria maxima* (day [d] 14) and *C. perfringens* (d 19) to assess the effectiveness of a sulfate polysaccharide extracted from marine algae in mitigating the adverse effects of NE in broilers. A total of 600 d-old Ross 708 male broilers were randomly assigned to one of four treatment groups: NC (negative control, fed a corn-soybean meal diet); PC (positive control, fed NC + 15 ppm Avilamycin and 125 ppm Amprolium); AGS (Algoguard Standard, fed NC + Algoguard added at 0.1% of the diet); and AGH (Algoguard High, fed NC + Algoguard added at 0.2% of the diet). Average daily feed intake (ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated and adjusted for daily mortality. On d 21, the jejunum and ileum of four birds per pen were examined for NE lesions. On d 14, 21, and 42, jejunum samples from one bird per pen were collected to measure mRNA abundance of claudins (CLDN) 1 and 3, zonula occludens (ZO) 1 and 2, and occludin (OCLDN). Data were analyzed using JMP, and significance between treatments was determined by LSD ($P \leq 0.05$). Overall, PC, AGS, and AGH had sig-

nificantly lower mortality, ADFI, and FCR, and greater ADG compared to NC. Additionally, PC, AGS, and AGH significantly reduced NE lesions compared to the NC group on d 21. There were no significant differences in mRNA abundance of CLDN1, CLDN3, ZO2, and OCLDN on d 21 among all treatments. However, on d 42, AGS and AGH showed greater mRNA abundance of CLDN1, ZO1, and ZO2 ($P < 0.05$) compared to NC and PC groups. Collectively, the enhancements in performance, reduction in lesion scores, and increased post-infection expression of tight junction protein mRNA demonstrate the potential of this marine algae-derived dietary supplement as an effective alternative to antibiotic growth promoters. This approach has the potential to alleviate the negative impacts of the disease yet further investigations into its mode of action under various enteric challenges are warranted.

Key Words: Broiler, Performance, Necrotic enteritis, Tight junctions, Marine algae

T161 Major histocompatibility complex B15 haplotype and alloantigen types D and E exhibit association with resistance traits to coccidiosis challenge in chickens Abhisek Niraula*^{IGS}, Robert Taylor, Jr.², Janet Fulton³, Rami Dalloul¹ *University of Georgia, ²West Virginia University, ³Hy-Line International*

Major histocompatibility complex (MHC) genes are associated with disease resistance traits in poultry. Recent evidence points to other blood alloantigen types exhibiting similar associations. Coccidiosis, a major protozoal disease in poultry caused by different *Eimeria* species, inflicts major economic losses due to poor gastrointestinal health and predisposition to secondary infections. Pedigreed progeny from four sires each mated to four dams segregated for five alloantigen systems (A, B, E, D, I) including the MHC, were used in a 26-day (d) study investigating resistance to coccidiosis. Chicks were hatched, individually wing-tagged, and assigned to different cages according to their pedigree. Birds were weighed on d 0, 7, 19, and 26 to calculate body weight gain (BWG). On d 19, all birds were inoculated with sporulated oocysts of *E. acervulina* (23,000), *E. maxima* (5,000), and *E. tenella* (10,000). All birds were euthanized on d 26 and scored for gross coccidiosis lesions in the duodenum, jejunum, and ceca. Additionally, duodenal, jejunal, and cecal mucosal scrapings were collected to score microscopic lesions. Fecal samples were collected to assess oocyst shedding. Similarly, blood was collected from each bird for SNP genotyping of all five alloantigen systems. The data were analyzed using a generalized linear model and one-way ANOVA with significance ($P \leq 0.05$) between treatments determined by Tukey's test. The B15 haplotype showed a negative association with cecal gross lesions ($P \leq 0.05$) whereas B21 had a positive association with BWG (d 0-7) plus gross lesions in the jejunum and ceca ($P \leq 0.05$). Alloantigen D had a positive association with BWG during d 0-7, 7-19, 0-19, and cumulatively on d 0-26, as well as with gross lesions in the jejunum ($P \leq 0.05$). Conversely, there was a negative relationship of alloantigen E with cecal gross lesions ($P \leq 0.05$). The MHC B15 haplotype and alloantigen types D and E showed major association with resistance to this mixed *Eimeria* infection. These genetic markers have potential application in selection programs to improve resistance and productivity in chickens exposed to coccidiosis.

Key Words: major histocompatibility complex, alloantigen, coccidiosis, chicken, resistance

T162 Increasing dietary indigestible protein may exacerbate effects of coccidia challenge in broiler chickens Jung Sung*^{GS}, Olayiwola Adeola *Purdue University*

The objective was to determine if increasing dietary indigestible protein concentration exacerbates detrimental effects of coccidiosis in broiler chickens. Non-autoclaved and 180-min autoclaved poultry meals were included at 200 g/kg in the standard diet and high-indigestible protein diet, respectively, to induce differences in intestinal indigestible protein concentrations. Digestible essential amino acid concentrations were equalized

between diets by adding crystalline amino acids. Crude protein concentration was also equalized by adding glutamic acid. A total of 192 broiler chickens (Cobb 500; initial body weight = 310 ± 30 g) on d 11 post hatching were allotted to 4 treatments in a randomized complete block design. The treatments were based on a 2 (standard diet vs. high-indigestible protein diet) \times 2 (control vs. coccidia challenge) factorial treatment arrangement. On d 12 post hatching, birds were orally gavaged with either 1 mL saline or 1 mL solution containing 25,000, 25,000, and 125,000 oocysts of *E. maxima*, *E. tenella* and *E. acervulina*, respectively. On d 18 post hatching, birds were euthanized and cecal mucosa was collected from the bird with median body weight in each cage. The coccidia challenge decreased body weight gain in both diets but the degree of the decrease was greater in birds fed the high-indigestible diet (interaction; $P < 0.05$). There was a tendency for an interaction between coccidia challenge and diet ($P = 0.052$) for feed intake with a greater intake of high-indigestible protein compared with the standard diet in non-challenged birds, whereas difference in feed intake was observed between the two diets in the challenged birds. Coccidia challenge or increasing dietary indigestible protein concentration decreased ($P < 0.05$) ileal nitrogen digestibility, which increased ileal indigestible nitrogen concentration. Coccidia challenge increased ($P < 0.05$) the mRNA expression of TNF- α and IL-1 β , whereas feeding the high-indigestible protein diet decreased ($P < 0.05$) occludin mRNA expression in cecal mucosa. In conclusion, increasing dietary indigestible protein has the potential to exacerbate detrimental effects on growth performance, and decrease the gene expression of cecal tight junction proteins during coccidia challenge.

Key Words: Indigestible protein, Coccidiosis, Broiler

T163 Impact of dietary inclusion of a yeast-based carbohydrate blend on the gut microbiota and horizontal transmission of *Histomonas meleagridis* in turkey poults Aaron Forga^{*1GS}, Alessandro Rocchi¹, Kyle Teague¹, Carl Jones², Charlie Elrod², Mariana Nascimento³, Theresia Lavergne², Danielle Graham¹ ¹Division of Agriculture, Department of Poultry Science, University of Arkansas, ²Natural Biologics, Inc., ³Sapiens

Provillus (Natural Biologics, Inc., Newfield, NY) is derived from complex carbohydrates, postbiotic compounds, and fermentation extracts from yeast of different substrates. To assess the effect of Provillus on *H. meleagridis* horizontal transmission and performance in turkey poults, 240 day-of-hatch poults were assigned to treatments (n=8 replicate cages/group; n=8-10 poults/cage). Treatments were: 1) Non-challenged control (NC), 2) Challenged, control (PC), and 3) Challenged + Provillus (1 lb/ton). Poults were fed a starter diet from d0-7 and a wheat middlings-based diet from d7-30. Individual BW was recorded at d0, 10, and 30 to determine BW (g) and BWG (g) of contact poults. Seeders (n=2 poults/pen) received 100,000 *H. meleagridis* (PHL strain) cells/0.5 mL/poult via intra-coecal inoculation at d10. Seeder poults were commingled with contact poults and wax paper was placed in the cage during the challenge period to promote horizontal transmission. Horizontal transmission was determined by the presence of liver and cecal lesions at d30. ANOVA was used to determine differences for performance and lesion scores. Chi-square test was used to assess differences for mortality and horizontal transmission rate. Contact poults fed Provillus had numerically reduced *H. meleagridis* horizontal transmission and mortality (66.7%; 2.08%) compared to the PC (73.9%; 8.33%) ($P > 0.10$). BW at d10 (129.44 ± 2.02) and BWG from d0-10 (73.03 ± 1.94) were ($P < 0.05$) higher for poults fed Provillus compared to the NC (119.23 ± 1.99 ; 62.34 ± 1.97) or PC (120.52 ± 2.10 ; 64.20 ± 2.06). BW at d30 and BWG from d0-30 were numerically higher for the Provillus group (376.11 ± 11.70 ; 319.09 ± 11.58) compared to the PC group (369.41 ± 12.03 ; 312.59 ± 12.04). Poults fed Provillus had a more favorable microbiome, with an increase in positive biomarkers (+18.13%) compared to NC. Microbiome richness (37,190 identified bacterial strains) and diversity (195 identified genera) were better for Provillus fed poults than PC (35,983; 186). Poults fed Provillus were more resilient and better able to withstand the disease challenge. Dietary inclusion of Provil-

lus may reduce *H. meleagridis* transmission without negatively affecting performance and could be used to improve health status of *H. meleagridis* challenged poults.

Key Words: *Histomonas meleagridis*, Provillus, histomonosis, blackhead, turkeys

T164 Comparison of the standard operation and output metrics of two commercial field spray vaccinators Cory Yarbrough^{*GS}, Brian Jordan *University of Georgia*

Infectious Bronchitis Virus (IBV) is one of the primary viral respiratory pathogens in chickens and practically all poultry raised in the U.S. are vaccinated with an IBV vaccine. Pullets are vaccinated via field application multiple times throughout rearing, yet testing of vaccine application repeatedly shows shockingly low or negative results after field spray vaccination. Previous research showed that modifications in application parameters improved hatchery spray application, therefore the same parameters need to be evaluated for field spray vaccination equipment. This study evaluated two commercial field spray applicators, a Stihl gas-powered, single-wand sprayer provided by Merck Animal Health and a SoloVac battery-powered, double-wand sprayer provided by Boehringer Ingelheim. Several parameters were measured for each sprayer including spray distance, output volume, output rate, and lateral spread of the spray, as well as the standard product specifications for each machine. The SoloVac sprayer has a tank capacity of 5 gallons and weighed a total of 74.8 lbs at full capacity. The Stihl sprayer has a tank capacity of 3 gallons and weighed 54.7 lbs at full volume. As delivered by the manufacturer, the SoloVac has a tighter spray pattern with larger, more coarse droplets compared to the Stihl sprayer which disperses diluent in a wider area with finer droplets, even on the coarser spray setting. The SoloVac nozzle only has two settings and is either a fine mist or coarse droplet, while the Stihl sprayer has 6 nozzle settings and 4 blower attachments and can be adjusted from a very fine mist to a more coarse droplet. The SoloVac dispersed diluent up to 10 feet with an average flow rate of 4.2ml/sec per wand, 8.4ml/sec total, while the Stihl sprayer extends the distribution distance to 15 feet with an average flow rate of 24.4ml/sec. At these rates, it would take 37.5 minutes to empty the 5-gallon SoloVac tank and 7.75 minutes to empty the 3-gallon Stihl sprayer tank. Understanding the parameters of available commercial vaccine sprayers will aid in improving vaccination success of IBV and other vaccines that are mass-applied to breeders and layers during rearing and lay, in a field vaccination scenario.

Key Words: Infectious Bronchitis Virus, Field Spray Vaccination

T165 Evaluation of spray application with *Bacillus amyloliquefaciens* spores as an alternative to formaldehyde fumigation during the hatching phase Mitchell Rowland^{*GS}, Aaron Forga, Makenly Coles, Billy Hargis, Christine Vuong, Danielle Graham *University of Arkansas*

In commercial hatcheries, fumigation with formaldehyde, a known carcinogen, is used to mitigate the microbial bloom during hatch. The purpose of the present study was to investigate the effects of spray application of Bacillus spore-based probiotics on the microbial load in the hatch cabinet, pioneer colonization of the gastrointestinal tract (GIT), and early performance compared to formaldehyde fumigation. An environmental challenge model was used to simulate the microbial bloom to compare the application of two *B. amyloliquefaciens* strains (MCR002 and MCR009) to formaldehyde fumigation during the hatching phase. Three experiments were conducted. Treatment groups included: 1) negative control (NC), 2) challenged with pathogen mix, non-treated control (PM), 3) challenged, formaldehyde control (PM+F), 4) challenged, MCR002 treated (PM+002), and 5) challenged, MCR009 treated (PM+009). To evaluate the microbial load in the hatch cabinet environment during the hatching phase, air samples were collected using the open agar plate method on DOE20 at ~20%, 50%, 80% hatch, and immediately prior to hatch pull. GIT samples were collected for enumeration of relevant enteric pathogens. Each sample was

plated on non-selective or selective agar plates to enumerate recovery of various bacteria present. Pen and feed weights were recorded at hatch and d7 for Exp 1 and 2 and also at d14 for Exp 2 to assess body weight gain (BWG) and feed conversion ratio (FCR). ANOVA was used to determine significant differences at $P < 0.05$ with means further separated using Student's t test. Combined analysis of all experiments resulted a significant reduction in coliform recovery from the GIT for PM+F, PM+MCR002, and PM+MCR009 compared to PM with both *Bacillus* treated groups being similar to PM+F. Compared to PM, coliform and *Enterococcus* recovery from environmental samples was significantly reduced in PM+F and PM+MCR002. No significant differences in performance were observed. Spray application with MCR002 or MCR009 shifted the microbial load in the hatch cabinet and GIT of chicks similar to formaldehyde and without negatively impacting performance. This suggests that MCR002 and MCR009 could be used as potential alternatives to formaldehyde fumigation.

Key Words: *Bacillus*, Probiotic, Formaldehyde, Hatchery, Broiler

T166 Development of a horizontal transmission model to assess the effects of exposure to *Enterococcus cecorum* during the hatching phase on performance and organ involvement in young broiler chickens James Higuaita^{*IGS}, Marcela Arango¹, Aaron Forga¹, Mitchell Rowland¹, Jundi Liu², Ross Wolfenden², Danielle Graham¹ ¹Department of Poultry Science, University of Arkansas Division of Agriculture, ²Animal Nutrition BU, Eastman Chemical Company

Due to limited information regarding early *Enterococcus cecorum* (EC) infections, the present study focused on development of a horizontal transmission model to simulate exposure to virulent EC during the hatching phase. In Exp 1, a subset of the embryos was inoculated by *in ovo* injection into the amnion (10^4 CFU/embryo) at DOE19 with EC5, EC7, or EC11B (seeders; S) and comingled with non-infected embryos (contacts; C) until hatch. Treatment groups included: 1) non-challenged control (NC), 2) EC5-C, 3) EC5-S, 4) EC7-C, 5) EC7-S, 6) EC11B-C, and 7) EC11B-S. EC colonization of the gut was elevated in contact chicks at hatch indicating transmission was successful. Based on Exp 1 results, a 26-day study was conducted (Exp 2). Individual body weights were recorded at hatch, d7, d14, d21, and d26 to assess body weight gain (BWG) and liver, spleen, and free thoracic vertebra (FTV) samples were collected to evaluate EC colonization using Chromagar Orientation agar followed by MALDI-TOF for confirmation. ANOVA was used to determine significant differences ($P < 0.05$) for BWG with means separated using Student's t test. Chi-square was used to evaluate significant differences for macroscopic lesions and EC incidence. Compared to NC, BWG was significantly reduced for EC7-S throughout the study and at d0-21 and d0-26 for EC5-S, EC5-C, and EC7-C. No differences in BWG were observed for EC11B-S or EC11B-C. At hatch, EC recovery was significantly higher in the gut across all groups and in the FTV of EC5-S, EC-7C, EC7-S, and EC11B-S compared to NC. At d26, EC recovery from the FTV was markedly elevated in all groups. Increased EC recovery from the spleen, but not the liver, was observed for EC5-S, EC5-C, EC-7C, and EC7-S. Prominent lesions included focal heart necrosis, splenomegaly, and hepatomegaly, with the heart being the most affected organ. These results suggest that exposure to EC via direct injection into the amnion during late embryogenesis or during the hatching phase as infected chicks emerge from the egg, could increase colonization of extraintestinal tissues and affect performance as early as 7d post-hatch depending on the strain. This horizontal transmission model could be used to evaluate methods to mitigate EC infections pre- and post-hatch.

Key Words: *Enterococcus cecorum*, horizontal transmission, broiler, performance, model

T167 Isolation of *Enterococcus faecalis* from hatchery samples and yolk sacs of pullets and broiler chickens Hugo Ramirez^{*GS}, Amari Shields, Marcela Arango, Jay Kay Thornton, Martha Pulido - Landinez *Mississippi State University*

Yolk sac infections are one of the main causes of increased mortality in chicks during the first week and the presentation of chronic bacterial infections during the growing period. Inappropriate practices in breeding flocks lead to fecal-contaminated eggs and promote environmental contamination at the hatchery, which could be the main reason for yolk sac infections. This study aimed to establish the frequency of isolation of *Enterococcus faecalis* from samples collected at the hatchery and the yolk sac of pullets and broiler chickens.

The information about ninety-three, 1- to 7-day-old broiler and pullet breeder cases, and 45 hatchery cases was compiled using a convenience sampling method. The cases were received at the PRDL of Mississippi State University between January and October 2023. Yolk sac samples were streaked on Blood, CNA-Columbia, and MacConkey Agars. The samples of the yolk sac and cecal tonsils were inoculated in tetrathionate broth with iodine for *Salmonella* isolation. Agar plates and broths were incubated overnight at 37°C under aerobic conditions. Hatchery samples were inoculated initially in BHI broth and incubated overnight at 37°C. After incubation, samples were streaked on CNA agar and incubated overnight (all plates and broths from Remel™ Thermo Fisher Scientific™, Lenexa, KS). Bacterial identification was performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) - Vitek® MS instrument (bioMérieux, Inc). *Salmonella spp* was genotyped by Intergenic Sequence Ribotyping (ISR).

Enterococcus spp was isolated from 100 % of yolk sac samples. *E. faecalis* was isolated from most of the analyzed samples (n=90; 96.77 %). The association with *E. coli* was observed in 55 (61.11 %) cases. *S. Enteritidis* was isolated from 1 case (1.11 %). *Enterococcus spp*, different than *E. faecalis*, was isolated from 7 cases. Concerning hatchery samples, *E. faecalis* was isolated pure from 39/45 cases. In six cases, it was mixed with *E. coli* and *Salmonella spp*.

These results emphasize the strong relationship between the hatchery sanitary conditions and the health of one-week-old chickens. The sources of *E. faecalis* causing contamination at the hatchery and yolk sac infections must be identified and controlled.

Key Words: *Enterococcus spp*, *Escherichia coli*, *Salmonella spp*, yolk sac infection

T168 Identification and characterization of *Enterococcus cecorum* recovered from commercial poultry cases. Marcela Arango^{*GS}, Rebecca Mackey, Jay Kay Thornton, Natalie Manginsay, Martha Pulido-Landinez *Mississippi State University*

Enterococcus cecorum (EC) is a Gram-positive bacterium that recently has been identified as an important cause of systemic disease and lameness in broilers. Research data related to the differentiation between pathogenic and commensal EC obtained from field outbreaks is limited. Phenotypic and genotypic characteristics of EC, including the lack of the ability to metabolize mannitol and the presence of the *cpsO* gene have been associated with pathogenicity.

Using a convenience sampling method, 299 cases received at Mississippi State University's Poultry Research and Diagnostic Laboratory (PRDL) from January to October 2023 were analyzed. The goals of this study were: 1) to analyze the distribution of *Enterococcus spp*. cases and 2) to assess the pathogenicity by the determination of mannitol metabolism and the detection of the *cpsO* gene in selected EC isolates (n=75).

Samples from birds were plated onto Columbia (CNA) agar. Samples from the hatchery were inoculated in enrichment broth and plated onto CNA plates. All plates and broths were incubated at 37°C overnight (CO₂-enriched atmosphere). Bacterial identification was performed by MALDI-

TOF MS using a VITEK®MS instrument (bioMérieux, Inc). On selected EC isolates, mannitol metabolism was evaluated using a Gram-positive plate identification (Thermo Fisher Sensititre® ARIS HiQ® complete automated system). Conventional PCR was performed by targeting the *cpsO* gene.

The distribution of *Enterococcus spp.* cases analyzed was as follows: broilers (n=127), breeder pullets (n=85), hatchery (n=50), broiler breeders (n=29), commercial layers (n=3), and others (n=5). EC represented 62% of *Enterococcus spp.* isolates from broilers (mainly from heart and femoral head samples), and 90% from broiler breeders, (mainly from intestines). 82% of the isolates were PCR-positive for the *cpsO* gene. 100% of the breeder isolates recovered from the intestine were *cpsO* negative, suggesting a commensal status. 96% of pathogenic isolates were unable to metabolize mannitol.

These results indicate a high prevalence of pathogenic EC in broilers, and the presence of commensal EC in breeders. Additionally, our results support previous research indicating that the mannitol metabolism may be an additional reliable tool to assess EC pathogenicity.

Key Words: Enterococcus, cecorum, broilers, infection, bacteria

T169 Avian pathogenic Escherichia coli (APEC) a versatile pathogen - but what have we learned? Catherine Logue*, Klao Runacharoon, Julia Inenes Lima, Yu-Yang Tsai, Lisa Nolan *University of Georgia*

Avian Pathogenic *Escherichia coli* (APEC) is the leading cause of colibacillosis in the poultry industry resulting in multimillion dollar losses worldwide as a result of morbidity, mortality and carcass loss (partial or whole) at processing. Infection associated with APEC causes a range of symptoms including respiratory distress, reduced appetite and growth and a range of syndromes including perihepatitis, airsacculitis, pericarditis, egg peritonitis, salpingitis, coligranuloma, omphalitis, cellulitis, and osteomyelitis/arthritis. While most often recognized as a secondary, to another infection, viral challenge or stressor, APEC can also be a primary pathogen and is often overlooked as the sole cause of disease in production birds. Here, we provide an overview of this versatile pathogen from the perspective of its development into a significant pathogen, the type of disease it causes, its virulence and resistance traits, zoonotic potential and intervention strategies. We also show the evolution of the pathogen in light of the effects of vaccination and various interventions and the changing landscape of APEC which is redefining high risk strains causing disease. We will also show some of our recent findings on APEC and its role in disease in Georgia poultry production.

Key Words: Escherichia coli, poultry, disease, APEC, pathogen

T170 Evaluation of Enterococcus cecorum Tissue Prevalence when Broilers receive two different Direct Fed Microbials Matthew Jones*¹, Charles Hofacre¹, Jennie Baxter¹, M. Suyemoto², Roy Berghaus³, Enid McKinley⁴, Jodi Delago⁴, Alexandra Smith⁴ ¹*Southern Poultry Research Group*, ²*Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University*, ³*Department of Population Health, College of Veterinary Medicine, University of Georgia*, ⁴*Arm and Hammer Animal Nutrition and Food Production*

Nonpathogenic *Enterococcus cecorum* (EC) is a common commensal bacterium in the intestines of broilers. However, pathogenic EC strains can escape the gut and cause septicemia resulting in infections of the free thoracic vertebrae (FTV) and femoral head. In recent years, some producers have observed an increased incidence of septicemia and osteomyelitis caused by EC. Interventions reducing the translocation of these bacteria from the gastrointestinal tract into circulation may help decrease these observed lesions. Two Direct Fed Microbials (DFM) were evaluated for efficacy against EC infection. Each product was fed at a rate of 3.0 x 10⁵ CFU/g (0.20%) from Day of Test (DOT) 0 – 35 and 7.5 x 10⁴ CFU/g (0.05%) from DOT 35 – 42. DFM groups also received a probiotic gel

at hatch. All groups received a 1x dose of commercial coccidiosis vaccine at placement. Each treatment had 8 replicate floor pens containing 25 male Ross broiler chicks. Each bird was orally gavaged with a known pathogenic EC strain (1.0 x 10⁷ CFU/chick) on DOT 0. Four birds were necropsied from each replicate pen on DOT 21, then at termination 100 birds were sampled from each treatment. Spleen prevalence of EC was evaluated at each time point and FTV prevalence was assessed on DOT 42. Birds and feed were weighed on DOT 0, 21, 35, and 42 to evaluate performance metrics. Prevalence data were subjected to a GEE logistic regression and comparisons were performed using the Bonferroni procedure with significance set to 0.05. On day 21, prevalence of EC in the spleens of the challenge control was 22% with no significant differences among groups. On DOT 42, there was a significant reduction in EC prevalence in spleens from 67%^B positive in the challenge control to 46%^A in DFM-1 and 33%^A in the DFM-2 group. FTV infection prevalence mirrored the spleens with 40%^B positive EC samples in the challenge control group and 23%^A and 19%^A in the DFM-1 and DFM-2 groups, respectively. There were no significant changes in feed conversion or body weight data at study termination. The DFM appeared to reduce translocation of EC into circulation which significantly decreased infection in target tissues. In a typical broiler farm this decreased osteomyelitis may lead to fewer cull birds in the days leading up to processing.

Key Words: Enterococcus cecorum, Femoral Head Necrosis, Direct Fed Microbial, Free Thoracic Vertebra, Kinkyback

T171 Genetic analysis of tylosin-resistant and tylosin-susceptible Mycoplasma synoviae from the USA Eniopo Oluwayinka*, Naola Ferguson-Noel *Department of Population Health, University of Georgia*

Key Words: Mycoplasma synoviae, Tylosin, 23s rRNA, Resistance, Proteome

T172 Development of an anti-HRV Chicken Monoclonal Antibody using Single B-cell Cloning Jill Skrobarczyk*¹, Cameron Martin¹, Suresh Pillai², Luc Berghman¹ ¹*Department of Poultry Science, Texas A&M AgriLife Research*, ²*Department of Food Science and Technology, Texas A&M AgriLife Research*

The unique mechanism of chicken antibody production presents an interesting model for therapeutic antibody development to treat and prevent enteric infectious disease. Rotavirus (RV) is the leading cause of severe gastroenteritis in infants and young children. One of the primary structural features of human rotavirus (HRV) is the VP4 spike protein. Cleavage of VP4 by trypsin into the VP5* and VP8* subunits is required for host cell attachment and subsequent infection. The objective of this study was to develop a new single B-cell cloning methodology for therapeutic chicken monoclonal antibody production using the HRV VP8* protein as a case study. Single B-cell cloning was used to develop the antibody, a technology that has yet to be used in the production of chicken monoclonal antibodies. Peripheral blood mononuclear cells were harvested from white Leghorn hens hyperimmunized with electron beam inactivated HRV particles. Chicken B-cells were sorted using flow cytometry with a fluorescent labeled antibody against chicken IgY and three synthetic, biotinylated HRV peptides derived from neutralizing epitopes of the VP8* protein. Single B-cells were maintained in vitro in the presence of chicken CD40 ligand and IL-4 for one week before screening for HRV-specific antibody secretion by ELISA. The variable regions of the chicken heavy and light - chain were amplified from positive B-cell clones and assembled into a functional single-chain variable fragment (scFv). The scFv was expressed in Chinese hamster ovary (CHO) cells and analyzed for binding and neutralization of HRV. The scFv demonstrated recognition of intact HRV particles in an ELISA with a signal to noise ratio greater than 2 (SNR >2). Competition between whole viral particles and VP8* peptides indicated that the scFv was also specific for the given VP8* epitope (SNR >2). The scFv was ineffective at preventing HRV infection in an in vitro virus

neutralization assay, however, further optimization may help improve the

stability of the antibody. This study reports on the first use of single B-cell cloning to develop chicken monoclonal antibodies.

Key Words: monoclonal, antibody, IgY, chicken, rotavirus

Metabolism & Nutrition VIII Feed Additives

T173 Development of a bacteriophage cocktail with high lytic efficacy against *Salmonella enterica* in vitro and in vivo Daniel Castillo*, Nicolás Cifuentes, Matias Aguilera, Soledad Ulloa, Luis Leon, Christian Pieringer, Constanza Sandoval, Hans Pieringer, Pablo Cifuentes *PhageLab SpA*

Salmonella enterica is a major human pathogen that can cause up to 7.8 millions of foodborne diseases and 59,153 deaths worldwide annually. The major route of infection is through fecal-oral transmission, and a variety of food matrices have been reported as sources and vehicles of *Salmonella* spp. The most frequent meat involved in salmonellosis are associated with poultry (chicken, turkeys, ducks and geese). However, the use of antimicrobials as a primary tool to control *Salmonella* in poultry is highly restricted in Europe and the US. In this context, bacteriophage-based cocktails have arisen as an alternative to antibiotics to reduce the growth of *Salmonella*.

Here, we isolated and sequenced 47 bacteriophages that showed variable degrees of lytic activity against 258 *Salmonella* isolates obtained from a Brazilian commercial chicken company. These bacteriophages were microbiologically and genomically characterized, and three were selected to assemble a cocktail. The lytic activity of the cocktail was tested in conditions that more closely resembled the chicken gut such as anaerobiosis, 42 °C and *Salmonella* mono-strain biofilms. Finally, the formulated cocktail was tested in a large field trial involving 46 aviaries from a Brazilian commercial chicken company and the total load of *Salmonella*, and specific loads of serovars Newport, Minnesota, Heidelberg, Agona, Mbandaka and Braenderup were measured from faecal swabs by qPCR.

The results showed that *in vitro* quantitative assays, the cocktail had an efficacy of 97% against the collection of *Salmonella*, showing high activity against the most prevalent serovars including Minnesota, Heidelberg and Agona. For the large-scale trial, the formulated-cocktail was applied in each aviary per cycle, in four consecutive cycles that involved a total of 3.3 million treated chicken and 1.1 million untreated chicken. The qPCR results showed a significant reduction of up to 3 log in *Salmonella* loads in the aviaries at days 28 and 42 (the day of slaughter) upon treatment with the bacteriophage cocktail.

Our results highlight the relevance of an optimized *in vitro* development of bacteriophage cocktails with high lytic efficacy against *Salmonella* and strong potential to be applied *in vivo*.

Key Words: Salmonella, Cocktail, Bacteriophage, Poultry

T174 Effect of a next generation phytogenic blend on broiler growth performance under challenged conditions Meghan Schwartz*, Prashant Mishra, Stacie Crowder *PMI*

Two studies were conducted to evaluate the effects of a next generation phytogenic feed additive on broiler performance with used litter challenges. Both trials were complete randomized block designs where block was based on pen location within the research facility. Treatments were 1) Control (C), 2) Novel phytogenic product (PHYTO) at 142 mg/kg (PMI, Arden Hills, MN). In each trial, 1,300 day-old Cobb 500 male broilers were assigned to 13 replicate pens per treatment (50 birds/pen). Body weight gain (BWG), feed intake (FI), and feed conversion ratio adjusted for mortality (mFCR) were evaluated at 14, 28, and 42 days of age. A commercial coccidiosis vaccine was administered according to manufacturer's recommendation at chick placement. Statistical analysis was performed using Proc Mixed procedure in SAS and significant means were

compared by Least Significant Difference ($P < 0.05$). Throughout both trials, no significant effect on BWG, FI, and mortality were observed. In trial 1, PHYTO treatment significantly improved mFCR at 28 (1.452 vs 1.410) and 42 days of age (1.739 vs 1.691). In trial 2, PHYTO treatment significantly improved mFCR at 42 days of age (1.572 vs 1.544). Inclusion of this next generation phytogenic blend improved mFCR in both trial 1 and trial 2. Based on these results, including this novel phytogenic blend in broiler diets improved growth performance. More specifically, feeding this novel phytogenic blend improved broiler feed conversion ratio.

Key Words: feed additive, broilers, phytogenic, performance

T175 Effect of phytogenic feed additives on live performance and meat quality of older broilers Megan Koppen*¹, Rob Payne¹, Manuel Da Costa¹, Jorge Urrutia², Kelley Wamsley², Li Zhang², Dalton Dennehy² ¹Cargill Animal Nutrition, ²Mississippi State University

Challenges and demand in the poultry industry paired with volatile feed ingredient costs and varying meat prices continue to persist. Thus, focusing on value-add feed additive selection is vital, especially with birds reared longer. Phytogenic feed additives have shown to improve nutrient digestion and utilization as well as meat quality properties. Based on several digestibility studies, Delacon developed nutritional matrix values for Biostrong™ 510 (BSG), called the Performizer™ solution (PS). The objective of this trial was to evaluate the impact of feeding BSG (on-top) and via PS matrix values (AME, CP, and digestible AA) by measuring production performance and meat quality of Ross 708 male broilers in a RCBD. Four dietary treatments were tested: 1) control, 2) 150 g/MT BSG 0-42 days, 3) 150 g/MT BSG 0-56 days, and 4) 150 g/MT BSG 0-56 days (PS from 42 to 56 days) with 16 replicates/treatment at 25 birds/replicate from 0 to 56 days of age. Birds were fed four phase corn/soybean meal based basal diets formulated to meet or exceed breeder recommendations (0-14, 14-28, 28-42, 42-56 d). Data were analyzed using ANOVA and means separated using Fisher's LSD at $p \leq 0.05$. Feed intake, body weight, and feed conversion ratio were measured during each feed phase. At 0-42 d, birds fed BSG exhibited a significantly lower FCR than the control ($p < 0.05$). A trend ($p = 0.103$) was observed for 0-55 d BW gain, wherein birds fed TRT 3 and 4 had numerically higher gain versus 1 and 2. At 56 days, birds were processed. Live body weight, carcass, breast meat (fillet, tender & total), leg weight yield (drumstick, thigh & total), and wing processing metrics were measured. The total antioxidant capacity (TAC) was also measured at 55 days. Across all metrics excluding leg yields, TRT 3 and 4 were heavier than 1 and 2 ($P < 0.05$). TRT 2 had significantly lower total leg yields than 1 and 3 ($P < 0.05$). Higher TAC levels were observed in TRT 3 and 4 compared to 1 and 2 ($P < 0.05$). BSG and PS yielded the most profitable results in this experiment based on current US meat prices. Based on the trial results, the dietary supplementation of BSG when fed on top or utilizing matrix values can improve meat yield and quality metrics of older broilers while also optimizing production costs.

Key Words: phytogenics, broilers, feed cost, performance, digestibility

T176 Effect of dietary blends of short chain fatty acids and phytochemicals on performance and oocyst shedding in broiler chickens subjected to repeat challenge with a live coccidiosis vaccine Aaron Forga¹, James Higuera¹, Snehal Tawde², Antonia Tacconi², Jonathan Broomhead², Samuel Rochell³, Danielle Graham*¹ ¹Department of Poultry Science, University of Arkansas Division of Agriculture, ²Perstorp Animal Nutrition, ³Department of Poultry Science, Auburn University

Eimeria cycling is required for live coccidiosis vaccines to induce immunity to protect against subsequent infections. This oocyst cycling may cause reduced feed intake and weight gain and increased feed conversion during the first three weeks of age in broiler chickens. The purpose of the present study was to evaluate the effectiveness of different combinations of short chain fatty acids (SCFA) and phytochemicals on growth performance and oocyst shedding in a coccidiosis vaccination challenge model. On d0, n=672 broiler chickens (7 treatments × 8 replicate cages × 12 birds per cage) were randomized, weighed, tagged, and placed in battery cages for 21 days. Treatment groups included: 1) negative control (NC), 2) positive control (PC), 3) Blend A, 4) Blend B, 5) Blend C, 6) Blend D, 7) Blend E. Groups 2-7 were orally vaccinated with a 1X, 10X, and 100X dose of a commercially available live coccidiosis vaccine at d0, d7, and d14, respectively, to simulate *Eimeria* cycling. Pen weights were recorded at d0, d7, d14, and d21 to determine average body weight (BW) and body weight gain (BWG) in grams. Feeder weights were also recorded to calculate feed conversion ratio (FCR). Litter pans were cleaned on d5, d12, and d19, and total feces was collected at d7, d14, and d21 for oocyst per gram (OPG) analysis. ANOVA was used to determine significant differences at P<0.05 and means were further separated by Student's t test. There were no differences in BW at d0 or d14 or d0-14 BWG across all groups. At d7, NC (151.23±2.08) and Blend E (144.36±2.56) BW was significantly greater than PC (136.10±2.69). At d21, NC (930.89±11.27) and Blend B (919.00±15.11) BW was significantly higher than PC (880.02±13.69). BWG from d0-7 was significantly greater in NC (113.69±2.08) and Blend E (106.69±2.51) than PC (98.74±2.64) and BWG d0-21 was markedly increased in NC (893.48±11.23) and Blend B (881.39±15.05) compared to PC (842.45±13.67). FCR was significantly improved from d0-7 for Blend B (1.17±0.02) compared to PC (1.24±0.02). Dietary treatments did not significantly affect OPG at any time point evaluated. In summary, Blend B of SCFA and phytochemicals negated performance reduction from coccidiosis vaccine, without a negative response on vaccine efficacy, based on OPG results.

Key Words: SCFA, phytochemicals, feed additives, coccidiosis, broiler

T177 Effect of liver conditioner pronutrients to prevent and ameliorate the effects of liver stress in layers David Diez*¹, Connie Gallardo², Cecilia Lugo¹, Anna Tesouro¹ ¹Biovet S.A., ²Universidad Científica del Sur

Energy imbalance and heat stress are two of the main predisposing factors to liver stress which can trigger fatty liver syndrome (FLS). Liver stress generates serious alterations in the liver and results in a negative impact on egg production and quality in laying hens. Liver conditioner pronutrients (PN) are active molecules from plant extracts used to prevent or ameliorate liver stress and recover productive parameters in the face of these predisposing factors.

A trial was carried out for 17 weeks to evaluate PN as a preventive tool for liver stress. 140 Dekalb Brown layers were distributed into 4 groups with 7 repetitions each and 5 birds per repetition. The treatments were a negative control (T1) fed with a standard isocaloric diet, the rest of the groups (T2, T3, T4) were challenged with a hypercaloric diet in order to induce a moderate liver challenge. The positive control (T2) with no PN; group (T3) supplemented with PN throughout the whole trial, and group (T4) supplemented with PN from week 23 (when negative liver effects from the challenge began) until the end of the trial. Differences with P<0.05 were considered statistically significant.

T3 obtained better weight gain, accumulated consumption, feed conversion, hematological and biochemical values, compared to the other hens fed with hypercaloric diets (T2 and T4) (P<0.05), obtaining similar values to T1 (negative control) throughout the whole trial. T4 showed that the birds were able to recover productive parameters after being challenged with a hypercaloric diet and supplemented from weeks 23 to 28 compared to T2 (P<0.05). In relation to the average weight of the egg, the laying rate and the kg of eggs produced per hen, T3 obtained statistically significant better results than T2 (P<0.05). T4 significantly improved the laying rate and kg of eggs produced per bird compared to T2 (positive control) (P<0.05). PN prevented these alterations when administered as a preventive measure (T3) and ameliorated them when administered when the problem was detected (T4).

In summary, PNs are effective in preventing and ameliorating liver stress in conditions of energy imbalance in the diet and heat stress. PN is a natural tool that does not generate residues and does not create resistance.

Key Words: Liver stress, Pronutrients, Performance, Feed imbalances, Liver health

T178 Effects of a commercial triple-strain Bacillus-based probiotic on cecal colonization with Salmonella Enteritidis in commercial layer pullets Eric Sobotik*¹, Kay Russo¹, Steve Lerner¹, Dorthe Sandvang¹, Antoine Meuter¹, Hannah McBride², George Girgis² ¹Chr. Hansen, Inc., Animal and Plant Health & Nutrition, ²Nevysta Laboratory, Iowa State University Research Park

Salmonella Enteritidis (SE) in layers represents a significant challenge in the poultry industry, directly impacting food safety for consumers. As a primary source of Salmonellosis outbreaks from egg consumption, effective control of SE is critical. A commercial triple-strain *Bacillus*-based probiotic was tested to determine its effect on *Salmonella* colonization in the ceca of commercial layer pullets. Potential for reduction in *Salmonella* colonization was assessed by prevalence and enumeration following oral inoculation of a selected nalidixic acid resistant SE strain. Two treatments were tested, each containing 128 day-of-hatch LSL layer pullets. On top of a standard diet, the treatments were: 1) No supplement (Control, CON), and 2) Probiotic (PRO, 1.6×10⁶ CFU/g of finished feed). Environmental swabs were collected from each experimental group and tested to ensure freedom from SE prior to challenge. At 21 days of age, the SE challenge strain was inoculated orally at a dose of 3.3×10⁸ CFU/bird. Pullets from each experimental group (n=32) were euthanized at 6-, 10-, 14-, and 18-days post infection (dpi). Contents from the ceca were aseptically collected for prevalence and enumeration of SE. Data were analyzed using GraphPad Prism 10.0.2 (GraphPad Software LLC, San Diego, CA). Significant differences (P<0.05) were identified by ANOVA of log transformed SE counts. No differences in prevalence of SE positive ceca following oral inoculation were observed between treatment groups at 6-, 10-, 14-, and 18-dpi (P>0.05). Cecal SE counts in the PRO group were not significantly different from CON at 6- or 10-dpi. However, significantly lower SE counts in the ceca of the PRO group were observed at 14-dpi (P=0.038) and 18-dpi (P=0.019) compared to CON. SE counts were 1.24 and 1.34 logs lower than CON at 14- and 18-dpi, respectively. In conclusion, supplementation of the triple-strain *Bacillus*-based probiotic resulted in lower cecal counts of SE compared to those birds not on an effective probiotic. SE counts were reduced to 5.6% and 4.7% of the control group at 14- and 18-days post inoculation, respectively.

Key Words: Bacillus, probiotic, layer, food safety, Salmonella

T179 The association of probiotic and prebiotic products in the feeding of laying hens minimizes the negative effects caused by heat waves. Tiago Urbano*¹, Fabrizio Matté¹, Patrick Roieski¹, Paulo Oliveira², Marcel Boiago², Antony Comin² ¹*Vetanco Brasil*, ²*University of Santa Catarina State*

The use of pre and probiotics has proven to be interesting in the poultry production, especially in hot regions, as it minimizes the negative effects caused by heat on the birds' digestive tract, with consequent improvements in nutrient absorption. The objective was to evaluate the use of probiotics, prebiotics and their combinations on the performance, egg quality, oxidative stress and intestinal permeability of laying hens challenged by heat waves (37°C) for three consecutive days, eight hours a day. One hundred 30 week-old Isa Brown laying hens were distributed in a completely randomized design, in a 4 X 2 + 1 factorial arrangement (four treatments X 2 moments – before and after the stress + negative control treatment, non-stressed birds) with five replications of four birds each. Treatments consisted of Positive Control (basal diet and heat-stressed birds); Stressed birds + probiotic (300 g.ton-1); Stressed birds + prebiotics (1000 g.ton-1); Stressed birds + probiotics (300 g.ton-1) + prebiotics (1000 g.ton-1). The probiotic and prebiotic products were composed of *Bacillus subtilis* (5 x 10⁹ cfu. g-1) and yeast wall lysate (50 g.kg-1). Productive parameters during the stress period, internal and external egg quality before and after stress, intestinal permeability (FITC-dextran method) and serum superoxide dismutase and glutathione peroxidase after stress were evaluated. The heat waves negatively affected feed consumption, egg production, feed conversion and the qualitative indexes of the eggs (specific gravity, shell thickness and Haugh unit). The stress also caused higher oxidative stress in birds, through greater activity of the enzyme superoxide dismutase (P<0.001). The use of products composed of pro and prebiotics had no significant effects, but their combination minimized the negative effects caused by hot waves on intestinal integrity (P=0.0027). Consequently, the better intestinal integrity of these birds led to better feed conversion (P=0.016) and improved the internal and external quality of the eggs, in addition to reducing the activity of the SOD enzyme (P=0.050). Based on these results, we can conclude that the association of probiotics and prebiotics is a good tool to minimize negative effects caused by heat waves in laying hens.

Key Words: Egg Quality, Intestinal Absorption, Mannan-oligosaccharide, Oxidative Stress

T180 Double-buffered sodium butyrate addition improved egg mass and feed efficiency of layers fed low energy-protein diet Julián Melo*¹, Matías Voelker¹, Agustín Ablanedo², Clemence Marecaille³, Xavier Roulleau³ ¹*Universidad Nacional de Luján*, ²*Universidad de Buenos Aires*, ³*Dietaxion SAS*

In a context of rising prices of feed ingredients, the improvement of the overall feed digestibility and maximum utilization of nutrients in the feed-stuffs should be a priority. Previous studies have shown the benefit of a double-buffered sodium butyrate (DBSB) on the performance and intestinal health of broilers fed low energy and protein diets (LEP). The aim of the study was to evaluate the effect of this uncoated form of sodium butyrate (DBSB) on the performance and feed efficiency of layers. A total of 384 Hy-line W-80 layers were allocated in individual cages in a completely randomized design. For each treatment there were 8 repetitions, being each line of 24 individual cages an experimental unit. The layers were weighted at the beginning and at the end of the trial. From week 56 to 59 of age two diets were used for dietary treatments. LEP diet was formulated to obtain a reduction in energy and protein considering the standard recommendations (Hy-line, 2019): -3% of AME, -2,5% of Crude Protein and the same amount reduction for digestible lysine, threonine, and sulfur amino acids (M+C). LEP+DBSB group was fed with the LEP diet supplemented with 600ppm of DBSB (Butylin 54, Dietaxion SAS). The measured parameters were feed intake (FI), number of collected eggs (EggN) and their weight (EggW), enabling to calculate the mass of eggs produced

(EggM) and the feed conversion ratio (FCR). Mortality was used to correct the feed intake. Data were statistically analysed using ANOVA, followed by a Duncan test for comparisons of means. No difference in FI, EggN and EggW was observed between treatments (P>0.05). However, hens in LEP group laid significantly (P<0.05) less EggM (3,1%). Regarding the FCR, significant differences were obtained between the groups (P<0.05) for feed efficiency per kg of egg mass, with the LEP diet presenting the worst performance (3,7%). There was no significant difference in FCR per dozens of eggs (P>0.05) but tended to be improved with DBSB (2,8%). The use of DBSB in layers fed LEP diets improved egg mass and feed efficiency per kg egg mass, so could be considered an important strategy to feed cost savings and excretion reduction.

Key Words: layers, sodium butyrate, egg mass, feed efficiency

T181 Enhancing performance and egg quality of commercial laying hens through fiber-rich diets supplemented with stmbiotic Amanda Lima¹, Adiel Lima¹, Paloma Souza¹, Carlos Nascimento¹, Matheus Ramalho Lima², Fernando Perazzo Costa¹, Gilson Gomes³, Alexandre Brito⁴, Xavière Rousseau*⁵, Thiago Tedeschi dos Santos⁶ ¹*Federal University of Paraíba | UFPB*, ²*Federal University of the Semi-Arid Region | UFRSA*, ³*AB Vista | EUA*, ⁴*AB Vista | Brazil*, ⁵*AB Vista | France*, ⁶*AB Vista | UK*

Fiber has characteristics that can alter its viability in poultry diets. Therefore, understanding its main aspects allows for the improvement of its nutritional utilization, as previously, fiber was not used to its full potential due to the lack of available technologies for practical application. Thus, the study aimed to evaluate the variation in fiber and its source and its interaction with stmbiotic (STB – Signis, AB Vista, UK) supplementation in the diet of commercial laying hens. A total of 1200 Bovans White hens aged 23 w, with 1,46 ± 0.04kg, and egg production at 95.95 ± 3.19%. The experimental design used was a completely randomized design with 12 treatments and 10 replicates with 10 birds each, for 5 cycles of 28 days. The treatments were based on diets with variations in the use of wheat, corn, and its by-products, isolated or combined, and were supplemented or not with 0.01% STB. Nutritional requirements followed the guidelines from the breed. Treatments consisted of a Control, commercial diet, and two basal diets, one with Wheat and Wheat Bran (100W), and another with Corn and corn germ meal (100C). These 100W and 100C diets were diluted to obtain the treatments 75W|25C, 50W|50C, and 25W|75C. All diets were supplemented with STB. The results were subjected to normality and error tests, and then a 2x6 factorial analysis was performed with two factors (STB and Fiber, and their interaction) with significance accepted when P ≤ 0.05. Among the fiber levels, and interactions, the means were compared by the Tukey test. Data was analyzed using R software. There were significant interactions for FCR and Haugh units. FCR dozen eggs was improved with STB in the diet with 75W|25C (P<0.01), while STB improved Haugh units in the Control diet, 75W|25C, and 25W|75C, but did not influence the other diets. STB improved shell thickness, especially in diets with a higher wheat content, reducing this variable when there was more corn than wheat in the diet. The quality of the shell was improved with STB in all evaluated diets. In conclusion, STB supplementation improved performance and egg quality of white-laying hens in diets with a high dietary fiber content.

Key Words: Dietary Fiber, Laying Hens, Stmbiotic Supplementation

T182 Benefits of supplementing breeder hens with a standardized dry grape extract on their bones and eggs' quality Paul Engler*¹, Dominique Chavatte², Mohammed BENARBIA¹ ¹*Nor-Feed SAS*, ²*DLJ Consultant*

Amongst the aspects of poultry reproduction, bone health, immune transfer and egg quality represent key elements. Previous published studies have shown that a supplementation with a commercial standardized dry grape extract (SDGE) could improve bone health in pullets, antibody titers

as well as egg quality in layers. The aim of this study was to evaluate the effect of a supplementation with a commercial SDGE on hens' bones, the specific immune protection transfer through the eggs and the quality of the eggs.

21660 breeding hens were randomly divided in two groups of a similar size and housed in identical conditions in two adjacent barns of the same farm. CTL group received a classic breeder feed. NG group received the same diet, fortified with 30ppm of SDGE (Nor-Grape®, Nor-Feed, France) from the start of lay to W60. At W60, 50 hens were randomly sampled, euthanized and sent to an independent laboratory to analyze their bones. 90 eggs were sample per group to study the following parameters: whole egg weight, fresh yolk mass, yolk and shell CEIL*a*b* parameters and Haugh units. Additionally, 30 eggs per group were used to analyze specific anti-Newcastle IgG in the eggs. Bones, egg quality parameters and egg IgG titers were then compared between groups using Student t-test.

Bones analyses showed that tibia density tended to be higher in the NG group (+2.2mg/mm, $p < 0.10$) and was correlated with higher bone stiffness (+4N/mm). Despite no egg weight difference between groups, NG eggs presented a significantly higher yolk mass (+0.9g of yolk/egg, $p < 0.05$), with NG yolks showing a significant improvement in color for both a* (+0.4, $p < 0.01$) and b* parameters (+1.2, $p < 0.01$). NG eggs also had a numerically higher Haugh unit (+1.2, ns) and IgG titers against Newcastle vaccine (+1760, ns.). Interestingly, NG eggs' IgG titers tended to be more homogeneous ($p < 0.10$), with a higher proportion of eggs with very high anti-ND titers (80% vs. 63% for NG vs. CTL respectively).

Overall, these results evidenced the beneficial impact of the supplementation with a SDGE on the hens' bone health, their egg quality and immune transfer. Further research would be of interest to assess its potential on older hens, as the demand for increased laying persistence is on the rise.

Key Words: Breeder, Immunity, Bone health, Polyphenols, Pigmentation

T183 Growth performance of turkey poults fed diets containing lysophospholipids Chan Sol Park*¹, Dan Moore², Hilary Pavlidis³, Janet Snow³, Jae Hark Jeong¹ ¹Pathway Intermediates USA, ²Colorado Quality Research, ³CSA Animal Nutrition

A study was conducted to test the hypothesis that the supplementation of dietary lysophospholipids (LPL) improves the growth performance of turkey poults fed diets with reduced metabolizable energy (ME) contents. A total of 1,050 turkey poults with an average initial body weight (BW) of 58 g were allocated to five dietary treatments in a randomized complete block design with the location of pen as a blocking factor. There were 15 replicate pens per treatment. Dietary treatments consisted of positive control (PC), negative control (NC), and NC with graded concentration of dietary LPL supplement at 0.25, 0.50, and 0.75 g/kg. Lipidol® Ultra was used as the LPL supplement in experimental diets. Dietary treatments were prepared using a two-phase feeding program: Phase 1, from Day 0 to 21; Phase 2, from Day 21 to 42. The PC diets were prepared as commercial corn-soybean meal-based diets, whereas NC diets were prepared with reduced ME contents at 150 kcal/kg from PC diets. Data were analyzed using the mixed linear model with the fixed effect of dietary treatment and the random effect of block. There was a tendency ($P = 0.077$) showing that BW of birds on Day 42 linearly increased with increasing concentration of dietary LPL supplements. From Day 0 to 21, birds fed the PC diet had reduced ($P < 0.001$) feed intake compared to the NC diets. Similarly, feed intake of birds fed the PC diet was less ($P = 0.014$) than those fed the NC diets from Day 21 to 42; however, BW gain and gain-to-feed ratio (G:F) of birds linearly increased ($P < 0.05$) as the concentration of LPL supplements increases in the NC diets. During the overall experimental period, birds fed the PC diet had less ($P < 0.001$) feed intake than those fed the NC diets, and the graded concentration of LPL supplements linearly improved ($P = 0.032$) G:F of birds and tended to linearly increase ($P = 0.076$) BW gain of birds. Regression analysis between G:F of birds from Day 0 to 42 and the concentration of LPL supplements confirmed that G:F of birds fed the PC diet was not different from the predicted value at 0.75 g/kg LPL supplement. In conclusion, results of this study confirmed that the growth performance of turkey poults fed diets with reduced ME contents is improved by feeding LPL supplements during brood phase.

Key Words: Growth performance, Lysophospholipid, Poultry, Turkey

Metabolism & Nutrition IX Feed Additives

T184 Microbiome Modulation by a precision biotic leads to increased production of short-chain fatty acids in the intestine of broiler chickens Cristiano Bortoluzzi*, Mick Watson *DSM-Firmenich*

The objective of the present studies was to evaluate: (1) the impact of the supplementation with a Precision Biotic (PB) on the modulation of the cecal microbiome collected from broiler chickens submitted to a necrotic enteritis challenge model (Experiment 1); (2) to measure the cecal production of short-chain fatty acids (SCFA; Exp. 2). In Exp. 1, day-old chicks were placed on a completely randomized block design with 3 treatments, 10 replicates, and 25 birds/rep. The treatments consisted of a control group, a challenged control, and a challenged group supplemented PB at 0.9 kg/MT. The challenge consisted of a coccidia vaccine (CocciVac B52) at d 0 applied to all treatments, and *Clostridium perfringens* via drinking water to the challenged groups on d 15, 21, and 28. On d 22 and 46, 1 bird/pen was selected, and the cecal content was collected for microbiome analysis. In Exp. 1, it was observed that the supplementation of PB significantly ($P < 0.05$) improved the growth performance of the challenged birds. In the microbiome, an increased relative abundance of several species related to SCFA production was observed at both d 21 and d 42, including increased relative abundance of several *Faecalibacterium* species (a known butyrate producer) on day 42 ($P < 0.05$). This was paired with an increased relative abundance of both the propionate ($P < 0.05$) and butyrate pathways in birds with PB supplementation at both d 21 and d 42.

In Exp. 2, day-old chicks were placed on a completely randomized block design with 2 treatments, 8 replicates, and 25 birds/replicate. The treatments consisted of a control group, and a group supplemented with PB at 0.9 kg/MT. At d 14, all the birds were challenged with 30X of a coccidiosis vaccine (Zhengdian, Fushan, China) via oral gavage. On d 21 and 43, 2 birds/pen were selected, and the cecal content was collected for SCFA analysis. PB led to an increase in the production of propionate ($P = 0.02$; 47%) and valerate ($P = 0.08$; 29%) on d 21, and numerically increased acetate, propionate, and butyrate on d 42. Taken together, the microbiome metabolic shift observed with the supplementation of PB, plus the observations with increased SCFA production, may explain the improvement in growth performance obtained with the supplementation of PB.

Key Words: Broilers, microbiome, metabolism, precision biotic, metagenome

T185 Evaluation of increasing doses of Eastman Entero-Nova™ 220C on broiler performance in a 48-day floor pen trial Jundi Liu*¹, Ross Wolfenden¹, Matthew Jones², Charles Hofacre² ¹Eastman Chemical Company, ²Southern Poultry Research Group, Inc.

A 48-day floor pen trial was conducted to evaluate the dose response of a short-chain fatty acid monoglyceride blend (Eastman Entero-Nova™ 220C) on broiler performance. A total of 2000 commercial Ross 708 male

broilers were obtained from a local hatchery and were randomly allocated into 5 groups with 25 chicks/pen and 16 replicates/treatment group. Broilers were raised on re-used litter and vaccinated at day 1 with 1x dose of a commercial coccidiosis vaccine. The Entero-Nova™ 220C was supplemented in feed at 0, 1500, 2000, 3000, 5000 mg/kg throughout the 48-day trial period. A 3-phase feeding program of starter (d 1-14, crumble), grower (d 14-28, pellet), and finisher (d 28-48, pellet) diets was utilized. BW gain and feed intake were measured on d 14, 28 and 48. Performance data were analyzed by ANOVA model with significance deemed at $P \leq 0.05$. Means were separated using Tukey's HSD test. The supplementation of Entero-Nova™ 220C at 2000 and 5000 mg/kg significantly improved BW gain at 303 g and 297 g, respectively, compared with the non-supplemented control group at 272 g on d 14 ($P \leq 0.05$). From d 1 to 28, the Entero-Nova™ 220C groups significantly increased BW gain as compared to the control ($P \leq 0.05$). Mortality-adjusted FCR was not statistically different between treatments on d 28. On d 48, the use of Entero-Nova™ 220C at 2000, 3000 and 5000 mg/kg showed significantly higher BW gain ($P \leq 0.05$) of 3414 g, 3429 g, and 3445 g, respectively, compared with the control group at 3300 g. A significant improvement in mortality-adjusted FCR was also observed in broilers fed Entero-Nova™ 220C at 3000 mg/kg (FCR = 1.51) and 5000 mg/kg (FCR = 1.50) compared to the control group (FCR=1.53). In conclusion, use of the monoglyceride blend Entero-Nova™ 220C can improve BW gain and feed conversion ratio in broilers raised on used litter and vaccinated with commercial coccidiosis vaccine.

Key Words: monoglyceride blend, Entero-Nova™ 220C, performance, broiler

T186 The use of PFA with nutritional matrix values can decrease feed costs and maintain the production performance of broilers Manu De Laet*, Caroline Donaldson, Yann Fournis, Roberto Montanhini Neto *Cargill Animal Nutrition - Global SMT Additives*

For broilers, feed costs are critical and account for 60–70% of the total production costs. Raw material prices have increased versus historical norms. Thus, it is necessary to optimize broiler diet formulations. Phyto-genic feed additives (PFA) have been shown to improve nutrient digestion and utilization. Based on several digestibility studies run by Delacon, nutritional matrix values were developed for PFA. This trial evaluated the effect of adding a PFA based on a combination of spices, bitter substances, essential oils, and saponins (Biostrong™ 510, referred to hereafter as PFA) used with a nutritional matrix. The trial measured the performance of 828 Ross308 male broilers (12 replicates/treatment; 23 birds/replicate) up to 42 d. The aim was to evaluate whether the final performance may be maintained with a lower feed cost per kg output. A 3-phase feeding program was used: starter (1–14d), grower (15–28d), and finisher (29–42d). Three dietary treatments were tested: 1) Positive control (PC); 2) Negative control (NC, Comparable to PC diet except that respective nutrients were reduced by the nutritional matrix values: 45 kcal/kg, 3 g/kg CP, 0.15 g/kg dLys and other amino acids), and 3) PFA (NC+150 g/MT PFA). Data were analyzed using ANOVA in R. In the starter phase, PFA performed comparably to the NC (equal FCR and BW). Birds of both treatments were significantly lighter than the PC ones ($P < 0.05$). From d14, birds fed PFA maintained and improved performance compared to PC and NC, with no difference in BW or FCR at d28. At d42, birds fed PFA had the highest BW and lowest FCR compared to the PC and NC (BW: 3483g, 3436g, and 3412g; FCR: 1.67, 1.68, and 1.70, respectively; $P > 0.05$). The trial showed that the proposed nutritional matrix values of the PFA can be used without any detrimental effect on bird performance in the grower and finisher phases. Using the PFA with nutritional matrix values allowed for a 2% feed price reduction at a similar performance, leading to a ROI (improved value/cost of investment of adding PFA with nutritional matrix values) of 3.6. Therefore, adding PFA on-top in the starter phase and applying

nutritional matrix values in the grower and finisher phases may be recommended to achieve cost-effective broiler production.

Key Words: phyto-genic, matrix values, cost-effective, broiler, performance

T187 Effects of a triple-strain Bacillus-based probiotic in ameliorating stress-related impacts on broiler gut microbiota for improved well-being and productivity Antoine Meuter*, Guilherme Borchardt¹, Gisle Vestergaard¹, Eric Sobotik² *Chr. Hansen A/S, ²Chr. Hansen Inc.*

Heat stress can negatively affect gut microbiota by disrupting the balance and composition of the microbial community. It can lead to a decrease in beneficial bacteria and an increase in pathogenic bacteria, compromising the intestinal barrier function. Probiotics can help mitigate the effects of heat stress in poultry by modulating the gut microbiome. 720 day-old male chicks were randomly assigned to two houses of 12 pens each and three treatment groups with the same basal diet: Negative Control (NC); Probiotic (PRO, triple-strain Bacillus-based probiotic at 1.6×10^6 CFU/g); and Positive Control (PC) containing lincomycin. While broilers of the first house (NS) were raised in thermoneutral conditions ($< 24^\circ$ C) from D0 to slaughter (D35), all birds from the second house (HS) were submitted to heat stress conditions (32° C, 8-10h / day) from D21 to D35. Data were analyzed using Statsmodels library for Python and Tukey method, with significance determined at a level of $P < 0.05$. At D35, NS birds had a more diverse gut microbiota (16S rRNA sequencing method) and better zootechnical results than HS birds. HS PRO showed tendency for higher microbiome alpha-diversity and significantly higher abundance of butyrate-producing bacteria such as Faecalibacterium, Turicibacter and Romboutsia versus HS NC. HS PRO had higher final body weight (2.304 vs. 2.228 kg) and increased breast weight compared to HS NC and PC. The improved performance in PRO can be correlated with welfare biomarkers, intestinal functionality, and carcass yield. HS PRO birds demonstrated higher concentrations of serotonin in bloodserum and intestine at D35. Reduced oxidative stress as measured by higher expression of the superoxide dismutase gene in liver was also observed. Additionally, improved gut integrity as indicated by increased intestinal expression of the mucin gene (MUC2) at D7, D28 and D35 was noted. Finally, there was a significant increase in breast meat yield in HS PRO and upregulation of the MyoD (myoblast determination protein 1) gene compared to NC. In conclusion, the use of a triple-strain Bacillus-based probiotic to modulate the gut microbiota effectively helps poultry withstand stressful conditions, optimizing productivity and promoting hormone balance associated with well-being.

Key Words: Microbiome, Probiotic, Stress, Productivity, Well-being

T189 Feed fiber profile and the use of stimbiotic influence broilers productivity and hindgut fermentation Xavière ROUSSEAU*, Dimcho DJOUVINOV, Gilson A. Gomes *AB Vista*

Environmental concerns around soybean production and willingness to reduce nitrogen (N) excretion led to replace soybean meal (SBM) with other ingredients or to the use of more synthetic amino acids. Rapeseed (RSM) and sunflower meal (SFM) are alternatives but will bring more fiber with different profile. This study aimed to evaluate the impact of SBM replacement by the inclusion of RSM and/or SFM on performance and hindgut fermentation, with and without stimbiotic (STB, Signis, ABVista) for birds fed low crude protein diets. Birds were allocated to 64 pens (16 birds/pen) in a 2x4 factorial design (STB: with or without; and 4 diet formulations differing by their Insoluble to Soluble Non-Starch Polysaccharides [NSPs] ratio). Trial lasted 35d, diets formulated on a 3 feeding-phase with 1) SBM based diet (ratio: 2.9/3.0/3.0 for starter, grower, finisher phase, respectively); 2) RSM diet (3.6/3.8/3.8); 3) SFM diet (5.6/6.0/5.8); and 4) 50% RSM & 50% SFM. Data were submitted to a 2-way ANOVA using JMP 16.2. Differences among means were separated by Tukey's test and significance accepted at $P \leq 0.05$. Livability was analyzed by non-paramet-

ric test and not affected by the treatments. On the overall, interactions were observed ($P \leq 0.05$) between STB and diet formulations on average daily gain (ADG), average daily feed intake (ADFI) and body weight corrected feed conversion ratio (bwcFCR). STB reduced broilers ADFI when fed RSM and SFM ($P \leq 0.05$) while increasing in RSM-SFM ($P \leq 0.05$) and had no effect on SBM ($P > 0.05$). ADG was improved by STB in RSM-SFM reaching same than SBM fed birds ($P \leq 0.05$) but was not affected in RSM, SBM and SFM fed birds ($P > 0.05$). STB improved bwcFCR for all diets but at different magnitudes ($P \leq 0.05$; -6pts in RSM; -11pts in RSM-SFM; -4pts in SBM and -1pt in SFM). Diets affected cecal short chain fatty acids (SCFAs) concentration ($P \leq 0.05$) with RSM fed birds showing lowest concentration. STB increased SCFAs regardless basal diet ($P < 0.05$). Litter N was reduced by STB regardless of diet ($P \leq 0.05$) and incremental decrease observed from RSM>SBM>RSM-SFM>SFM ($P < 0.05$). The use of alternative ingredients influenced performance and hindgut fermentation. STB improved performance irrespective of the diet but with varying magnitudes of improvement depending on diet fiber profile

Key Words: fiber, stimbiotic, SCFAs, Litter

T190 Effects of organic acids blend supplementation via water on the performance of broiler chickens and on return of the investment Andrea Silvestrim^{*1}, Lane Pineda¹, Karolina von Zuben Augusto¹, In Ho Kim², ¹Selko, ²Dankook University, Department of Animal Resource and Science

Acidification of water in broiler chickens' production is commonly used to improve the zootechnical performance. Thus, the objective of this study was to evaluate the effect of using a synergistic blend of free and buffered organic acids (OA) to reduce pH in water supporting digestion and intestinal health, on performance (1 to 35 d-old) of broiler chickens. A total of 969 1-d-old male Ross 308 chicks were distributed in a completely randomized design: a control group without any additives in feed and water (T_1); a blend of OA applied via water during the entire cycle (1L/m³ water) (T_2); and a blend of OA applied via water for 6 hours per day on week's 1-2-4-5, except on week 3 (1L/m³ water) (T_3) with 17 replicates per treatment of 19 birds each. The diets were based on corn-soybean according to NRC (1994) in two phases: Initial (d1-d14), Grower (d15-d35), free of additives. Daily, the water intake per pen was measured and on day 35 the performance was evaluated. Data were analyzed using a RCBD using GLM procedures of SAS (1996), with each pen as the experimental unit. Variability was expressed as the pooled SE and the selected level of significance at $P \leq 0.05$. Broilers significantly decreased FCR when received a blend of OA via water during the entire cycle ($T_2 = 1.607$) or during a reduced period ($T_3 = 1.638$) compared to the control broilers ($T_1 = 1.772$) ($P < 0.05$), result of significant increase in final BW and ADG ($P < 0.05$). There was no treatment effect on water intake (135.1, 131.7, and 132.5 mL/d for T_1 , T_2 , T_3 , respectively, $P > 0.1$). Based on local feed, product, and livestock prices, the application of T_2 (full cycle) leads to a net return of € 0.17 per broiler (ROI 13.3) compared to T_1 . T_3 (reduced application) resulted a net return of € 0.12 per broiler (ROI 12.4) compared to T_1 . When comparing both OA treatments, T_2 exhibited a net return of 0.04 (ROI 2.9) compared to T_3 . In conclusion, the results indicate that water supplementation with a blend of OA enhance the broiler performance without negatively affecting the water consumption. And despite the lower initial investment with the reduced application of OA via water compared to the use full cycle, the economic return is considerably better for a continuous use strategy, making this choice viable.

Key Words: Feed conversion ratio, Organic acids, Weight gain, ROI, Water consumption

T191 Practical application of multi-mycotoxin biomarker analysis from dried blood spots to assess mycotoxin exposure risk and performance impacts of a novel mycotoxin intervention in broilers Don Ritter^{*1}, Arnau Vidal² ¹Innovad USA, ²Innovad Global

Determining the real risk from mycotoxin exposure is challenging. An UHPLC-MS/MS method targeting 36 mycotoxin biomarkers via dried blood spots (DBS), based on previous work (Lauwers et al, 2019) was used to determine actual mycotoxin exposure in samples obtained from commercial broilers in several USA locations. To establish a complete mycotoxin risk assessment, feed consumed at the time of blood collection was also analysed for 16 mycotoxins by LC-MS/MS. The combined results demonstrated a persistent exposure of broilers to multi-mycotoxins in the cases examined. The DBS analysis specifically revealed that mycotoxins produced by *Alternaria* and *Fusarium* spp. were most common.

This methodology was used in a pen study designed to evaluate performance impacts of an intervention to control mycotoxins, Novin P[®] (Innovad). Novin-P[®] is a proprietary feed additive containing clays, yeast, plant extracts and natural antioxidants with the intended effects of detoxifying consumed mycotoxins, improving liver function and maintaining immunocompetence. One-day old ROSS 708 male chicks (n=1040) were vaccinated with Coccivac[®]-B52 (Merck) and randomly placed in 20 floor pens (52 per pen) and assigned to 2 treatments: T1 Control (no additives) or T6) Novin-P[®] 2.00 lbs/ton during starter (0-14 days), 1.50 lbs/ton during grower (15-32 days) and 0.50 lbs/ton in finisher till the end at 45 days of age. Feed and DBS samples were collected (1 sample per pen) for mycotoxin analysis at the end of each feed phase on days 14, 32 and 45. Birds and feed were weighed at 45 days to assess body weight (BW), daily growth (ADG), feed efficiency ratio (FCR). Data were analysed using SAS 2020-2021, with pen as the experimental unit. Treatment effects were compared using a two way t-test. Novin-P[®] fed birds had higher BWT ($P < 0.0229$) and ADG ($P < 0.0229$) and tended to improve BWT and Mortality adjusted FCR ($P < 0.0995$). DBS analysis for biomarkers of mycotoxins showed less numerous and lower concentrations of mycotoxins in blood from Novin-P[®] fed birds at 14 and 32 days, while feed mycotoxin results were similar. These results demonstrate that Novin-P[®] reduces systemic levels of mycotoxins in broilers and allows greater growth performance.

Key Words: mycotoxins, biomarkers, blood, broiler, performance

T192 A synergistic in-feed Technology enhances the intestinal epithelial barrier via the upregulation of cytoprotective & antioxidant proteins in broilers under dietary challenges in real farming conditions. Alireza Khadem^{1,2}, DON Ritter^{*2}, Konstantinos C. Mountzouris³, Christos Gougoulias² ¹Ghent University, ²Innovad SA, ³Department of Animal Science, Agricultural University of Athens

In broilers, dysfunction of the intestinal barrier is associated with increased microbial translocation and disease risk, impaired nutrient absorption and reduced growth performance. Here, we evaluated the protective gut barrier effects of esterified butyrate, combined with plant extracts and essential oils a) *in-vitro*, via an established permeability model (Trans-epithelial Electrical Resistance: TEER in IPEC-J2 cells, n=5) and, b) *in-vivo*, in a dietary-induced chronic inflammation model (high NSP diet: 60% Wheat + 5% rye without NSPase and coccidiostats), via the jejunal mRNA gene expression of cytoprotective & antioxidant proteins in broilers. The impact on growth promotion was also monitored under two concentrations (1 or 2 kg/ton of feed) (n=8 pens/treatment; n=30 birds/pen). Importantly, the pens were housed inside a commercial production unit of 55,000 broilers so that the experimental birds could get exposed to real farming conditions. Through the enhancement of TEER, in feed technology completely reverted LPS-induced barrier dysfunction at 12 hours post-challenge and increased TEER by 2-fold at 24 hours post-challenge ($P < 0.01$). One broiler per pen was randomly selected on D28 and D35 for the determination of the intestinal expression of cytoprotective & antioxi-

dant proteins. ANOVA with Tukey posthoc analysis revealed that 1 and 2 kg/ton, when compared to the control treatment, increased significantly the BW of broilers at D35 by 3 and 4%, respectively ($P = 0.040$) and reduced the FCR (1.64, 1.62 and 1.57, respectively; $P = 0.01$). FI was not affected by the treatments. Importantly, 1 kg/ton and 2 kg/ton resulted in a profound increase of intestinal expression of Nrf2, Keap1, Glutathione peroxidase-2 and Glutathione transferase-2 compared to the control, both at D28 and D35 ($P < 0.05$ in all cases). In this study, broilers fed with an in-feed Technology of plant extracts, fatty acids, and essential oils conferred a superior protective effect on gut barrier function, accompanied by enhanced growth performance.

Key Words: Intestinal barrier function, gene expression, Growth performance

T193 Effect of a probiotics on body weight, feed conversion, and mortality of Cobb 500 and Ross 708 broilers challenged with a coccidiosis vaccine at 20 times the recommended dose John Schleifer*, Kristy Dorton, Gary Reznik, Shameer Rasheed, Mark LaVorgna, Mahmoud Masadeh *Devenish Nutrition LLC*

Four studies were conducted to determine the effects of probiotics on growth performance and mortality of Cobb 500 (Study 1, 2, and 3) or Ross 708 (Study 4) broilers challenged with a 20x dose of coccidiosis vaccine (Coccivac B-52; Merck Animal Health, Madison, NJ) on d 3, 4 or 14. Day-old broiler chicks (as hatched; $n = 900$ to 2,880) were randomly allocated to floor pens (9 to 12 reps/treatment; 50 to 60 birds/pen; 0.73 to 1.08 ft²/bird). Study 1 and 2 had four treatments: Control (CON; no probiotics),

ValuPro A (VPA; *B. amyloliquefaciens*, 5.1×10^5 CFU/g; Devenish Nutrition, Fairmont, MN), Product A (ProA; *B. subtilis*, 6.0×10^5 CFU/g), and Probiotic B (ProB; a blend of *Bacillus* spp., 1.6×10^6 CFU/g). Study 3 had three treatments: CON, VPA, and ProB. Study 4 had two treatments: CON and VPA. Broilers were fed corn/soy based pelleted starter, grower, finisher, and withdrawal (study 4 only) diets. Body weight (BW), feed intake, and mortality were measured. Feed conversion ratio adjusted for mortality (FCR_M) and to a common body weight (FCR_{M+BW}) were calculated. Data were analyzed as a one-way ANOVA using the Mixed procedure of SAS with pen as the experimental unit. Means were separated by Fisher's protected least significant difference. Differences were considered significant at $P \leq 0.05$ and a trend at $P \leq 0.10$. In only study 1, BW was greater ($P = 0.03$) for broilers supplemented with ProA than CON and VPA. For broilers receiving the test probiotics, FCR_M was better compared to CON in Study 2 ($P = 0.003$), Study 3 ($P = 0.07$; tendency), and Study 4 through d 31 ($P = 0.003$). Broilers receiving ProA in Study 1 tended to have a better ($P = 0.08$) FCM_{M+BW} compared to CON and similar FCM_{M+BW} compared to VPA and ProB. In study 4, broilers supplemented with VPA had better FCM_{M+BW} from d 0 to 31 ($P = 0.01$) and tended to have better FCM_{M+BW} from d 0 to 57 ($P = 0.09$). Mortality was also lower when broilers received test products. Although, the effect was only significantly different in Study 2 ($P = 0.05$) and Study 3 ($P = 0.03$), results showed that supplementation of VPA and the other test probiotics can have a positive impact on feed conversion and mortality in broilers during a challenge. This could result in lower feed costs and improved profitability for the producer.

Key Words: Probiotics, Broiler, Eimeria, Feed conversion, mortality

Metabolism and Nutrition X Vitamins and Minerals

T194 Would correlative elemental imaging explain the assimilation of essential trace elements in eggshell? Iris Valido^{1,2}, Rosa Mary Lopez Alvarez¹, Florent Penen¹, Maria Angels Subirana¹, Stéphane Faucher³, Pascale Senechal³, Peter Moonen³, Sandra Mounicou¹, Yron Joseph Manaig⁴, Dirk Schaumlöffel¹ ¹CNRS, Université de Pau et des Pays de l'Adour IPREM UMR, ²Universitat Autònoma de Barcelona, Centre GTS, Departament de Química, ³CNRS, Université de Pau et des Pays de l'Adour, DMEX, IPRA FR, ⁴Animine

Broken eggs represent an important source of economic loss for the egg industry. Shell defects are even more often in old layer hens, which have eggs with a thinner shell. Trace mineral (TM) supplementation can help to improve the eggshell quality, thanks to the role of TM on shell formation. The objectives of this study were to evaluate the effect of TM supplementation on eggshell quality and understand how TM are incorporated into eggshell. A total of 936 Leghorn layers (60 weeks of age) were distributed in three treatments with 12 replicates of 26 birds. Treatments were: negative control (NC) diet, without TM supplementation (54 ppm Zn, 60 ppm Mn); Zn diet, NC + 90 ppm of Zn from HiZox; Mn diet, NC + 110 ppm of Mn from ManGrin (Animine, Annecy, France). After 20 weeks trial, eggs were collected and analyzed for fracture force, shell thickness and correlative imaging (X-ray tomography, Laser ablation LA-ICPMS, and NanoSIMS). Shell thickness was improved ($P < 0.05$) by both Zn and Mn supplementation (0.35 mm) in comparison to NC (0.34 mm). However, fracture force was improved ($P < 0.05$) when hens received Mn diet (37 N) than Zn or NC diets (35 N in average). Correlative imaging results (X-ray tomography and LA-ICPMS) showed that Mn and Zn are located in the outer membrane of the eggshell and in the cuticle, whereas Ca is mainly found in the palisade and mammillary layers. NanoSIMS images revealed that Ca in eggshell seems to be more present in Mn supplemented diets, which may be related to the impact of Mn on calcium-binding proteins, and would explain the better fracture force in these eggs. In conclusion,

TM supplementation improves eggshell quality but the effect of Mn seems to be more important than Zn.

Key Words: egg quality, manganese, zinc, trace minerals

T195 Effect of chromium propionate on insulin sensitivity and performance of turkeys J. Spears^{*1}, K. Lloyd¹, K. Krafka², J. Hyda², J. Grimes¹ ¹North Carolina State University, ²Kemin Agrifoods North America, Inc.,

Chromium (Cr) functions to enhance insulin action. Experiment 1 was conducted to determine the effect of dietary Cr, from Cr propionate (Cr Prop; KemTRACE Cr Prop) on insulin sensitivity. Day-old Nicholas female poults ($n = 336$) were randomly assigned to treatments consisting of 0 (control), 0.2, 0.4, or 0.6 mg supplemental Cr/kg diet. Each treatment consisted of 12 replicate cages. Orthogonal contrasts were used to test the linear and quadratic effects of dietary Cr. Experimental diets were fed for 35 d. Blood samples were collected on d 35 in a fed state from 2 birds per cage for plasma glucose, nonesterified fatty acids (NEFA), and liver glycogen concentrations. The remaining turkeys were fasted for 24 h. Two turkeys per cage were bled in a fasted state, and 2 additional birds were refed diets for 4 h following fasting. Liver glycogen and plasma NEFA concentrations were not affected by treatment. Turkeys supplemented with 0.2 or 0.4 mg Cr/kg and refed after fasting had lower (quadratic, $P = 0.04$) plasma glucose concentrations than controls. The lower plasma glucose in Cr-supplemented turkeys following refeeding is consistent with Cr enhancing insulin sensitivity. Experiment 2 was designed to determine the effect of Cr Prop supplementation on performance of turkeys from 1 to 84 d of age. Male Nicholas white turkey poults were randomly assigned to treatments consisting of 0 (control), 0.2, or 1.0 mg Cr/kg diet. Each treatment consisted of 8 floor pens with 12 poults per pen. Turkeys were weighed initially, and at the end of the starter 1 (d 21), starter 2 (d 42), grower 1 (d 63), and grower 2 phase (d 84). Orthogonal contrasts were used to compare: 1) control vs Cr-supplemented treatments, and 2) 0.2 vs

1.0 mg Cr/kg. Turkey performance was not affected by treatment during the starter 1 phase. Gain was greater ($P = 0.01$) and feed/gain was lower ($P = 0.03$) for Cr-supplemented turkeys compared to controls during the starter 2 phase. Over the entire 84-d study turkeys supplemented with Cr had greater ($P = 0.01$) ADG and tended ($P = 0.07$) to gain more efficiently than controls. Performance did not differ among turkeys receiving 0.2 and those fed 1.0 mg Cr/kg. These studies indicate Cr Prop increases insulin sensitivity and improves performance in turkeys.

Key Words: chromium, insulin, turkeys

T196 Role of Vitagene-Regulator for comprehensive stress and immunosuppression management in poultry flocks Woo Kim¹, Shivi Maini^{*2} ¹*Poultry Science Department*, ²*Indian Herbs*

Stress-induced immunosuppression is one of the most common hazards in poultry intensive production, which often leads to vaccination failure & severe economic losses. Stress reduces feed intake, growth, impairs immune response, resulting in high disease susceptibility. In recent years, there has been a re-evaluation of the concept of cellular antioxidant defense, emphasizing the critical role of cell signaling. The antioxidant systems within living cells are structured on three primary levels of defense, encompassing several options for protection. Activation of vitagenes in response to stress conditions is regarded as a fundamental adaptive mechanism. The vitagene family includes a diverse set of genes responsible for

synthesizing protective molecules such as thioredoxins, superoxide dismutase (SOD), sirtuins, and heat shock proteins (HSP70).

An experiment was undertaken at University of Georgia, USA to evaluate efficacy of phyto-genic feed supplement and vitagene regulator-PhytoVita on growth performance, antioxidant defense and immune responses in broilers exposed to heat stress. Day old chicks were randomly allocated to 2 groups: control (C) & treatment (T1). T1 was supplemented with PhytoVita@200g/ton of feed. Body weight, body weight gain, feed intake, FCR & mortality were recorded weekly. All experimental data were analyzed statistically by one-way ANOVA using SAS software (Version 9.4) at $P \leq 0.05$. Differences between means were determined using Duncan's Multiple Range test. At 5th week gene expression of HSP70 and SOD were determined by qPCR method. Mean BW at 6th week in T1(2611g) was significantly higher than C (2539.2g). Cumulative FCR in T1(1.45) was significantly lower than C(1.51). HSP70 is the master regulator of stress and supplementation of PhytoVita significantly downregulated gene expression of HSP70 & improved stress tolerance in birds through HSP70 vita-regulation. PhytoVita supplementation significantly increased fold change value of antioxidant gene SOD & strengthened antioxidant defense in broilers under high THI. It was concluded on basis of trial findings that PhytoVita is a vitagene regulator- phyto-genic feed supplement that helps to prevent stress & immunosuppression in poultry birds and helps to boost antioxidant defense system.

Key Words: stress, immunosuppression, vitagene regulation, stress tolerance, antioxidant defense

Metabolism & Nutrition XI Vitamins and Minerals

T197 Increasing the dietary inclusion of a soluble limestone has an impact on the digestibility of calcium and phosphorus and phytase efficacy in broilers hengxiao zhai¹, Carrie Walk², Cristiano Bortoluzzi^{*2}, Qian Zhang¹ ¹*dsm-firmenich (China) Animal Nutrition Research Center*, ²*dsm-firmenich Nutritional Products*

The objective of this study was to evaluate the effect of increasing levels of limestone solubility on the apparent ileal digestibility (AID) of Ca and P in broilers fed diets supplemented with or without 1,000 phytase units (FYT)/kg of phytase. Nine hundred and sixty, Cobb 500, male broilers on day 9 post-hatch were used in a completely randomized design. There were 8 dietary treatments arranged as a 2×4 factorial with two phytase doses (0 and 1,000 FYT/kg) and 4 concentrations of soft, soluble limestone (chalk; 0.0, 0.40, 0.80, and 1.20%) in place of the same concentrations of hard, low soluble limestone (marble). The birds were fed the experimental diets for 2 days. On day 11, ileal digesta was collected from all birds per pen and pooled. Titanium dioxide was included at 3 g/kg feed as an indigestible marker. The main effects of chalk limestone concentration, phytase and their interaction were tested by GLM, and the linear and quadratic effects of chalk limestone concentration were checked using orthogonal contrasts. The results showed that in the absence of phytase, the AID of Ca quadratically ($P < 0.01$) decreased as the marble limestone was replaced with 0 to 100% of the chalk limestone. Phytase supplementation increased the AID of Ca ($P < 0.01$), but there was no effect of chalk limestone inclusion level (interaction, $P < 0.05$). Similarly, in the absence of phytase, the AID of P quadratically ($P < 0.01$) decreased as marble limestone was replaced with 0 to 100% of the chalk limestone. Phytase supplementation increased the AID of P quadratically with the greatest effect in broilers fed 33.3% of the chalk limestone (interaction, $P < 0.05$). The digestible Ca and P equivalence of phytase increased both linearly and quadratically with increasing chalk limestone levels. In conclusion, digestibility of Ca and P as well as the relative phytase efficacy were affected by the solubility of limestone in broilers. The current results highlight the importance of considering limestone quality when measuring Ca digestibility of lime-

stone, evaluating phytase efficacy, and meeting the animal's requirement for digestible Ca instead of total Ca.

Key Words: Calcium, Limestone, Phosphorus, Phytase

T198 Broiler and layer performance and health under LPS challenge when fed different sources of minerals. Gavin Boerboom* *Selko*

Stressors can be major threats to poultry as they impact overall health and can reduce overall performance. Trace minerals are essential for health and productivity, especially during increased immune response demand. To establish mineral effects on the immune response in broilers, a total of three trials were designed, two in broilers and one in layers.

In the first trial, 80 30-week-old layers were randomly distributed to one of 4 treatments: Low sulphate (LS) (50 ppm Zn, 45 ppm Mn), high sulphate (HS) (100 ppm Zn, 90 ppm Mn), low IntelliBond (LI) (50 ppm Zn, 45 ppm Mn) and high IntelliBond (HI) (100 ppm Zn, 90 ppm Mn). Birds were injected with either PBS or 500 ug/kg BW LPS at 41/42 weeks of age. In the second trial 60 1-d-old Ross 708 broilers were distributed to one of 6 treatments: 50 ppm Zn+45 ppm Mn from IntelliBond or sulphate, 100 ppm Zn+90 ppm Mn from IntelliBond or sulphate or only 100 ppm Zn or 100 ppm Mn from IntelliBond. At 21 days of age, birds were injected with LPS. In the third trial, 1920 1-d-old Cobb 500 birds were randomly distributed to a 2X3 design with Cu at low (15 ppm) or high (150 ppm) levels and Mn fed at 40, 80, or 120 ppm from IntelliBond source. The two controls contained Cu at 15 or 150 ppm and Mn at 80 or 120 ppm. Birds were challenged with LPS injection at 35 days.

In the first trial, feeding LS Zn and Mn resulted in a reduced superoxide dismutase response in the macrophages in blood, while feeding LI resulted in an elevated level of SOD, comparable with HS and HI ($P < 0.05$).

In the second trial, especially Zn was determined to be driving SOD response, with higher inclusion of IntelliBond Z leading to an equal response as high sulphate ($P < 0.05$). Only increasing Mn did not lead to an increase in SOD activity ($P > 0.05$).

In the third trial, supplementation of 150 ppm of IntelliBond Cu resulted in a higher respiratory burst by macrophages ($P < 0.05$). The superoxide dismutase response also indicated lower SOD levels with the use of low sulphates ($P < 0.05$).

Overall, the results indicate that minerals are critical in broilers and layers under increased immune response demand. These studies also show that a trace mineral source with higher quality and availability better supports the immune response, reducing the impact of immune challenges.

Key Words: Minerals, Health, LPS, Immunity

T199 Feeding methionine hydroxyl analogue chelated trace minerals reduces mineral excretion to the environment in broiler chickens Thi Hiep Dao¹, Hoang Duy Nguyen¹, Amy Fay Moss¹, Frances Yan^{*2}, Hugo Romero² ¹University of New England, ²Novus International, Inc.

This study investigated the effects of feeding mineral methionine hydroxyl analogue chelates (MMHAC) zinc (Zn), copper (Cu) and manganese (Mn) (MINTREX® Zn:Cu:Mn, Novus International, Inc.) on growth performance, carcass yield, and excreta mineral levels of Ross 308 males. A total of 384 one-day-old chicks were randomly distributed to 4 dietary treatments with 8 replicates of 12 birds per pen per treatment. The treatments consisted of (1) Inorganic trace mineral ZnSO₄ 110 ppm, CuSO₄ 16 ppm, MnO 120 ppm (ITM), (2) MMHAC Zn 40 ppm, Cu 10 ppm, Mn 40 ppm (MMHAC10), (3) Inorganic trace mineral ZnSO₄ 110 ppm, tribasic copper chloride 125 ppm, MnO 120 ppm (TBCC125), and (4) MMHAC Zn 40 ppm, Cu 30 ppm, Mn 40 ppm (MMHAC30). Three feeding phases including starter (d0-10), grower (d10-21) and finisher (d21-42) were used. Growth performance were calculated as per feeding phase. Data were subject to one-way ANOVA and means were separated by Tukey's post-hoc test using R Commander with P-value ≤ 0.05 considered significant. The results showed that birds fed the MMHAC10, MMHAC30 and TBCC125 diets tended to have higher feed intake with the highest feed intake observed for TBCC125 treatment ($P = 0.052$), and higher weight gain with the highest weight gain observed for MMHAC30 group ($P = 0.063$) compared to those fed the ITM control diet over the entire study. Although, a significant difference was not obtained, birds fed MMHAC10 had numerically higher overall liveability and European productivity index over the entire study compared to the other groups. Thigh and drumstick weight was higher in birds fed MMHAC30 compared to the ITM control group at d42 ($P = 0.050$). At d16 and d21, feeding MMHAC at both levels significantly decreased Zn and Mn levels while feeding TBCC125 increased Cu level in the excreta compared to the other dietary treatments ($P < 0.001$). Thus, dietary supplementation of MMHAC at the reduced levels could serve as a nutritional strategy to improve growth performance of broilers while reducing Zn, Cu and Mn excretion, therefore reducing the environmental impacts of broiler production.

Key Words: chelated trace minerals, broiler, sustainability, environment

T200 Supplementation of Cu Methionine-Hydroxy-Analogue Chelate maintains chickens' performance and reduces mineral excretion and foot pad dermatitis Kelen Zavarize^{*1}, David Torres¹, Gabriela Cardoso¹, Jovanir Fernandes², Sabrina Palma² ¹Novus, ²Universidade Federal do Paraná

High doses of copper (Cu) are extensively used as a growth promoter in poultry production, with Cu sulfate & TBCC as the traditional source. Usage of lower dosages of Cu in the form of Methionine-Hydroxy-Analogue Chelate is an alternative due to its higher bioavailability. The objective of this study was to evaluate sources of Cu (Cu Methionine-Hydroxy-Analogue Chelate and Cu Sulfate) on performance, litter and incidence of footpad lesions (FPL) in broilers. A total of 1,274 Cobb day-old male chicks were used in this study and randomly distributed into 3 treatments with 13 replicates per treatment of 42 birds per pen. The diets were formulated to meet nutritional requirements, with Cu sources added to the diet to replace inert substances. The treatment groups were as follows:

T1 10 ppm Cu sulfate (Cu-Sulf10); T2. 125 ppm Cu sulfate (Cu-Sulf125) and T3. 30 ppm Cu Methionine-Hydroxy-Analogue Chelate (Cu-HMT-Ba30). New litter was used, and a water spray program was adopted to increase litter moisture and promote FPL. On 42 days, feed intake (FI), body weight (BW) and feed conversion ratio (FCR) were evaluated. Feet from 3 birds/pen were frozen on the slaughter day (43d) and evaluated for the incidence of FPL. FPL was evaluated according to 0 to 5 score scale by the same person. Litter samples were collected on day 44 from 5 locations of each pen approximately 300g of sample. Data were subjected to analysis of variance (ANOVA-GLM) and means were compared by the Tukey test at 5%. The results showed that from 1 to 42 days, there was no statistical difference ($P > 0.05$) in FI and BW across treatments. FCR was significantly ($P < 0.05$) lower in the Cu-Sulf125 (1.627) and Cu-HMT-Ba30 (1.636) in comparison to the Cu-Sulf10 (1.667). Birds fed with Cu-Sulf125 and Cu-HMTBa30 diets presented lower FPL incidence ($p < 0.05$) than those fed 10 ppm of Cu sulfate. Litter mineral content was affected ($p < 0.05$) by treatments, with a lower concentration of Cu observed in the Cu-HMTBa30 and Cu-Sulf10 treatments. The results indicate that high dosage of Cu sulfate (125ppm) can be replaced by 30ppm of Cu Methionine-Hydroxy-Analogue Chelate with no lose in performance or benefits in foot pad dermatitis, and additionally brings sustainability to the poultry operation by reduction of Cu excretion.

Key Words: organic minerals, footpad dermatitis, performance, poultry, copper

T201 Evaluation of supplemented zinc amino acid complexed mineral on performance of broilers fed diets with different calcium and phosphorous recommendations Cibele Torres^{*1}, Kyle Venter², Roselina Angel³, Peter Plumstead², Leonardo Linares¹ ¹Zinpro Animal Nutrition, ²Neuro Livestock Research, ³Department of Animal and Avian Sciences, University of Maryland,

This study investigated the performance of broilers fed diets formulated to optimize phytate hydrolysis in the upper gut and supplemented with reduced levels of Zn in the form of Zn amino acid complexed minerals (ZnAA). A total of 3,600 d-old straight-run Ross 308 broilers were randomly distributed across 72 floor pens. Birds received 1 of 6 diets (corn-soybean meal based) from day one to 37 d with 12 replicate pens per treatment, in a randomized block design. The control diet (T1) was formulated following Ca and P Aviagen 2021 Breeder recommendations supplemented with 90 ppm ZnSO₄. The other 5 diets followed University of Maryland (UMD) Ca & P recommendations. The UMD diet was supplemented with 90 ppm Zn from ZnSO₄ (T2) or 20 (T3), 40 (T4), 60 (T5), 80 ppm Zn (T6) as a ZnAA. All diets were supplemented with 2000 FTU phytase/kg feed. Performance data were analyzed by one-way ANOVA, using JMP 16.0. Means were separated by Tukey test when $P \leq 0.05$. There was a stepwise improvement ($P < 0.05$) with increasing Zn for BW, FCR adjusted to mortality (cFCR) and hot carcass weight at 37d was observed at 20, 40, 60, and 80 ppm ZnAA. Breast yield (g and %) was highest in birds fed 40, 60, and 80 ppm ZnAA compared to the lowest breast yield at 20ppm ZnAA. Birds fed 80 ppm ZnAA had greatest body weight at d 37 compared to birds fed T1 and T2, which were similar to each other. The FCR, however, was negatively affected in control fed birds compared to the UMD counterpart with birds fed T2 or T5 diets having 3.2 and 4.4 points improved FCR, respectively. Zinc in the form of ZnSO₄ did not affect any of the carcass parameters measured. Birds fed ZnAA at 60ppm, had fewer scratched carcasses and produced a larger breast yield (g and %) compared to UMD ZnSO₄ at 90 ppm and numerically to Control. Under an environment in which absorption and digestion of inorganic Zn from raw materials is maximized, the impact of supplementing diets with ZnAA as the only source of trace mineral goes beyond the performance at farm level. ZnAA at 60 ppm not only led to heavier and more efficient

birds at the farm, it yielded birds with fewer scratches in their carcasses and improved breast yield.

Key Words: Zinc amino acid, broiler performance, breast yield, inorganic Zn

T202 Effect of chelated trace minerals in laying hens during a second production period after molting HUGO ROMERO-SANCHEZ*¹, David Sanchez Torres¹, Ingrid Martinez¹, Frances Yan¹, Maria Castaneda^{2,3}
¹Novus International Inc, ²AB Vista Mexico, ³Universidad Autonoma de Mexico

Trace mineral minerals Zn, Cu and Mn play important roles in laying production and egg quality particularly in second production phase. The objective of this study was to study the effect of supplementation of bis-chelated trace minerals on egg production and egg quality in hens during a second production period after molting. Two hundred eighty-eight Bovans White layers were molted at 80 weeks of age and randomly assigned to two treatments with twelve replicates. The treatments were the inorganic (ITM) source from Zn and Cu sulphates and Mn oxide and the organic source from mineral methionine hydroxy analogue chelates (MMHAC), with 90:6:110, and 40:10:40 ppm Zn:Cu:Mn, respectively. Treatments were evaluated after 85 weeks of age, through sixteen weeks for productive parameters and eggshell quality, including the breaking strength and

the incidence of translucent egg was evaluated through ovoscopy. Data were subject to repeated measures two-way ANOVA with a general linear mixed model using Autoregressive covariance structure (AR1). Treatment, week and the interaction between them were defined as fixed effects and replicates as random effect. There was an interaction between diet and week in egg production ($p=0.007$) and egg mass ($p=0.019$). Layers fed with MMHAC had higher egg production ($P<0.05$), resulting in more cumulative eggs, higher egg mass and lower feed conversion ratio, especially in the last 6-8 weeks of the study accounting for the interaction. However, Egg weight was not affected by treatments ($p>0.05$) supporting that MMHAC is a source of methionine. The MMHAC also significantly increased thickness ($355\mu\text{m}$ vs $351\mu\text{m}$) and breaking strength (4.71Kgf vs 4.62Kgf) compared to inorganic source. A negative correlation ($P<0.001$) between breaking strength and - translucent eggs was found. Ovoscopy analyses resulted in higher number of eggs scored 0 (no presence of translucent eggs) for MMHAC; there was similar incidence for both treatments in score 1 (light translucent) and higher number in score 2 and 3 for ITM (moderate translucent). In conclusion, supplementation of MMHAC at the reduced levels supported a better second phase after molting in layers, increasing the egg production and eggshell quality.

Key Words: trace mineral, laying hen, molting, egg production, eggshell quality

Environment Management V Animal Well-Being

T203 Transcriptional profiling of stress response genes in poultry broilers exposed to environmental heat stress Woo Kim¹, Shivi Maini*²
¹Poultry Science Department, UGA, ²Indian Herbs

In chickens, transcriptomic approaches have been applied in studying heat stress. Use of recently developed whole genome technologies gives insight into molecular responses of cells to external challenges. It is reported that heat stress in chickens is associated with 317 differentially expressed genes in liver. In this study, we have investigated gene regulation (vitagens) response to acute heat exposure. Gene microarrays were exploited to analyze effects of heat stress in Cobb 500 broilers & protective effect of supplementing phyto-genic vitagene regulator for stress mitigation. An experiment was undertaken at University of Georgia, USA, day old chicks were randomly allocated to 2 groups: control (C) & treatment (T1). T1 was supplemented with PhytoVita@200g/ton of feed. At 5th week gene expression of Heat Shock Protein 70 (HSP70), BCL2-associated athanogene 6 (Bag6), Caspase1 (CASP1) and interferon- γ (IFN- γ) were determined by qPCR method. Under acute stress, BAG3 in concert with molecular chaperones heat shock protein (HSP70) is activated under pathophysiological conditions to stress stimuli. This represents a pivotal adaptive safeguarding from stress & ensuring cellular proteostasis. Dietary phyto-antioxidants suppress mRNA expression of apoptotic gene BAG and HSP70 via stabilizing antioxidant status in birds under summer conditions. CASP1 plays important role in the inflammatory immune response. It is highly expressed in immune organs such as liver, kidney, spleen during stressful stimuli. IFN- γ is immunoregulatory cytokine that has a critical role in immune regulation in face of stress induce immunosuppression. It was recorded that acute heat stress triggered & upregulated expression of HSP-70 and apoptotic gene BAG6. HSP70 & BAG 6 is the master regulator of stress and supplementation of PhytoVita significantly downregulated gene expression of HSP70, BAG6, CASP1 & improved stress tolerance in birds through HSP70 vita-regulation. PhytoVita supplementation significant upregulated gene expression of IFN- γ & lead to immuno-regulation in broilers under high THI. It was concluded on basis of trial findings that PhytoVita is a vitagene regulator-phyto-genic feed

supplement that helped to prevent stress & immunosuppression in poultry birds and regulated vitagens.

Key Words: vitagens, stress, immunoregulation, cellular proteostasis, vitagene regulator

T204 Light-emitting diodes can modulate a chicken's embryonic growth, hatching performance and chick quality Muhammad Faisal Riaz*^{1,2}, Jibran Hussain², Sohail Ahmad², Atif Rehman¹, Muhammad Asif Raza¹, Junaid Ali Khan¹ ¹Department of Poultry Science, Muhammad Nawaz Shareef University of Agriculture, ²Department of Poultry Production, University of Veterinary and Animal Sciences

Light is an important factor that affects the establishment of the biological clock and circadian rhythms of birds through physiological changes. According to standard incubation, eggs are incubated without light. In the last decade, many studies have shown that white light and different monochromatic light colors have a positive impact on hatchability and chick quality traits. To this end, a total of 500 broiler breeders (Ross 308; 44 weeks) and 500 layer breeders (Bovans White; 43 weeks) were randomly divided into 8 groups ($n = 25$) with five replicates (trays) to evaluate the effect of different combinations of dichromatic light: Green \times Red (GR), Green \times Blue (GB), Blue \times Red (BR) and dark (D) that were provided for 12 hours a day during the whole incubation period. Embryo index, hatch window, hatching traits, and chick quality characteristics were evaluated in this study. The eggs incubated under GR showed accelerated ($p \leq 0.05$) embryonic growth at different intervals of the incubation period in genotype, color combination, and their interaction. Hatch window was also improved ($p \leq 0.05$) in lighting treatment and interaction under GR and GB. Hatchability and dead in shell showed a significant difference ($p \leq 0.05$) in broilers and interaction, while hatch of fertile improved ($p \leq 0.05$) in response to GR lighting. Heart and liver weight percentages were higher in broilers ($p \leq 0.05$). By evaluating the results of this study it was concluded that the GR color combination is best to get a higher hatch of fertile with an improved hatch window along with comparatively better chick quality.

Key Words: LEDs, Poultry, Incubation, Embryo, Hatchability

T205 Rose colored glasses: impact of colored LEDs and lighting types on Pekin duck visualization of their environment. Gregory Fraley*, Patrice Baumhardt, Darrin Karcher, Esteban Fernandez-Juricic *Purdue University*

Poultry scientists have studied the impacts of colored lights on production, behavior, and welfare on many poultry species, including ducks. However, no one has considered how these colored lights impact the birds' ability to visualize their environment. Mathematical modelling of vision has been a standard practice for wild birds; however, this field has been largely ignored in poultry science. In order to address this issue, we first measured the spectral qualities of red (peak at 618-638 nm; total of 0.128 $\mu\text{mol photons/m}^2/\text{sec}$), green (peak at 534-566 nm; total of 0.133 $\mu\text{mol photons/m}^2/\text{sec}$), blue (peak at 457 nm; total of 0.143 $\mu\text{mol photons/m}^2/\text{sec}$), fluorescent white (peaks at 437, 489, 545, 588, and 613 nm; total of 0.163 $\mu\text{mol photons/m}^2/\text{sec}$) and incandescent lights (peak at 700 nm; total of 0.157 $\mu\text{mol photons/m}^2/\text{sec}$). We also measured the reflectance properties of all resources in the barn, the flooring and walls, and of several points on the bodies of the ducks. We then euthanized 26 adult ducks with pentobarbital, collected the eyes, measured eye size, light transmission properties of the eye, then immediately removed the retina. Retinas were then assayed for absorbance of visual pigments and oil droplets, types and distribution of oil droplets, and the relative density of photoreceptors of the eye. These visual system characteristics were then used to build a perceptual model of the duck through use of the receptor-noise limited model. This model has been used for decades in behavioral and ecological studies and serves as a means to determine the visual contrast of an object against a background from the point of view of the ducks' eyes. Results revealed that some objects (eg. red spigot base) cannot be resolved through chromatic or achromatic contrast under any lighting type due to their color and reflective properties. Similarly, it appears that the light peach-colored bill of Pekin ducks also cannot be resolved against numerous backgrounds under any lighting types. The visual acuity model suggests that after 5 meters, object resolution is greatly diminished in Pekin ducks. Our results are beginning to suggest to us how barn resources should be recolored or how lighting systems can be redesigned based upon the visual properties of poultry.

Key Words: visual perception, object recognition, retinal physiology

T206 Effects of enrichment hut on natural behavior and leg-health of broilers in light-enriched commercial houses Seong Kang*, Karen Christensen² *University of Arkansas, Department of Poultry Science, Tyson Foods, Inc*

Enriched lighting program affects natural behavior, welfare, and performance of broilers in commercial houses. The objective of this study was to evaluate the effects of enrichment hut (EH) on the dustbathing behavior and the number of leg-problem culled birds in light-enriched broiler houses. Two trials (Ross 308 and Cobb 500) were performed to see the effect of different number of EHs. Day old chicks (mixed sex) were placed in four commercial broiler houses (12.8 m x 122 m, wood-shavings). Each quadrant of the house was placed with 4,800 chicks with all source flocks equally represented in each quadrant (section). Enriched lighting program house was average about 40 lux light intensity over the feed lines and dimmer light intensity at the sidewalls (2-5 lux). Different numbers of EHs were randomly placed in each house (Control; no hut, 1x hut (1/1000 sqft), 3x hut (3/1000 sqft), 6x hut (6/1000 sqft)). Dust-bathing holes as evidence of dust-bathing behavior were counted at 5, 12, and 19 days of age in 12 parts of each section, and the number of holes per 5 square meters was determined. The total number of leg-problem culled birds in each trial (Ross 308 and Cobb 500) were counted at the end of experiments. Differences among EH treatment groups were analyzed using one-way ANOVA followed by mean separation using the Tukey's HSD test using JMP 14. Significance level was $p < 0.05$. The numbers of dust-bathing holes in 3x hut and 6x hut houses on day 5 of age was significantly higher compared to control and 1x hut houses in both trials (Ross 308 and Cobb

500) ($p < 0.05$). On day 19 of age, Ross 308 trial showed significant higher number of dustbathing holes in 1x hut house compared to control house (no hut, $p < 0.05$). Effects of 3x hut and 6x hut were not significantly different ($p > 0.05$). Result indicates that voluntary natural behavior, dustbathing, of broilers induced by light-enriched program was further increased by EHs (3x and 6x huts) in commercial houses. The number of leg-problem culled birds in 3x and 6x hut houses were reduced average 47% and 34% compared to controls in Ross 308 and Cobb 500 trials, respectively. Taken together, results indicate the beneficial effects of EHs on broilers natural behavior and mortality in light-enriched commercial houses.

Key Words: Broilers, Welfare, Enrichment, Hut, Leg-health

T207 Effect of a calcium and magnesium source on organ physiology, blood mineral profile, footpad, and general performance of broilers chickens. Fausto Solis*, Rajasekhar, Kasula., Christopher Olinger *Wenger Nutrient Science and Innovation Center (WNSIC), The Wenger Group,*

Ca⁺ is a macro-mineral that animals need for proper bone metabolism, and general metabolism. Similarly, magnesium (Mg⁺) is an essential cation that is involved in many cellular functions and as a cofactor in all major metabolic pathways. The purpose of this study was to evaluate a product with 30% calcium, and 4.5% magnesium (CalMagCarb) compared to a typical calcium carbonate that has 38% calcium and 0.5% magnesium or dolomite with 30% calcium and 10% magnesium. The importance of testing this intermediate CarMagCarb is the determination of its feasibility as ingredient for livestock and evaluate its effects on organ patho-morphology, blood mineral content, gut health, animal welfare and general performance of broiler chickens. Three treatments of CalMagCarb, 0 (control), CarMagCarb50 (replacing 50% of calcium carbonate) and CarMagCarb100 (replacing 100% of calcium carbonate) in the broiler diets for a period of 42 days in a Completely Randomized Design (CRD). Each treatment was replicated eight times for a total of 24 floor pens of 25 chicken each (3 treatments x 8 replicates = 24 floor pens x 25 chickens = 600 chickens). The results data were analyzed with the General Linear Model (GLM) procedure of the Statistical Analytical Software (SAS) with a probability of error of 5%. There was not consistent effect of CarMagCarb on the liver, kidneys, and spleen histo-pathology; similar results were observed for the content of calcium, phosphorus, magnesium and protein in the blood. CarMagCarb had an inconsistent effect on footpad lesions and in the litter moisture score. On day 7, feed intake was reduced ($P < 0.05$) from 0.51 lb to 0.46 lb when calcium carbonate was 50% and 100% replaced with CalMagCarb, respectively. However, on day 41, there was no negative effect of CalMagCarb on feed intake. Body weight was not significantly ($P > 0.05$) affected by the inclusion of CalMagCarb. In conclusion the results of the present study suggest that CalMagCarb is safe to be included in broiler diets as a partial or total substitute of calcium carbonate without impacting the physiology and performance parameters.

Key Words: calcium, magnesium, CarMagCarb, performance, chickens

T208 Ghrelin synthesis and receptors in regions of the chick gastrointestinal tract and putative cholinergic control Colin Scanes*, Klaudia Jaszczak², Krystyna Pierzchala-Koziec² *University of Wisconsin Milwaukee, University of Agriculture in Krakow, Poland*

is the octanoylated "hunger" peptide produced enteroendocrine cells in gastric oxyntic mucosa. The critical stages in its synthesis are expression of the ghrelin and ghrelin O-acyltransferase (GOAT) genes. It acts by binding to growth hormone secretagogue receptor type 1a (GHSR-1a). The present studies examined ghrelin synthesis and receptors in regions of the chick gastrointestinal (GI) tract. It was hypothesized that ghrelin synthesis would be limited to the proventriculus while expression of its receptors, GHSR-1a, would extend to other GI regions. Explants of crop, proventriculus and duodenum of newly hatched chicks were incubated *in vitro*. Expression of ghrelin, GOAT and GHSR-1a were determined by real-time PCR. release *in vitro* was determined by ELISA. was synthesized

in the chick crop, proventriculus and duodenum with expression of both ghrelin and GOAT together with release of ghrelin *in vitro* from explants of these tissues. Data were analyzed by one- or two-way ANOVA with means separated by Tukey's test. Levels of ghrelin and GOAT expression were similar in the three GI regions. There was also expression of GHSR-1a in the three GI regions with much higher expression in the crop of day-old chicks. It was further hypothesized that expression of ghrelin, GOAT and GHSR-1a together with release of ghrelin *in vitro* would be influenced by cholinergic neurons. This was investigated using explants of incubated *in vitro* in the presence or absence of cholinergic antagonists. There were effects of both atropine (a muscarinic antagonist) and hexamethonium (a nicotinic cholinergic antagonist) on expression of ghrelin, GOAT and GHSR-1a together with on the release of ghrelin *in vitro* from explants of crop, proventriculus and duodenum tissue from newly hatched chicks. The effects were either stimulatory or inhibitory and varied in different GI regions. This is the first report of ghrelin synthesis and release in the chicken crop and the first report of cholinergic control of ghrelin synthesis and release.

Key Words: ghrelin, crop, proventriculus, neuropeptide, duodenum

T209 Effects of unlimited access to alfalfa straw on performance and behavior in broiler breeder pullets Rick van Emous* *Wageningen Livestock Research*

Over the last 50 years, the growth potential of broilers and the parent stock, has increased impressively. For this reason, the amount of feed that broiler breeders receive during the rearing (and laying) phase is restricted. Alfalfa straw is a common roughage variant with low energy and high fiber content. The fibers cause a feeling of satiety for the chicken, because

they remain in the gizzard longer, and it is assumed that it can reduce stereotypical pecking behavior. Therefore, an experiment was conducted to investigate the effect of whether (ALF+) or not (ALF-) unlimited access to alfalfa straw on performance and behavior in broiler breeder pullets. To determine BW and weight gain, all birds were weekly weighed as a group, in the morning before feeding. Individual BW of all hens was recorded at 5, 10, 15, and 20 week of age (WOA) for determine BW uniformity (CV%). Water intake was measured weekly per pen and water-to-feed ratio was calculated. Dry matter (DM) content of the litter below the nipple drinker and fresh feces was determined at 7, 10, 12, 15, 17, and 20 WOA. At 5, 10, 15, and 20 WOA, home pen behavior (eight observation sessions per day) was observed by scan sampling and the novel object test was performed. Data were analyzed using ANOVA. No differences were observed on BW between the ALF+ and ALF- pullets during the entire rearing phase. Between 3 and 18 WOA no differences in feed allocation between the ALF+ and ALF- pullets were found. Feed allocation was, however, 0.9 g/b/d lower in week 19 and 20 WOA for the ALF+ compared the ALF- pullets. Average BW uniformity (CV%) was worse (11.8% vs. 10.4%; $P = 0.042$) for the ALF+ pullets. Water usage, water-to-feed ratio, DM% of the litter, DM% of the fresh feces, friability score of the litter, and wetness score of the litter was not affected by whether or not access to unlimited alfalfa straw. Pullets with unlimited access to alfalfa straw showed a tendency to less stereotypic object pecking behavior (22.1% vs. 24.9%; $P = 0.066$). The ALF+ pullets were less eager to approach the novel object. It is therefore concluded, that unlimited access to alfalfa straw during the rearing phase can reduce feed allocation and stereotypic object pecking, however, BW uniformity can be impaired.

Key Words: breeder pullets, alfalfa straw, performance, behavior

POSTER ABSTRACTS

Physiology, Endocrinology and Reproduction: Broilers, Turkeys

P211 Experimental infection of turkeys with *Clostridium septicum* strains and assessment of immune responses during Clostridial dermatitis disease development Valeria Criollo Vinuesa*^{1GS}, Ravi Kulkarni¹, Carissa Gaghan¹, Rocio Crespo¹, Oscar Fletcher¹, Anil Thachil² ¹North Carolina State University, ²Rollins Lab

Clostridium septicum is one of the major causative agents of clostridial dermatitis (CD), a re-emerging disease of turkeys, characterized by necrotic dermatitis and sudden death. Despite its economic burden on the poultry industry, immunopathological changes and pathogen-associated immune responses are poorly understood. Here, we used three strains of *C. septicum*, named Str. A1, B1 and C1, isolated from CD field outbreaks to experimentally infect turkeys to evaluate local (skin and muscle) and systemic (spleen) pathological and immunological responses. Results showed that while all three strains produced an acute disease, strains A1 and B1 caused statistically significant higher mortality. A statistical comparison of gross and histopathological score evaluations showed that A1 and B1 groups had significantly increased inflammatory, edematous, granulomatous and necrotic lesions in the skin, muscle and spleen when compared to Str. C1. Immune gene expression in these tissues by real-time PCR showed that Str. B1-infected birds had statistically significant higher expression of Interleukin (IL)-1b, IL-6 and Interferon (IFN) γ genes compared to uninfected control, suggesting a robust inflammatory response locally as well as systemically, followed by Str. A1 infection. Additionally, strains A1 or B1-infected groups also had significantly higher ($p < 0.05$) IL-4 transcription in these tissues, while birds infected with all three strains developed *C. septicum*-specific serum antibodies. Furthermore, splenic cellular immunophenotyping in the infected turkeys using flow cytometry analysis showed a marked reduction ($p < 0.05$) in CD4⁺ cells. In summary, it can be inferred that host immune response against *C. septicum* involves marked inflammatory changes in local and systemic tissues,

coupled with serum antibody production and that the disease severity and its associated immune responses seem to depend on the strain-specific virulence of the pathogen.

Key Words: Clostridial dermatitis, Turkey, *Clostridium septicum*, immune response, immunopathology

P212 Coinfections Effects of Histomoniasis (Blackhead) with *Eimeria* spp. and *E. coli* on Mortality, Growth Performance, Lesion Scores, and Gut Permeability in Turkey Poults Hamid reza Rafieian-Naeini*^{GS}, Catherine Fudge, Chongxiao Chen, Hamid Reza Rafieian Naeini *UGA*

In the turkey industry, Histomoniasis (HM) remains a persistent challenge. Existing research suggests that HM may exploit synergies with other pathogens, such as *E. coli*, or capitalize on the vulnerabilities induced by invading coccidiosis. A study was conducted to evaluate the coinfection dynamics of HM with *Eimeria* spp. and *E. coli*, aiming to elucidate their combined impact on mortality rates, growth performance, liver and ceca lesion scores, *E. coli* colonization, and liver histopathology. A total of 360 one-day old male turkey poults were allocated into six treatments with five replicates during the 28 days of the experiment. Treatments consisted of NC, negative control; HM, challenged with 100,000 histomonads at d 18; *E. coli*, challenged with 10⁹ *E. coli* at d 18; EM, challenged with 8,000 oocysts of *E. meleagridis* and *E. adenoides* at d 22; HM+*E. coli*, challenged with 100,000 histomonads and 10⁹ *E. coli* at d 18; and HM+EM, challenged with 100,000 histomonads at day 18 and 8,000 oocysts of EM at d 22. Mortality, feed intake, recorded daily and body weight were measured at d 18, d 24, and d 28. All the dead birds were necropsied, and liver and ceca were scored (1-4). At d 28, the gut permeability test was per-

formed. The data were analyzed using JMP Pro 16, treatment groups were tested by 1-way ANOVA, and Tukey post-hoc analysis was employed.

The results showed that HM-challenged groups had higher mortality ($P < 0.01$). Body weight at d 24; and feed intake during 19-28 were significantly decreased in HM+ME group ($P < 0.01$). HM+EM and NC had the highest and lowest FCR respectively ($P < 0.03$). Cecal and liver lesion score significantly increased in the HM+EM ($P < 0.0001$). Gut permeability was significantly higher in all groups compared to NC ($P < 0.001$). Liver *E. coli* colonization in HM+*E. coli* significantly increased compared to other groups ($P < 0.001$). The liver histopathology of HM challenge groups showed hepatocyte necrosis and vacuolar degeneration of hepatocytes. In Turkey, it is evident that HM, EM, and *E. coli* led to increased gut permeability; concurrent infection of HM with *Eimeria* increased FCR, and simultaneous infection of HM with *E. coli* exacerbated *E. coli* penetration into the liver.

Key Words: Histomoniasis, *Eimeria*, *E. coli*

P213 Differences in phenotypic traits and fatty acid composition of conventional and slow-growing broiler chicks. Lindsey Eppinger^{*1GS}, Lindsay Brown², Brynn Voy¹ ¹University of Tennessee, Department of Animal Science, ²University of Tennessee, Department of Chemistry

Increased efficiency in modern broilers has contributed to a perceived decrease in welfare by consumers due to increased incidence of musculoskeletal disorders, breast muscle myopathies, and compromised organ function. Consumer concern has led to push for a slower growing, healthier bird with increased welfare and a decreased prevalence of these ailments. The purpose of this study was to compare phenotypic and fatty acid composition differences between a conventional line and a slower-growing line of broiler chicks selected for multiple characteristics to allow for more robust genetic differences to detect variation in growth traits. Eggs (n=60) from a conventional (Cobb 700) and a slower-growing (Redbro) line were incubated to embryonic day 17 (E17) or hatched and raised to 5, 10, or 14d under the same diet. Breast muscle (BM) and adipose (subcutaneous; SQ) fat pad were collected and weighed at E17 and at all ages after hatch. Heart, abdominal and neck fat pads were collected and weighed at D10 and D14. Adipose at D10 was snap-frozen and used for analysis of fatty acid composition. Tissue weights normalized to body weight were analyzed by t-test ($P < 0.05$). Cobb 700s weighed significantly more at each test day than Redbros. At days E17 and D5, Cobbs showed a significantly higher relative SQ weight. At D10, Cobbs showed a significantly higher relative BM weight. Relative weights of abdominal fat, neck fat, or heart did not differ significantly between lines at any day. Fat pads from D10 were further analyzed for differences in fatty acid methyl esters (FAMES) composition between chick line and depot type. Results were analyzed using a two-factor ANOVA with interaction to determine statistical significance ($P < 0.05$). Results showed no difference in composition of short-chain fatty acids between chick line or line and depot interaction. Both medium-chain and long-chain fatty acids differed in composition by line and the interaction of line and depot, including long chain n-3 PUFA species. The marked differences in performance do not appear to result from differences in early life development. Differences in fatty acid composition are not diet related and may point to genetic variation in fatty acid metabolism that could influence growth performance.

Key Words: broiler, chick, slow-growing

P214 Effect of growth rate in the physiology of thyrotropic and corticotropic axes in a commercial broiler strain Nabin Neupane*, Laura Ellestad, Shailes Bhattra, Colin Barcelo, Prafulla Regmi *University of Georgia*

Genetic selection has led to a remarkable increase in growth rate and reduced time to market body weight of broilers. These changes are paralleled by modifications to activities of the corticotropic and thyrotropic axes regulating growth and metabolism. Studying these axes in chickens

with varying growth rates may provide insights to mitigate genetic selection's negative impact on organ system development and broiler welfare. This study examined how partial feed restriction and slow growth affect the corticotropic and thyrotropic axes in broilers. Four hundred and eighty male Ross 708 chicks were randomly assigned to either *ad libitum* (ADLIB) or feed-restricted (RES) treatments (8 pens/treatment; 30 birds/pen) in floor pens. *Ad libitum* feed was provided to all birds during Wk 1 whereas from Wk 2 onwards, the RES group received 80% feed compared to the ADLIB group. Tissue (*pectoral major* and liver) and blood samples were collected when the ADLIB and RES birds reached the target body weights (TW) of 1kg, 2.5kg, and 4kg. Plasma levels of corticosterone (CORT) and triiodothyronine (T_3) levels were measured with ELISA and radioimmunoassay respectively. Gene expression was assessed in muscle and liver via RT-qPCR. Data were analyzed using one-way ANOVA in R-3.6.1. and differences were considered significant at $P \leq 0.05$. Plasma concentrations of T_3 and CORT were lower in RES birds compared to ADLIB birds at 2.5 kg and 4kg TW, respectively ($P \leq 0.05$). No significant differences were found in the expression of the corticotropic and thyrotropic axes-related genes in liver, as well as the corticotropic axis-related genes in the muscle between treatments ($P > 0.05$). The pectoral muscle of RES birds exhibited lower levels of deiodinase (DIO) 2 and 3 compared to ADLIB birds both at 2.5kg and 4kg TW ($P \leq 0.05$). Additionally, levels of thyroid hormone receptor (THR) b were reduced in pectoral muscle of RES birds compared to ADLIB birds at 4kg TW ($P > 0.05$). These results suggest that during partial feed restriction in broilers, reduced expression of thyroid hormone-metabolizing deiodinase enzymes may regulate T_3 bioavailability in muscle. Furthermore, growth rate is depressed in RES broilers without apparent alteration of thyrotropic and corticotropic activity in the liver.

Key Words: Broilers, Slow growth, feed restriction, corticotropic and thyrotropic, Corticosterone

P215 Host defense peptide response of heritage and modern broiler breeds during subclinical necrotic enteritis Ali Calik*, Candice Blue, Laney Froebel, Rami Dalloul *Department of Poultry Science, University of Georgia*

In broilers, necrotic enteritis (NE) is caused by toxin-producing *Clostridium perfringens* often due to predisposing factors such as *Eimeria* infection. Variation in susceptibility to NE can be attributed to genetic backgrounds among different breeds. In this comparative study, we evaluated mRNA abundance of cathelicidins (CATH), avian beta-defensins (AvBD), and NK-Lysin (NK-LYS) in the spleen and cecal tonsils (CT) of Athens Canadian Random Bred (ACRB) and Cobb broilers in response to subclinical NE. The design was a 2x2 factorial with breed (ACRB and Cobb) and challenge (no challenge and NE) as main factors. On day (d) of hatch, 96 male chicks (48 ACRB and 48 Cobb) were allocated to four experimental groups with 8 replicate cages and 3 birds/cage. On d 14, birds in the NE-challenged groups were orally gavaged with 3,000 *E. maxima* sporulated oocysts followed by two doses of approximately 1×10^8 CFU/mL of *C. perfringens* on d 19 and d 20. On d 21, spleen and CT samples were collected to measure mRNA abundance of CATH-1, -3, -B1, AvBD-1, -12, -14, and NK-LYS. The data were analyzed by 2-way ANOVA (JMP Pro 16) and significance ($P \leq 0.05$) between treatments was evaluated by LSD test. No interaction effect of breed and challenge was observed on mRNA abundance of CATHs in both spleen and CT. However, all CATHs exhibited greater mRNA levels in CT of NE-challenged birds ($P \leq 0.05$) compared to non-NE birds. While mRNA abundance of AvBD1 in CT was significantly greater ($P < 0.001$) in NE-challenged Cobb birds in contrast to non-NE Cobb and NE-challenged ACRB birds, that of AvBD12 was significantly greater ($P = 0.029$) in non-NE ACRB birds compared to non-NE and NE-challenged Cobb birds. Moreover, NE-challenged ACRB birds showed higher ($P = 0.004$) NK-LYS mRNA abundance compared to all other treatment groups in the spleen. These findings indicate that the innate immune response to subclinical NE differs between ACRB and Cobb

birds. This distinction could play a crucial role in regulating inflammation and the immune response to intestinal infections such as NE. Nevertheless, additional research is warranted to explore both innate and adaptive

immunity along with the changes in the microbiota in response to acute/clinical disease models.

Key Words: necrotic enteritis, avian beta defensins, cathelicidins, ACRB, broilers

Physiology, Endocrinology and Reproduction: Layers, Breeders

P216 Evaluating the vertical transmission potential of *Salmonella* Reading outbreak and non-outbreak strains Abubakar Isah*^{1GS}, Reshma Ramachandran¹, Hossam Abdelhamed², Anuraj Sukumaran³, Aaron Kiess⁴, Claudia Castañeda⁵, Tim Boltz¹, Kenneth Macklin¹, Li Zhang¹ ¹Department of Poultry Science, Mississippi State University, ²Department of Comparative Biomedical Sciences, College Veterinary Medicine, Mississippi State University, ³Freshpet, 146 North Commerce Way, ⁴Prestage Department of Poultry Science, North Carolina State University, ⁵Engrain LLC

Salmonella Reading (*S. Reading*) recently emerged as a major foodborne pathogen causing large multistate outbreaks in North America by consuming contaminated poultry products mostly from turkeys. Understanding this newly emerged pathogen's transmission and tissue colonization potential in poultry is crucial for mitigating future outbreaks. Hence, this study aimed to understand the ability of *S. Reading* to colonize reproductive tissues and contaminate the eggs of broiler breeder hens. For this purpose, two *S. Reading* strains previously tagged with bioluminescence marker gene were used; a recent outbreak strain RS330 and a reference non-outbreak strain RS326. Thirty-two commercial broiler breeder hens of 34 weeks of age were tested to ensure they were negative for *Salmonella*. The *Salmonella* negative hens were randomly placed in individual cages and assigned to one of the two treatments (16 hens/strain) within the animal biosafety level-2 facility. Each hen was intravaginally challenged with 10⁸ CFU of the respective strain on day 1 and rechallenged on day 4. Post challenge, eggs were collected daily to recover bioluminescent *S. Reading* strains from external eggshell surface and internal egg contents. On day 7 post-challenge, ten (n=10) hens from each treatment were euthanized and ovaries, oviduct, and ceca were aseptically collected to identify bioluminescent *S. Reading* colonization. The percent positive for all treatment samples were calculated. Results showed that 70.5% and 34.5% of external eggshell surfaces and 4% and 1.8% of the internal egg contents tested positive for the outbreak and non-outbreak strains respectively. A chi-square test revealed that these differences in contamination rates were statistically insignificant (p>0.05). Further, 40% of ovaries, 70% of oviduct, and 70% of ceca samples from the hens challenged with the outbreak strain and 20% of ovaries, 70% of oviduct, and 80% of ceca from the non-outbreak strains tested positive. Overall, the results showed that *S. Reading* has the capability to colonize the reproductive tissue of hens, leading to egg contamination and cecal translocation. Future investigations are essential to determine whether *S. Reading* can remain viable within the egg throughout the incubation period until hatching.

Key Words: *Salmonella* Reading, Foodborne pathogen, Egg contamination, Reproductive tissue colonization, Bioluminescence imaging

P217 Determining whether *Campylobacter* can invade the avian ovum and where in the hen reproductive tract it may occur Justin Lowery*^{GS}, Mary Mendoza, Christina Sigmon, Rebecca Wysocky, Aaron Kiess, Kenneth Anderson, Lin Walker North Carolina State University

Campylobacteriosis affects roughly 1.5 million people annually in the United States. *Campylobacter*, the causative agent of campylobacteriosis, naturally resides in poultry intestines. Prior research suggests that *Campylobacter* may invade the hen reproductive tract and, potentially, the egg. The purpose of this research was to investigate the ability of *Campylobacter* to persist in the hen reproductive tract and determine whether *Cam-*

pylobacter can invade the ovum. The outcome of this research has relevance to the laying hen and broiler breeder industries for the assessment of *Campylobacter* in table eggs and breeder progeny eggs. Forty-eight, 30-week-old commercial laying hens were placed into ABSL-2 battery cages. After two weeks, each hen was intravaginally inoculated with a 10⁸ CFU cocktail of antibiotic-resistant *Campylobacter* marker strains. Video monitoring was used for one week to target ovum location in each hen during its lay cycle and define sampling times. The hens were calmly euthanized and sterilely necropsied for the removal of the reproductive tract and ovum after another week. Each reproductive tract section and ovum were enriched, and *Campylobacter* species were confirmed using BAX qPCR and antibiotic resistance testing. The assessment of *Campylobacter* presence relied on the calculation of percentages and direct comparison of recovered wild-type and inoculated *Campylobacter* from each part of the reproductive tract and ova. Overall, *Campylobacter* was recovered from the reproductive tracts and/or ova of 9 hens, with 4 others testing positive via PCR. Two isolates matched the marker strains, and 7 wild-type isolates were collected. Overall, 10 reproductive tracts (5.2%), two follicles (4.2%), and one ovum (2.2%) contained *Campylobacter*. This study showed that laying hen reproductive tracts harbor *Campylobacter* naturally. Wild-type *Campylobacter* was recovered from follicles and an ovum, indicating natural invasion of *Campylobacter* into the developing egg. Future research is needed for the exploration of the presence of *Campylobacter* in the reproductive tract over a longer period and the age of the bird as egg quality decreases with more advanced age. This would assist in better-targeted food-safety interventions at the live-production level.

Key Words: *Campylobacter*, Reproductive Tract, Egg, Laying Hen

P218 Potential epigenetic effects of heat stress on ACTH response in F1 offspring of breeder Pekin ducks. Esther Oluwagbenga*, Victoria Tetel, Jenna Schober, Gregory Fraley Purdue University

Stressors in breeders can elicit phenotypic changes in their offspring, potentially through deposition of glucocorticoids (GC). We set out to determine if heat stress (HS) was related to phenotypic changes in the F₁'s response to ACTH. Breeder hens and drakes were randomly allocated into 2 treatments: control and HS groups. The HS group were subjected to cyclic HS of 35°C for 10h/day and returned to 29.5°C for the remaining 14h/day for 3 weeks while the control room was maintained at 22°C. Eggs (N = 10/treatment) were collected weekly and tested for GC in albumen and yolk using mass spectrometry. Duck level analyses were completed using 1-, or 2 -way ANOVA or Student's T-test as appropriate. A p < 0.05 was considered significant. Student's T-test analyses showed that eggs from HS hens showed significantly (p < 0.05) higher levels of cortisol in albumen compared to eggs from control hens. To determine effects of HS on the F₁, eggs from the last 3 days of the treatment period were incubated, and hatchlings placed into pens by treatment in a single room to minimize environmental differences. The HS F₁ ducks had a lower hatch weight (p < 0.05) compared with the CON F₁ ducks but no significant difference in growth rates during the 5-week period. At 3 weeks of age, an ACTH challenge was given to 10 ducks per treatment/sex. Circulating levels of corticosterone were significantly (p < 0.05) elevated at hours 1 and 2 after ACTH injection in the HS F₁ group compared to controls. Circulating levels of cortisol were significantly (p < 0.05) depressed at hours 1, 2, 3, and 4 in the HS F₁ group compared to controls. Further, novel object tests were done weekly to access behavioral response. The HS F₁ showed

significantly ($p < 0.001$) higher fearfulness compared to the controls. Our results suggest that there are epigenetic effects of HS, and that these effects may be mediated through cortisol deposition in the egg.

Key Words: Parental hormone, Phenotypic plasticity, Behavior, Epigenetic effect, Maternal effect

P219 Determination of Eggshell Translucency as a Novel Non-invasive Predictive Tool for the Hatch of Fertile Eggs in Broiler Breeders Jinquan Wang^{*1}, Chongxiao Chen¹, Austin Jasek², Colwayne Morris², Raquel Burin², Duarte Neves² ¹University of Georgia, ²Zinpro Corporation

This study aims to evaluate shell translucency as a novel eggshell quality parameter to predict hatchability of fertile eggs. A total of 22,140 eggs were collected from 738 broiler breeder flocks between 25 to 65 wks of age, from multiple commercial hatcheries in the US. Translucency was completed with Zinpro[®] BlueBox[™] using a 3-point score system: TS1 = none or few small translucent spots; TS2 = several small translucent spots; TS3 = many large translucent spots. All eggs were individually evaluated for shell coloration lightness (L), thickness (Th), breaking strength (BS), translucency score percentage (TS1, TS2, TS3), and total translucency score (TS, the sum of the product of score and percentage). Pearson's correlation was calculated using the `rcorr` function in the `Hmisc` library in R. Significance level was set at $P < 0.05$. Pearson's correlation showed

that HOF is negatively correlated with age ($r = -0.45$, $P < 0.05$), L ($r = -0.25$, $P < 0.05$) and Th ($r = -0.08$, $P > 0.05$), while a positive correlation was observed with BS ($r = 0.15$, $P < 0.05$). To assess the effect of age and blocking its effect on HOF, a linear regression model with both linear and quadratic terms was completed with `lm` function in R, revealing that HOF peaked at 90.5% when breeders were 37-38 wks old. Shell quality parameters were individually regressed against HOF using age as a co-variable. Results from the fitted models showed that Th is quadratically ($P < 0.05$, $R^2 = 0.760$) correlated with HOF, lowest at 0.418mm, and L showed a quadratic correlation ($P < 0.05$, $R^2 = 0.769$) with HOF, peaking at 75%. However, BS showed no significant correlation with HOF ($P < 0.05$, $R^2 = 0.750$). The TS1 showed positive linear ($P < 0.05$, $R^2 = 0.764$) correlations with HOF, while TS3 was negatively correlated with HOF ($P < 0.05$, $R^2 = 0.764$). Moreover, the total TS score exhibited a negative linear correlation with HOF ($P < 0.05$, $R^2 = 0.755$). Results from translucency related parameters indicated a less TS1 or more TS3 score in eggs is correlated with a lower HOF. In summary, eggshell coloration lightness, thickness and translucency scores can be used as indicators of HOF. In conclusion, TS1 and TS displayed straightforward linear correlations with HOF, showing that translucency can be used as a novel eggshell quality parameter to predict HOF.

Key Words: Translucency, Eggshell quality, Hatch of fertile, Breeder, Hatchery

Processing and Products

P220 Application of ultrafast gas chromatography electronic nose to characterize warmed-over flavor in chicken muscle and skin Jacob Dees^{*1UG}, Linda Barahona¹, Suyeong Bak², Sungeun Cho¹ ¹Auburn University, ²ChunbguK National University

Electronic sensing technologies (e.g., electronic nose) have significantly improved over the last decades. It recognizes aroma patterns, providing fast aroma analysis. In this study, we used the Heracles Neo II Alpha MOS electronic nose (e-nose) (Alpha MOS, Toulouse, France) to characterize warmed-over flavors (WOF) developed in reheated oven-grilled chicken muscle and skin. On Day 0, chicken breast, thigh, and skin were grilled in a commercial oven at 190°C for approximately 30 min until the internal temperature reached 80°C. Over the 3-day storage period, the chicken samples were stored in Ziploc bags[®] at 4°C. The stored chicken samples were reheated until the internal temperature reached 80°C using either a microwave (SHARP[®] 1200W Commercial Microwave) at 180°C or an air fryer (Kaloric[®] 5-Quart Digital Air Fryer). The aroma analysis using the e-nose was carried out in triplicate on each chicken sample on Days 0, 1, 2, and 3. Overall, Principal Component Analysis (PCA) of the e-nose data showed the differences in the volatiles of all chicken samples reheated using microwave or air fryer from the 3-day storage period with a total variance of 97.0%. The different reheating methods over the 3-day storage period affected the WOF of breast, thigh, and skin samples with a discrimination index of 93%, indicating good discrimination. The volatile analysis found more alkanes (e.g., 3-ethylpentane, 3-ethylhexane, and 4-methyl-octane) in skin samples when compared to breast and thigh samples. These alkanes were formed from the lipid oxidation from the skin, which prompted the development of lipid oxidation over storage time. This study confirmed that the e-nose was able to characterize and differentiate WOF between chicken muscle and skin in a quick time frame.

Key Words: electronic nose, warmed over flavor

P221 Convergence Science: Merging Meat Science and Lean Six Sigma Approach to Improve Further Processing Efficiency using Burger Manufacturing as a Model Process. Luis Guzman^{*GS}, Amit Morey Auburn University

Production efficiency of a further processing plant is highly dependent on meat quality, equipment and operation procedures. A unique study converged the Lean Six Sigma approach and Meat Science to improve efficiency of a further processing plant (burger manufacturing as a model) by reducing waste and shrinkage (yield loss). The research was initiated with an assessment of current processes, conducting a systematic in-plant study, and discussing the outcomes with the operations team to ensure process improvement. After process assessment, four key production stages were identified for data collection: (1) the receiving room scales: calibration, organization, cleanliness standards, and operational training; (2) combo dumping: purge and leak from the hopper; (3) water misting: functioning of the misters (coverage of misting), weighing patties before and after misting and after freezing; (4) empty box weights: measurements taken on boxes from different lots. The research employed Lean tools such as Define, Measure, Analyze, Improve, and Control (DMAIC), Kaizen, Poka-Yoke, 5S, Statistical Process Controls (SPC), and linear regression models using Minitab. The receiving room scales displayed a considerable variance ($n = 345$), with 79% of meat combos weighing heavier than vendor-provided weights. This section used 5S methodology for sorting, setting in order, shining, standardizing, and sustaining the area, in addition to Poka-Yoke for visual management on instructions and guides. The combo dumping process ($n = 145$) findings showed a direct correlation (0.42) between the lean percentage and the purge volume. Kaizen methodology combined with a combo pull system was recommended to improve the machinery design. The water misting procedure ($n = 372$) showed that 38% of patties lost weight at post-freezing. DMAIC engaged with SPC, linear regression models were used to measure the process, and the Kaizen methodology was recommended for continuous improvement. Finally, the standardized tare weight for the boxes ($n = 1200$) used SPC to determine

an individual box weight of 0.92 lb. A Convergence Science approach will improve production efficiencies of further processing operations.

Key Words: Further processing, Meat Science, Lean Six Sigma, Continuous Improvement, Kaizen

P222 Antimicrobial interventions effectively reduce Salmonella serovar complexity in chicken carcasses at processing Amber Richards*^{GS}, Amy Siceloff, Nikki Shariat *University of Georgia*

Approximately one in five salmonellosis cases is attributed to consumption of contaminated chicken. A limitation to traditional *Salmonella* culture is that often a single colony is picked and characterized. This favors the most abundant serovar in a sample, effectively masking other serovars that are also present. CRISPR-SeroSeq is a deep serotyping approach that resolves the relative frequency of multiple serovars in a sample. The objective here was to use deep serotyping to assess *Salmonella* serovar complexity during broiler processing and to determine the impact of antimicrobial interventions on serovar dynamics. Hot rehang and post-chill chicken carcasses were collected from processing plants across the United States from August to November 2022. CRISPR-SeroSeq was performed on *Salmonella*-positive broiler carcasses collected at hot rehang (n = 153) and post-chill (n = 38). This data set included 31 paired hot rehang and post-chill carcass rinses, 122 unpaired hot rehang, and 7 unpaired post-chill carcass rinses (n=191). Multiple serovars were detected in 48.4% (74/153) rehang and 7.9% (3/38) post-chill samples. Rehang carcasses had more serovars per sample (1.6 serovars/carcass) than post-chill carcasses (1.1 serovars/carcass) (Mann Whitney U, p=0.00932). There was 98.4% (188/191) concordance between traditional isolation with serotyping and CRISPR-SeroSeq. Comparison of serotyped colonies showed 71.0% (22/31) of serovars matched between paired rehang and post-chill samples. This discrepancy was partially explained when CRISPR-SeroSeq data was included as the concordance increased to 87.1% (27/31). Nineteen different serovars were identified; serovar Kentucky was the most common in rehang (72.5%; 111/153) and post-chill (73.7%; 28/38) samples. Serovar Infantis had a higher prevalence in rehang (39.9%; 61/153) than in post-chill (7.9%; 3/38) samples. These data demonstrate that processing interventions effectively reduce *Salmonella*, as serovar complexity was reduced in nearly all post-chill samples. This study exhibits how deep serotyping is an effective technique for profiling *Salmonella* populations and could be used to guide the development of serovar-specific interventions.

Key Words: Salmonella, Processing, CRISPR-SeroSeq, Broilers, Surveillance

P223 Application of Electron beam (eBeam) technology to reduce foodborne pathogens and spoilage organisms in ground turkey. Tanmaic Kalapala*^{IGS}, Jacqueline Prince², Jayla Andrews², Anna Luiza Fachetti V Assumpcao¹, Geetha Kumar-Phillips¹, Komala Arsi², Annie Donoghue², Tomi Obe¹, Palmy Jesudhasan² ¹UNIVERSITY OF ARKANSAS, ²Poultry Production and Product Safety Research Unit, ARS, USDA

Foodborne outbreaks continue to impact public health and the economy annually in the United States and worldwide. According to recent estimates, *Salmonella* and *Campylobacter* cause approximately 2.9 million illnesses annually and economic losses of up to \$2.8 billion and \$6.9 billion, respectively, in the United States. *Salmonella* and *Campylobacter* continue to be significant contributors to foodborne illness through poultry meat products despite standard food safety protocol. FDA-approved electron beam (eBeam) technology can be used to inactivate these pathogens in the meat, so the objective of this study was to evaluate the reductions of multiple *Salmonella* serovars, *Campylobacter jejuni* strains, and background spoilage bacterial populations through eBeam technology on ground turkey samples and to determine the most effective dosage for killing these foodborne pathogens. There were seven groups of ground turkey samples, each consisting of 3 replicate meat samples, that were inoculated with 2.3×10^7 CFU/g of *Salmonella* (mixture of 5 serovars)

and 5.4×10^7 CFU/g of *C. jejuni* (mixture of 5 strains). Samples were exposed to 6 levels of eBeam dosage: 0 kGy, 1 kGy, 2 kGy, 3 kGy, 4 kGy, and 5 kGy. In addition, we had a non-spiked control group to determine the background bacterial counts, which did not get spiked with *Salmonella* or *Campylobacter* and did not receive eBeam treatment. After eBeam treatment, the samples were stored at 4°C and subsequently stomached in buffered phosphate diluent. The samples were serially diluted and plated on Xyline Lysine Deoxycholate with novobiocin for *Salmonella*, Campylobacter Enrichment Agar for *Campylobacter*, and Tryptic Soy Agar for total aerobic bacteria. Results revealed that 2 kGy eBeam dose reduced the populations of *Salmonella* and 1 kGy eBeam dose reduced the populations of *Campylobacter* bacterial cultures by 6 logs CFU/g in ground turkey meat. After 4 kGy, spoilage bacteria in ground turkey were not detectable, indicating that applying 4 kGy of eBeam treatment to ground turkey meat could significantly increase shelf-life and reduce foodborne illnesses due to *Salmonella* and *Campylobacter* contamination.

Key Words: Salmonella, Campylobacter, Spoilage organisms, Electron beam technology

P224 Rapid flavor assessment of warmed-over deep-fried chicken and quality changes over time Linda Barahona*^{GS}, Ainsley Jessup, Michelle Hayden, Sungeun Cho *Auburn University*

Warmed-over flavor (WOF) has long been recognized as a flavor defect in cooked, refrigerated, and reheated chicken meat, which is a challenge in the poultry industry. For a better understanding of WOF over time, this study assessed the aroma profiles of deep-fried chicken reheated in a microwave (MWV, Sharp® Carousel 1200W, Sakai, Osaka, Japan) and air fryer (AF, Kalorik® 5-Quart Digital Air Fryer, Belgium) over a period of 5 days. An electronic nose (Heracles Neo II, Alpha MOS, Toulouse, France), a powerful tool for rapid assessment of aroma profiles, was used in this study. For this experiment, chicken drumsticks were deep-fried in canola oil for approximately 8 min until an internal temperature of 73°C (165°F) was reached. Two grams of fibularis longus muscle were collected to characterize WOF of each drumstick after deep-frying (Day 0) or reheating for 5 min in MWV or AF for a 5-day storage period. On Day 1, samples reheated in MWV developed more volatile compounds compared to samples reheated with AF (9 vs 5 new volatile compounds), different from those found on Day 0, including 2-methyl-hexane, methyl butanoate, and pentanoic acid. On day 3, MWV and AF reheated samples developed a single common volatile, 4-methyl-octane. Interestingly, the development of lipid oxidation products (aldehydes, ketones, and alcohols) was delayed in the AF samples compared to the MWV samples. For example, 3-methylbutanal (aldehydic, cheese, fatty, etc.) was identified in MWV samples on Day 1, but on Day 3 in AF; 1-propanol (alcoholic, ethanol, fermented, etc.) and 1,1-dichloropropene appeared on Day 3 in MWV samples, but on Day 5 in AF. Volatile compounds such as dibromochloromethane and n-nonanal (aldehydic, chlorine, fatty, etc.) were identified on most days in both AF and MWV samples over a 5-day period except on Day 1 in MWV samples. Principal component analysis of the sensors confirmed the differences in volatile profiles of the MWV and AF during the 5-day storage period accounting for a total variance of 99.3%. In conclusion, this study confirmed that both storage time and reheating method affected the development of the WOF-related volatiles. With the appropriate heating guidelines, consumers will have a better experience with reheated deep-fried chicken meat.

Key Words: Warmed-over-flavor, deep-fried chicken, E-nose, Volatile composition

P225 Comparison of egg functionality between conventional and aviary rearing systems Skye Freeland*^{1GS}, Zhiyuan Wu², Masahiko Taniguchi², Christina Sigmon¹, Aaron Kiess¹, Yan Campbell¹ ¹*Prestage Department of Poultry Science, North Carolina State University;* ²*Department of Chemistry, North Carolina State University*

More consumers pursue cage free (aviary) eggs instead of conventional (cage) eggs. The purpose is to investigate the impact of rearing systems on the functionality of liquid egg white. 30-40 eggs were collected from the two systems from three different time points covering separate production phases. Cage (C) and Aviary (A) had a non-heated (NH) and a heated (H) treatment (trt) with 3 replicates. The functionality was determined by evaluating protein content, dry matter content, surface hydrophobicity, free and total sulfhydryl (SH) groups, and protein denature temperature and enthalpy. Data was analyzed by SAS 9.4, with a p-value < 0.05. The results demonstrated that there was no difference (P > 0.05) detected between cage and aviary liquid egg whites in both H and NH trts in PEAK sampling point for protein concentration, particle size diameter, zeta potential, protein denature temperature between both recognized peaks, and the first peak of protein enthalpy. A_H had greater (P < 0.05) dry matter content than the C_NH. C_H had lower (P < 0.05) surface hydrophobicity than A_H, but were not different from the other two trts. C_NH had less (P > 0.05) free SH groups, and C_H had the most free SH groups, A_H and A_NH had less but not different from each other. A_NH had the most (P < 0.05) total SH groups, C_NH had the least amount, the other two trts were not different. C_NH in Peak 2 had greater protein enthalpy compared to A_H and the other two were not different. In conclusion, dry matter content, SH groups, protein denaturation, differed between systems and heat trt. A_NH egg white exhibited more hydrophobicity and less solubility. Heat increased the protein denaturation and decreased hydrophobicity for aviary and cage LEW.

Key Words: liquid egg white, LEW protein functionality, aviary rearing systems, cage rearing systems

P226 Evaluating the microbial ecology of a small-pastured poultry processing environment. Eniola Betiku*, Tolulope Tiwa Ogundipe, Tomi Obe *University of Arkansas*

Pastured poultry production is on the rise and persistence of foodborne pathogens such as *Salmonella* and *Campylobacter* at post-harvest despite intervention strategies, remains. Conducting a bio-mapping from time to time to know the microbial status of processing operations and equipment could give processors better insight into improving interventions. Hence, in this preliminary study of a year survey of pastured poultry operation, the microbial populations of processing rinsates (carcasses and water) and equipment pre and post sanitation was evaluated. The plant was visited twice (V1 and V2) at different times representing different flock at each visit. Ten rinsate samples from live receiving to chiller and eight equipment swabs each at pre and post sanitation were collected (N=52). All samples were analyzed for *Salmonella* and *Campylobacter* using culture method and BAX quantification, along with total aerobic count (AC) and *Enterobacteriaceae* (EB) using Petrifilms. Results were analyzed using ANOVA and means separated with Fisher's LSD (p ≤ 0.05). At both visits, *Salmonella* prevalence differed for rinsates (P=0.02) and swabs (P=0.007). Rinsates had 20% positives at V1 and 70% at V2 while swabs significantly reduced (P=0.008) between pre- and post-sanitation for V1 (23% - 0%) and V2 (87% - 38%), respectively. Similarly, *Campylobacter* prevalence differed for rinsates (P=0.04) but not for swabs (P=0.62). Rinsates increased from 30% to 70% between V1 and V2, with a quantity that ranged from 1.30 to 4.65 Log CFU/ml. For swabs, sanitation significantly (P=0.002) reduced *Campylobacter* prevalence between visits (63% - 0%, V1 and 50% - 25%, V2). AC population in rinsates reduced by 9.0 Log CFU/ml from live receiving to evisceration at V1 whereas at V2, only by 1.33 Log CFU/ml, while EB was detected in only 40% of the samples. Microbial population post-sanitation was impacted by visits (flocks) for AC (P=0.5) but not for EB (P=0.04). Importantly, *Salmonella*

and *Campylobacter* were not detected from rinsates after chilling, but not from swabs post-sanitation signifying possibility for cross-contamination. This routine and persistent bio-mapping could help small processors identify high microbial population flocks and hotspots within the facility for effective mitigations.

Key Words: Salmonella, pastured poultry, Campylobacter, indicators, bio-mapping

P227 Assessment of Salmonella and Campylobacter from pullet through final raw product of a conventional broiler complex Yagya Adhikari*¹, Matthew Bailey¹, Luis Munoz¹, Leticia Galindo¹, Pankaj Gaonkar², Steven Kitchens², Stuart Price², Jeff Buhr³, Dianna Bourassa¹, Kenneth Macklin⁴ ¹*Department of Poultry Science, Auburn University;* ²*Department of Pathobiology, Auburn University;* ³*USDA ARS Poultry Microbiological Safety and Processing Research Unit;* ⁴*Department of Poultry Science, Mississippi State University*

Salmonella and *Campylobacter* are foodborne pathogenic bacteria that can cause diarrhea in humans. They are frequently associated with the consumption or handling of raw poultry meat and meat products. With an objective to isolate these pathogens, a total of 1699 environmental samples were collected from inside and outside farms and facilities of a commercial broiler complex. This study was conducted in partnership with a company that uses antibiotics as a standard feed additive. Altogether 8 pullets, 10 breeders, 20 broiler houses, a hatchery, 5 trucks, and a processing plant were sampled for this study. Samples were pre-enriched with buffered peptone water and 3M Campy enrichment broth for *Salmonella* and *Campylobacter* respectively and pre-screened with 3M Molecular Detection System (MDS). The suspect positives were further processed for confirmation. Data were analyzed using R version 4.3.1 using Generalized Linear Model for binomial distribution. Odds ratio and confidence limits were calculated for stages of production and sample types based on MDS results. The level of significance was measured at $\alpha=0.05$ and 95% Confidence Limits. Altogether 17.32% (167/964) of samples were positive on MDS for *Salmonella* and 32.52% (239/735) for *Campylobacter*. Interestingly, the odds of occurrence of *Salmonella* in hatchery, transport and processing plant samples was significantly higher (P < 0.05) than breeder, pullet and broiler farms while the odds of occurrence of *Campylobacter* in broiler farms, transport and processing plant samples were significantly (P < 0.05) higher than breeders, pullets and the hatchery. Similarly, boot swab, sponge-stick swab samples and carcass rinses (post-picking) were useful for predicting the occurrence of both pathogens. Among 167 MDS *Salmonella*-positive samples, 137 were positive on culture while among 239 MDS *Campylobacter*-positive samples, 133 were positive on culture. Both pathogens were recovered from all stages of production and sample types, which indicates that surroundings of poultry houses and facilities are contaminated with these pathogens which can potentially contaminate food products and can cause people sick. Further study in further processing and retail meat markets and antibiogram of isolated pathogens would be noteworthy.

Key Words: Salmonella, Campylobacter, Sample types, Stages of production, broiler

P228 Peak-period egg quality traits of local and pure line laying hens housed in a non-cage housing system Metin Petek*, Eyuphan Petek *Bursa Uludag University*

Consumer interest in eggs produced from non-cage housing systems and backyard poultry is leading to a constant increasing trend in the number of local pure line or cross line hens used in table egg production. The early stage of egg production including peak period plays an important role in how a poultry flock will perform and how long laying hens can be kept in egg production in a sustainable way. This period may also be a good indicator of what egg quality will be like in the later stage of the production. Therefore, this study was made to investigate the peak period

internal and external egg quality characteristics of a local laying hen, a commercial laying hen, a pure line layer and a local cross-line layer, all of similar age. All flocks were raised in a commercial farm in standard conditions for non-cage egg production. A total of 60 eggs from each genotype were collected randomly from all eggs laid in a particular day at 34 and 36 weeks of age (30 eggs in each week from each genotype, 240 eggs in total). The egg quality evaluation included egg weight, egg length, egg width, shell color (L^* , a^* , b^*) traits, shell thickness, yolk height, thick albumen height and yolk color. Data was evaluated statistically using two-way analysis of variance (ANOVA) by the general linear model procedure of the SPSS program. Mean separation was conducted using the Tukey test. There were significant differences for the egg weight of different genotype laying hens and cross line layers had significantly the lowest egg weight. Commercial laying hen had significantly the greatest egg shell thickness and the lowest L^* values. There were significant differences for a^* and b^* color traits of eggs produced by different genotypes of layers. The eggs of commercial layer and pure line laying hens had significantly greater HU values than those of local layer and cross-line hens. But HU values of egg from all genotypes were found to be acceptable for AA grade standard of table eggs at a score of 72 or above. Although peak period egg quality of commercial laying hens was slightly superior than the other genotypes it can be said that the overall egg quality characteristics of pure line and cross line hens were competitive with commercial laying hens.

Key Words: Laying hen, peak period, egg quality, non-cage housing

P229 Breast meat myopathies in local and commercial female broiler chickens Metin Petek*, Erdem Küpeli, Eyuphan Petek *Bursa Uludag University*

This study was carried out to investigate effects of genotype on breast meat myopathies in female broiler chickens. The breast meat samples of a local and two commercial broiler genotypes slaughtered at 37 and 44 days of age were analysed for occurrence of white stripping and woody breast by visual examination and palpation of fillets by a trained person as normal, mild, moderate, and severe. Then, the samples were evaluated for pale, soft and exudative meat according to their lightness values (L) as being dark, normal or pale. The breast meat samples were also analysed for occurrence of deep pectoral myopathies, spaghetti meat and dark-firm-dry meat. Differences in the frequency of myopathies were compared using the Kruskal-Wallis test, and Dunn's multiple range test was used for post-hoc multiple comparisons. There were a different degree of macroscopic appearance, basically mild or medium, of white stripping and woody breast myopathies in some of the breast meat samples of all genotypes. No occurrence of deep pectoral myopathies, spaghetti meat and pale-soft-exudative myopathies was seen in all genotypes. The incidence and severity of myopathies increased with increasing slaughter age and its severity was more higher in those with higher body and breast meat weight. The severity of white stripping and woody breast were found to be slightly higher in commercial genotypes than local broilers. In conclusion, it can be said that the occurrence and severity of these breast meat myopathies increased with age and live body weight in all broiler lines included in this study.

Key Words: Broiler, breast meat myopathies, female, genotype

P230 Quality characteristics of broiler breast meat affected by overlapping white striping, woody breast and spaghetti meat myopathies Jaroslav Valenta*¹, Laura Garner¹, Aftab Siddique², Katherine Sierra¹, Vianca Tashiguan¹, Luis Guzman¹, Telah Black¹, Jacob Doster¹, Payten Leeds¹, Amit Morey¹ ¹Auburn University, ²Fort Valley State University

The poultry industry is witnessing an increase in overlapping breast myopathies, woody breast (WB), white striping (WS), and spaghetti meat (SM), in different severities leading to quality issues which should be investigated. This study evaluated the impact of overlapping myopathies on breast meat quality. A total of 400 freshly deboned (2.5 h post slaughter)

whole butterfly breasts (Ross 708; all males; 55 days old) were randomly selected at a commercial processing plant from four flocks (n=100/flock). Right-side fillets were analyzed and scored into different severity categories for WB and WS (0-normal, 1-mild, 2-moderate, 3-severe), and for presence (1) or absence (0) for SM. Based on the theoretical 32 overlapping combinations (from WB0WS0SM0 to WB3WS3SM1), the fillets were divided into different groups. Further, those fillets were analyzed for bioelectrical impedance (resistance and reactance), color (CIE $L^*a^*b^*$), water holding capacity (drip and cook loss), and texture (BMORS). Data from 7 prominent groups were selected for further statistical analysis using GLIMMIX procedure with Tukey-Kramer test to separate means ($P<0.05$). A total of 41.71% of samples (out of 400) had a combination of two or three myopathies, 36.18% had only one myopathy, leaving only 22.11% without any myopathies. WB1WS1SM1 (6.03%) was the most common overlapping myopathy group. Bioelectrical properties (resistance) of normal breast fillets (WS0B0SM0) were higher ($P<0.05$) compared to WB3 + WS1, however there was no difference ($P<0.05$) from WB2 + WS1 or SM fillets. Weight of normal and SM fillets were lower ($P<0.05$) than WB3 + WS1 fillets. In addition, WB3 + WS1 combination had higher values ($P<0.05$) for redness, fillet height and peak count than normal or SM fillets. The WB1WS1SM1 fillets had lower values for redness ($P<0.05$), yellowness ($P<0.05$), and peak count ($P<0.05$) than WB3WS1SM0. While there was no statistical difference in cook loss or reactance between the groups, the WB2 and WB3 groups were trending with higher cook loss and lower reactance values. The presence of WB3 showed the most difference in the measured parameters compared to the normal and/or SM fillets and the overlapping of WS and SM myopathies had the least impact on fillet quality characteristics.

Key Words: breast meat quality, overlapping breast myopathies, woody breast, white striping, spaghetti meat

P231 Removal of inoculated Salmonella Kentucky^{NR} and Campylobacter coli^{GR} from broiler carcasses using heated water (60 C) at high pressure (450 psi) Michael McIntyre*¹, Douglas Cosby², Latoya Wiggins², C. Crozier², Aaron Jordan¹, Josh DeVoll¹ ¹Spraying Systems Company, ²USDA, U.S. National Poultry Research Center

Salmonellosis and Campylobacteriosis continue to be major foodborne human illnesses attributed to consumption of poultry and/or poultry products. Industry and regulatory personnel constantly strive to reduce or eliminate these enteropathogens from finished poultry products. This study was designed to evaluate the ability of a novel wash cabinet using heated water (60 C), low-volume fluidic nozzles, and high pressure (450 psi) to remove artificially inoculated Nalidixic Acid Resistant *Salmonella* Kentucky (SK^{NR}) or Gentamicin Resistant *Campylobacter coli* (Cc^{GR}) from full-feathered, pre-scald carcasses. Ten carcasses were obtained from a local processing plant, placed into individual plastic bags, and transported to the Pilot Processing Plant at the U.S. National Poultry Research Center. Ten mL of approximately 10⁵ cfu/mL of SK^{NR} or Cc^{GR} was spread along the keel of individual carcasses from the vent to the crop area and allowed to attach for 60 minutes. Four replications were conducted for SK^{NR} (n=40) and Cc^{GR} (n=40). After 60 min, the carcasses were placed into shackles and pre-wash breast swabs were collected. The carcasses were processed through the wash cabinet at a line speed of 52 birds/min. After the cabinet, post-wash breast swabs were collected. All swabs were maintained on ice until serially diluted and plated onto either Brilliant Green Agar with Sulfapyridine and 200 ppm Nalidixic Acid (BGS+NA) for the SK^{NR} inoculated carcasses or Campy Cefex agar with 200 ppm Gentamicin (Cefex+G) for the Cc^{GR} inoculated carcasses. BGS+NA plates were incubated at 37 C for 24 h, and Cefex+G plates were incubated micro-aerobically for 48 h. Colonies were counted, log₁₀ converted and recorded as cfu/carcass. Paired t-tests were conducted with p<0.05 for significance. The average initial loads on the carcasses were 3.87 and 3.23 log₁₀ cfu/carcass for SK^{NR} and Cc^{GR}, respectively. The final levels on the carcasses after the wash were 2.29 and 1.62 log₁₀ cfu/carcass for SK^{NR} and Cc^{GR},

respectively. This correlates to a 1.58 log₁₀ cfu/carcass reduction for SK^{NR} (p<0.00006) and a 1.61 log₁₀ cfu/carcass reduction for Cc^{GR} (p<0.0007). Use of the novel wash cabinet was effective to remove approximately 1.5 log₁₀ cfu of pathogenic bacteria from carcasses.

Key Words: Salmonella, Campylobacter, Processing, High Pressure, Heated Water

P232 Effect of a Botanical Solution on Performance and Fecal Score in Eimeria-challenged Turkey Poults Sebastian Decap¹, Rosa Navarro¹, Victoria Tapia^{*1}, Hans Konsens¹, Brett Lumpkins² ¹*Plantae Labs SpA*, ²*Southern Poultry Feed and Research, Inc*

Introduction: Coccidiosis is a major poultry disease caused by protozoan parasites of the genus *Eimeria* also called coccidia. Coccidia infects the turkey's intestinal tract, leading to a significant impact on their health. With the rise of antimicrobial resistance, there is a pressing need for natural solutions able to mitigate the widespread dissemination of resistance. Thus, the objective of the study is to evaluate the anticoccidial efficacy of a natural encapsulated feed additive against current turkey coccidia field isolates.

Methodology-In vivo study: A total of 480 day-of-hatch Nicholas Select male turkey poults were randomly allocated with 6 birds/cage. 5 treatments: unchallenged/untreated control (CON); challenged/untreated control (POS); Monensin at 81 ppm (MED); and Quillaja saponaria extract

powder (QS) at 45 ppm, and a combination of QS at 45 ppm and monensin at 81 ppm (QSMED). On Day 14, all birds except CON were individually inoculated by oral gavage with 30,000 oocysts/ml with a mixture of *E. meleagridis*, *E. adenoids*, and *E. gallopavonis* field isolates (1:1:1). Feed intake (FI) and body weights (BW) were recorded on Days 0, 14, and 28. Fecal samples were collected on Day 21 for evaluation using a Visual Dropping Pan (VDP) score. The data were analyzed by Analysis of Variance and Tukey's Test, using Statistix software.

Results: Supplementation with QS between D0-28 improved BWG and FCR by 7% compared to POS. However, QS was not highly effective as MED, which increased BWG and reduced FCR significantly (p<0.05) compared to the untreated group at D0-28. QSMED supplementation increased BWG by 26% and significantly improved FCR by 21% compared to POS. Performance at D14-28 was similar. BWG in QS-supplemented birds was significantly (p<0.05) higher than in the POS group, with an 11% increase. In the same period, QSMED supplementation showed a 40% and 24% improvement in BWG and FCR, respectively, compared to the untreated/challenged group. Compared to POS, QS decreased VDP scores by 7%, while QSMED has a significant (p<0.05) effect in the scores.

Conclusion: QS not only improved the performance of turkey poults under *Eimeria* challenge conditions but also enhanced the effects of MED under the same challenge conditions.

Key Words: Coccidiosis, Performance, Turkey

Pathology

P233 Profile of blood microbiota in broiler chickens with different foot pad dermatitis scores Janghan Choi^{*1,2}, Doyun Goo¹, Byungwhi Kong², Brian Bowker², Hong Zhuang², Woo Kim¹ ¹*University of Georgia*, ²*US National Poultry Research Center*

While blood has traditionally been considered sterile, blood microbiota could be an important indicator of systemic health in broiler chickens. Foot pad dermatitis (FPD) is a common condition in broiler chickens raised in floor pens. It is hypothesized that bacteria from the litter can infiltrate the foot pad lesion, leading to secondary infections by altering blood microbiota and increasing blood bacterial toxins in broiler chickens. The purpose of this study was to investigate the profile of blood microbiota in broiler chickens with different FPD scores. On D 42, blood was collected from broilers (N = 4) with different FPD scores (0: no lesion, 1: FPD covers less than 50% of the food pad, and 2: FPD covers more than 50% of the food pad), and DNA was extracted from the buffy coats and analyzed to determine blood microbiota by using 16s rRNA sequencing. PROC MIXED in SAS was used for group comparison, and orthogonal polynomial contrasts were used to evaluate the significance of linear or quadratic effects of FPD. Alpha and beta diversity of blood microbial communities was not affected by FPD (P > 0.1). The phyla Proteobacteria, Cyanobacteria, Actinobacteria, Firmicutes, and Bacteroidetes in this order were the most abundant groups in the blood microbial communities of broiler chickens without FPD. In the blood microbial communities of broiler chickens with FPD score 1 and 2, the phyla Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, and Cyanobacteria in this order were the most abundant groups. The FPD tended to linearly decrease the relative abundance of the phylum of Cyanobacteria in the blood microbial communities of broiler chickens (P = 0.058). However, no statistical differences were observed in the other phylum groups and in the family groups (P > 0.1). The serum endotoxin level tended to be linearly increased due to FPD in broiler chickens (P = 0.059). In conclusion, FPD did not significantly influence blood microbiota in broiler chickens.

Key Words: Blood microbiota, endotoxins, foot pad dermatitis, broiler chickens

P234 Blood pictures indicate cryptic plasmacytosis can complicate a nutrition study Paul Cotter* *Cotter Laboratory*

Principle: How nutritional treatments affect performance sometimes includes data on blood cytology. Stress levels measured by the heterophil/lymphocyte ratio (H/L) are typical. However, without the total white cell count (TWBC), descriptions of atypical cells, and information on cellular behavior, the H/L is a weak proxy for the blood picture. **Methods:** To illustrate the principle, blood from apparently healthy ducks in a diet study was examined microscopically. 6 wk samples were stained with Wright-Giemsa. SDCs, H/L ratios, and TWBCs were determined on an average of 2 x 200 individual cells per slide. **Results:** Cellular aggregates called "reactive clusters" (RC) ranging from 3-10 members were composed of a single series (plasmacytoid) or a mixed series (lymphoid/granulocyte). Some plasma cells shared features with monocytes suggesting they are transitional types (Mn/PC). Türk cells, proplasmacyte indicators of inflammation, were in several samples. They were recognized by their giant size [cell area, A_c 170 μm², nuclear area, A_n 130 μm², nuclear/cytoplasmic ratio N/C 0.75, ploidy ratio (PR) ~4; where a PR of 1.6 indicates a diploid nucleus. Their cytoplasmic Hofs (Golgi) and coarse chromatin configuration are plasmacytoid features. In other cases, several Mn/PC were grouped into RC surrounding a central lymphoid cell. When an RC was found in a blood film atypical heterophils or thrombocytes were nearby. The study-wide TWBC ranged from 50K (leukocytosis) to 200K/μL (leukemoid reaction). H/L ratios ranged from 1 to > 50 without considering RCs. **Conclusions:** Cryptic plasmacytosis occurred during a study designed to evaluate the effects of diets on duck performance. The diagnosis is based on cytology analysis of blood samples collected at 6 wk, at processing, where leukocyte clustering and other atypia were observed. H/L ratios alone failed to capture the complexity of the blood picture. These observations reinforce the notion that adding detail to leukograms is superior to reliance on the simple H/L ratio when blood data are considered.

Key Words: duck, cryptic plasmacytosis, blood picture, H/L ratio, leukogram

P235 In vitro virulence characterization of avian pathogenic Escherichia coli isolates from broiler breeders with colibacillosis using HD11 cells Jiddu Joseph^{*GS}, Reshma Ramachandran, Sabin Poudel, Linan Jia, Pratima Adhikari, Li Zhang *Mississippi State University*

Avian pathogenic *Escherichia coli* (APEC) is a gram-negative extra intestinal pathogenic bacterium that causes colibacillosis in poultry. Controlling these bacteria in broiler breeders is critical as it potentially causes vertical and horizontal transmission; however, there is only limited information about the isolates collected from breeders. Therefore, characterizing the isolates *in vitro* and comparing them with phenotypic and genotypic virulence characteristics would provide the virulence potential of broiler breeder APEC isolates. This study evaluated the adhesion and invasion potential of nine broiler breeder APEC isolates which were selected from 28 isolates based on their phenotypic and genotypic virulence factors towards chicken macrophage HD11 cells. The HD11 cell line was grown at 37°C with 5% CO₂ until cells reach a confluency of 80-90%. Each *E. coli* isolate was grown to reach 10⁸ CFU/mL and subsequently challenged to the HD11 cells having a final count of 10⁶ cells/mL, giving a Multiplicity of Infection (MOI) of 1:100. After 3 hrs. of infection, cells were washed and collected to determine adhesion potential using an adhesion assay, and invasion potential using a gentamycin protection assay. Results showed that there were approximately about 8 log₁₀ CFU/mL for adhesion for all isolates except one (P15) while invasion concentration varied between 3 to 7 log₁₀ CFU/mL except for one isolate (P27) which had no invasion at all. Moreover, the adhesion and invasion potential of the isolates were not correlated to the genotypic and phenotypic virulence factors tested. Overall, we were able to identify the adhesion and invasion potential of the nine broiler breeder APEC isolates. However, more studies based on a whole genome aspect would provide factors responsible for this adhesion and invasion and can be used to identify the characteristics of these isolates further, to identify vaccine targets, or to develop an *E. coli* challenge model in broiler breeders.

Key Words: APEC, broiler breeder, adhesion, invasion, HD11 cells

P236 Characterisation of resistant variants of Escherichia coli exposed to essential oils mix and an antibiotic Nathalie Bonal^{*1}, Claire Carlu¹, Claire Girard¹, Sylvain Kerros¹, Diego Garcia-Gonzalo² *¹Phytosynthese, ²Universidad de Zaragoza-CITA*

The intense use of antibiotics has been associated with the rise of antimicrobial resistance. Moreover, cross-resistance can occur, leading to the emergence of multidrug-resistant pathogens. Alternatives have been studied to replace these substances in animal production. Essential oils have demonstrated antimicrobial properties with an increase of use in poultry production.

This study aims to compare phenotypically antimicrobial resistant variants (RVs) of *Escherichia coli* MG1655 after extended exposure to subinhibitory doses of an essential oils mixtures (EOMix = SentinelTM, Phytosynthese) and Amoxicillin through evolutionary assays and mutagenesis frequency.

Minimum Inhibitory Concentration (MIC) was determined by inoculating *E. coli* (5x10⁵ CFU/mL) in presence of different concentrations of EOMix (50-500 µL/L) and amoxicillin (0,5-32 µg/mL). Resistant *E. coli* strains were obtained through their submission to 20 cyclic exposures at sub-inhibitory doses (½ MIC). Subsequently, RVs were isolated and characterized. To assess the extent of direct resistance increase, the MIC values of RVs and the wild type strain against EOMix were compared through MIC determination. In order to evaluate the mutation frequency, the rifampicin-based selection method was used (Merino *et al.*, 2023). Exponential phase culture of *E. coli* was inoculated (1:3) in presence of ½ MIC of EOMix or amoxicillin. Aliquots of the culture were serially diluted. Plates were incubated and colonies counted. Mutation rates were calculated.

EOMix MIC was 200 µL/L and amoxicillin MIC was 8 µg/mL. RV from *E. coli* isolated from evolution experiment with EOMix showed 28%

increase in MIC value against EOMix after 20 cycles. In comparison, Amoxicillin showed 200% increase in term of MIC values. Incubation of *E. coli* with ½ MIC of EOMix did not lead to a higher proportion of mutants (p>0.05). A higher mutation rate was observed in presence of amoxicillin which increased the mutation rate of *E. coli* by ~100 times (p<0.05). These results indicate that exposure to subinhibitory doses of EOMix does not lead to significant increase in resistance. In conclusion, EOMix could serve as safe alternatives to antibiotics against the emergence of antimicrobial resistant bacteria and to preserve the efficacy of antibiotics.

Key Words: Essential oils, *Escherichia coli*, mutation rate, resistance, alternative

P237 Temporal Expression of Proinflammatory Cytokines in Poultry Lung Tissues Following Avian Pathogenic Escherichia coli Infection Xin Ye^{*1}, Chuan-Yu Hsu², Linan Jia¹, Xue Zhang³, Christopher Magee⁴, Stephanie Whitham⁴, Spencer Leigh⁴, Jeffrey Evans⁴, Li Zhang¹, Kelsy Robinson⁴ *¹Department of Poultry Science, Mississippi State University, ²Institute for Genomic, Biocomputing, and Biotechnology, Mississippi State University, ³Department of Food Science, Nutrition and Health Promotion, Mississippi State University, ⁴Poultry Research Unit, Agriculture Research Service, United States Department of Agriculture (USDA)*

APEC (Avian Pathogenic *Escherichia coli*) is a pathogen that is detrimental to the United States poultry industry, causing respiratory infections and systemic colibacillosis. Understanding the influence of APEC infection on the immune response is crucial for the development of more effective prevention and treatment strategies. The study evaluates the effect of APEC infection on chickens' immune systems by tracking proinflammatory cytokines and identifying genes related to infection progression. Seven-day old male broiler birds were divided into control and experimental groups, with control group receiving tryptic soy broth, and experimental group receiving 7.5 × 10⁷ CFU APEC via intratracheal inoculation to simulate infection. On days 1, 7, 15, and 21 post-infection, lung tissue samples were collected from eight birds in each group for RNA extraction and subsequent gene expression examination. Gene expression analysis focused on four key proinflammatory cytokines, including *IFN-γ*, *IL-1β*, *IL-8*, and *IL-6*, using *18S rRNA* as the reference for normalization to determine the host immune response to APEC infection. Interactions between treatment and inoculation times were analyzed with SAS PROC GLM, and gene expression fold changes between control and treatment groups at the same times were compared using the Mann-Whitney test. Our findings demonstrated that three of the four studied cytokines (*IFN-γ*, *IL-1β*, and *IL-8*) were significantly modulated (P < 0.05) over time in response to APEC infection, while *IL-6* showed no significant changes. The most notable observation was the marked early surge in cytokine levels, particularly evident with *IFN-γ*, which displayed a significant increase from the first day of post-infection and persisted through day 15. This suggests *IFN-γ* plays a critical role in the host's initial defense mechanisms against APEC. Conversely, *IL-8* and *IL-1β* showed significant elevations in expression only on day 1 post-infection, with no differences observed by day 7 and later, indicating a rapid but transient inflammatory response to APEC infection. These results highlight *IFN-γ* as a potential biomarker for early detection of APEC infections and as a key target for therapeutic intervention.

Key Words: Avian Pathogenic *E. coli*, broiler chick, proinflammatory cytokine, immune response, gene expression

P238 High-throughput multilocus sequence typing (MLST) for a large-scale avian *Escherichia coli* study in Mississippi Linan Jia*¹, Mark Arick II², Chuan-Yu Hsu², Daniel Peterson², Jeffrey Evans³, Kelsy Robinson³, Anuraj Sukumaran⁴, Reshma Ramachandran¹, Pratima Adhikari¹, Li Zhang¹ ¹*Department of Poultry Science, Mississippi State University*, ²*Institute for Genomics, Biocomputing, and Biotechnology, Mississippi State University*, ³*USDA, Agriculture Research Service, Poultry Research Unit*, ⁴*Freshpet*

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis, and accurately distinguishing infectious isolates is critical for controlling APEC transmission. Multilocus sequence typing (MLST) is an accurate and efficient strain identification method for epidemiological surveillance. This research aimed to develop a fast, cost-effective, and high-throughput MLST workflow that simultaneously sequences gene alleles from multiple *E. coli* isolates using the Oxford Nanopore Technology (ONT) for large-scale avian *E. coli* study. The seven housekeeping genes used in the Achtman MLST scheme were amplified from extracted gDNA samples for each of the 66 *E. coli* isolates collected from broiler farms. The amplicons were pooled for each strain and sequenced on an R9.4 MinION flow cell using the Nanopore GridION sequencer (named ONT-MLST Beta). The workflow was further optimized by introducing 96-well column DNA extraction and multiplex PCR (ONT-MLST Pro) and large-scale sequencing of 274 more isolates was conducted using a total of three Flongle flow cells on the Nanopore MinION Mk1C sequencer. The ST assignment accuracy, along with the reagent and labor costs, were compared between Sanger sequencing, ONT-MLST Beta, and ONT-MLST Pro. Finally, the ST results of all 313 isolates collected from infected chickens and poultry farm environments were reported, and phylogenetic analysis for ST allele sequences was conducted. Data showed that the most frequent sequence types among disease-related isolates were ST 1594 (52 isolates), ST 8578 (37 isolates), and ST 355 (34 isolates), accounting for 39.3% of all disease-related *E. coli* isolates. Additionally, data also suggest these frequent *E. coli* STs might have the potential to infect multiple organs including the spleen, yolk sac, heart, liver, lung, and air sac. Three specific STs (ST 69, ST 10, and ST 38) detected from this study have also been reported in other research as isolates from patients diagnosed with urinary tract infections, indicating a possible zoonotic concern of avian *E. coli*. With the advantages of the high-throughput and low cost of ONT, this study provides a rapid and cost-effective workflow for *E. coli* typing, which ensures the long-term and large-scale epidemiologic surveillance of infectious diseases.

Key Words: Avian *Escherichia coli*, Oxford Nanopore, large-scale, high-throughput, field study

P239 Intra-tracheal infection model to evaluate the effects of virulence genotypes of avian pathogenic *Escherichia coli* on layer pullets Fozol Ovi*^{1GS}, Dan Wilson², Kelsy Robinson³, Christopher Magee³, Douglas Cosby⁴, Jeff Evans³, Li Zhang¹, Pratima Adhikari¹ ¹*Mississippi State University*, ²*Wilson Veterinary Co.*, ³*Poultry Research Unit USDA*, ⁴*USDA-Agriculture Research Service*

The objective of this study was to establish the effect of virulence-associated genes (VAGs) on the colonization of extra-intestinal tissue by *Escherichia coli* (*E. coli*) and the immune response of Hy-Line W-36 chicks. We investigated an avirulent (T1) and a virulent (T2) isolate of *E. coli* that were previously categorized based on the presence of 5 VAGs (*iroN*, *ompT*, *hlyF*, *iutA*, and *iss*). A known colibacillosis-causing isolate was also used as a positive control (PC) and sterile phosphate buffer solution served as the negative control (NC). These isolates were made resistant to 100 ppm chloramphenicol. The experiment was performed in a BSL-2 animal facility. The facility was partitioned into 4 rooms to prevent cross-contamination. Each room housed a battery of 5 cages with 10 chicks/cage, totaling up to 200 chicks. These chicks were inoculated intratracheally with approximately 10⁶ CFU of different *E. coli* isolates at 7 days of age. On the 7th day post-inoculation (dpi), 5 random chicks from each

cage were euthanized to evaluate the bacterial concentration in lung, liver, heart, and spleen, as well as tracheal and airsac swab. Additionally, total leukocytes count and different leukocyte concentration in peripheral blood were investigated. The data were analyzed by Kruskal Wallis test using PROCNPAR1WAY function of SAS 9.4 and means were separated by DUNN's test. We found the concentration of T1 isolate in tracheal swabs subsided by the 7-dpi compared to T2 and PC isolates (P<0.001; 3.93 vs 4.80 and 4.79 log CFU). The PC resulted in a higher colonization in the spleen than the T1 and T2 isolates (P=0.007; 1.23 vs 0.97 and 0.92 log CFU/gm). *E. coli* colonization in the liver progressively increased from T1 to T2 and PC isolate (P<0.001; 0.65 vs 0.70 vs 0.77 log CFU/gm). Isolate T2 and PC resulted in a higher concentration of total leukocytes in blood compared to avirulent isolates (P<0.01). The population of $\gamma\delta$ T-cells was elevated by PC and T2 isolates compared to the T1 isolates (P<0.01). Thus, we conclude that *E. coli* isolates of different combinations of VAGs have distinct colonization patterns and they result in a varying degree of $\gamma\delta$ T-cell mediated immune response.

Key Words: Colibacillosis, Colonization, Extra-intestinal, Leukocytes, $\gamma\delta$ T-cells

P240 Linking norepinephrine production and performance to diet-induced low-grade, chronic inflammation in the intestine of broilers Michael Kogut*¹, J Byrd¹, Kenneth Genovese¹, Haiqi He¹, Christina Swaggerty¹, Karrie Daniels², Mark Lyte² ¹*Southern Plains Agricultural Research Center, USDA-ARS*, ²*College of Veterinary Medicine, Iowa State university*

Maintenance of intestinal health is critical to successful poultry production and one of the goals of the poultry production industry. For decades the poultry industry has relied upon the

inclusion of antibiotic growth promoters (AGPs) to achieve this goal and improve growth performance. With the removal of AGPs, the emergence of chronic, low-level gut inflammation has come to the forefront of concern in the poultry industry with the diet being the primary source of inflammatory triggers. We have developed a dietary model of low-grade, chronic intestinal inflammation in broilers that employs feeding a high non-starch polysaccharides (NSP) diet composed of 30% rice bran to study the effects of this inflammation on bird performance and physiology. For the present studies, we hypothesize that the low-grade chronic inflammation causes neurons in the intestinal enteric nervous system to secrete neurochemicals that activate immune cells that drive the inflammation and negatively affect bird performance. To test our hypothesis, one-day-old broiler chickens were weighed and divided into two dietary regimes: a control corn-soybean diet and a group fed a high NSP diet (30% rice bran). At 7-, 14-, 21-, and 28-days post-hatch (PH), birds were weighed, fecal material collected, and 5 birds were sacrificed and sections of duodenal and cecal tissues excised, and duodenal and cecal contents collected for ultra-high performance liquid chromatography analyses. UHPLC revealed 1000s-fold increase in the concentration of norepinephrine (NE) in birds fed the high NSP diet compared to the control fed birds. Further, the fecal concentrations of NE were also found to be significantly elevated throughout all time points. There were no differences in weight gain nor feed conversion from 1-14 days PH, but birds fed the high NSP diet had significantly reduced weigh gain and feed conversion from 14-28 days PH. The results revealed that a dietary-induced low-grade chronic inflammatory response increased NE production in the gut which negatively affected bird performance. This study suggests that neuroimmune pathways may serve as a mechanistic target for the development of new interventions to decrease the incidence of chronic inflammation and thereby benefit performance.

Key Words: Chronic inflammation, Norepinephrine, performance, intestinal health

P241 Transformation of *Dunaliella salina* by *Agrobacterium tumefaciens* for the Expression of the Hemagglutinin of Avian Influenza Virus H5 Inkar Castellanos-Huerta^{*1}, Gabriela Gómez-Verduzco², Guadalupe Ayora-Talavera³, Bernardo Bañuelos-Hernández⁴, Víctor Petrone-García⁵, Gilberto Velázquez-Juárez⁶, Guillermo Tellez-Isaias¹ ¹Department of Poultry Science, University of Arkansas Division of Agriculture, ²Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, ³Centro de Investigaciones Regionales, Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán (UADY), ⁴Escuela de Veterinaria, Universidad De La Salle Bajío, ⁵Departamento de Ciencias Pecuarías, Facultad de Estudios Superiores Cuautitlán UNAM, Cuautitlán Izcalli, ⁶Departamento de Química, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara

Avian influenza is one of the main threats to the poultry industry worldwide. Vaccination efforts are based on inactivated, live attenuated, and recombinant vaccines, where the virus hemagglutinin is the main component of any vaccine formulation. This study uses the microalgae *Dunaliella salina* to express the AIV HA protein of an H5 virus. *D. salina* offers a system of feasible culture properties, generally recognized as safe for humans (GRAS). The synthetic H5rD gene, with appropriate promoters and the terminator, was based on reference strain A/chicken/Hidalgo/28159-232/1994 (H5N2) and subcloned into the binary vector pCAMBIA-1301. For protein recombinant protein expression, a strain of *D. salina* from the Culture Collection of Algae at the University of Texas (Austin, TX, USA) was used. Genetic transformation of *D. salina* was performed by co-culture of *D. salina* cells with *Agrobacterium tumefaciens*. A PCR protocol confirmed the integration of the H5HA gene named H5rD. Total soluble protein samples were obtained and evaluated for protein expression with SDS-PAGE and Western Blot protocols. The hemagglutination assay evaluated the hemagglutination activity of the recombinant protein. *D. salina*-system for antigen production offers industrial advantages such as low production costs, low risk of biological contamination with animal pathogens, the capacity for post-translational in proteins like human glycoproteins, and the possible oral administration of the antigen. This study aimed to establish the expression of the recombinant H5rD protein of AIV in the nucleus of *D. salina*. PCR, SDS-PAGE, and Western blot confirmed expression of HA5r protein expression, and a hemagglutination assay biological activity of the HA5r protein confirmed H5HA-gene. These findings suggest that a complex protein such as HA5r from AIV (H5N2) can be successfully expressed in *D. salina*.

Key Words: *Dunaliella salina*, avian influenza, vaccine

P242 Efficacy (Novela Plus) a multi-strain *Bacillus Subtilis* and *Bacillus Pumilus* Probiotic via Drinking Water on Growth Performance of Broiler Chickens during Fowl Adenovirus Infection in the United Arab Emirates Merghani Adam* *ARABIAN FARMS DEVELOPMENT LLC*

Fowl adenoviruses (FAdVs) are non-enveloped paramyxoviruses with double-stranded DNA molecules and belong to the Avi adenovirus genus. There are also several diseases caused by fowl adenoviruses in broiler chickens, including inclusion body hepatitis, hydropericardium hepatitis syndrome, and adenoviral gizzard erosion caused by fowl adenoviruses. In recent years there has been an increasing concern about developing antibiotic resistance in pathogenic bacteria in humans and poultry. This study aimed to determine the efficacy of Novela ECL Plus administered via drinking water on the growth performance of broiler chickens during the infection with Fowl Adenovirus. This study was carried out from September 27, 2023, to October 27, 2023, at a commercial broiler floor system in Abu Dhabi, UAE. A total of 90,000 Ninety thousand one-day-old, commercial broiler Ross 308 breed chicks were brought from a commercial hatchery and randomly divided into three treatment groups in three broiler houses, 30,000 birds per house. Treatment 1, as a control, received antibiotic with a combination of (Gentamycin + Doxycycline) with a dose

of 0.5 gm per liter in the first three days from 1 to 3 days of age and received the second dose of antibiotic with a combination of Tilmicosin + Colistin for three days from 18 to 20 days of age with the dose 0.5 gm per liter. In treatments 2 and 3 the birds in this group received Novela ECL via drinking water for 8 hours with a dose of 0.05 gm per liter without usage of antibiotics from 0 days till the slaughtering at 28 days. At 15 days of age all treatment flocks had been infected with fowl adenovirus serotype 8b and postmortem findings showed an enlarged liver with necrotic foci, splenomegaly, and necrotic enteritis. The infection had been confirmed with an insulated isothermal PCR-specific strain kit for fowl adenovirus serotype 8b 8b. Feed intake and body weight, live body weight gain, and feed conversion ratio a European Production Efficiency Factor were measured. The results obtained from this study showed a significant reduction in mortality rate, improved weekly live body weight, enhanced dressed weight, and improved feed conversion ratio, EPEF, and dressing percentage, whereas as no significant difference in feed intake. In conclusion, the administration of the multi-strain probiotic Novela ECL Plus to broiler chickens from 0 days to 28 days of age plays a major role in and reduces the usage of antibiotics as antimicrobial substances, in addition to also improves the growth performance parameters and enhances the profitability.

Key Words: Fowl Adenovirus, novela, probiotic, Growth, Performance

P243 Prevalence of coccidiosis in Brazilian commercial poultry and its relation to non-specific enteritis according to ISI Sys, a collaborative monitoring platform for animal health. Igor Soares^{*1}, Bruna Belote¹, Otto Figueiró¹, Elizabeth Santin² ¹ISI Institute, ²Kupono Consultancy

Compiling necropsy data from routine sanitary monitoring provides valuable epidemiological data for poultry professionals to make decisions on animal health. This study aimed to survey the prevalence of coccidiosis and non-specific enteritis in Brazilian poultry farms applying the ISI Sys, a monitoring platform for animal production, from January 1st, 2022, to August 31st, 2023. The survey relied on necropsy data from 1,037 broiler flocks raised in four Brazilian industries, totalizing 6,590 chickens from 20 to 35 days of age (5 to 6 birds per flock). The necroscopy checklist on the ISI Sys mobile app included the scoring of lesions compatible with *Eimeria acervulina* (ACE), *E. maxima* (MAX), and *E. tenella* (TEN). For non-specific enteritis, the survey considered the scoring of general intestinal alterations, such as presence of mucus, desquamation, hyperemia, and undigested feed. Routinely, the parameters are evaluated through scores (S) of alteration from 0 to 3 (0 = absent; 3 = severe). For each parameter, an impact factor (IF) from 1 to 3 is fixed according to reductions on the organ functionality (3 = greatest reduction). The system calculates the birds' ISI Score by multiplying the IF of each parameter by the respective necropsy score and summing the results, or, briefly, ISI Score = $\Sigma(IF * S)$. In 2022 and 2023, respectively, the mean prevalences were 10.5% and 9.0% for ACE; 15.8% and 4.9% for MAX; and of 5.2% and 1.8% for TEN. A pattern was observed on the mean prevalence behavior comparing the years. In 2022 and 2023, the prevalence decreased, respectively, 7.3 and 3.8 points from April to May; increased 2.1 and 1.6 points up to July; and decreased 2.1 and 1.6 points in August. In 2022, the ISI Scores of MAX lesions and of non-specific enteritis waved similarly with respective decreases of 58.6% and 7.0% from April to May; increases of 58.8% and 14.6% in June; and reductions of 35.4% and 23.5% up to August. Our results exemplify what can be achieved by compiling necropsy data from different industries. Increasing the sample size would allow the patterns observed in our survey to be validated or new ones to be identified – given the wide geographic distribution of the Brazilian production – but for that, more collaborative work and tools are necessary.

Key Words: poultry epidemiology, prevalence, sanitary monitoring, coccidiosis, ISI

P244 Evaluation of the Poulvac® ST vaccine given in ovo to commercial broiler chickens: hatch success, early post-hatch mortality, and antibody production Katie Elliott*, Jeffrey Evans, Joseph Purswell, Christopher Magee *USDA-ARS Poultry Research Unit*

Following experiments testing dosages of Poulvac® *E.coli* vaccine for use *in ovo*, there was an interest to test the related Poulvac® ST vaccine *in ovo*. Ross 708 hatching eggs from Salmonella-vaccinated breeders were incubated in NatureForm 1600 series incubators with 3 (90 egg) trays/treatment in each of 2 incubators (6 replicate trays/treatment). Eggs were *in ovo*-vaccinated with Poulvac® ST vaccine as a 50µL volume using an Inovoject M vaccinator at 18 d of incubation. Treatments included non-injected eggs and eggs infected with diluent-only, 1 of 4 dosages of the vaccine to include 10⁻⁷ dilution (3.23 CFU/egg), 10⁻⁵ dilution (323 CFU/egg), 10⁻³ dilution (32,300 CFU/egg) and 10⁻² dilution (323,000 CFU/egg). Two eggs from each tray were injected with Coomassie blue dye to determine injection location. Following hatch, 4 pens/treatment (15 birds/pen) were reared as a randomized complete block design. Blood samples were collected from chicks at hatch and at 3 wk of age for ELISA testing for detection of IgY antibody levels. Most embryos were injected in the amnion (90.3%). Hatch of injected eggs was not significantly different between non-injected (95.5%), diluent-injected (91.9%), and the 3.23 CFU/egg dosage (91.3%) with 323 CFU/egg (83.7%), 32,300 CFU/egg (81.8%) and 323,000 CFU/egg dosages (79.5%) having decreased hatch which did not differ from each other ($P < 0.001$). No control birds died. The 32,300 CFU/egg and 323,000 CFU/egg experienced 22% and 8% mortality, respectively, by day 4 post-hatch and were thus removed from the study. The 3.23 and 323 treatments experienced 5% and 3% mortality, respectively, during the first week with only 1 further bird loss beyond the first wk. Most newly hatched chick antibody results were positive in all treatments (all treatments >83.3% positive), thought to be due to maternal antibodies. Most ELISA results at 3 wk were negative in the 4 remaining treatments (non-injected 1.7% positive; diluent-injected 5.1%; 3.23 CFU/egg 12.3%; and 323 CFU/egg 0% positive). These results, like Poulvac® *E.coli*, indicates that a very low dose (3.23 CFU/egg) when administering Poulvac® ST *in ovo* would be required to limit an impact on hatchability. Further immune response research would be needed to determine protection against a challenge.

Key Words: in ovo, broiler, bacteria, incubation, hatch

P245 Competitive inhibition of pathogenic Enterococcus cecorum by avirulent strains Grayson Walker*, M Suyemoto, Chalise Brown, Matthew Browning, Luke Borst *North Carolina State University College of Veterinary Medicine Department of Population Health and Pathobiology*

Pathogenic *Enterococcus cecorum* (EC) cause mortality in broiler flocks from pericarditis and sepsis early in the growing period and lameness and paralysis late in the growing period. The first step of EC infection is gastrointestinal colonization. Competitive inhibition of pathogenic EC gut colonization was hypothesized as an intervention strategy to reduce clinical EC-related disease. Avirulent EC mutants with a deletion in the core region $\Delta cpsC$ or the variable region of the capsular locus $\Delta cpsO$, or a cocktail of three commensal EC strains (CE123) were administered to 120 broiler chicks per group orally upon hatching or *in ovo* at 17 d of incubation in a series of experiments with 20 birds randomly allocated to 6 replicate litter floor pens. Broilers were orally challenged with pathogenic EC three days post-hatching. A positive control group of untreated broilers and an unchallenged negative control group were included in each experiment. Spleen samples from 4 and 16 birds per pen were collected at 14- and 35-days post-infection, respectively, and cultured to measure the prevalence of sepsis caused by pathogenic EC. Sepsis prevalence among groups was compared using a Fisher's Exact test of significance at each sampling time point. There was a significant reduction in the sepsis prevalence in CE123 broilers at 2 weeks post challenge with pathogenic EC ($P \leq 0.05$); however, this difference did not persist for the 5-week duration of the study. The attenuated deletion mutants $\Delta cpsC$ and $\Delta cpsO$ did not

disrupt long-term colonization of the gut by pathogenic EC as no decrease in sepsis prevalence was seen at week 5 among treatment groups. When $\Delta cpsC$, $\Delta cpsO$ or CE123 were administered *in ovo*, neither group was effective in decreasing sepsis at 14- or 35-days post-challenge. Commensal EC strains may be useful in the competitive exclusion of pathogenic EC and prevent subsequent EC infection consistent with a probiotic control strategy. However, the dose, frequency, and route of administration warrant further investigation.

Key Words: Enterococcus cecorum, Probiotic

P246 In vitro evaluation of efficacy of Bacillus amyloliquefaciens to minimize pathogens using an optical density experimental model Shameer Rasheed*¹, Charles Hofacre², Jennie Baxter², John Schleifer¹, Mark Lavorgna¹, Mahmoud Masadeh¹, Gary Reznik¹, Kristy Dorton¹ ¹Devenish Nutrition LLC, ²The Southern Poultry Group

A study was conducted to evaluate the efficacy of a probiotic to minimize pathogens *in vitro* using an optical density experimental model performed in microtiter plates. The probiotic was a *Bacillus amyloliquefaciens* product (VPA; ValuPro A, Devenish Nutrition, Fairmont, MN) and was tested at an equivalent inclusion to mimic providing 5.1 x 10⁵ CFU/g of finished feed. Two controls were included in the experiment: Non-Challenge Control (100 µL of sterile media and 100 µL of sterile 0.85% saline) and Challenge Control (100 µL of test organism (1.0 x 10⁶ CFU/mL) and 100 µL of sterile media). Test organisms included: *Salmonella* Enteritidis (SE), Avian Pathogenic *Escherichia coli* (APEC), and *Clostridium perfringens* (CP). A 100 µL aliquot of the test organism in Tryptic Soy Broth (1.0 x 10⁶ CFU/mL) and 100 µL aliquot of cell free supernatant were dispensed into individual microtiter plate wells. Prior to adding into the wells, VPA was solubilized in water and then the solids removed by centrifugation. The supernatant was then added to the test wells at 100 µL. Optical density (OD) was measured using a microplate reader. Microtiter plates were read at 630 nm and 37°C (98°F). For SE and APEC test plates, measurements were taken every 2 h through 8 h and then again at 24 h. To maintain anaerobic conditions, test plates for CP were measured only at 18 h. Results were expressed as the average OD of three microtiter wells. A lower OD value indicated a lower concentration of SE, APEC, and CP. All results are numerical differences as statistical analysis was not conducted due to the nature of the test. Compared to the Challenged Control, VPA was able to minimize the growth of SE and APEC *in vitro* as indicated by lower OD values. Lower OD values began 4 h into the test for both SE and APEC and continued through the 24-h period with the difference being greater through the end of the test at 24 h. After 18 h of incubation, VPA was also able to help minimize the growth of CP *in vitro*. In conclusion, test results provide evidence of the ability of VPA to minimize the growth of *Salmonella* Enteritidis, Avian Pathogenic *E. coli*, and *Clostridium perfringens* *in vitro*.

Key Words: Probiotics, In vitro, Salmonella, E. coli, Clostridium perfringens

SCAD

P247 Impact of a postbiotic containing saponin, with or without vaccination, on the mitigation of colibacillosis in broilers challenged with avian pathogenic *Escherichia coli* serotype O78 William Chaney*¹, Timothy Johnson², Alicia Meyer¹, Teresia Maina¹, Vivek Kuttappan¹, S. Naqvi³, Matthew Jones⁴, Charles Hofacre⁴ ¹Cargill Animal Nutrition, Cargill, Inc., ²University of Minnesota, Department of Veterinary and Biomedical Sciences, ³Engineering and Data Sciences, Cargill, Inc., ⁴Southern Poultry Research Group, Inc.

Avian pathogenic *Escherichia coli* (APEC) cause colibacillosis in poultry operations resulting in significant flock health and economic burden, creating the need for additional solutions to promote resiliency against infection. This study evaluated a postbiotic feed additive containing a saponin ingredient (SCFP+; Dia-V™ PT_{PLUS} prototype, Diamond V, Cedar Rapids, IA), alone or in combination with vaccination, to ameliorate the severity of colibacillosis in broilers intratracheally challenged with APEC O78. Six hundred male broilers were randomly allocated across five treatment groups consisting of 4 pen replicates with 30 birds per pen. Birds were fed a basal diet and challenged birds fed the diet either supplemented with SCFP+ or not and administered a vaccine or not with challenge on Day 28. Necropsy, lesion scoring, and tissue samples were collected on Day 35 (n=5/pen) and remaining birds on Day 42. Tissues were analyzed for *E. coli* load with random isolate characterization by PCR. Data were analyzed in SAS v9.4 with Treatment as fixed effect and pen nested within block as a random effect. Day 35 mean lesions scores for perihepatitis, pericarditis and air sacculitis were numerically improved by all treatments. SCFP+ alone significantly reduced perihepatitis lesions over the challenged control (1.15 vs. 1.80; $P=0.043$) and approached significance for pericarditis (0.95 vs. 1.55; $P<0.10$) with numerical improvement in air sacculitis (1.25 vs. 1.75). Vaccination alone numerically improved perihepatitis (1.35) and pericarditis (1.30) and approached significance for air sacculitis (1.20; $P<0.10$). SCFP+ with vaccine numerically improved perihepatitis (1.25), pericarditis (0.90) and air sacculitis (1.20) scores all approaching significance ($P<0.10$). Cumulative mean lesion scores were lowest for SCFP+ with and without vaccine both approaching significance. SCFP+ treatment resulted in lower mean APEC load in liver and lung tissue and % positive heart swabs. No treatment differences were observed in recovered birds at D42 and all treatments numerically improved livability over challenged control. These data suggest SCFP+, alone or in combination with vaccination, may be a viable, non-antibiotic solution to support reduction in the severity of colibacillosis in broilers.

Key Words: colibacillosis, postbiotic, saponin, broiler, vaccination

P248 Antimicrobial resistance pattern of *E. coli* strains from clinically healthy chickens from one integrated company Yosef Daniel Huberman*¹, Natalia Villarino², Laura Méndez¹, Ramón Alejandro González Pasayo¹ ¹Animal Health Group, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible (IPADS, CONICET-INTA), ²Phileo by Lesaffre

Escherichia coli is the causative agent of colibacillosis and is responsible to localized or systemic infections in poultry. Multiple Drug Resistant (MDR) strains are continually identified, even in clinically healthy chickens. To evaluate correct antimicrobial treatment, the resistance pattern of 54 *E. coli* strains considered avian pathogenic *Escherichia coli* (APEC) from one poultry integration with a history of colibacillosis, was monitored.

These strains were isolated from broiler breeders (n=13), meconium (n=13), and broilers of 7 (n=22) or 34 (n=6) days old. According to CLSI, the disk-diffusion method was used to test 13 antimicrobials from 7 classes: tetracyclines (tetracycline, doxycycline, and oxytetracycline), quinolones (enrofloxacin and norfloxacin), trimethoprim/sulfamethoxazole, florfenicol, aminoglycosides (neomycin and amikacin), β -lactams (amoxicillin, amoxicillin + clavulanic acid, and ampicillin), and fosfomy-

cin. Intermediate and resistant isolates were grouped as resistant. MDR strain was defined as resistant to three or more antimicrobials classes.

There were 9 (69%) MDR strains from breeders, 7 (54%) from meconium, 17 (77%) from 7-day-old broilers, and only 1 (17%) from 34-day-old broilers. High resistance rates to quinolones in breeders (92%), meconium (84%), and 7-day-old chicks (73%) were lowered in 34 days old broilers (17%) while resistance to tetracyclines was more even (77%, 54%, 68%, and 67%, respectively). A high rate of resistance to β -lactams and amphenicols in 7-day-old chicks (100% and 95%, respectively) was not observed in the other categories.

According to these results, the antimicrobials of choice are fosfomycin, florfenicol, and trimethoprim/sulfamethoxazole. The high resistance rates and variation in antimicrobial susceptibility patterns between strains from different stages of broiler production emphasize the importance of active, ongoing monitoring of antimicrobial susceptibility of *E. coli* isolates that are considered APEC, especially from apparently healthy chickens.

Key Words: *Escherichia coli*, antimicrobial resistance, poultry

P249 Molecular screening of *E. coli* virulence-associated genes from healthy chickens from broiler production Ramón Alejandro González Pasayo¹, Natalia Villarino², Jorgelina Lomónaco¹, Yosef Daniel Huberman*¹ ¹Animal Health Group, Instituto de Innovación para la Producción Agropecuaria y Desarrollo Sostenible (IPADS, CONICET-INTA), ²Phileo by Lesaffre

Escherichia coli is a commensal bacterium widespread in the intestines of warm-blooded animals and humans, although may induce enteric and extraintestinal diseases. As both types of *E. coli* isolates have been found in the intestines of birds, it has been proposed that this organ serves as a reservoir for virulent bacteria. Based on the virulence of the *E. coli* strain and the host's health, a disease may develop known as colibacillosis that affects all stages of poultry production causing significant economic losses worldwide. There is little information on the presence of pathogenic strains based on their virulence traits in healthy chickens in poultry production systems. To elucidate this matter, we aimed to investigate the presence of virulence-associated genes (VAGs) in clinically healthy chickens (CHC) from four different stages of production.

A total of 181 cloacal swab samples were recovered from CHC in one poultry integration that had a history of colibacillosis. These strains were collected from broiler breeders (n=41), meconium (n=21), and broilers of 7 (n=64) or 34 (n=55) days old. The isolates were analyzed for the presence of 5 known VAGs: *iroN*, *ompT*, *hlyF*, *iss*, and *iutA*; using a multiplex PCR. Isolates are considered to be potential avian pathogenic *E. coli* if they possess at least 4 VAGs.

Results showed that the broilers of 34 days old exhibited the highest percentage of isolates that totally lacked the VAGs studied (75%) compared with other stages: broiler breeders (61%), meconium (19%), and broilers of 7 days old (42%). These isolates could be considered commensal strains originally from the intestines and are categorized as avian fecal *E. coli*. From the rest, the number of isolates harboring 4-5 VAGs among broiler breeders, meconium, broilers of 7, and 34 days old were 81, 82, 59, and 57%, respectively. The presence of *ompT* was detected in all the stages, whereas *iutA* was only detected in meconium.

Variation in the number of VAGs-free isolates was observed in these 4 production stages, which was likely caused by the variation in the number of samples collected in the initial sampling with isolation of *E. coli*. Nevertheless, the high percentage of VAGs found in CHC is a risk factor that needs to be further investigated to prevent colibacillosis.

Key Words: Broiler chicken, *Escherichia coli*, Virulence genes

P250 The impact of a Bacillus-based direct-fed microbial on growth performance and in vivo Salmonella dissemination in broiler chickens

Kevin Bolek^{*1}, Sahil Kalia², Brooke Humphrey¹, Elizabeth Bobeck²
¹Phibro Animal Health Corp., ²Iowa State University

Direct-fed microbials (DFMs) are used by broiler producers to improve performance and mitigate pathogen challenges such as *Salmonella*. An experiment was conducted to determine the effects of MicroLife[®] Prime (MLP) DFM, containing four *Bacillus* species, on performance and *Salmonella* recovery in broiler chickens. 560 Ross 308 broiler males were wing banded at 1 day of age and distributed among 40 cages, with 14 birds/cage. Birds were allocated into two dietary treatments ± *Salmonella* Enteritidis, inoculated at 7 d of age, for a total of 4 treatments: Treatment 1 (T1): control corn-soy diet; Treatment 2 (T2): control corn-soy diet plus oral gavage with 1 x10⁸ CFU of *Salmonella* Enteritidis; Treatment 3 (T3): corn-soy diet plus 500,000 CFU/g MLP; or Treatment 4 (T4): corn-soy diet containing MLP at 500,000 CFU/g of feed plus oral gavage with 1 x10⁸ CFU of *Salmonella* Enteritidis. Growth performance was measured weekly through d21. Tissue and swabs were taken from CO₂ asphyxiated birds at several timepoints post inoculation (pi). Analysis of growth performance data was conducted using the PROC ANOVA procedure in SAS (Carey, NC) and present/absent plate counts were analyzed via the chi-square test. On a weekly basis through d14, body weight (BW) was not different based on treatment; however, birds in T4 remained numerically the heaviest throughout. *Salmonella* significantly reduced BW gain in T2 and T4 from d14-21 compared to uninoculated T1 and T3 due to reduced feed intake ($P<0.05$). At d21, BW was significantly different due to treatment, where birds receiving *Salmonella* in T2 and T4 were lightest, T1 was intermediate, and T3 was heaviest ($P<0.05$). At 4dpi, 10 birds per treatment were cloacal swabbed and samples were streaked on an XLT4 plate. T2 resulted in 7 out of 10 plates that were *Salmonella* positive and T4 resulted in 4 out of 10 positive ($P<0.0006$). At 4dpi, liver and spleen cultures in T2 resulted in 6 out of 10 positive plates and T4 resulted in 4 out of 10 positive ($P<0.002$). The main effect of *Salmonella* was reduced body weight, while MLP was associated with increased body weight in unchallenged chicks at d21. MLP was associated with significantly fewer positive plates in *Salmonella* challenged birds vs. birds challenged with *Salmonella* alone.

Key Words: direct-fed microbial, Salmonella, Bacillus, performance

P251 Characterization of the Salmonella enterica serovar Kentucky Type III Secretion System SPI-2 Secretome Jill Skrobarczyk^{*1}, Lindsey Wythe¹, Yuhua Farnell¹, Michael Kogut², Kenneth Genovese², Morgan Farnell¹
¹Department of Poultry Science, Texas A&M Agrilife Research, ²Agricultural Research Service, United States Department of Agriculture

Salmonella enterica serovars are leading causes of human gastroenteritis. To mediate virulence, *Salmonella* rely on type III secretion systems known as *Salmonella* Pathogenicity Islands 1 and 2 (SPI-1 and SPI-2). The SPI-1 secretion system facilitates host cell entry and invasion of the gut mucosa while SPI-2 proteins regulate intracellular survival and pathogenesis. In a previous study, *S. enterica* serovar Enteritidis SPI-2 proteins induced high levels of serum IgG in vaccinated hens and reduced SE colonization. The objective of this study was to generate and characterize the *Salmonella enterica* serotype Kentucky (SK) SPI-2 secretome for potential vaccine applications in poultry. *Salmonella* Kentucky is the most isolated *S. enterica* serovar from contaminated poultry carcasses. The bacteria were first cultivated in LB broth to log phase growth, washed, and subcultured in an acidic, low-phosphate, low magnesium medium (LPM) for 16 hours at 37°C to induce secretion of SPI-2 proteins. The LPM culture supernatant containing the SPI-2 secretome was harvested, buffer exchanged, and analyzed against known *S. enterica* SPI-2 proteins. Polyacrylamide gel electrophoresis analysis revealed that the protein profile obtained from the SK inoculated LPM culture differed from that of the unstressed SK culture propagated in LB broth. The low molecular weight associated with SPI-2 secretome proteins was observed in the LPM supernatant and suggested

that the stress response was triggered. Core SPI-2 effector proteins such as SseB, SseC, and SseL were identified by mass spectrometry analysis. Other SPI-2 related effector proteins were detected including PipB2 and SopB. These have been linked to SPI-2 secretomes in *S. enterica* serovars. Overall, this work describes the generation and proteomic analysis of the SK SPI-2 secretome. Further studies are warranted to compare the secretome to other *S. enterica* serovars and to analyze the efficacy of the SK SPI-2 secretome in a vaccine application.

Key Words: Salmonella, Kentucky, SPI-2, secretome, vaccine

P252 Deep serotyping highlights Salmonella surveillance challenges in turkey production Emily Cason^{*1GS}, Anna Carlson², Nikki Shariat¹

¹Department of Population Health, University of Georgia, ²Cargill Inc.

Despite extensive *Salmonella* mitigation strategies used during turkey processing, 5.9% of human salmonellosis cases are linked to the consumption of contaminated turkey. The purpose of this study was to determine *Salmonella* prevalence and serovar diversity through turkey live-production and processing by sampling 22 flocks on the farm and during four different stages of processing. Bootssocks (n=22) were collected on the farm immediately after load-out. At processing, pre-scald wingtips (n=6 composites of 10 per flock), pre-chill wingtips (n=6 composites of 10 per flock), mechanically separated turkey (MST; n=6 bins per flock) and ground turkey (n=6 bins per flock) samples were collected. *Salmonella* prevalence was determined using a commercial PCR assay and then confirmed by selective enrichment and culture. Deep serotyping by CRISPR-SeroSeq was performed on *Salmonella*-positive samples (n=167). At the farm, nine flocks were *Salmonella* positive, compared to 21 flocks that were positive in at least one location during processing. *Salmonella* prevalence decreased from pre-scald (56.1%) to pre-chill (18.2%) ($p<0.05$, non-parametric Mann-Whitney U test) then increased in MST (65.2%; $p<0.05$) and ground turkey (48.5%; $p<0.05$). In total, 34.7% of samples contained multiple serovars and deep serotyping detected one or more serovars in products that were absent before chilling in 15 flocks. In total, 19 serovars were detected; Uganda (28.4%), Hadar (24.3%), and Typhimurium (24.3%) were most prevalent. Almost half of samples containing serovar Typhimurium (20/42) corresponded to a live-attenuated Typhimurium vaccine. This study demonstrates that *Salmonella* prevalence and serovars at load out and at pre-scald are not indicative of that in final product samples, underscoring the complexity of tracking serovars in turkey production. This ultimately limits the applicability of developing serovar-specific controls such as vaccination based only on pre-harvest data. This work highlights the challenge of identifying on-farm surveillance samples that accurately represent *Salmonella* in final products.

Key Words: Salmonella, turkey, food safety, live production, processing

P253 Reevaluating ceca as the gold standard for Salmonella surveillance in ground turkey Emily Cason^{*1}, Grace Bannister², Marvin Tzirin², Allen Byrd³, Anna Carlson⁴, Nikki Shariat¹, Jessie Vipham²

¹Department of Population Health, University of Georgia, ²Department of Animal Science and Industry, Kansas State University, ³USDA-ARS Southern Plains Agricultural Center, ⁴Cargill Inc.

Ceca have long been considered the gold-standard for *Salmonella* prediction within the turkey industry. Through two experiments, this study sought to evaluate correlation between *Salmonella* detected in ceca and ground turkey. In the first experiment, ceca (n=360) and grind (n=72) samples were collected from 36 flocks. *Salmonella* prevalence and quantification was molecularly determined using a commercial qPCR assay. In the second experiment, ceca (n=132) and grind (n=132) samples were collected from 22 flocks. *Salmonella* prevalence was determined by qPCR and plating, with positive samples serotyped by serum agglutination.

In Experiment 1, *Salmonella* was detected in the ceca of 22.2% (8/36) of flocks and 13.9% (4/36) of total ceca samples. In grind samples from the same flocks, 36.1% (13/36) of flocks and 30.6% (22/72) of total samples

were *Salmonella* positive. In this experiment, we found concordance between grind and ceca prevalence in 53.1% (17/32) of flocks. *Salmonella* quantity in ceca samples was between 0.45 and 1.34 log CFU/ml (n=5) while grind samples ranged from -1.4 and 2.54 log CFU/ml (n=12). These results show an important contrast between *Salmonella* prevalence and quantification in ceca and grind samples.

In Experiment 2, *Salmonella* was detected in ceca of 22.7% (5/22) flocks and 9.1% (12/132) of total samples. In grind samples 59.1% (13/22) of flocks and 25.8% (34/132) of samples were *Salmonella* positive. As seen in Experiment 1, *Salmonella* prevalence in ceca was significantly lower than in grind samples ($p = 0.0353$, two-sample t-test), with grind samples being 2.8 times more likely to contain *Salmonella* than ceca. Additionally, in 45.5% (10/22) of flocks, serovars were detected in grind samples that were not present in ceca from the same flock. Overall, three serovars were found in ceca but seven serovars were found in ground turkey, demonstrating that ceca fail to predict the serovar complexity and makeup observed in grind samples.

The results of this study demonstrate the underperformance of ceca in predicting ground turkey *Salmonella* prevalence and serovars, and therefore may indicate that the turkey industry's reliance on ceca as a predictive sample of *Salmonella* is inadequate.

Key Words: *Salmonella*, Turkey, Ceca, Processing, Surveillance sampling

P254 Assessment of environmental sampling for determining *Salmonella* prevalence and load in broiler houses Marco Reina*^{GS}, Emily Cason, David Ayala-Velastegui, Nikki Shariat *University of Georgia*

New poultry industry initiatives have introduced "directed processing" as a logistical strategy to reduce *Salmonella* cross-contamination in processing plants. This risk-based approach relies on accurate on-farm testing to determine the associated risk of each flock and determines slaughter processing order (from lower-risk to higher-risk). The aim of this iterative study was to evaluate various environmental sampling methods to assess *Salmonella* prevalence and load in broiler houses. In Phase 1 (24 houses, 10 farms), boot-socks (n=2/house), electrostatic pads wrapped on a paint roller (n=2/house), feather swabs (n=12 birds) and cloacal (n=12 birds/house), and grabs of feces and litter (n=2 composites each/house) were evaluated. In Phase 2 (16 houses, 7 farms) boot-socks (n=2/house), boot-socks wrapped on a paint roller (n=2/house), and feather swabs (n=16 birds/house) were evaluated. *Salmonella* prevalence was determined by qPCR in parallel with selective culture. *Salmonella*-qPCR-positive samples were quantified by qPCR from a 10-hour primary enrichment, and Ct values reported. Fisher's Exact Test was performed to establish differences in prevalence and ANOVA with Tukey's HSD was used for Ct mean separation. For Phase 1, there was no difference among sample types in prevalence by culture ($p=0.44$) whereas there were differences when molecular screening was used ($p=0.015$). The best performing sample types were: boot-socks (42/48 by culture and 41/48 by qPCR), feather swabs (42/48 and 36/48), and electrostatic pads (35/48 and 34/48). *Salmonella* load differed across sample types ($p=0.014$) but not on these three sample types. Similarly, for Phase 2, there were no differences by culture ($p=0.35$) but were by qPCR ($p=0.0004$). Here, boot-sock rollers (31/32 and 28/32) and traditional boot-socks (31/32 and 28/32) were the best performing sample types. The qPCR screen showed lowest prevalence from feather swabs (56/64 and 45/64) in this phase. For *Salmonella* load, there was no significant difference between sample types ($p=0.47$) for phase 2. These findings demonstrate that sample type can influence the recovery of *Salmonella* and underscores the importance of considering sample type and screening methodology for *Salmonella* assessment in broiler houses.

Key Words: *Salmonella*, sampling, prevalence, broilers, qPCR

P255 Investigation of the immune responses of *Salmonella* Enteritidis challenged broiler chickens fed a dietary yeast fermentate Allison Milby-Blackledge*^{1GS}, Yuhua Farnell¹, Audrey McElroy¹, Christi Swaggerty², Leticia Pacheco³, Robson Barducci³, Anderson Santos³, Joao Koch³, Thaila Putarov³, Morgan Farnell¹ ¹*Department of Poultry Science, Texas A&M AgriLife Research*, ²*United States Department of Agriculture, Southern Plains Agricultural Research Center*, ³*Biorigin Brazil*

The development of avian immune tolerance to *Salmonella* is characterized by a network of host-pathogen interactions, including modulation of immune cells and cytokine responses. Understanding tolerance is crucial for developing strategies, like antibiotic alternatives, to combat *Salmonella* infection in poultry. In this study, we hypothesized that the administration of a second-generation prebiotic, HyperGen[®] (HG), would improve immune function in young broilers challenged with *Salmonella* Enteritidis (SE). The objectives of this study were to assess the effects of the dietary yeast fermentate product on serum cytokines, IgM, and lipopolysaccharide (LPS) levels of SE-infected broilers (n = 10/treatment). Day-of-hatch broilers were randomly assigned into a two by four factorial design of unchallenged and challenged treatments across eight floor pens assigned to specific diets. The diets consisted of: 0 kg/MT (control), 0.5 kg/MT, 1.0 kg/MT, and 1.5 kg/MT of HG. On day seven of age, broilers were orally gavaged with either 0.5 mL of sterile PBS for unchallenged treatments or 0.5 mL of 9.70×10^6 CFU/mL of SE for challenged treatments. One week post challenge, birds were euthanized, and blood was taken by cardiac puncture for serum extraction. Data were analyzed via a one-way ANOVA with an $\alpha = 0.05$. Increased expression of *pro-inflammatory cytokines*-interferon gamma (IFN γ ; $p = 0.0108$), interleukin-6 (IL-6; $p = 0.0138$), and IL-16 ($p = 0.0088$); *anti-inflammatory cytokines*- IL-10 ($p = 0.0064$) and IFN α ($p = 0.0506$); *chemokines*- macrophage inflammatory protein-1 β (MIP-1 β ; $p < 0.0001$), and MIP-3 α ($p = 0.0050$); and *growth factors*-vascular endothelial growth factor (VEGF; $p = 0.0317$) were observed in unchallenged and challenged HG inclusions. No significant differences ($p > 0.05$) were observed in IL-2, IL-21, MIP-3 α , IgM, and LPS. However, IL-10 is currently understood to function as a regulatory cytokine, implying that the observed increase in expression may indicate the suppression of the pro-inflammatory response. Notably, the heightened concentration of VEGF suggests potential tissue regeneration. Overall, these data suggest that HG modulates the host immune response.

Key Words: antibody, immune response, poultry, *Salmonella* Enteritidis, yeast fermentate

P256 Core genome-based phylogeny of *Salmonella* isolated from various stages of No-Antibiotics-Ever (NAE) broiler complex Yagya Adhikari*^{1GS}, Matthew Bailey¹, James Krehling², Luis Munoz¹, Pankaj Gaonkar², Steven Kitchens², Stuart Price², Jeff Buhr³, Dianna Bourassa¹, Kenneth Macklin⁴ ¹*Department of Poultry Science, Auburn University*, ²*Department of Pathobiology, Auburn University*, ³*USDA ARS Poultry Microbiological Safety and Processing Research Unit*, ⁴*Department of Poultry Science, Mississippi State University*

Salmonella is the leading bacterial cause of foodborne infections in the United States. With the aim to isolate and genetically evaluate isolated pathogens, a total of 840 samples were collected from 2 pullet farms, 4 breeder farms, a hatchery, 9 broiler farms, transport and a processing plant of a No-Antibiotics-Ever (NAE) broiler complex. Samples were analyzed with a 3M-Molecular Detection System (MDS) for rapid screening of *Salmonella* and suspect-positive samples were further processed for confirmation. From pure isolates, DNA was extracted, and sequencing was performed using Illumina Miniseq. Pre-processing of raw reads was conducted with Fastqc, BBduk tools, and SPADEs. In addition to genomes from this study, 39 *Salmonella* genomes were considered from NCBI based on the environmental sources such as swab samples, feces, soil samples and water samples to build a core genome-based phylogeny. Data were analyzed using R version 4.3.1 using Generalized Linear Model for binomial distribution. The Odds ratio and confidence limits were calcu-

lated for stages of production and sample types based on MDS results. The level of significance was measured at $\alpha=0.05$ and 95% Confidence Limits. Altogether 4.52% (38/840) of total samples were positive on MDS and 16 of which were also positive on culture. The odds of occurrence of pathogen were not statistically significant ($P>0.05$) among stages. However, boot swabs and sponge-stick swab samples were useful in predicting the occurrence of pathogen. The serotypes identified were Rough_O:r:1,5, Barranquilla, Kentucky, Liverpool, Luciana, Enteritidis and Typhimurium. Interestingly, from phylogeny results, *S. Liverpool* and *S. Kentucky* isolates obtained from water sample near the entrance of broiler houses were genetically related to isolates from fly paper placed in live birds loading dock of processing plant and from sponge-stick swab sample of floor of transport truck respectively. Moreover, four *S. Barranquilla* isolates that were obtained from fly papers inside and outside the broiler houses were genetically related. With these findings, it can be inferred that surroundings of poultry-houses and facilities include various risk factors that can transmit *Salmonella* into the poultry complex, and hence, potentially to the consumers.

Key Words: Core genome, phylogeny, Salmonella, broiler, serotype

P257 Effect of diet glucose oxidase and Bacillus-DFM on vaccination against *S. Enteritidis* in broiler chickens Rubén Merino-Guzmán^{*1}, G. Madeleine Cardoso-Luna¹, Juan Latorre², Roberto Señas-Cuesta², Billy Hargis², Guillermo Téllez-Isaias² ¹*Departamento de Medicina y Zootecnia de Aves, Facultad de Medicina Veterinaria y Zootecnia, UNAM, 2*Department of Poultry Science, University of Arkansas

Feed additives are used in poultry with the aim of improving the chicken's performance and reducing the use of antibiotics. Direct-feed microbial (DFM) and glucose oxidase have been used with those purposes. Salmonella infection is a constant threat for poultry, so vaccination is a measure to diminish the potential transmission of Salmonella to the human consumer. The objective of this study was the evaluation of the effect of glucose oxidase (GO) supplementation and a Bacillus probiotic applied directly to the feed (Bacillus DFM) on Salmonella Enteritidis infection and intestinal integrity in broiler chickens vaccinated against Salmonella. Broiler chickens, A total of 126 one-day-old broiler chickens were randomly assigned to four groups: a control group receiving a standard commercial diet, a positive control group that also received a standard commercial diet and was challenged with 10^8 *Salmonella* Enteritidis, one treatment group receiving the Salmonella vaccine at 1 d-o and one group receiving the Salmonella vaccination at 1 day of age and the diet supplemented with GO (100 U/Kg) - Bacillus-DFM. The experiment spanned over 21 days. At 20 days old, the positive and both vaccinated groups were orally challenged with *Salmonella* Enteritidis to a concentration of 10^8 cfu. Twenty-four hours later, samples for Salmonella colonization (cecal tonsils, CCT) and invasion of internal organs (liver and spleen, LS) were taken. Salmonella Enteritidis was absent in samples from the negative group, whereas the positive group had 92% of CCT positive for *S. Enteritidis* (mean= 2.47 Log CFU/g) and 25% of LS Salmonella-positive (mean= 0.67 Log CFU/g). When compared to the control, in the vaccine-only group *S. Enteritidis* incidence decreased ($P < 0.05$) by 25% and 1.71 Log CFU/g in CCT. No difference in Salmonella counts was seen between the positive group and the one receiving the Salmonella vaccine and the dietary GO-Bacillus DFM supplementation. A single Salmonella vaccination reduced the number of positive broiler chickens after challenge as well as the pathogen concentration; however, the simultaneous dietary supplementation of glucose oxidase and Bacillus DFM didn't improve the protection conferred by the vaccine.

Key Words: Glucose oxidase, Bacillus DFM, Salmonella Enteritidis, broiler chickens

P258 Assessment of the effects of feed sanitizer Finio and organic acid Fortrol on *S. typhimurium* contamination in soybean meal, canola meal, and meat and bone meal Callie Selby*, Enrique Montiel, Cheryl Shaffer, Nicole Holcombe, Jason Sands, Jose Ramirez *Anitox Corporation*

Protein meals are a co-product from other agricultural sectors that are often utilized in monogastric diets. These products increase sustainability while also providing economically advantageous protein sources for feed formulation. Soybean meal (SMB), canola meal (CM), and meat and bone meal (MBM) are utilized globally as protein meals in monogastric diets and are typically a target for contaminants, including *Salmonella*. Many feed producers treat finished feed products with sanitizers or organic acids functioning as feed acidifiers. However, treating raw materials at intake could enhance microbial control in finished feed and prevent cross contamination within the feed mill. Different protein meals have different chemical composition and physical forms, therefore, efficacy could differ between meals. In the current study, Finio, a synergistic blend of phytochemicals and carboxylic acids and Fortrol, an organic acid utilized to reduce microbial loads in feed, were evaluated for their efficacy to reduce *Salmonella* in different protein meals and create a separate dose curve for each raw material. 990 g of each raw material was inoculated with a dried *S. typhimurium* (ST) inoculum at 1×10^8 cfu/g. Post inoculation, the raw material was treated with selected product and dose. 24 hrs post treatment, a 10 g feed sample was collected ($n=10$ samples/jar), diluted in sterile saline, and spread plated on XLT-4 media. ST cfu/g were reported and analyzed via ANOVA in JMP Pro 17. Means were further separated via Tukey's Multiple Range Test. While significant differences ($P<0.0001$) were observed in both products in all raw ingredient substrates, different trends were observed in each raw materials dose response curve. These data lead researchers to hypothesize that different raw ingredients or ratios of ingredients within formulations could respond to different chemistries differently, possibly leading to customizable feed sanitation programs based upon geographic location and nutritional needs of the animals. Research is to be continued to validate these findings and investigate possible combinations of products to enhance feed sanitation in monogastric diets

Key Words: raw materials, feed sanitizer, organic acid, salmonella

P259 Longitudinal Salmonella surveillance in broiler breeder flocks Amy Siceloff^{*1GS}, Sean Nolan², Nikki Shariat¹ ¹*Poultry Diagnostic and Research Center, University of Georgia, 2*Nolan Integrated Pest Control and Management (NIPCAM) Group

Successful *Salmonella* control during broiler production relies on robust biosecurity and an appropriate surveillance platform, which includes monitoring breeder flocks. CRISPR-SeroSeq is a PCR-based, next-generation sequencing tool that exploits the native *Salmonella* CRISPR sequences to detect and determine the relative frequency of multiple serovars in a sample. This deep serotyping approach overcomes significant limitations of traditional *Salmonella* isolation.

Eight breeder flocks from two complexes were sampled over a 65-week production period. Pullets were sampled at weeks 14 and 21 and breeder flocks were sampled every four weeks, and weekly during peak production (weeks 29-31). Two bootsock pairs were collected for each pullet and breeder house and *Salmonella* prevalence determined by culture isolation. Rodents (mice and roof and Norway rats) were captured from breeder farms and analyzed for *Salmonella*. A generalized additive mixed effects model showed the expected marginal mean *Salmonella* prevalence for pullet and breeder flocks peaked around 20 and 38 weeks, respectively, indicating that these time points would be optimal for surveillance. Deep serotyping showed that 38% (59/154) samples contained multiple serovars (average of 1.4 serovars/sample), and six and 16 serovars were identified from Complex A and B, respectively. Using the Hutcheson t-test, the Shannon diversity index based on serovar identity was significantly different between the two complexes ($p < 0.05$). In rodents, three and six

serovars were found in Complex A and B, respectively, including three serovars also found on bootsocks from the same farms/houses.

This is the first longitudinal study of *Salmonella* incidence in breeder flocks in the United States. The data show that multiseroovar populations often occur, and the increased resolution of CRISPR-SeroSeq can support development of improved *Salmonella* control strategies based on transmission patterns. The difference in serovars found across complexes may be attributed to management practices as Complex A employs a 3rd party integrated pest control company while Complex B relies on growers for pest control. This study highlights the importance of maintaining on-farm biosecurity to limit *Salmonella* introduction.

Key Words: *Salmonella*, breeders, CRISPR-SeroSeq, surveillance, rodents

P260 Evaluation of acetylsalicylate acid in turkeys infected with coccidia Aaliyah Gore*^{GS}, Rocio Crespo *North Carolina State University*

Acetylsalicylate acid (aspirin) is a common NSAID used in human and veterinary medicine in ill patients. This drug has been previously used in poultry production for its antipyretic, anti-inflammatory, and analgesic properties. There is limited information on the effectiveness of acetylsalicylate acid application in turkey production. The objective of this study was to investigate the effect of acetylsalicylic acid on the physiological and behavioral response of turkeys with induced coccidial enteritis. A total of 24, five-week-old, female turkeys were divided into 4 groups: NN=no coccidia or aspirin, NA= aspirin only, CN=coccidia only, and CA=coccidia + aspirin. On day 1 groups CN and CA were given 100x the dose of a commercial turkey vaccine and groups NN and NA received water via gavage. Starting 48 hours post inoculation and every 12 hours for 7 days, the turkeys from groups NA and CA were given aspirin (50mg/kg) via gavage, and groups NN and CN were gavage with water based on their group assignment. All the birds were weighed, and cloaca temperatures were collected daily. From each group, blood was collected from two randomly selected birds daily. At the end of the trial, a necropsy was performed, and tissue samples were collected for histology. Data were analyzed via ANOVA in JMP Pro 16, with a significance level of $P < 0.05$. No significant differences were observed in the temperature, or blood parameters between the groups. Daily body weight change was significantly highest in the NN group, followed by NA and NA, and lowest in the CN group. Multifocal lymphocytic infiltration in the parenchyma and tubular mineralization were observed in the turkeys from NA and CA groups. These changes suggest that aspirin has a toxic effect in the turkeys. From this study, it can be concluded that the dose of aspirin, at 50mg/kg BID had minimal to no effect on reducing inflammation associated with enteritis in turkeys. Additionally, the administration of aspirin can have a negative effect on bird health and result in toxic changes to the kidneys.

Key Words: Turkey, Acetylsalicylate acid, Aspirin

P261 Chicken cecal enteroids as a model for studying *Eimeria tenella* development and host-parasite interaction Bingqi Dong*^{GS}, Alberta Fuller, Rami Dalloul *University of Georgia*

Coccidiosis is a major enteric disease caused by apicomplexan parasites of the genus *Eimeria* and significantly impacts the poultry industry. *Eimeria tenella* is one of the most prevalent and problematic field species and causes severe lesions in cecal tissues leading to reduced performance, predisposition to secondary enteric infections, and bird mortality. Due to its complexity, the completion of the life cycle of *E. tenella* in intestinal or other epithelial cells is not well-established. Therefore, we developed an apical-out chicken cecal enteroids system to investigate *E. tenella* development and host-parasite interaction ex vivo. Cecal tissues were collected from day of hatch broiler chicks and their crypts/villi were isolated and frozen in liquid nitrogen until further analysis. Approximately 100/mL of cecal crypts/villi were cultured floating in organoid-specific medium in a 24-well plate and half of the medium was replenished every other day.

Approximately 1×10^5 *E. tenella* sporozoites were added to each well on the second day of culture. The enteroids were collected for immunohistochemistry at 2, 5, and 8 days post-inoculation, fixed with 4% paraformaldehyde, blocked with 10% normal donkey serum, and then stained with primary antibody at 4°C overnight. The next day, goat anti-mouse FITC secondary antibody, 4',6-diamidino-2-phenylindole (DAPI) and Alexa Fluor conjugated Phalloidin were incubated with the fixed enteroids to visualize *E. tenella*, nuclear DNA and F-actin, respectively. Enteroids were mounted in ProLong® Diamond Antifade Mountant and visualized using LSM 900 Confocal Microscope and analyzed using ImageJ. Our results indicated that chicken cecal organoids have viability and functionality for at least 9 days in culture. Further, the enteroids were successfully infected with *E. tenella*. Immunohistochemistry results revealed the presence of intermediate life stages of *E. tenella* but without definitive identification of the specific life stages; stage-specific antibodies are needed for further analysis. These results demonstrate that the chicken cecal enteroids make a suitable model to study host-parasite interaction ex vivo, and could be a useful tool for vaccine testing and therapeutics development for coccidiosis control.

Key Words: enteroid, *Eimeria tenella*, host-parasite interaction, coccidiosis, chicken

P262 In ovo administration of a postbiotic regulates the differential expression of key intestinal genes in broilers during subclinical necrotic enteritis Bingqi Dong*, Rami Dalloul *University of Georgia*

Necrotic enteritis (NE), an enteric disease caused by *Clostridium perfringens*, results in damage to the intestinal epithelial lining disrupting its function, nutrient absorption and utilization. This study evaluated the effects of in ovo administration and post-hatch water application of a postbiotic on the mRNA abundance of tight junction proteins, mucin-2 (MUC2), and nutrient transporter genes in jejunal tissues of broilers during subclinical necrotic enteritis. At embryonic day (D) 18, Ross 708 eggs were injected with 0.2 mL of either water or postbiotic. Male chicks (n=288; 12 birds/cage; 6 replicate cages/group) were assigned to one of the following post-hatch water treatments: 1) NC (in ovo water injection, drinking water, no challenge); 2) NC+ (NC with NE challenge); 3) PIW (in ovo postbiotic injection, postbiotic in drinking water, no challenge); and 4) PIW+ (PIW with NE challenge). On D14, all birds in NC+ and PIW+ were orally gavaged with 3,000 *E. maxima* sporulated oocysts followed by two doses of $\sim 1 \times 10^8$ CFU/mL of *C. perfringens* on D19 and D20. Jejunal tissues were collected to assess mRNA abundance of target genes from one bird/cage on day of hatch (DOH), D7, D14, and D21. From DOH to D14 and on D21, data were analyzed by student's t-test, and two-way ANOVA (water treatment, NE challenge, water treatment by NE challenge interaction), respectively, with significance set at $P < 0.05$. mRNA abundance of CLDN1 and OCLN, PepT1, and GLUT2 were significantly greater in birds with postbiotic supplementation compared to NC on DOH, D7, and D14, respectively ($P < 0.05$). On D21, a significant interaction was observed in mRNA abundance of ZO1 between water treatment and NE challenge with a greater mRNA in PIW+ compared to the other treatments. This challenge potentially indicates the role of ZO1 in stabilizing the tight junction barrier in mitigating tissue damage from the NE challenge. mRNA abundance of MUC2 and the nutrient transporters GLUT2 and SGLT1 was greater ($P < 0.05$) in the non-challenged groups (NC and PIW) compared to the challenged groups (NC+ and PIW+) on D21. Overall, in ovo administration followed by water supplementation of this postbiotic helps to improve gut integrity, mucin secretion, and nutrient absorption in the jejunum.

Key Words: postbiotic, necrotic enteritis, tight junction, nutrient transporter, in ovo

P263 Phytogetic blends elicit positive effects on broiler immune response during a necrotic enteritis challenge model Candice Blue*¹, Ashley Wagner², Bertrand Médina², Ivan Girard², Rami Dalloul¹ ¹University of Georgia, ²Probiotech International Inc.

In broilers, natural feed additives can effectively mitigate necrotic enteritis (NE) by improving feed conversion and helping maintain the integrity of the epithelial barrier. This study aimed to evaluate the effects of phytogetic blends on mRNA abundance of pro-inflammatory and regulatory cytokines during a co-infection with *Eimeria maxima* (day [d] 14) and *Clostridium perfringens* (d 19). Day-old Ross 708 male chicks (n=600) were allocated to one of four treatment groups (6 replicate pens, 25 birds/pen): negative control (NC) fed a corn-soybean meal diet; positive control (PC) fed NC + 15 ppm Avilamycin and 125 ppm Amprolium, and two phytogetic additive groups PHY1 (Alterna®) and PHY2 (Alterna® + Synbiotec®). PHY1 was fed NC + Alterna at 0.4 kg/MT during the starter and grower phases and 0.3 kg/MT during the finisher phase. PHY2 was fed NC + Alterna® and Synbiotec® at an inclusion rate of 0.4 and 0.5 kg/MT, respectively, during the starter and grower phases, 0.3 and 0.25 kg/MT during the finisher phase. On d 14, all birds were orally gavaged with 2,000 *E. maxima* sporulated oocysts, followed by one dose of approximately 1×10⁸ CFU of *C. perfringens* on d 19. On d 14, 21, and 42, jejunal samples were collected to assess mRNA abundance of interleukins (IL) 1β, 10, and 12B, interferon (IFN) γ, tumor necrosis factor (TNF) α, and annexin (ANXA) 1. The data were analyzed using ANOVA (JMP, Pro 16) and significance (P ≤ 0.05) was determined by the LSD test. mRNA abundance of TNFα, IL10, and ANXA1 was significantly lower in PHY2 compared to all treatments on d 21. Meanwhile, on d 42, PHY1 and PHY2 showed greater mRNA abundance of IFNγ, TNFα, IL10, ANXA1, and IL12B. During peak infection (d 21), a decrease in various inflammatory cytokines could enhance tolerance against infection, while the release of anti-inflammatory mediators can resolve inflammation and restore homeostasis. Previously, incorporating Alterna® and Synbiotec® resulted in enhanced performance, reduced pathology, and a positive impact on tight junction proteins. The current findings further demonstrate the usefulness of phytogetic blends in this NE model with their potential to diminish the intrusion of pathogens and enhance broilers' ability to counteract the adverse effects of NE.

Key Words: Phytogetics, Necrotic enteritis, Immune response, Cytokines, *C. perfringens*

P264 Immunization of broiler chickens with CpG-Oligonucleotide Recombinant HVT–Laryngotracheitis in ovo Vaccine confers protection against a viral challenge Carissa Gaghan*¹IGS, Matthew Browning¹, Abdelhamid Fares¹, Mohamed Abdul-Careem², Isabel Gimeno¹, Raveendra Kulkarni¹ ¹North Carolina State University, ²University of Calgary

In recent years, incidences of Infectious laryngotracheitis (ILT), primarily in the broiler flocks, have been on the rise leading to significant economic losses. Although the live attenuated chicken-embryo-origin (CEO) vaccine can effectively control ILT, the vaccination is associated with the risk of inducing mortality and severe respiratory reactions as the CEO vaccine strain can regain virulence through bird-to-bird passages. The recombinant ILT vaccines such as recombinant herpesvirus of the turkey-Laryngotracheitis (rHVT-LT), which are approved for *in ovo* delivery, can reduce clinical signs; however, their failure to protect chickens against ILT as effectively as CEO vaccine warrants the need for more efficacious rILT vaccines. Here, we adjuvanted rHVT-LT *in ovo* vaccine with CpG-Oligonucleotide (CpG-ODN) and evaluated the protective efficiency against a wild-type ILT virus (ILTV) challenge at 28 days of age in broiler chickens. Additionally, a CEO vaccine administered in water at 14 days of age was used as a comparative control for protection assessment. The splenic immune gene expression as well as cellular responses were also assessed. The results showed that both the rHVT-LT adjuvanted with CpG-ODN and the CEO vaccinations provided significant protection against the

ILTV challenge, and that the level of protection induced by both vaccines was found to be statistically similar. Furthermore, the protected birds had a significantly upregulated expression of interferon (IFN)γ or interleukin (IL)-12 cytokine genes, as well as a significantly higher frequency of γδ T cells and activated CD4+ or CD8+ T cells compared to the unvaccinated-ILTV challenge control, indicating a Th1 mediated response. Together, our findings suggest that CpG-ODN can be used as an effective adjuvant for rHVT-LT *in ovo* vaccination to induce protective immunity against ILT in broiler chickens.

Key Words: CpG oligonucleotide, adjuvant, chickens, infectious laryngotracheitis, vaccine

P265 Study investigating the interaction of IBDV (AL2) and IBV (DMV/1639) challenges in commercial broilers based on infectious bronchitis vaccination status Kalen Cookson*, John Dickson, Jon Schaeffer Zoetis

Infectious bursal disease virus (IBDV) infections within the first 2 weeks of age cause significant immune suppression while infections after 3 weeks are less severe and more transient. The purpose of this study was to see if a 3-week IBDV challenge could significantly reduce immunity against an infectious bronchitis virus (IBV) challenge given 4 days later. Study Design: Ross 708 broilers with high maternal antibodies to IBDV (7,480 Idexx-XR) were vaccinated at day of hatch (Vax) with a full dose of Mass + GA08 IB vaccines by coarse spray or (half) remained unvaccinated (NoVax), then placed into a dozen horsfal isolators. At 21 days of age, half of all birds were challenged with IBDV AL2 (3.5EID50) resulting in four groups: Vax/noIBD, NoVax/noIBD, Vax/AL2, NoVax/AL2. At 25 days of age all birds were challenged with 3.5EID50 DMV/1639. At 31 days, birds and bursas were weighed, bursas were measured using bursameters and tracheas were collected for real-time IBV PCR and histopathology scoring. All hypotheses were conducted at the p≤0.05 level of significance with the Shaffer simulated method used to adjust for multiple comparisons. Results: The 21-day AL2 challenge “took” as mean bursa to body weight ratios (B:BW) and bursameter scores were significantly lower than controls (1.8 vs. 1.0 and 5.8 vs. 4.4, respectively). Mass + GA08 vaccination resulted in significant reductions in IBV infections after DMV/1639 challenge based on Mean Ct (36.1 vs. 27.4) and Ct-35 protection (52% vs. 0%). Vaccinated birds also had significantly higher values if they were not also IBDV challenged based on Mean Ct (37.4 vs. 34.7) and Ct-35 protection (74% vs. 30%). Tracheal protection from inflammation and damage was also significant in IB vaccinates. AL2 challenge resulted in significantly enhanced IB lesions in non-vaccinated birds and numerically enhanced them in IB vaccinates. Conclusions: In this study, a 21-day IBDV challenge in commercial broilers with high day of age maternal antibodies resulted in bursal atrophy and led to increased tracheal inflammation and viral loads from an IBV challenge happening 4 days later. Given how crucial IBV control is today—especially during winter respiratory season—it’s easy to see why IBD vaccination of broilers continues to grow.

Key Words: IBD, AL2, vaccination, IBV, DMV/1639

P266 Evaluation of Feed Sanitation using feed sanitizers Termin-8 and Finio on the control of Necrotic Enteritis in Broiler Chickens Cheryl Shaffer¹, Dan Moore², Enrique Montiel¹, Nicole Holcombe¹, Callie Selby*¹ ¹Anitox Corporation, ²Colorado Quality Research

Necrotic enteritis (NE) continues to be one of the most economically impactful diseases for commercial poultry globally. Caused by the pathogen *Clostridium perfringens*, NE has been associated with the disease in broiler breeder pullets, broilers, and laying hen pullets. *C. perfringens* is a common microbe in the gastrointestinal tract of poultry, however, overgrowth results in dysbiosis and clinical disease. While previous management techniques included antibiotics, researchers and poultry producers now turn to alternative practices to prevent NE in the field. In a previous

study, feed sanitation using Termin-8 was shown to improve performance in broiler chickens under a necrotic enteritis challenge model. In the current study, two feed sanitizers, Termin-8 and Finio, were utilized to evaluate the impact of broiler performance under a necrotic enteritis challenge model. Broiler chickens were vaccinated against coccidiosis on day-of-hatch and challenged with *C. perfringens* on d17. Birds were fed a starter and grower diet sanitized with with Termin-8, a formaldehyde + propionic acid + terpene -based feed sanitizer or Finio, a synergistic blend of phytochemicals and carboxylic acids. All data was subjected to ANOVA via JMP pro17. Significant differences between means were determined by Tukey's Multiple Range Test at $P < 0.05$ as the significance level. Broiler performance, mortality, and lesion scores were measured up to d28. Significant reductions in feed conversion ($P < 0.0001$), mortality ($P < 0.0001$), and NE lesion scores ($P < 0.0001$) and increases in BWG ($P = 0.0003$) were observed in broilers fed a sanitized diet under challenge conditions. These data suggest that feeding broiler chickens a diet with reduced microbial loads can lessen the impact of NE, further increasing performance and livability in broiler chickens

Key Words: feed sanitation, broiler, necrotic enteritis

P267 Screening dusts for potential activity against Litter Beetles Carla Guardado*^{1GS}, Ruediger Hauck^{1,2}, Kenneth Macklin³, Arthur Appel⁴ ¹Department of Poultry Science, Auburn University; ²Department of Pathobiology, Auburn University; ³Department of Poultry Science, Mississippi State University; ⁴Department of Entomology and Plant Pathology, Auburn University

Litter beetles (*Alphitobius diaperinus*) are the most prevalent insect species in poultry houses. They are capable of acting as both a vector and a reservoir for a variety of pathogens. Using traditional insecticides for control has limitations due to health concerns and the development of insecticide resistance. This study investigated the impact of various dust treatments on the behavior of larvae and adult beetles as potential alternatives to traditional insecticides.

Ten larvae or adult beetles were placed in each of six plastic cups (5 1/2 oz). Dusts were tested by applying 2.5 mL dosages of each dust treatment to the experimental cups. To ensure uniform dust covering, the cups were sealed and gently shaken. Additionally, a control group of six cups that were not treated was included. Observations were made every 8 hours for 72 hours. Dead beetles were counted, and any disruption of the dust or unusual behavior of the larvae and beetles was noted. If appropriate, the median lethal time (LT₅₀) was calculated by probit analysis.

Larvae were killed by zeolite clay, talc, 85% diatomaceous earth, morrocon rhassoul clay, and silicon dioxide insecticide. The LT₅₀ of these dusts was between 24 and 72 hrs. Some beetles were killed when zeolite clay powder was used. Some larvae were killed as well when kaolin clay and boric acid were tested with an LT₅₀ between 40 and 72 hrs. When beetles were tested with 85% diatomaceous earth, boric acid, and powdered morrocon rhassoul clay, few were killed with an LT₅₀ between 32 and 72 hrs. Bentonite clay, biochar, ground gypsum, and walnut powder did not show any activity against either larvae or beetles; neither did the talc powder against the beetles nor the kaolin clay against the larvae. The experiments showed that various dust treatments had an impact on the activity of the beetles over each phase of the observation period. While some treatments produced less noticeable activities, others seemed to discourage insect activity.

These results indicate the potential of dust treatments for beetle infestation control, advancing eco-friendly pest management.

Key Words: dust treatments, behavioral impact, pest control, poultry, parasites and diseases

P268 Assessment of Two Enterococcus cecorum Challenge Strains when administered after a Respiratory Vaccine in Broiler Chickens

Matthew Jones*¹, Charles Hofacre¹, Jennie Baxter¹, M. Suyemoto², Enid McKinley³, Jodi Delago³, Alexandra Smith³ ¹Southern Poultry Research Group, ²Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University; ³Arm and Hammer Animal Nutrition and Food Production

As the US broiler industry shifted towards less antibiotic usage one observed change was an increase in leg issues including femoral head necrosis and infection of the free thoracic vertebra (FTV). While multiple bacteria can infect these tissues, *Enterococcus cecorum* (EC) is often isolated from these lesions. EC has also been isolated from the pericardium of young chicks. To better understand the manifestation of this disease, two strains of pathogenic EC were evaluated side-by-side in one BSL-2 room to monitor septicemia, gross lesions, and mortality. Forty male Ross broilers were placed in each pen on fresh pine shavings. Each of three pens was vaccinated by coarse spray with a commercial infectious bronchitis and Newcastle virus vaccine at placement. One group served as an unchallenged control, one group was orally gavaged with EC strain 5872 isolated from an infected pericardium from a field case (3.0×10^7 CFU/bird), and the last group was orally gavaged with the well characterized SA3 EC isolated from an FTV lesion (1.5×10^7 CFU/bird). Spleens were aseptically collected from 5 birds in each group on day of test (DOT) 10, 14, and 17. On DOT 21, FTV swabs and spleens were collected from all remaining birds. All samples were evaluated for EC prevalence.

Splenic EC prevalence in the unchallenged control and the SA3 group increased from DOT 10 to DOT 17. Overall, the SA3 group had the highest prevalence 12/35 (34%) followed by the unchallenged group 10/36 (28%), and the 5872 EC at 10/39 (26%). Prevalence in the FTV samples was lower than the spleens at 20%, 15%, and 12% for the control, SA3, and 5872 groups, respectively. EC was isolated from the femoral head of two mortality in the 5872 group. Six birds had pericarditis at termination and three (one in each treatment) cultured EC positive. The presence of EC in the unchallenged treatment indicates lateral spread despite biosecurity protocols. This EC transmission within the same airspace has been observed by N.C. State researchers in the past. The field pericarditis strain did not result in increased pericarditis over the SA3 strain in this study. The SA3 strain had numerically greater EC prevalence in the spleen and FTV than the field pericarditis isolate.

Key Words: Enterococcus cecorum, Femoral Head Necrosis, Septicemia, Free Thoracic Vertebra, Pericarditis

P269 A comparison of direct-fed microbials containing multiple or single Bacillus strains on the performance and health of broiler chickens exposed to a mixed disease challenge model

Saheed Osho*¹, Kevin Bolek¹, James McNaughton², Kari Saddoris-Clemons¹, Brooke Humphrey¹, Miriam Garcia-Orellana¹ ¹Phibro Animal Health Corporations, ²AH Pharma

In modern poultry production, direct-fed microbials (DFM) are often used to optimize gut health, performance, and meat yield. This study compared the effects of three DFMs: Microlife® Prime (MLP), which contains four *Bacillus* species, and two single-species *Bacillus* products (*Bacillus licheniformis*-BL and *Bacillus subtilis*-BS), each provided at 4 inclusion levels: 0.5×10^6 , 1×10^6 , 1.5×10^6 , or 2×10^6 cfu/g of complete feed. Using a randomized complete block design, birds (n=8,736) were allocated to 14 treatment groups, across 168 pens (12 pens/trt) with 52 mixed-sex broilers per pen. The study consisted of 12 DFM treatments, a non-medicated unchallenged control (NEG), and a non-medicated challenged control (POS). Diets were corn-soybean meal-based, fed in mash form. Birds in the DFM and POS groups were challenged on d 1 post hatch with *Clostridium*, coccidia oocysts (150,000 oocysts per bird via water), and naturally present bacteria from build-up litter obtained from a high mortality farm. Feed intake (FI), body weight (BW), body weight

gain (BWG) and feed conversion ratio were calculated over the 42-d experimental period. On d 42 of age, lesion scores were determined, excreta were collected for oocyst enumeration, and pre-chill weight of breast yield were measured. Data were analyzed with GLIMMIX procedure of SAS. Birds in the POS group, compared to the NEG group, showed ($P<0.05$) reduced BWG (10%), whole breast yield (9%), higher mortality (6 \times), lesion scores (6.7 \times) and oocyst shedding (1.5 \times). At the highest inclusion level, MLP fed birds outperformed ($P<0.05$) single-strain competitors in final BW, BWG, whole breast yield, lesion scores, and oocyst count, and were similar to NEG controls for all these parameters. At the lowest inclusion level, MLP had the lowest ($P<0.05$) mortality, lesion scores, and

oocyst counts, when compared to single strain competitors, highlighting better health. At the 2 intermediate inclusion levels, MLP also had the lowest mortality, lesion scores, and oocyst counts than BS, and was at least numerically better than BL. In conclusion, the multi-strain formulation MLP consistently exhibited enhanced efficacy over single-strain products, translating to improved broiler performance and health indicators, under challenge conditions.

Key Words: Bacillus, broiler chickens, direct-fed microbial, MicroLife Prime, performance

Environment, Management: Animal Well-Being (stress, welfare, behavior)

P270 Automatic detection and scoring of footpad dermatitis in poultry utilizing YOLOv8-FPD models Ramesh Bist^{*IGS}, Xiao Yang¹, Sachin Subedi¹, Bidur Paneru¹, Keshav Bist², Lilong Chai¹ ¹*Department of Poultry Science, College of Agricultural & Environmental Science, University of Georgia,* ²*Department of Electronics and Computer Engineering, Institute of Engineering, Tribhuvan University*

Footpad dermatitis (FPD) stands as a prominent welfare concern, inducing distress, decreasing food intake, and potentially resulting in lameness among laying hens. Detecting FPD in its early stages is essential for identifying strategies to enhance footpad health. This research introduces an innovative method for addressing FPD and automatically assigning scores on a 0-2 scale, ranging from 0 (representing normal footpad health) to 2 (indicating severe FPD). This study's aims were a) to develop an automated FPD assessment model using advanced machine learning techniques, b) to test and compare new YOLOv8 models, and c) to compare the performance of the final model in detecting FPD on original and thermal images. This study used 700 Hy-Line W-36 hens raised in four cage-free housing systems where Electrostatic Particle Ionization (EPI) and bedding material (BM) treatments were ongoing. The GoPro cameras were used to capture the FPD of hens. A thermal camera was used to record and compare thermal and original images, enabling comprehensive data collection for a week through data preprocessing techniques that enhance dataset quality by filtering, separating, and augmenting data. Statistical analysis was done using One-way ANOVA to compare different models and between different FPD scoring. The results highlight YOLOv8I-FPD as the top-performing model, with exceptional precision of 92.8%, recall of 96.6%, mean average precision (mAP@0.50) of 97.0%, and an impressive F1-score of 95.0%. Furthermore, the YOLOv8I-FPD model achieves high mAP@0.50 scores for FPD scoring with FPD 0 (98.0%), FPD 1 (95.0%), and FPD 2 (97.9%), along with an outstanding F1-score of 95.0% for all FPD scores. Importantly, the study explores the potential of thermal imaging for FPD detection, highlighting its superiority over original images. This research underscores the importance of YOLOv8I-FPD as a reliable tool for FPD detection, offering valuable insights to enhance poultry health management. The implications of this study have the potential to transform FPD detection and scoring in poultry farming, leading to improved animal welfare and increased production efficiency.

Key Words: Cage-free housing, Footpad dermatitis, Footpad scoring, Animal welfare, Machine learning

P271 Tracking dustbathing behavior of cage-free laying hens with machine vision technologies Bidur Paneru^{*GS}, Ramesh Bist, Xiao Yang, Sachin Subedi, Lilong Chai *University of Georgia*

Dustbathing (DB) is a natural and functionally important maintenance behavior in birds that realigns feather structures and removes lipids from the skin, which helps to control parasites and prevents feathers from becoming too oily. Among different natural behaviors, DB is one of the important behaviors contributing to hen welfare in the cage-free (CF) housing sys-

tem. DB is a critical indicator for assessing hens' welfare and litter quality in the CF houses. However, the manual observation of DB behavior is laborious, time-consuming, and sometimes imprecise. Therefore, an automated precision method is needed to detect DB in CF-laying hens. The objectives of this study were to a) develop and test a deep learning model for detecting DB behavior; and b) evaluate the performance of the method on hens with different ages. In this study, new deep learning models, i.e., YOLOv8s-DB, YOLOv8x-DB, YOLOv7-DB, and YOLOv7x-DB networks, were developed, trained, and compared in tracking DB behavior in 4 CF rooms (180 hens/room). Statistical analysis was done using one-way ANOVA to compare detection accuracy between different models and between different ages at a significance level of 5%. Results indicate that the YOLOv8x-DB model detected DB behavior with a precision of 88.6%, recall of 89.8%, and mean average precision (mAP_0.50) of 93.8%; the YOLOv8s-DB model detected the DB behavior with a precision 87.6%, recall of 86.1%, and mAP_0.50 of 91.8%; the YOLOv7-DB model detected the DB behavior with a precision of 83.3%, recall of 86.2%, and mAP_0.50 of 89.7%, and the YOLOv7x-DB model detected DB behavior with a precision of 86.2%, recall of 87.2%, and mAP_0.50 of 90.9%. All models performed with over 83% detection precision; however, model performance was affected by equipment interference like drinking lines, perches, and feeders. The YOLOv8x-DB model detected DB behavior with higher accuracy, precision, mAP, and recall than other models. This study provides a reference for CF producers to evaluate DB behavior automatically.

Key Words: Cage-free, dust-bathing, deep learning, precision farming, laying hen

P272 Tailoring feed strategies: A study on breeder pullet behavior with modified diets and probiotic supplements Mazette Croom^{*IGS}, Courtney Daigle², Rosemary Walzem¹ ¹*Poultry Science Department, Texas A&M University,* ²*Animal Science Department, Texas A&M University*

At 3 weeks post-hatch, breeder pullets typically commence feed restriction (FR) in an effort to promote welfare by increasing hen livability, and chick yield. Modified diets and anorexigenic probiotics (P) have been observed to decrease feed intake (FI) during ad libitum consumption. Our goal was to characterize the impact of strain and diet on the feeding behavior of pullets during daily FR. Breeder pullets from two commercial strains (n = 200 birds/strain) were randomly assigned to 1 of 2 dietary treatments (e.g., usual & vehicle P (US-P) or high oleate & active P (OL+P) at a dose of 1x10⁸CFU *E. coli* Nissle 1917/mL/bird/day) and placed into 1 of 4 pens (2.32 m²) resulting in 25 pullets/pen. Daily FR started on d 21 and hen behavior in the pen was video recorded from d 30-32. Video recordings were decoded for feed clean-up time (CT; min) and feeder departure rate (FDR; birds/min) using the BORIS software. A Generalized Linear Mixed Model (PROC GLIMMIX) tested the effects of strain, treatment, and their interactions on the hen behavior. A strain by dietary treatment interaction was observed for CT ($p < 0.05$). Pullets from Strain 1 that were consuming the OL+P diet had a CT 58-min longer than the US-P pullets. The opposite

was observed in Strain 2 where pullets consuming the OL+P diet a CT 13-min shorter compared to the US-P pullets. Pullets from Strain 1 left the feeder more quickly (e.g., a more negative FDR) compared to Strain 2 ($p < 0.05$). These results indicate that pullets from Strain 1 had an FDR that was 68% faster than Strain 2 ($p < 0.05$). The dietary treatment had both a greater and opposite effect on the clean-up times of Strain 1 pullets as compared to Strain 2 pullets. The association (PROC CORR) between CT and FDR ($r = 0.6151$; $p = 0.0112$) illustrated that pullets with prolonged CT departed from the feeder faster. The study underscores genetic differences existing between these two strains with regards to feeding behavior and suggests that monitoring FDR provides a novel parameter to characterize differences in feeding behavior. These observations can guide more effective and tailored feeding strategies for different breeder strains in the future.

Key Words: Broiler Breeders, Feed Restriction, Behavior, Welfare

P273 Comparison of traditional LED lighting program with a Dynamic lighting program on broiler stress, fear, health and growth Parker Watson^{*UG}, Gabrielle House², Aaron Stephan², Gregory Archer¹
¹Texas A&M Agrilife Extension, ²Once by Signify

Poultry are known to be sensitive to light spectrum, intensity, flicker, and photoperiod. Limited research has been conducted on how species-specific lighting programs affect poultry production and welfare. The objective of this study was to evaluate the effects of a dynamic lighting program designed specifically for broilers compared to a traditional lighting program using a standard LED lighting source on stress, fear, health, and growth. Cobb 500 broilers ($n=960$) were reared in environments illuminated either by a white LED (3000K 0-14d; 5000k 15-42d) light using the Cobb guide lighting recommendation (CON) or a dynamic lighting program using a multispectral LED luminaire (Once by Signify, USA) (DYN). The study consisted to two in time replications in which treatments were rotated between experimental rooms. Stress susceptibility was measured using heterophil to lymphocyte ratio (HL) and physical asymmetry of three bilateral traits (ASYM). Behavioral fear response was determined using tonic immobility (TI) and inversion (INV) tests. Production parameters were measured using body weight, feed consumption, pen feed conversion ratio (FCR) and weight corrected FCR (WCT FCR) and average daily gain (ADG). To determine the effects on general health tibial breaking strength (BS) and ash (BA), foot pad scores (FP), eye dimensions (weight, EWT; depth, ED; and width, EW), and complete blood cell count (CBC) were measured. Data were analyzed using ANOVA or Kruskal-Wallis, and $P < 0.05$ was considered a significant difference. DYN had lower stress susceptibility as indicated by both ASYM and HL as values were lower DYN in both compared to CON ($P < 0.05$). D42 BW and ADG were higher in the DYN ($P < 0.05$). FCR and WTC FCR were lower in the DYN ($P < 0.05$). No differences in BS, BA, EWT, ED, or EW were observed between treatments ($P > 0.05$). D42 FP were worse in the CON ($P < 0.05$). No differences ($P > 0.05$) were observed between treatments in CBC measures apart from heterophils and lymphocytes which are related to the stress susceptibility differences observed. The results of this study indicate that DYN lighting programs can improve productivity and welfare compared to traditional lighting programs.

Key Words: Broiler, lighting, behavior, stress, welfare

P274 Impact of perch provision timing on musculoskeletal health of laying hens Shaunie Cruz^{*UG}, Mallory Anderson, Alexa Johnson, Mireille Arguelles-Ramos, Ahmed Ali *Clemson University*

Laying hens can experience a progressive increase in bone fragility due to the ongoing mobilization of calcium from bones for eggshell formation. Over time, this escalates their susceptibility to bone fracture, which can reduce their mobility and cause pain. The provision of perches as an exercise opportunity could potentially enhance bone strength, but the timing of exposure to perches during their development may modulate its

impact. The objective of this study was to investigate the enduring impacts of perch provision timing on the musculoskeletal health of laying hens. A total of 810 pullets were kept in different housing conditions (7 pens/treatment, 29 birds/pen) with either continuous access to multi-tier perches from 0-40 weeks (CP), no access to perches (NP), access to perches during the rearing phase from 0-17 weeks (EP), or solely during the laying phase from 17-40 weeks of age (LP). At 40 weeks of age, 3 birds per pen ($n=84$) were euthanized for computed tomography scans to obtain tibial bone mineral density (BMD) and cross-sectional area (CSA), with further analysis including muscle deposition, tibial breaking strength, and tibial ash percent. Differences within measured parameters across time points and groups were assessed using GLMM with Tukey's Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. Total bone CSA did not differ between treatments ($P > 0.05$), but CP had greater total BMD than NP ($P < 0.05$) with no differences between EP and LP treatments. CP and LP had larger biceps brachii, pectoralis major, and leg muscle groups, as well as greater tibial breaking strengths than EP and NP treatments ($P < 0.05$). CP pens had higher tibial ash percentages compared to EP, LP, and NP ($P < 0.05$). Our results indicate that providing continuous perch access improves musculoskeletal health at 40 weeks of age compared to no access and that late access to perches has a beneficial impact on muscle deposition and bone strength. The possible beneficial impact of access to perches during rearing is not observed at 40 weeks of age, suggesting early access does not have a long-term impact on the musculoskeletal health of laying hens.

Key Words: laying hen, perch provision, musculoskeletal health

P275 Effect of late chick placement on growth performance, relative organ weights, and breast muscle of broiler chickens raised to 21 days of age Jordan Smith^{*UG}, Oscar Tejada *Southern Arkansas University*

An experiment was conducted to evaluate the effects of late chick placement on the growth performance, relative organ weights, and breast muscle of broiler chickens. A total of 80 chicks were randomly allocated into 2 experimental treatments ($n = 4$ pens per treatment; 10 birds per pen). Treatment 1 consisted of chicks placed in the farm 24 hours after hatching and treatment 2 consisted of chicks placed in the farm the same day of hatching. All birds were fed a common corn and soybean meal diet formulated to meet or exceed nutritional requirements. Growth performance, relative organ weights, and breast muscle weights were evaluated on days 7, 14, and 21. No statistical differences were observed in the growth performance during the rearing period. However, treatment two had a non-significant better feed conversion ratio compared to treatment 1 during the whole rearing period ($P > 0.05$). Relative organ weights showed that birds placed in the farm the same day of hatching (T2) had heavier relative weights of liver on days 14 and 21 ($P < 0.05$). Furthermore, the relative weights of the breast muscle of birds from treatment 2 also tended to be heavier compared to the relative weights of the breast muscle from birds from treatment 1 ($P = 0.096$). In conclusion, shorter periods of time between hatching and placement can improve development of internal organs, breast muscle growth, and performance of broiler chickens.

Key Words: Broiler chicken, Relative organ weights, Breast muscle, Chick placement, Growth performance

P276 Comparison between farm hatching and traditional hatching on post-hatch growth performance of broilers raised to 17 days of age Grace Rutherford^{*UG}, Mallory Landreth, Hannah Walters, Oscar Tejada *Southern Arkansas University*

In the broiler industry, it is standard that birds are hatched in a hatchery and then transported to the farm within the first few days of their life. Early in life, traditionally hatched chicks are exposed to the stress of transport, and pathogens, and remain without food and water until 1 to 3 days of age. Therefore, we conducted an experiment to compare traditional hatching versus on-farm hatching of broiler eggs. We had a total of 86 eggs of

a breeder flock that were divided into T1 that were eggs hatched under traditional systems: 18 days in incubator and 3 days in hatcher. T2 eggs were incubated for 18 days and then transfer to hatch at the farm. After hatching weight gain, feed intake, and FCR were measured on days 7, 14, and 17. Statistical analyses were conducted using SPSS version 21. Chickens from eggs hatched at the farm had heavier body weight compared to those hatched under traditional conditions used by the industry ($P = 0.045$). The FI was significantly higher in chickens from T2 associated with higher weight gain ($P = 0.029$). No statistical differences were observed in the rest of parameters measured ($P > 0.05$). The feed conversion ratio was numerically poorer in chickens hatched under traditional conditions compared to those hatched at the farm. These results indicate that on farm hatching is a viable alternative to improve post-hatch performance of broiler chickens.

Key Words: On-farm hatch, Broiler chicken, Growth performance, Incubation

P277 Vocalizations as a flock welfare indicator in the poultry industry. Gregory Fraley, Jenna Schober*, Jeffrey Lucas, Tze Yao Yap, MacKenzie Ulrey, Jill Merritt *Purdue University*

Sounds have been shown to affect the behavior and welfare of captive birds. We wish to build upon this knowledge implement duck vocalizations as a type of environmental enrichment, however, we first need to identify their full repertoire. To accomplish this goal, we utilized Pekin ducks (19 hens and 4 drakes) 35-45 woa to develop a vocal repertoire. We placed 1-4 ducks of varying sexes into a sound chamber for several minutes to record initial vocalizations and to acclimate the ducks to the sound chamber. Once acclimated, various stimuli were used to encourage new vocalizations. Vocalizations were recorded for at least 20 minutes. We found that ducks can produce up to 20 different vocalizations. We designated each call with a unique name that reflected physical sonic attribute using Adobe Audition: e.g., AM, pip, pip-harmonic, honk, and honk-AM. Our results suggest that ducks are significantly affected by environment in their repertoire of calls, and in the spectral properties of calls. The stimuli that were placed in the chamber with the ducks had a significant effect on the rate of calls given by the ducks (repeated measures ANOVA: $F_{6,31} = 8.55, p < 0.0001$). The diversity of their vocal repertoire increased with an increase in the number of hens in the anechoic chamber ($F_{3,26} = 4.66, p = 0.0097$). There was no effect of the number of drakes on the repertoire diversity ($F_{3,26} = 1.39, p = 0.2686$). The implication is that social interactions are more important for females than for males, and this is reflected in the amount and type of information they convey to each other. We ran a canonical discriminant analysis (Proc Candisc, SAS 9.4) to evaluate whether the properties of the calls used by birds changed in different environments. The analysis showed that the spectral properties of the pip-harm call, a vocalization made only by hens, was significantly influenced by the stimuli the hens were given (repeated measures ANOVA, $p = 0.0152$). Within sex, the spectral properties of honks differ among environments. Our results suggest that the hen 'voice' is qualitatively different than the drake 'voice.' Overall, our data imply that there is variation in the spectral analysis of calls that ducks generate under different conditions.

Key Words: Pekin duck, vocalizations, repertoire, welfare

P278 When is a peep a fear peep? Characterization of chicken vocalizations during an isolation fear test. Gregory Fraley¹, Jeffrey Lucas¹, Jenna Schober*¹, MacKenzie Ulrey¹, Anne Troyer¹, Karen Christensen² ¹*Purdue University*, ²*Tyson Foods*

The isolation fear test is widely used to determine welfare states in poultry. The belief is that if a chicken with negative welfare is placed in an isolated environment, the bird will vocalize for a longer period of time compared to a chicken from a good welfare environment. However, we observed that broilers make multiple types of vocalizations during these tests. Thus, we set out to characterize the types of vocalizations made by 12 broil-

ers when placed in an isolated environment. Broiler chicks, raised under brooder conditions and fed a standard commercial diet as recommended, were individually placed for 2 minutes in an anechoic chamber at 7, 14, and 21 days old. The anechoic chamber was a plywood box raised off the ground with soundproofing foam attached to the inside of the chamber to ensure that we recorded the purest sound without any unnecessary noise from the barn. The lack of both noise and reverberations due to the soundproofing foam made the chamber an isolated environment. Each bird went through an isolation fear test in the chamber. If a bird did not vocalize for the first 20 seconds, they received a score of 0. If they initially vocalized but stopped for 5 seconds, we recorded the duration of the initial time vocalizing. Audio recordings were analyzed using Adobe Audition, Adobe Premiere Pro, and Praat, a phonetics software. Vocalizations were characterized based on the following criteria: number of pulses, amplitude, frequency, and the shape of any frequency modulation. A canonical discrimination analysis was used to further cluster similar calls to account for individual variability. We identified 24 different vocalizations (based on spectral criteria) with 3 birds making only 1 type of vocalization during the testing period while 5 birds failed to vocalize for the first 20 seconds. Our results showed that there is variation in call diversity among chicks, possibly implying a diversity in motivational states across individuals. Thus, more analyses should be conducted to confirm that the "isolation fear test" is truly a test of fear alone.

Key Words: broiler chicks, vocalizations, isolation fear test, fear

P279 Preening cups in duck pens are associated with an increase in central dopamine and decrease in serotonin activity indicative of increased aggression Melanie Bergman*¹, Jenna Schober¹, Drew Frey², Heidi Parnin², Gregory Fraley¹ ¹*Animal Sciences, Purdue University*, ²*Culver Duck Farms, Inc.*

Preening cups (PCs) are a type of open water source used as environmental enrichment for Pekin ducks to allow for natural behaviors. We set out to determine if PCs can impact the affective state of Pekin ducks. We evaluated the effect of PCs on serotonin (5-HT) and dopamine (DA) turnover via mass spectrometry and their respective synthetic enzyme gene expression via qRT-PCR. Low activity levels of central 5-HT and DA are affiliated with unwanted behaviors in animals, like aggression. Due to an increase in aggressive pecking associated with PCs, our study investigated the link between aggressive pecking and brain 5-HT and DA. Pekin meat ducks (n=260) were housed at Purdue and raised per industry standards. Day 18 brains were collected from pens before PCs were placed (PRE, n=6) and, day 43 brains were collected from pens with PCs (PC, n=6) and pens without PCs (CON, n=6). Brains were dissected into 4 brain areas: caudal mesencephalon (CM), rostral mesencephalon (RM), diencephalon (DI), and forebrain (FB). Mass spectrometry used the right hemisphere to determine the neurotransmitter concentration (ng/mg of tissue) and apply them to synapse turnover equations. A 2-way ANOVA (age x treatment) was run for each brain area. There was a trend to significance ($p = 0.08$) that showed an increase in 5-HT turnover for CON when compared to PRE and PC treatments. There were significant decreases in DA turnover across age ($p = 0.0067$) and treatments ($p = 0.003$) within the CM and RM. The left hemisphere of the brain was used to perform qRT-PCR on the genes for 5-HT and DA synthetic enzymes, tryptophan hydroxylase (TPH1 and TPH2) and tyrosine hydroxylase (TH), respectively. A pairwise Wilcoxon rank sum test was used to compare treatments. Within the CM, day 43 duck brains had significantly increased gene expression for TPH1 mRNA ($p = 0.022$) and TH mRNA ($p = 0.022$). All other brain areas showed no significances. Our data suggest that ducks within PC pens that showed increased feather pecking are associated with high dopaminergic activity. The increased dopamine in the brain may lead to predisposing the ducks to a negative affective state with increased aggression in the form of feather pecking.

Key Words: Pekin duck, serotonin, dopamine, open water, aggression

P280 Assessing behavior of three Naked Neck genotypes reared under different production systems Arif Hameed¹, Shahid Mehmood¹, Sohail Ahmad¹, Arshad Javid² ¹*Department of Poultry Production, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore, Pakistan, ²Department of Wildlife and Ecology, Faculty of Fisheries and Wildlife, University of Veterinary and Animal Sciences, Lahore, Pakistan*

Present study evaluated the behavioral repertoires of three Naked Neck genotypes reared under different production systems. For this, 90 cockerels were selected from three Naked Neck genotype i.e., Full Feathered (FF), Partial Feathered (PF), and Naked Neck (NN) that were reared under three production systems i.e., aviary, backyard, and open sided (30 birds from each production system and 10 birds from each genotype). These birds were wing tagged and their behaviors were recorded daily for 15 minutes between the hours of 9:00 am and 2:00 pm for the duration of 6 to 16 weeks. Experiment was set up as a completely randomized design with 3 × 3 factorial arrangement of treatments. Behaviors included scratching, preening, perching, standing, drinking, running, jumping, walking, sitting, aggressiveness, feeding, wing flapping, dust bathing. Significant interactions between production systems and genotypes were noted in scratching, preening, perching, standing, drinking, feeding, and dust bathing. In general, bird reared under backyard system were involved in exploratory behavior and showed more grooming behavior than those of aviary and open sided system. In conclusion, maintenance, grooming, and exploratory behaviors were more pronounced in Naked Neck genotypes when reared under backyard type system.

Key Words: Naked Neck genotypes, Production systems, Scratching, Preening

P281 Effect of stocking density on production performance of Ross 708 and Cobb 700 broilers Shengyu Zhou*, Yang Zhao, Tanner Thornton, Mustafa Jaihuni *University of Tennessee, Knoxville*

This study comparatively evaluated the production performance of Ross 708 and Cobb 700 broilers at 4 stocking densities (SD) during a 56-day production cycle in 2 trials. In each trial, 432 chicks were randomly placed into 32 pens (1.5 m² of available space per pen) to simulate SD 27 (10 chicks per pen), 29 (12 chicks per pen), 32 (14 chicks per pen), and 44 (18 chicks per pen) kg/m² based on a projected final BW of 4 kg. Stocking densities were selected based on standards set by welfare and industry organizations. The results showed that by reducing the SD from 44 to 27, the final BW of Cobb 700 broilers increased from 4.15 to 4.38 kg (P=0.03), but the FCR decreased from 1.44 to 1.66 (P<0.01), while the stocking density had no significant effect on the final BW (3.55 kg, P=0.68) or FCR (1.81, P=0.50) of Ross 708 broiler chickens. There was no significant effect on final body weight (3.55 kg, P=0.68) and FCR (1.81, P=0.50). These results indicate that reducing stocking density can slightly increase the final body weight of Cobb 700 broilers, but it will greatly reduce the FCR and economic returns.

Key Words: Stocking density, Production performance, Broiler, Ross, Cobb

P282 Reducing pecking in debeaked hens Jean-Francois Gabarrou* *Laboratoire Phodé*

Debeaking is a traumatic practice for laying hens. But keeping a beak intact is also a risk factor. The use of pecking blocks is sometimes proposed, with variable results. Certain plant extracts have been shown to reduce deviant behavior. The aim of this trial is to measure the impact of the combined use of pecking blocks with a plant extract based mainly on Citrus Cinensis essential oil.

300 non-debeaked Novogen hens have been placed in 12 different pens. All pens offered pecking blocks. After 14 days of testing, only 6 pens received a 14-day drinking water treatment with a plant extract based

mainly on Vegetal essential Oil of Orange (VeO). The other 6 control flocks (Ctrl) received nothing in their drinking water. Feed intake and egg production was monitored daily. The number of pecks on the pecking blocks was measured by video (18 x 2 min, twice a week). The quantity of pecking blocks consumed was measured weekly by weight loss of the blocks. The number of hens having received too many pecks to be maintained in the test was also monitored throughout the experiment (28 days). Feed intake and egg production were analysed using ANOVA GLM, other indicators were analysed using Mann-Whitney U test. P<0,05 was considered as significant.

No significant differences were observed in feed consumption or egg production. The total number of pecks observed on the pecking blocks was similar between the VeO and Ctrl groups before treatment (662 vs. 657). The number of pecks decreased significantly (p<0.05) over time (R=0.55). After treatment, the VeO group showed renewed activity on these blocks (1036 pecks observed per pen vs. 542). Pecking block consumption did not differ between PeO and Ctrl before treatment (1.80±0.24 vs. 1.71±0.26g/d). After treatment, consumption in the treated group tended (p=0.08) to increase (2.41±0.15 vs. 1.81±0.22 g/d). The number of hens dead or discarded due to severe pecking was 9 for the PeO group vs. 35 for the Ctrl one.

The consistency of the results between the use of pecking blocks and mortality suggests that VeO can play a part in the management of deviant behavior on the farm. Further studies are needed to better understand the product effect on animals' behavior.

Key Words: Citrus Cinensis, debeaking, pecking, pecking blocks, layers

P283 Effect of cocoa bean shell (Theobroma cacao) as partial replacement for maize on growth performance and haematological indices of broiler chicken Rasheed Hamzat*, Alagbe John² ¹*Department of Animal Nutrition and Biochemistry, Sumitra Research Institute Gujarat India, ²Department of Animal Science, University of Abuja Nigeria*

This experiment is tailored towards exploring the use of agro-industrial waste as partial replacement for corn. This research will further reduce the competition between human and animals for conventional feedstuffs and address the increasing cost of corn recently experienced in Nigeria. Therefore, this study was to investigate the effect of Cocoa bean shell (CBSL) as partial replacement for maize on the growth performance and haematological indices of broiler chickens. A total of 200 1-day old broiler chicks (Ross 308) of mixed sex were reared in a galvanized battery cage measuring: 100 m × 60 cm × 50 cm and were randomly distributed to 5 groups of 40 birds each. Birds in diet 1 (Corn based diet without CBSL, CBSL was used to partially replace maize at 10 %, 20 %, 30 % and 40 % in diet 2, 3, 4 and 5 respectively). The experimental diet were adequate in all nutrients and fed ad libitum for 42 days in a completely randomized design. Using Statistical Package of Social Sciences (SPSS version 23.0), one way analysis of variance (ANOVA) was used to examine all of the data. Using Duncan's multiple range test of the same package, means were sorted where P ≤ 0.05. CBSL contained crude protein (6.44 %), crude fibre (14.40 %), ether extract (1.75 %), ash (7.20 %) and energy (2933.7 kcal/kg). Average weight gain, average daily feed intake and feed conversion ratio were not influenced (P>0.05) by dietary treatments. Pack cell volume, red blood cell, platelet, mean platelet volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, white blood cell, neutrophils, basophils, monocytes and lymphocytes were higher (P<0.05) in T3, T4 and T5 than in T1 and T2. All values were within optimum ranges for healthy birds. Result showed that CBSL can be used to partially replace maize up to 40 % without negatively affecting the performance and health status of broiler chickens.

Key Words: Maize, Cocoa bean shell, Broilers, Performance, Blood

P284 Evaluating the EEG of broilers in response to VSD plus Heat with humidity injected to accelerate depopulation methodologies Kari Harding¹, Ramon Malheiros¹, Sanjay Shah², Kenneth ANDERSON*¹ *1*Prestage, Dept. of Poultry Sci., North Carolina State University, *2*Dept. of Bio and Ag Engineering, North Carolina State University

In 2022, the poultry industry dealt with what has now been categorized as the worst highly pathogenic avian influenza outbreaks in U.S. poultry history. Timely and humane depopulation continues as the primary and critical measure to contain the outbreak. In 2022 depopulation methods utilized for broilers included foaming, Ventilation Shutdown Plus (VSD+), and CO₂ to mitigate prolonged suffering of infected birds. The objective was to enhance the effectiveness of VSD+ by increasing the relative humidity (Rh) in the system thereby reducing latent heat loss reducing the Time of Death (TOD) for depopulating broilers. TOD was evaluated by the Electroencephalogram (EEG), and recording environmental parameters. Four single bird chambers constructed to control environmental conditions were used. Each method was repeated 4 times with one bird per chamber for a total of 12 broilers equally representing males and females. EEG electrodes were affixed in the subcutaneous skin around the skull of the birds to measure brain activity. Data was analyzed with JMP 15 Pro using GLM with full factorial effects for VSDH, VSDHRh and VSDCO₂ fit to each of the response variables and all pairwise comparisons used Tukey's HSD to separate means. The transformed EEG analysis used the integral area under the curve calculated using the Trapezoidal method; using a NPARM analysis. Neither the slopes of the transformed EEGs nor the integral area under the curve were significantly different between the treatments. Starting chamber temperature was lower (P<0.0001) in CO₂ while VSDH and VSDHRh were similar, the ending temperature was lower (P<0.0029) with CO₂ than VSDH and VSDHRh. VSDCO₂ had the highest CO₂ % than the other treatments. VSDH and VSDHRh were similar at 85.55% and 82.7%, respectively with VSDCO₂ the lowest (P<0.0005) at 65.25%. The starting core body temperature (CBT) was not different between treatments. At time of death (TOD) the CBT was highest (P<0.01) for VSDHRh and VSDH over the lower (P<0.0001) VSDCO₂ broilers. The TOD was longest in the VSDH followed by VSDHRh then VSDCO₂ (P<0.0001). The conclusion, in broilers there is no significant difference between the methods other than TOD and cause of death with VSDH and VSDHRh is hyperthermia while VSDCO₂ is hypoxia.

Key Words: Broiler, Depopulation, Heat nad Humidity, CarbonDioxide

P285 Litter consumption of broiler chickens raised in either floor pens or battery cages during the starter period Stephanie Whitham*, Christopher Magee, Joseph Purswell *USDA-ARS Poultry Research Unit*

Broilers frequently consume litter, which may affect digestive dynamics, feed intake, and growth performance of the birds. An experiment was conducted to determine quantity of litter consumption (LC) and the effects of LC on growth performance of broilers during the starter period. Broilers were provided with 5 treatments. Four treatments were arranged as a 2 × 2 factorial of housing (battery cages or floor pens) and litter condition (old or new). Broilers in battery cages were raised on wire floors and provided with a supplemental feeder containing litter. One additional treatment was housed in battery cages and was not provided with litter. Growth performance was evaluated at 7 and 14 D of age and litter disappearance was measured in battery cages. Additionally, 2 birds from each pen or battery were euthanized for collection of gizzards at 7 and 14 D of age. Empty gizzards were cleaned and weighed. The experiment was designed as a randomized complete block design with 8 replicates per treatment and 20 birds per replicate. Data were analyzed using two-way ANOVA for the factorial treatments. Contrasts were utilized to compare broilers provided with litter in batteries to those not provided litter. The relationship between LC and gizzard weight was assessed with simple linear regression. Growth performance was not affected (P ≥ 0.12) by litter condition or presence in battery cages. At 7 D of age, broilers raised in batteries and provided with old litter had heavier (P ≤ 0.0271) gizzards relative to

BW than those provided no litter, while broilers evaluated in the factorial arrangement had heavier (P ≤ 0.0198) gizzards relative to BW when provided with old litter compared with broilers provided new litter. Broilers housed in batteries consumed more old litter than new litter at 7 D of age (7.13 vs 4.56% of feed intake, relatively; P < 0.0001) and at 14 D of age (6.61 vs 4.43% of feed intake, relatively; P < 0.0001). No relationship (P ≥ 0.19, R² ≤ 0.12) was observed between determined LC and gizzard weight in broilers provided litter in battery cages. These results indicate that broiler chicks may consume litter equivalent to 4.4 to 7.1% of their feed intake during the starter period, and this may affect the development of the gastrointestinal tract.

Key Words: Battery, Broiler, Growth Performance, Litter, Management

P286 Effect of feeder area distribution on broiler performance to 56 days Joseph Purswell*¹, Hammed Olanrewaju¹, Yang Zhao² *1USDA-ARS Poultry Research Unit, 2University of Tennessee*

Feeder space availability has been shown to effect live performance in broiler chickens, especially during early life. Research trials evaluating this effect typically employ a constant number of pans with obstructed access and this arrangement may mask effects resulting from spatial distribution of feed. This experiment was conducted to determine if distribution, rather than amount, of available space affects live performance in broiler chickens. A total of 720 straight-run broiler chickens were obtained from a commercial hatchery on day of hatch. Chicks were randomly allocated to each of 16 floor pens in an environmentally controlled poultry house. Each pen was equipped with fresh pine shavings and nipple drinkers. Feeder space distribution treatments included a single feeder with 104 cm feeder space and three feeders with 34.7 cm feeder space. Birds were provided a four-phase corn-soy diet formulated to meet or exceed NRC requirements. Live performance was assessed at 14, 28, 42, and 56 days. Each treatment was represented by eight replicates. Data were analyzed via ANOVA using PROC MIXED in SAS. Placement chick weight was 42 g and was not different between treatments (P = 1.000). No differences were observed in BW, BWG, or FI in this experiment. FCR during the starter period was significantly lower for the single feeder treatment (P=0.014) but was not different for the remainder of the flock. Mortality was significantly increased for the single feeder treatment during the grower period (P = 0.008) but was not different at the end of the flock. In conclusion, distribution of feeder space may not be a critical factor for live performance if feeder space needs are met.

Key Words: broiler, housing, feeder

P287 Using a transect approach to assess behavior and welfare of laying hens in aviary tiers during peak lay Metin Petek*^{1,2}, Janice Siegford² *1Bursa Uludag University, Faculty of Veterinary Medicine, Department of Animal Science, 2Michigan State University, Department of Animal Science*

Transect approaches are commonly used to assess welfare in non-cage poultry flocks and consist of slowly walking through the barn while observing the flock for select indicators. We used a transect approach to investigate the behavior and welfare of laying hens under experimental conditions during peak lay housed in 2 styles of multi-tier aviaries (Natura-60 and Natura-step Big Dutchman®). Each aviary had three-tiers running the length of the pen, but the location of feeder, drinker, perches, and nests varied between styles. There were 4 pens along the length of each room, with 576 hens/room in Natura 60 and 656 hens/room in Natura-step aviaries. Behavioral observations were made of hens located in the bottom, medium, and top tiers while welfare indicators were assessed from hens in the bottom tier. The data collection was done at 27, 32, and 36 weeks of age over two consecutive days at each age between 1300 to 1530. Data collected during transect walks were used to calculate the frequency of birds with a particular welfare and behavior indicator as a proportion of the total number of observed (counted) birds. Generalized linear models

including aviary style, age, side, and tier as main factors were used to analyze behavioral indicators, while for welfare indicators aviary style and age were fixed factors. 61.50 and 13.50% of total hens housed were evaluated for behavioral and welfare indicators with transect walks, respectively. Perching was the most common behavior observed and was significantly affected by aviary style ($P<0.001$), side ($P<0.001$), and tier ($P<0.004$). Age ($P<0.01$), side ($P<0.001$), and tier ($P<0.001$) significantly influenced feeding behavior of hens. Sitting-laying behavior was affected significantly by tier ($P<0.001$), while walking behavior was affected significantly by side ($P<0.009$). Almost no adverse behaviors were observed, except a few instances of gentle feather pecking, and the prevalence of birds having slight feather damage on their back/rump and tail was $<10\%$. In conclusion, it appears a transect approach can be used effectively in experimental studies to assess behavior and welfare indicators of hens. Transect walks will continue as the hens age to further evaluate influence of aviary style on behavior and welfare outcomes.

Key Words: Laying hen, aviary, transect, behavior, welfare

P288 Sprinkler cooling broilers and performance Jonathan Moon^{*1}, Jessica Drewry¹, J. Purswell², Tom Tabler³, Yi Liang⁴ ¹Mississippi State University, ²USDA-ARS Poultry Research Unit, ³University of Tennessee, ⁴University of Arkansas

Maintaining broiler performance and limiting heat stress during summertime conditions can be challenging, and boosting performance under these same conditions can be virtually impossible. Therefore, the objective of this study was to determine if commercial sprinkler systems used in combination with a cool cell system (SSCC) can improve broiler performance

when compared to cool cell systems (CC). Two preliminary studies during (1) summer 2020, (2) summer 2023, were conducted. Each study consisted of two flocks with treatments alternated between two houses. Two lines of overhead sprinklers above outside feed lines spaced evenly 20 ft (6 m) apart and 7ft (2.1 m) above the litter surface intermittently applied controlled volumes of large water droplets onto birds. Study performance data collected consisted of feed conversion ratio (FCR), body weight, daily mortality, paw quality, core body temps, and pay per pound and was analyzed using PROC GLM SAS 9.4 to determine significance. For study 1, the SSCC treatment followed the manufacturers guidelines for setting up the sprinkler system and cool cells were set to come on at 90°F. Settings for CC treatment had a cool cell on-temp of 82°F. No significant differences were observed for FCR, body weight, daily mortality, and paw quality ($P>0.05$). However, a 0.12°F increase ($P=0.0004$) in core body temp, and a 0.155 cent per pound (CPP) increase ($P=0.021$) in pay when comparing SSCC over CC was observed. For study 2 SSCC treatment followed the SS manufacturer's new factory program with the addition of activity promotion mode, and cool cells were set to come on at 88°F. In addition, the same CC treatment setting for study 1 was utilized (on-temp 82°F). For study 2, we failed to detect a significant increase in core body temperature, average weight, or FCR. In conclusion when using a 2°F lower cool cell setting in the SSCC treatment, a modified program, and the activity promotion feature used in the SS controller, although not deemed significant ($P\leq 0.05$) numerical performance gains were observed during summertime flocks. Therefore, more data is needed to assess the effects of sprinkler use on performance.

Key Words: Broiler, Sprinkler, Cooling, Heat Stress, Performance

Environment, Management: Environmental Impacts

P289 Biomapping of a commercial poultry hatchery in the Southeastern United States Walid Al Hakeem^{*1}, Benjamin Zwirzitz², Xiang Li³, Ade Oladeinde³, Michael Rothrock Jr³ ¹Oak Ridge Institute for Science and Education, Athens, Georgia, United States, ²Institute of Food Science, University of Natural Resources and Life Sciences, Vienna, Austria, ³USDA-ARS, USNPRC-EPPSRU, Athens, Georgia, United States

Pathogenic microbes can find a suitable niche within poultry hatcheries designed to nurture early chick development. Despite this concern, limited knowledge exists regarding the microbiomes within these hatcheries. For this study, a commercial hatchery was bio-mapped using sponge swabs to sample air, water, bait boxes, and eggs from pre-hatch (egg inventory, pre-in ovo, and post-in ovo), hatch (chick processing), and post-hatch (chick transport) areas. To determine the bacterial composition, 16S rRNA gene sequencing (using the MilSeq platform) was conducted, and the sequence data was analyzed using QIIME2(v2022.2). One-way ANOVA with a sampling area of the hatchery as the main effect, followed by Tukey's multiple comparison method for pairwise comparison, was performed using R software. The highest bacterial richness was detected in the water samples during chick processing/transport compared to the other sample types, indicating a possible role of water in introducing microbes to the chicks. The beta diversity index (PCo) revealed that the eggshell harbors microbiomes distinct from the hatchery's environment prior to hatch; however, egg-related microbiomes (chicks fecal samples) from the transport area were more similar to the hatchery facility areas, indicating the hatchery environment's role in shaping the early gut microbiome post-hatch. *Salmonella* taxa were found throughout the hatchery (they represented a core taxa microbiome of all sample types and sampling areas) but predominantly found in the chick's feces post-hatch. SourceTracker analysis, which typically uses a reference database of microbial communities to compare against the different hatchery samples, indicated the break-room tables as one of the main sources of *Salmonella* within the hatchery. In conclusion, the hatchery's environment can be a potential reservoir for

foodborne pathogens such as *Salmonella*; however, a more comprehensive study investigating *Salmonella* serotypes present in the hatchery extending through grow-out is required to determine the role of hatcheries as a source for microbial populations entering commercial poultry houses.

Key Words: Salmonella, Broiler Commercial hatcheries, Biomapping

P290 Impacts of on-farm water sanitation practices on microbial hygiene in poultry waterlines and efficacy of sodium hypochlorite-based product on foodborne pathogens. Rabin Raut^{*1GS}, Agnes Kilonzo-Nthenge¹, Susan Watkins², Pramir Maharjan¹ ¹Tennessee State University, ²University of Arkansas

The microbial water quality of poultry water supplies directly impacts bird health, livability, and overall production. Two on-farm studies were conducted to assess the microbial hygiene of poultry waterlines in relation to water sanitation practices. In the first study, we examined Farm A and Farm B, that did not consistently practice water sanitation during the flock grow-out period. Swab samples were collected post bird harvest ($n=4$ lines, each farm) from waterlines on both farms and tested for the presence of specific pathogens, including *Salmonella*, *E. coli*, *Listeria*, and *Staphylococcus*. In the second study, we focused on a broiler farm with four barn units that consistently treated water with chlorine ($Cl \approx 1$ ppm) and flushed waterlines with concentrated chlorine solution (>1000 ppm) between flocks. Swab samples (1 from each barn, $n=4$; each sampling occasion) were collected on three occasions: before flushing, after flushing, and at the end of the grow-out period (day 42) to assess the nature of bacterial biofilm growth in poultry waterlines. To further understand the efficacy of a commonly used poultry drinking water sanitizer (sodium hypochlorite, 8.25%), a separate benchtop study was conducted. This study mimicked the application rates used in field practice, exposing *Salmonella enteritidis*, *E. coli*, and *Listeria* broth cultures (2.2×10^8 CFU/ml) to the product. Farm A and Farm B were found to be positive for *Listeria* and

Staphylococcus but negative for other pathogens. Biofilm regrowth exceeding 4 log₁₀ CFU/ml was observed by the end of the grow-out cycle despite regular waterline cleaning and sanitation. Results showed that the chlorine-based water sanitizer may not effectively kill pathogens and is affected by the pathogen load in the water. The overall results, on farm or *in-vitro*, indicated that poultry growers should firmly emphasize consistent poultry drinking water sanitation to keep the microbial levels low in poultry water system or minimize pathogen load in water supplies.

Key Words: water supplies, microbial levels, biofilm, sanitation

P291 Title: Patterns of perch use in relation to body weight in two strains of broiler. CHIOMA AKUKWU*^{GS}, CIRENIO HISASAGA, MAJA MAKAGON *Center for Animal Welfare, Department of Animal Science, University of California.*

Estimates of perch use are often made by scan sampling, which may miss perching events with short durations, and underestimate overall perch use. The objective of this study was to detail patterns of perch use in a slower- and faster- growing broiler strain in relation to body weight and bone mineral density (BMD) of the tibia. Ross 708 (23-24 birds/pen) and Ranger Gold (26-27 birds/pen) were raised on wood shavings in 9.3 m² pens (4 pen replicates/strain). A 1.2 m long mushroom-shaped plastic perch was placed in each pen at 2 weeks of age. The occurrence and duration of perching was recorded for eight individually marked birds/pen. Focal birds were observed continuously from 7:00 to 22:00 h on each of two consecutive days/week. At their target ages of 42 and 56 days, Ross 708 and Ranger Gold broilers weighed 3126.9 g and 2847.5 g, on average. Tibia BMD was estimated from DEXA scans. Due to differences in stocking densities and target weights, data from the two strains were not compared statistically. At 2 weeks, 10 of 32 Ross 708 broilers used the perch with 90.0% perching events lasting <60 s (average 35 s; range 5 to 348 s). At 5 weeks, 7 of 32 Ross 708 broilers used the perch, with 76.6% perching events lasting <60s (average 55 s; 2 to 322 s). Of the 32 Ranger Gold broilers, 24, 17 and 6 used perches at 2, 5 and 7 weeks, with average perching durations of 77 (1 to 1500 s), 281 (3 to 2812 s) and 512 s (5 to 1935 s), respectively. At 2, 5 and 7 weeks of age, 78.0, 37.7 and 16.5% of perching events lasted <60 s, while the number of perching events lasting over 5 minutes increased from 10.1 to 42.7 to 68.6%. On average, Ross 708 broilers accessed the perch 20 times across the 2 day observation period during week 2, and 7 times during week 5. Ranger Gold broilers accessed the perch 15, 17 and 20 times during weeks 2, 5 and 7, respectively. Overall perching frequencies did not correlate with carcass weight, or BMD of the tibiae for either broiler strain (all r² <0.1). The results of this preliminary study suggest that perches may lead to different welfare consequences for different broiler strains. Further studies are needed to investigate the most optimal enrichments for different broiler strains.

Key Words: Perch use, Broiler, Development, Slow growth, Fast growth

P292 Assessing activity of broilers in a commercial house using laser enrichments Reyna Mero*^{UG}, Katy Tarrant *California state University, Fresno*

Considering the increasing focus on welfare and sustainability in broiler production, adopting emerging technologies is crucial to improve welfare conditions while preserving productivity. Laser enrichment is a promising technology to consider as an environmental enrichment, which serves as an alternative means to increase activity and improve broiler performance without inducing or causing any distress. In our study, we evaluated the activity level associated with laser enrichments within a commercial broiler house. This study was accomplished utilizing a 1-meter density model with four separate cameras incorporated to evaluate movement before and after laser enrichment. The experiment consisted of a standardized acclimation period of 5 minutes prior to the laser intervention. After the acclimation period, a hand-held laser was positioned at the ground for 5 seconds, followed by moving the laser in a straight line covering a

distance of 1 meter in a 5-second interval. This process was repeated in four separate 1-meter blocks recorded on camera weekly during growout. The number of birds performing the following actions were recorded prior to and during introducing the laser: total bird count, # eating, drinking, and active. An ANOVA was performed and means were separated using Tukey HSD in JMP Pro v.16. Total bird count did vary by week, with the lowest count at week 2 (19.75±5.53) and the highest count was week 6 (60.50±5.53; P=0.0009). The week displaying the most active birds was week 5 (2.26±0.36), while the lowest level of activity prior to the laser was in week 2 (0.68±0.12; P=0.0003). Throughout all weeks, activity increased an average of 5.96 birds, and the number of birds eating immediately following the laser presentation increased by 2.17 birds. Weeks 5 and 6 had the highest number of birds engaging with the laser (14.25±2.322 and 14.00±2.483, respectively), while weeks 1 and 2 showed the least amount of interest (3.75±1.79 and 2.25±0.62; P<0.02). Our results are similar to percent of birds engaging or chasing the laser as age increases. While we did not evaluate production traits, this form of enrichment points to the promise of sustained or increased eating activity, which is opposite of what has been seen utilizing other enrichment types.

Key Words: broiler, laser, activity, enrichment

P293 Comparing surface temperatures of different broiler platform designs Brenda Hernandez Tapia*, Monica Franco, Katy Tarrant *California State University, Fresno*

Previously, we have demonstrated that broilers in commercial houses utilize platform enrichments of different designs at different rates. Platforms act as one method in which producers can meet enrichment requirements established by internal or external auditing tools. Choice in platform design may be influenced by a number of factors which have yet to be quantified, including comfort, proximity to preferred locations in the barn, etc. which may contribute to platform usage. In this study, we evaluated temperature variation at the surface level of platforms of different design types in a commercial broiler facility in an attempt to explore one possible facet of the variation in usage patterns. Three designs (a-frame, ramp, and box) were placed equal distances from heaters, waters, and feeders in a commercial broiler facility. Daily, surface temperature of each platform type and the adjacent litter, serving as a control, was recorded. Data were analyzed by week in JMP Pro v.16 using an ANOVA. Means separated using Tukey HSD. Combined, enrichment surface temperatures were significantly higher in weeks 1 (P = 0.0026) and 2 (P = 0.0191), while week 6 litter temperatures were higher than enrichment (P = 0.020). When comparing the surface temperature between enrichment designs, weeks 1-3 were not significantly different. The a-frame design had the highest temperature in week 4 (29.53 ± 0.144 C; P < 0.0001), week 5 (28.71 ± 0.561; P = 0.0211), and in week 6 (26.86 ± 0.509; P < 0.0001). The box design had the lowest recorded temperature of all designs weeks 2 through 6. While a number of factors may be contributing to enrichment surface temperature (material, airflow, usage), it is important to recognize the different micro-climates related to each design, as this will impact the enrichment's overall usage in a commercial facility.

Key Words: broiler, temperature, commercial, platform

P294 Machine learning algorithms predict *Listeria* prevalence and *Campylobacter* species on pastured poultry farms based on management practices and farm environment variables. Xiang Li*, Michael Rothrock, Adelumola Oladeinde *USDA USNPRC EPPSRU*

Listeria and *Campylobacter* infections from the consumption of poultry have significant public health and economic impacts, and there is a potential for these risks to increase as poultry management systems increase the exposure of broilers to the environment. To investigate how broiler management practices and the farm environment effect foodborne pathogen ecology, management, environmental, and microbiological data collected from 11 pastured poultry farms were analyzed using three machine learn-

ing (ML) algorithms: (1) random forest classification, (2) logistic regression classification, and (3) XGBoost. The goal of this work was to apply these ML methods using broiler management farm environment variables to: (1) Predict *Listeria* prevalence and compare the performance of these new ML models with the previously used predictive microbiological model, and (2) predict *Campylobacter* species recovered throughout pastured poultry production using a novel XGBoost algorithm. The random forest and logistic regression classification ML models identified the number of years of farming, broiler flock age, and sample types (e.g., feces, soil, whole carcass rinse) as the most significant parameters in predicting *Listeria* prevalence. In comparison to our previous non-ML based predictive models, we have primarily addressed the imbalanced sample types and applied newly developed transformation and normalization methods to improve the model predictive performance. Moreover, the logistic regression classification model confirms statistically significant management practice variables, which complement the tree-based random forest classification models, indicating the necessity of applying multiple ML methods simultaneously. Furthermore, our novel XGBoost model suggests that farm environment variables such as carbon-to-nitrogen ratio, moisture, conductivity, and zinc concentration are most predictive of *Campylobacter* species recovered within these pastured poultry farms. This work showcases how ML can be applied to pre-harvest poultry food safety research, and how these types of algorithms can be useful for stakeholders and researchers in developing new strategies to mitigate poultry-related zoonotic pathogens.

Key Words: machine learning, *Listeria*, *Campylobacter*, management practices, environmental variables

P295 Comparison of metagenomic sequencing methods using *Salmonella* Heidelberg infected broilers. Xiang Li*, Adelumola Oladeinde, Michael Rothrock *USDA USNPCR EPPSRU*

Shotgun metagenomics sequencing is frequently employed to determine the microbial populations in complex samples. As one of the newer molecular techniques, it exhibits the theoretical capability to capture all genes, making it more advanced when compared to other techniques like 16S rRNA gene sequencing, Polymerase Chain Reactions, cloning, blotting, microarrays, and so on, that are widely used in poultry-related research. However, one of the limitations of shotgun metagenomics sequencing is that it cannot accurately answer the question of “what bacterial genome harbors a mobile DNA/gene of interest”? The introduction of chromosome conformation capture (Hi-C), a novel method that reveals physical associations between two DNA fragments has the potential to link functional genes with their bacterial host/genome. Therefore, in this study, we applied both techniques (Shotgun and Hi-C metagenomics sequencing) to

sequence two cecal samples collected from broiler chickens infected with *Salmonella* enterica serovar Heidelberg. Our goal was to determine which method was more suitable for reconstructing bacterial genomes, and the antibiotic resistance and virulence genes present in the ceca of broiler chickens. The results demonstrated that shotgun and Hi-C metagenomics sequencing can efficiently assemble bacterial genomes that are present in high abundance in ceca, however, the shotgun method performed better when dealing with small bacterial genomes (approximately ~2.4 Mbp in size). Furthermore, both methods exhibited equal performance for the identification of antimicrobial resistance and virulence genes. In conclusion, our results support the notion that shotgun and Hi-C metagenomics are valuable tools for profiling bacterial communities and for surveying genes present in the gut microbiome of chickens.

Key Words: metagenomics sequencing, *Salmonella*, bacterial genomes, ARGs, Virulence genes

P296 Comparison effects of natural and artificial lighting on growth performance and carcass characteristics of broilers grown to 56 days of age. CHRISTOPHER MAGEE¹, Stephanie Collier¹, John Linhoss², Bethany Baker-Cook², Stephanie Whitham¹, Joseph Purswell¹, HAMMED OLANREWAJU*¹ *1USDA-ARS, 2Auburn University*

Light is one of the major environmental factors that support development, production performance, and welfare/well-being of broiler chickens. This study compared the effects of natural and artificial lighting on growth performance and carcass characteristics of broilers grown to 56 days of age. In each of the two trials, a total of 704 (352 males/352 females) 1-d-old Ross x Ross 708 chicks were obtained from a commercial hatchery on day of hatch. The design structure was a randomized complete block design with two treatments and eight replications. Birds were equally and randomly distributed into 16 environmentally controlled rooms in groups of 44 (22 M/22 F) per room. Temperature was set according to typical breeder recommendations. Feed and water were provided *ad libitum*. Birds were provided a four phase-feeding program (Starter: 0-14 d, Grower: 15-28 d, Finisher: 29-42 d, and Withdrawal: 43-56 d). Birds and feed were weighed on 1, 14, 28, 42, and 56 d of age for growth performance. On d 56, 12 (6 M and 6 F) birds from each room were processed to determine weights and yields. Data were analyzed using PROC MIXED procedure of SAS with significance level of $P \leq 0.05$. The BW and BW gain, FCR, and mortality were not affected by treatments. In addition, live BW and carcass characteristics were found to be unaffected at 56 days of age by both natural and artificial lighting.

Key Words: Natural-lighting, Artificial-lighting, growth performance, carcass yield, broilers

Metabolism and Nutrition: Enzymes

P297 THE USE OF FIBRE-DEGRADING ENZYME SUPPLEMENTATION IN CORN COB-BASED DIETS FOR BROILERS: EFFECTS ON GROWTH PERFORMANCE, AND CARCASS TRAITS Frank Idan*^{1,2}, Alberta Mante³ *1Kwame Nkrumah University of Science and Technology, 2Kansas State University, 3University of Ghana*

The current experiment was conducted to determine the effect of partial replacement of wheat bran with milled corn cob (CC) on growth performance and carcass traits in broiler chickens. A total of one hundred and seventy-five-day-old straight-run broiler chicks (Cobb 500) were used. The birds were assigned to one of eight dietary treatments in a 2 × 4 factorial design with two levels of enzymes and four levels of CC. The diets consisted of four levels of CC: 0%, 25%, 50%, and 75% with or without enzyme cocktail supplementation. Feed intake and body weight were measured weekly and used to calculate average daily feed intake (ADFI) and average daily gain (ADG). Mortality was recorded daily and used

to correct for FCR. Carcass parameters measured included live weight, dressed weight, full gizzard, empty gizzard, full intestine, and empty intestine. Data collected were analyzed using the GLM procedure of SAS and means with significant differences were separated using the Student Neumann Kuels (SNK) test. The results obtained showed interactions ($P > 0.05$) between enzyme addition and level of CC in the diet. Again, the main effects of corn cob or enzyme inclusion did not affect ADFI, ADG, and FCR as well as the live and dressed weights ($P > 0.05$). However, full gizzard weights significantly increased with the increasing levels of corn cob in diets ($P < 0.001$). Additionally, empty crop weights significantly increased with the increasing levels of corn cob in the diets ($P < 0.02$). In conclusion, corn cob can partially replace wheat bran (up to 75%) in broiler diets without adversely affecting growth performance and carcass parameters without using enzymes.

Key Words: Corn cobs, ADFI, Average Daily Gain, Feed Conversion Ratio, Carcass Traits

P298 Evaluation of multi-carbohydrase enzyme complex and phytase dietary supplementation on Pekin duck performance and carcass traits Hector Leyva-Jimenez^{*1}, Melinda Grimes², Jessica Rocha², Gregory Archer², Carlos Soto¹, Yemi Burden¹, Brian Dirks¹ ¹United Animal Health Inc., ²Texas A&M University

This study evaluated the effects of supplementing a multi-carbohydrase enzyme complex and a 6-phytase, in a combo product (MCEP), on performance and carcass traits of Pekin ducks. The study consisted of a complete randomized design of 3 treatments including positive control (PC), negative control (NC; -132 kcal/kg of ME compared with the PC), and the NC supplemented with the MCEP combo (Enspira[®]+Phytase; United Animal Health, US; EP) at 100 ppm. The control diets were supplemented with a commercially available 6-phytase at 1,000 FTU/kg. The EP treatment was supplemented with the proprietary MCEP combo to yield 1,000 FTU/kg. Both phytase products were expected to deliver 0.18% non-phytate P and 0.20% Ca. Each treatment included 10 replicate floor pens with 25 straight-run Pekin ducks per pen. The feeding program consisted of 2 dietary phases (starter 0-14 d; grower 14-35 d). Diets were pelleted and offered ad libitum. Feed intake, body weight (BW), body weight gain (BWG), and FCR (corrected for mortality) were determined. Five birds per pen were processed for the evaluation of carcass traits at 36 d. Data were subjected to one-way ANOVA and means were separated using Fisher's LSD ($P \leq 0.05$). After 14 d, the BW was lower ($P < 0.001$) in the NC compared to the PC and EP groups. Cumulatively after 35 days, the ducks fed the NC diet reduced ($P < 0.001$) BW and increased ($P < 0.001$) FCR compared to both the PC and the EP diet groups. The EP treatment maintained FCR to levels comparable ($P > 0.05$) to PC. Moreover, when the FCR was adjusted to a common BW (3.49 kg) a 13.7-point difference ($P < 0.001$) was detected between the PC (1.379) and NC (1.516) groups. When compared to the NC the EP treatment recovered 12.3 FCR points (1.393) and was not different ($P > 0.05$) from the PC. The whole carcass yield was numerically increased ($P = 0.062$) with MCEP combo supplementation compared to the NC. Breast weight was increased ($P < 0.001$) in the EP treatment when compared to NC. In conclusion, the supplementation of MCEP combo-maintained duck performance in energy-reduced diets with positive effects on carcass traits.

Key Words: multi-carbohydrase complex, enzyme, duck, performance, phytase

P299 Xylanase Supplementation: Influence on growth performance and carcass characteristics of broiler chickens Omar Al-Qudah, Kamel Mahmoud^{*}, Basheer Nusairat *Jordan University of Science & Technology*

Two studies were conducted sequentially to evaluate the effect of adding xylanase to the diets of broiler chickens on growth performance, carcass characteristics, and meat quality. Corn-soy based diets were used in both trials, but the second trial was formulated to contain 15% wheat. Seven hundred and fifty of one-day-old unsexed Ross 308 broiler chicks were randomly allocated into 6 dietary treatments with 5 replicates per pen of 25 chicks each. Control diets were formulated according to Ross 308 manual recommendation designated as positive control (PC and PW for corn-based diets and corn/wheat-based diets, respectively); control diet with 90 kcal/kg less energy designated as negative controls (NC and NW for corn-based diets and corn/wheat-based diets, respectively). Xylanase at 0.01% inclusion rate with 15 XU/g of feed was added to negative control diets (XC and XW for corn-based diets and corn/wheat-based diets, respectively). All diets were fed as mash, starter phase was from 1-14 days of age and the grower phase was from 15-28 days of age in experiment one and 15-35 in experiment two. Results showed that only body weight gain for the period of 15-28 d and overall period were higher in wheat-NC diet with enzyme addition. Meat lightness (L^*) was lower in meat from birds fed wheat PC, while water holding capacity was improved by adding xylanase to control diets. In conclusion, under the current experimental conditions, it can be concluded that xylanase supplementation in broiler diets can be used as a mean for reducing energy content of the diets. Re-

sults were better in wheat-based diets, possibly due to a higher substrate concentration.

Key Words: Broiler, wheat, xylanase, carcass

P300 In vitro evidence of synergistic effect of proteases and amylases on starch degradation Jacqueline de Souza^{*1}, Rafael Alves¹, Diogo Rosso², Eduardo Della Pia², Vitor Fascina³ ¹Novozymes Latin America Ltda, ²Novozymes A/S, ³dsm-firmenich Brasil

Nutrients in feed ingredients are often present in a complex matrix, consisting of proteins, starch and non-starch carbohydrates, lipids, and various minerals and vitamins. This complexity brings some challenges for animals to digest and absorb all the nutrients efficiently. Although broilers secrete their endogenous enzymes, the improvement in feed digestibility using exogenous enzymes is well described in the literature. Amylases are capable of solubilizing starch, while proteases can enhance the accessibility of amylases. The starch granules within cereal grains are surrounded by proteins. The degradation of these proteins is necessary to fully expose the starch and allow for complete hydrolysis by amylases.

The effect of two commercial enzymes, an amylase and a sphericase protease, on solubilization of starch from corn was evaluated. The effect of the enzymes applied alone or in combination was investigated. Incubations of the enzymes with the corn substrates were carried out *in vitro* simulating the intestinal portion of the gastrointestinal tract of broilers (40°C and pH 6 for 4 hours).

The inclusion of the amylase alone was found to increase the amount of solubilized starch (measured as glucose after acid hydrolysis) by 50% compared to a blank sample. The addition of the sphericase protease does not impact the activity of the amylase enzyme; in fact, when both enzymes were applied together, a synergistic effect on the solubilization of corn starch was observed and 23% more starch was degraded compared to the use of amylase alone.

Overall, our findings suggest that addition of the sphericase protease to animal feed can improve the digestibility of both protein and non-protein nutrients, and that the combination of the protease with an amylase can further enhance starch solubilization. These results indicate that combining different exogenous enzymes such as an amylase and a protease can be a powerful strategy to increase nutrients availability and improve animal nutrition.

Key Words: Enzyme, Amylase, Protease, Starch, Feed ingredients

P301 The effect of Zymbiotics[®] supplementation in Commercial feed on laying hens performance and table-egg quantitative and qualitative parameters Lucio Araujo^{*1}, Rasha Qudsieh², Sudhir Yadav², Sandra Rodrigues², Yun-mei Lin², Douglas Faria¹, Isabela Silva¹, Raimundo Netto¹, Vitor Pais¹, Cristiane Araujo^{1,3} ¹University of Sao Paulo - Faculty of Animal Science and Food Engineering, ²BioResource International, ³University of Sao Paulo, School of Veterinary Medicine and Animal Science

Since the international community has sought to ban the use of growth-promoting antibiotics (AGP) in animal feed, the poultry industry has been looking for alternatives regarded as safe for use in animal feed and do not promote bacterial resistance in humans, and several alternatives have been used, such as exogenous enzymes and probiotics. Enzymes, pre and probiotics are examples of feed additives that might be used as alternatives to AGPs. An experiment was conducted to evaluate the use of Zymbiotics[®][AL1] (EP) in the diets of commercial layers aged 20-40 weeks. A total of 168 Lohmann LSL white layers were used. Birds were divided into 3 treatments (T1, positive control diet; T2, positive control - 100 kcal (NC); T3, NC + 100g/MT EP) in a completely randomized design with 7 replicates/treatment and 8 hens per replicate. Animals were fed *ad libitum* on a maize and soybean meal-based diet. Performance data (egg production, feed consumption (g/day), FCR, kg/d) and egg quality (egg weight, shell strength, shell thickness, albumen height, and Haugh unit)

were evaluated. Data was analyzed as one-way ANOVA and means were separated by Tukey's test with significance set at $P < 0.05$. There was a numerical improvement when EP was added to the diet, although there were no statistical differences in egg production and the percentage of dirty eggs ($P > 0.050$). There was a downward trend in egg mass for NC-treated birds ($P = 0.0917$) and higher egg weight for PC birds ($P = 0.0121$). There were no significant differences in feed consumption and FCR (kg/d). Although the values for albumen height and Haugh unit were higher for eggs from hens fed PC treatment ($P = 0.0084$ and $P = 0.0410$, respectively), the values found were compatible with market requirements and do not necessarily imply a reduction in egg quality. An improvement in shell quality was also observed with the use of EP in the diet, with better resistance to cracking ($P < 0.0001$) and greater shell thickness ($P = 0.0064$). The results show that the use of the EP complex maintains the zootechnical performance of the birds fed a reduced energy diet and improves the quality of the eggshells.

Key Words: Egg Quality, Hen Production, Layer, Reduced Energy, Zymbiotic

P302 Effects of dietary enzyme supplementation on the performance and nutrient digestibility of turkey poults fed a low nutrient diet. Brendan Heinichen*^{GS}, Peter Ferket, Ramon Malheiros, Jesse Grimes, Tuoying Ao *Prestage Department of Poultry Science, North Carolina State University*

Dietary inclusion of phytase and carbohydrase enzymes is common practice to reduce feed cost and improve growth performance of turkeys. Allzyme[®] Spectrum (Alltech, Inc., Nicholasville, KY), produced through solid state fermentation, is an enzyme complex with both phytase and carbohydrase activities. Increasing dietary supplementation of this enzyme in a corn-soybean meal diet with low nutrient density was hypothesized to increase growth performance and nutrient (P and Ca) digestibility and AMEn of turkey poults. Nicholas Select turkey poults were randomly assigned to 48 battery cages (7 poults/cage; 8 cages/treatment) and raised to 28 d. Six pelleted dietary treatments included a positive control (PC) corn-soy diet that met breeder recommendations for nutrients; a negative control (NC) corn-soy diet with reduced nutrient content relative to PC (88 kcal/kg less ME and 0.15% less Ca and 0.15% less avP, 0.03% less dLys and 0.02% less dTSAAs); and NC diets supplemented with 100, 200, 300, and 400 mg enzyme/kg feed, respectively. Celite was included in the diet as internal marker for the determination of nutrient digestibility. Body weight and feed intake were determined weekly. At the end of trial, ileal digesta and excreta pooled by cage were collected and two birds per cage were sampled for tibias to measure bone ash and breaking strength. Data were subjected to ANOVA and PROC GLM regression analysis of NC treatments (SAS, Inc. Cary, NC). There were no significant treatment effects ($P > 0.05$) on growth performance indicators (BW, FCR, and mortality rate), tibia breaking force, and tibia ash content. However, significant difference ($P < 0.05$) in nutrient retention and energy digestibility was observed between the PC and NC treatment. As dietary supplemental levels of the enzyme product increased, AMEn increased linearly ($AMEn = 0.3779x + 3013.51$, $P < 0.0001$); ileal digestibility of Ca increased linearly ($dCa = 0.00025557x + 0.569$, $P < 0.005$), and ileal digestibility of P increased quadratically ($dP = 0.0000006263x^2 + 0.0000393291x + 0.4445001408$, $P = 0.0238$). Based on the results of this study, dietary supplementation of Allzyme[®] Spectrum could compensate for reduced levels of ME, Ca, and P in turkey diets by increasing nutrient digestibility and AMEn.

Key Words: turkey, enzyme, AMEn, Ca/P, digestibility

P303 Evaluation of a new generation of phytase genetically engineered for greater thermotolerance and pH tolerance in broilers Coleman Hatmaker*^{GS}, Thomas Jones, Reed Dillard, Adam Davis *University of Georgia*

A new generation phytase produced by a recombinant strain of *Aspergillus niger* and derived from *Escherichia coli* was evaluated in the current research. It was engineered with gene site saturation mutagenesis and specific amino acid substitutions to generate an enzyme with increased thermotolerance, enhanced stability to digestion in the gastric environment and improved performance based on its capability to withstand extremely acidic conditions and maintain activity over a broad pH range. In the current research, activity of this phytase, named Bestzyme phytase, was evaluated during feed manufacturing with a 30 second condition time at 175, 180, 185, 190 and 195° F. In addition, the effectiveness of this phytase was evaluated in broilers from day of hatch to 16-days of age in a battery brooder experiment. The 8 dietary treatments consisted of a negative, intermediate and positive control diet containing 0.20, 0.34 or 0.48% available phosphorus respectively, with 5 negative control diets with 250, 500, 1,000, 2,500 or 10,000 FTU/kg diet Bestzyme phytase added. Each dietary treatment had 12 replicate pens each containing 9 broilers. On day 14 of age, overall body weight gain and feed to gain were determined, and 5 birds per pen were sacrificed for tibia ash determination. The remaining 4 birds were utilized for the determination of apparent digestibility of amino acids and minerals over the next 3 days. Data were analyzed by ANOVA and differences were considered significant when $P < 0.05$. From 0 to 14 days of age, body weight gain, feed to gain and tibia ash content were equivalent between the positive control birds and the negative control birds supplemented with 1,000, 2,500, and 10,000 FTU/kg diet. Apparent digestibility of calcium and all the essential amino acids was increased ($P < 0.05$) for the broilers fed the negative control diet supplemented with 2,500 FTU/kg diet relative to those fed the negative control diet. When manufacturing feed at 195° F, this new phytase only had a 7% decrease in activity when examined across 4 different preparations of the enzyme. The results indicate that this phytase could be a suitable alternative for broiler production and would be advantageous in situations where broiler feed was manufactured at high temperatures.

Key Words: poultry, tibia ash, feed to gain

P304 Myo-inositol and phytase supplementation to low protein diets differentially influence growth performance and nutrient digestibility in broiler chickens Tobi Ogunribido*^{1GS}, Abiola Lawal¹, Mike Bedford², Olayiwola Adeola¹, Kolapo Ajuwon¹ *¹Department of Animal Science, Purdue University; ²AB Vista - A division of Ab Agri Ltd., Marlborough*

Myo-inositol is an end-product of complete phytate hydrolysis by phytase. It is known to have modulatory effects on metabolism. However, its influence in broiler chicken nutrition and growth is unclear. This study was conducted to investigate the impact of dietary supplementation with phytase and myo-inositol on performance, nutrient digestibility, gut morphology, serum metabolites and inflammatory markers in broiler starters. A total of 384 day-old Cobb 500 broiler chicks were housed in a battery cage and fed a common starter diet for 7 days. On day 8, the chicks were randomly allotted to six diets consisting of 8 replicate cages of 8 birds per replicate cage for 14 days. The diets were arranged as 2 x 3 factorial of 2 dietary crude protein (CP) concentrations consisting of a positive control (PC) with 20% CP or a negative control (NC) with 16% CP and 3 dietary supplements consisting of non-supplemented, supplemented with myo-inositol (5.0 g/kg), or phytase (3,000 FTU/kg). There was no effect of diet on average daily gain or feed intake. The main effect of CP was significant for feed efficiency (Gain: Feed) such that feed efficiency was decreased ($P < 0.05$) in the NC relative to PC. Irrespective of dietary CP concentration, feed efficiency was lowest in the myo-inositol supplemented group and highest in the phytase supplemented group ($P < 0.05$). Phytase increased ($P < 0.05$) apparent ileal digestibility (AID) of P in both PC and NC groups. The AID of P was also higher ($P < 0.05$) in NC vs. PC. Phy-

tase supplementation increased ($P < 0.05$) villi height (VH) and VH:crypt depth (CD) ratio (VH:CD) in the jejunum. Serum *myo*-inositol concentration was increased ($P < 0.05$) by both dietary *myo*-inositol and phytase supplementation and blood urea nitrogen (BUN) tended ($P = 0.06$) to be lower in the NC group compared to PC. The gene expression level of tight junction protein occludin was lower ($P < 0.05$) in the jejunal mucosal of the NC group compared with PC without an effect of the supplements. In conclusion, *myo*-inositol and phytase supplementation differentially influence growth performance, gut structure and nutrient digestibility in broiler chickens.

Key Words: Broilers, Myo-inositol, Phytase, Protein

P305 Evaluation of supplementing a multi-carbohydrase enzyme complex to Pekin duck diets on growth performance, and carcass traits Melinda Grimes^{*1GS}, Jessica Rocha¹, Hector Leyva-Jimenez², Yemi Burden², Carlos Soto², Brian Dirks², Gregory Archer¹ ¹Texas A&M Agrilife Extension, ²United Animal Health

This study evaluated the effect of supplementing a multi-carbohydrase enzyme complex (MCE) in the feed of Pekin ducks on the performance and carcass traits. A complete randomized design was used to distribute 5 dietary treatments, each consisting of 10 replicate floor pens and 25 straight-run day-of-hatch ducks/pen. The treatments were: Positive control (PC, no energy reduction); Negative control (NC, -132 kcal/kg energy reduction); NC + MCE (Enspira[®]+, United Animal Health, USA) at 75 ppm (E75); 100 ppm (E100); and 125 ppm (E125). Body weight (BW) and feed consumption (FC) of birds from 0-35d of age were measured for each pen. Mortality corrected feed conversion ratio (FCR) was calculated as well as weight adjusted FCR (WTCFCR) to the PC. On d36, 5 ducks/pen were processed, and carcass, breast, and leg quarter weights and yields were determined. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation ($P < 0.05$). At d14, the PC treatment had a lower ($P = 0.01$) FC (68.5 g/d) compared to all other treatments (avg. 74.4 g/d). No differences in FC between treatments were observed cumulatively or during 14-35d of age ($P > 0.05$). NC weighed less (0.78 kg and 3.30 kg) than all other treatments at d14 and d35 (avg. 0.84kg and 3.46 kg, $P < 0.001$). FCR at d35 was lower ($P < 0.05$) in PC (1.379) compared to NC (1.460) while the MCE treatments were intermediate (avg. 1.414). When FCR was adjusted to a common BW the NC had a higher WTCFCR (1.516) than all other treatments (avg. 1.413, $P = 0.001$). The carcass, breast, and leg quarter weights were the lowest in NC (2.19, 0.59, 0.56 kg) compared to all other treatments (avg. 2.33, 0.64, 0.59 kg, $P < 0.03$). No yield differences were seen between treatments in carcass or leg quarter yields ($P > 0.05$). E125 had higher ($P < 0.05$) breast yield (28.0%) than NC (26.9%) and E100 (27.0%) with all other treatments being intermediate. A linear dose response was observed in d14 and d35 BW, d35 WTCFCR, carcass, breast, and leg quarter weights, and breast yield ($P < 0.05$). In con-

clusion, these results suggest that the MCE supplementation in the feed can improve duck growth, feed efficiency, and yield and that increasing inclusion rates led to greater gains.

Key Words: carbohydrase enzyme complex, pekin, Duck, performance, yield

P306 Effect of a commercial xylanase supplementation on top of phytase on digesta viscosity, growth performance, and carcass yield in broilers fed a complex diet M. Grimes^{*1}, A. Bello², E. Vinyeta Puntí², Y. Dersjant-Li³, G.S. Archer¹ ¹Department of Poultry Science, Texas A&M, ²Danisco Animal Nutrition & Health, IFF, Wilmington, 19803, Delaware, ³Danisco Animal Nutrition & Health, IFF, 2342 BH Oegstgeest, The Netherlands

This study evaluated the effect of supplementing a phytase-containing complex diet (consisting of corn, wheat, barley, soybean meal, sunflower meal, and rape seed meal) with a commercial xylanase on digesta viscosity, growth performance, and carcass yield of broilers across 1-42 days of age. A complete randomized design was used to test 3 dietary treatments, each consisting of 13 replicate pens (30 Ross 308 male broilers/pen). The treatments were: 1) Positive control (PC) diet supplemented with a consensus bacterial 6-phytase variant at 1,000 FTU/kg and down-specified for its contribution (66-44 kcal ME, $\leq 0.04\%$ dig AA, 0.21% Ca, and 0.175% dig P) vs. breeders recommendation; 2) Negative control (NC, reduced by 85 kcal/kg ME and 0.02% dig AA vs. PC, without added enzyme) and; 3) XLY diet (NC + 1000 FTU phytase and 2440 U xylanase per kg of diet). Body weight (BW), body weight gain (BWG), feed consumption (FC), feed conversion (FCR), and livability were measured. On d42, 5 birds a pen were processed for carcass, breast, tender, wing, and leg quarter wts and yield. Data were analyzed using the Tukey HSD for comparisons between means ($P < 0.05$). On d10, d21, d35 and d42, the PC and XYL had greater BW than the NC ($P < 0.05$), as a result, the PC and XYL had greater BWG during each phase compared to the NC ($P < 0.05$). Overall, 0-42d, PC (3,123 g) and XYL (3,105 g) had greater BW than NC (2,910 g; $P < 0.05$) and FCR was not significantly affected. XYL reduced ileal digesta viscosity at day 10 compared to PC ($P < 0.05$), with the NC being intermediate. The NC had the smallest carcass weights compared to the PC and XYL ($P < 0.05$). While feed cost/BWG was only numerically lowered by xylanase usage (0.70 vs. 0.72 USD/kg; $P > 0.05$), total carbon footprint/BWG was significantly decreased by xylanase treatment (2166 vs 2298 g CO₂ eq / kg; $P = 0.003$). No other differences were observed in part weights or yield ($P > 0.05$). These results demonstrated that XLY used in this study was able to compensate the ME and dig AA reduction, maintain performance and carcass quality in a nutrient deficient complex diet similar to that of a commercial diet containing phytase in the background, to further improve environmental sustainability.

Key Words: Xylanase, Broiler, Performance, Complex diet

Metabolism and Nutrition: Amino Acids

P307 Effects of a coccidiosis challenge on dietary methionine recommendations in broilers Julianna Jaspersen^{*1GS}, Juliano de Paula Dorigam², Kaitlyn Sommer¹, Cameron White¹, Laney Froebel¹, Bailey Harsh¹, Ryan Dilger¹ ¹University of Illinois, ²Evonik Operations GmbH, Nutrition & Care

Broilers are commonly exposed to coccidiosis infections, and the use of dietary strategies to reduce losses in growth performance has practical implications for the poultry industry. Met is typically the first limiting amino acid for broilers and is involved in metabolic and immunological pathways; however, literature is conflicting on how dietary Met requirements are affected by environmental stressors. Our objective was to assess whether the Met requirement changes in birds experiencing coccidiosis based on results of growth performance, carcass traits, and health out-

comes. Two trials were conducted using a total of 780 male Ross 308 broiler chicks in floor pens randomly assigned to treatment. All treatments received common starter (d 0-10) and finisher (d 24-35, Trial 2 only) diets, and only differed based on their assigned experimental grower diet (d 10-24). Trial 1 consisted of 6 treatments with diets ranging from 2.61 to 6.21 g/kg digestible Met. Trial 2 consisted of 6 treatments arranged in a 2x3 factorial with health status (uninfected or infected) and experimental diet formulated to contain Met at 15% below, at, or 15% above the requirement determined in Trial 1. Birds were exposed to an *Eimeria* challenge on d 11, with blood and tissue collection on d 18 and carcass processing on d 35 in Trial 2. Data in both trials were analyzed using a 1- or 2-way ANOVA. A non-linear regression analysis was conducted in Trial 1 to estimate the Met requirement of 4.32 g of digestible Met/kg

of diet using body weight gain. Coccidiosis infection reduced ($P < 0.05$) growth performance during the grower and overall study periods in Trial 2. Increasing dietary Met from below requirement to meeting requirement during the grower period improved ($P < 0.001$) body weight gain and feed conversion ratio (FCR), but this effect was only significant between treatments below and above the requirement for the overall study period. There was an interactive effect ($P = 0.038$) on FCR for the overall study period and on relative rack weight ($P = 0.009$). These findings provide evidence that the Met requirement likely increased when optimizing FCR during a coccidiosis infection.

Key Words: Methionine, broiler, coccidiosis, performance, amino acids

P308 Effect of arginine supplementation on the production, intestinal health, and immune response of broilers during necrotic enteritis challenge Shahna Fathima*^{1GS}, Walid Ghazi Al Hakeem¹, Revathi Shanmugasundaram², Ramesh Selvaraj¹ ¹University of Georgia, ²Toxicology and Mycotoxin Research Unit, US National Poultry Research Center

Restrictions on the use of in-feed antibiotic growth promoters in poultry led to the reemergence of necrotic enteritis (NE) in poultry, necessitating the need to develop alternatives to antibiotic growth promoters. Arginine is a conditionally essential amino acid that is the substrate for nitric oxide and ornithine biosynthesis. Arginine can modulate the immune response of birds to the *Eimeria* challenge by regulating macrophage differentiation and subsequent inflammatory pathways. This study evaluated the effects of 125% and 135% L-arginine on production, intestinal integrity, and immune responses in NE-challenged broilers. A total of 480 day-old chicks were randomly allocated into four treatment groups- non-infected group fed basal diet, NE + basal diet, NE + 125% arginine diet, and NE + 135% arginine diet. NE was induced by inoculating 1×10^4 *Eimeria maxima* on day 14 and 1×10^8 CFU *C. perfringens* on days 19, 20, and 21 of age. All data were analyzed by ANOVA and the means were compared by Tukey's HSD and were considered significantly different at $p \leq 0.05$. NE infection significantly increased the feed conversion ratio ($p = 0.01$), intestinal permeability ($p = 0.01$), jejunal lesion score ($p < 0.01$), decreased body weight gain ($p < 0.01$), and the expression of tight junction proteins occludins ($p < 0.01$) and claudin-4 ($p = 0.01$). 125% arginine diet increased the feed intake by 30g ($p = 0.02$) and reversed the NE-induced loss in BWG by 70g ($p = 0.12$). 125% arginine diet significantly increased the bile anti-*C. perfringens* IgA concentration by 39.74% ($p < 0.01$). Arginine supplementation significantly increased the ratio of CD8⁺:CD4⁺ cells in the spleen on days 28 ($p=0.01$) and day 35 ($p < 0.01$). 125% arginine supplementation also significantly increased the mRNA expression of iNOS and IFN- γ on day 21. However, different levels of arginine supplementation did not reverse the NE-induced loss in intestinal permeability nor restore the loss in production performance caused by NE. It can be concluded that the supplementation of 125% arginine may have immunomodulatory effects during NE, which when used in combination with other feed additives might have the potential to replace antibiotic growth promoters in broilers.

Key Words: L-Arginine, Broilers, Necrotic enteritis, Nutraceuticals, Nutritional immunology

P309 Methionine supplementation in reduced protein diets altered bone quality of broilers under coccidia challenge assessed by DEXA and Micro-CT scanning. Guanchen Liu*^{GS}, Venkata Choppa, Milan Sharma, Yuguo Tompkins, Woo Kim ^{Department of Poultry Science, University of Georgia}

Methionine (Met) is crucial for broiler growth, but over supplementation of Met could lead to accumulation of homocysteine, a known risk factor for bone health. We aimed to investigate the effects of different dietary Met levels on bone health parameters of broilers under coccidia challenge. Birds were randomly assigned in a 5×2 factorial arrangement. They were fed either normal protein diets (20% crude protein, NPD) or reduced pro-

tein diets (17% crude protein, RPD) containing 2.8, 4.4, 6.0, 7.6, and 9.2 g/kg of Met. Birds were challenged with mixed *Eimeria* species on day 14. Dual X-ray absorptiometry was performed to assess the whole body bone mineral density (BMD) and bone mineral content (BMC), and microtomography was performed to assess the 3D structural characteristics of the cortical and trabecular bones of the femoral metaphysis and diaphysis sections. Data were analyzed by two-way ANOVA and orthogonal polynomial contrast. On 9 days post inoculation (DPI), whole body BMD and BMC linearly decreased ($P < 0.05$) as Met increased. For the cortical bone of femoral metaphysis: On 9 DPI, BMD linearly decreased as Met increased ($P < 0.05$). Number of closed pores (NCP), volume of closed pores (VCP), and closed pore percentage (CPP) linearly increased as Met increased ($P < 0.05$). For the trabecular bone of femoral metaphysis: On 9 DPI, bone volume (BV) and BV to tissue volume ratio (BV/TV) of the RPD groups and the connectivity density and trabecular number linearly increased as Met increased ($P < 0.05$). The trabecular thickness linearly decreased as Met increased ($P < 0.05$). For the cortical bone of femoral diaphysis: On 6 DPI, BMC was higher whereas BV/TV was lower in the RPD compared to the NPD groups ($P < 0.05$). BMD linearly decreased as Met increased ($P < 0.01$). The NCP increased and then decreased as Met increased following a quadratic trend ($P < 0.05$), with 7.6 g/kg Met group showing the highest NCP. On 9 DPI, BMD of the NPD groups and BV/TV linearly decreased as Met increased ($P < 0.05$). NCP, VCP, and CPP changed quadratically as Met increased ($P < 0.05$), with the lower values observed in the 2.8 and 9.2 g/kg Met groups. In conclusion, our results suggest that increasing dietary Met levels adversely affect bone quality of broilers under coccidia challenge.

Key Words: Methionine, DEXA, MicroCT, Coccidiosis, Bone health

P310 The dietary supplementation of L-arginine alleviates the adverse effects of coccidiosis in laying hens infected with mixed Eimeria species Milan Sharma*^{1GS}, Robert Gogal², Prafulla Regmi¹, Lilong Chai¹, Todd Applegate¹, Woo Kim¹ ¹Department of Poultry Science, University of Georgia, ²Department of Biosciences and Diagnostic Imaging, College of Veterinary Medicine, University of Georgia

Coccidiosis in laying hens has a substantial economic impact on the egg industry as it can either delay the onset of egg production or temporarily cease egg production when infected at pre-lay or at peak production, respectively. Dietary supplementation of L-arginine might help alleviate the negative effect of coccidiosis by modulating immune responses, pathogen suppression, or tissue repair. An experiment was conducted to evaluate the effect of dietary supplementation of L-Arginine on the performance and intestinal health of laying hens infected with coccidiosis. A total of 180 Hy-Line W-36 laying hens aged 23 weeks of age were allocated into four treatment groups: (1) non-challenged control (NCC), (2) pair-fed control (PFC), (3) challenged control (CC), and (4) CC with 50% above L-arginine requirement (0.89% vs. 1.35%, ARG). The laying hens grouped in CC and ARG were orally gavaged with 12,500 *E. maxima*, 12,500 *E. tenella*, and 62,500 *E. acervulina* sporulated oocysts at 25 weeks of age. The PFC hens received the exact amount of feed consumed by CC from 0-14 days post inoculation (DPI). Growth performance, feed intake (ADFI), and egg production (HDEP) were measured from 0-21 DPI. Gut permeability was measured using FITC-d at 5 DPI, and gross lesions were scored at 6 DPI. Growth performance and permeability data were analyzed using one-way ANOVA, whereas a split-plot in time for ADFI and HDEP and the Kruskal-Wallis test were used for lesion scores. On 6 DPI, hens grouped in PFC, CC, and ARG had negative BWG; however, supplementation of ARG improved the BWG by 32% compared to that of CC ($P=0.005$). Overall, ADFI was lower in PFC (75.31 g), CC (74.74 g), and ARG (78.76 g) than that of control (95.74 g; $P<0.0001$). During the challenge period, 0-21 DPI, ARG supplementation improved the HDEP by 9.79% and FCR by 0.66 per dozens of eggs compared to CC; however, they were still 13.68% lower and 1.02 higher than PFC ($P<0.0001$). Furthermore, ARG supplementation reduced the gut permeability and the

severity of the intestinal lesions compared to that of CC hens ($P < 0.05$). To conclude, dietary supplementation of L-arginine not only partially alleviated the adverse effects of coccidiosis but also improved the performance and intestinal health of laying hens.

Key Words: Coccidiosis, Laying hens, Arginine, Intestinal health, Gut Permeability

P311 Pellet die thickness and the use of a throughput agent interacted to demonstrate that high frictional heat increased apparent ileal amino acid digestibility but did not influence trypsin inhibitor activity or male broiler performance Lucas Knarr^{*1}, Kristina Bowen¹, Jon Ferrel², Sunhyung Kim³, Hari Krishnan⁴, Joseph Moritz¹ ¹West Virginia University, ²Azomite Mineral Products, ³University of Missouri, ⁴USDA Agricultural Research Service

The inclusion of Azomite® (AZM) in broiler diets containing dicalcium phosphate has been shown to increase apparent ileal amino acid digestibility (AIAAD). These findings are likely due to die-scouring and lubrication properties that decreased the frictional heat exposure of feed. Past research indicates that modifying pellet die thickness (PDT) affects the frictional heat exposure of feed. Therefore, it was hypothesized that PDT and AZM would interact to influence AIAAD and broiler performance. The study's objective was to determine the effect of AZM (0.0% or 0.25%) and PDT (32 and 45 mm with a common pellet diameter) on broiler performance and AIAAD from 0 to 21 days of age. Live performance did not differ due to the interaction or main effects ($P > 0.05$). However, AIAAD was influenced by AZM and PDT interactions ($P < 0.05$), with 11 amino acids demonstrating increased digestibility in the 45mm control treatment. The AIAAD increase was likely not enough to influence performance. The increased frictional heat was presumed to deactivate trypsin (TI) and chymotrypsin inhibitors (CTI), ultimately increasing AIAAD. Quantitative analysis of TI and CTI activity, utilizing a novel assay based on the current American Oil Chemists' Society and the American Association of Cereal Chemists International accepted procedure, showed no practically influential amount of either inhibitor before or after pelleting. The authors, therefore, speculate that the increased AIAAD was due to cell lysis of the corn aleurone layer via increased frictional heat exposure of the 45mm PDT and the absence of AZM.

Key Words: Pellet Die Thickness, Trypsin Inhibitor, Aleurone Layer

P312 Effects of graded levels of methionine on growth performance and digestive tract characteristics Kaitlyn Sommer^{*1}, Maci Oelschlager¹, Juliano de Paula Dorigam², Rose Whelan², Julianna Jespersen¹, Lancy Froebel¹, Ryan Dilger¹ ¹University of Illinois - Urbana Champaign, ²Evonik Nutrition & Care GmbH

Methionine is typically the first-limiting amino acid (AA) for broilers and plays a critical role in growth and nutrient allocation. This study determined the methionine requirement of chickens with a fixed cysteine level in maximizing both body weight gain and gain:feed. A total of 798 male broilers (Ross 308) were housed in battery cages and allotted to 1 of 7 dietary treatments, with 14 replicates of 6 birds per replicate. Diets were provided *ad libitum* in 2-phases with a common starter diet and experimental grower diets provided d 0-10 and 10-24, respectively. Grower diets included a low total sulfur AA (LTSAA), and a basal diet (0.16% total Met) supplemented with 0.360% L-Cys [0% suppMet], and 5 experimental diets with graded levels of suppMet (0.072% increments provided as DL-Met). To ensure the only limiting AA would be Met, other AA were included at 15% above AMINOChick 3.0 recommendations. On study d 10 and 24, birds and feeders were weighed, and one bird per cage was euthanized to collect organ weights and lengths on d 24. Data were analyzed via 1-way ANOVA using the MIXED procedure of SAS. Body weight gain during the grower phase was lowest ($P < 0.01$) in 0%DLM compared with 0.216%DLM or higher. Birds receiving 0%DLM had the poorest feed conversion ratio ($P < 0.01$) compared with treatments \geq

0.216%DLM). This effect corresponded to feed intake being lowest ($P < 0.01$) for 0%DLM compared with treatments containing 0.216% or more of supplemental Met. Birds receiving 0.288%DLM had the shortest ($P < 0.01$) relative duodenum compared with LTSAA and 0%DLM groups. However, birds receiving 0%DLM had the shortest ($P < 0.01$) relative ileum compared with 0.288%DLM. Relative duodenum and jejunum weight increased ($P < 0.01$) in birds fed LTSAA and 0%DLM, when compared with birds receiving 0.288% suppMet or more, whereas relative ileal weight was highest ($P < 0.01$) in birds receiving LTSAA compared with all other treatments. In conclusion, broilers receiving diets deficient in Met exhibited a reduced growth performance, which may have contributed to increased intestinal weights and lengths.

Key Words: broiler, methionine, growth performance

P313 Dietary arginine responses of Cobb 500 broilers reared under cyclic elevated temperatures. Annalise Anderson^{*1}, Jason Lee², Roshan Adhikari², Samuel Rochell¹ ¹Department of Poultry Science, Auburn University, ²CJ America, Inc.

The objective of this study was to evaluate the effects of dietary arginine level on performance and processing characteristics of broilers subjected to a cyclic heat stress (HS) challenge model. A total of 1,200 male Cobb 500 broiler chicks were fed common starter (0-14 d) and grower (14-27 d) diets before being fed 1 of 6 dietary digestible Arg:Lys ratios (80, 92, 104, 116, 128, and 140) across both finisher 1 (27-38 d) and finisher 2 (38-46 d) phases. Birds were reared in floor pens and treatments were replicated by 8 pens of 25 birds. At placement, barn temperature was set to 32°C and gradually decreased to maintain bird comfort until initiation of the HS challenge at d 28. From 28-47 d, barn temperature was maintained at 32°C for 12 h daily (7:30 h to 19:30 h) and reduced to 24°C each night. At 32, 39, and 46 d, cloacal temperatures were measured in 2 birds per pen at approximately 06:30 h (thermoneutral) and 2:30 h (HS). At 48 d, 8 birds per pen were processed and deboned to determine carcass weights, including parts weights and yields. Data were analyzed by one-way ANOVA with pen location as a random blocking variable. Linear and quadratic contrasts of Arg:Lys ratio were also performed. Statistical significance was determined at $P \leq 0.05$ and trends were considered at $P < 0.10$. Feed conversion ratio improved linearly with increasing Arg:Lys ratio in the diet from 27-38 d ($P = 0.018$) and tended to improve linearly ($P = 0.056$) from 27-46 d. However, no effects were observed ($P > 0.05$) for body weight gain or feed intake. Cloacal temperatures were approximately 1.6°C higher during the HS than during the thermoneutral period at each time point, but were not influenced by dietary Arg. For processing characteristics, there was a quadratic response for both fat pad weight ($P = 0.005$) and yield ($P = 0.019$) with increasing Arg and linear increases in the weight of thighs ($P = 0.009$) and drums ($P = 0.035$). Overall, the results of this study indicate that dietary Arg may improve feed efficiency and influence processing characteristics in a dose dependent manner in Cobb broilers subjected to a cyclic HS challenge, without influencing core body temperature.

Key Words: Arginine, Cyclic heat stress, Broiler, Processing characteristics, Cloacal temperature

P314 Effect of supplementation of DigeGrain Pro-6; a novel protease on performance, gut health and expression of growth-related genes in broiler chickens Utsav Tiwari^{*1}, Dipak Roda *Enzyme Innovation*

Around 10-40% of undigested proteins are fermented by cecal bacteria. Thus, to alleviate the birds gut health it is imperative to reduce undigested protein content in ileum and cecum. The objective of this study was to access the influence of DigeGrain pro 6 (DGP) on performance, digestibility and the expression of growth-related genes and amino acid transporters in broiler. DGP a unique protease helps to increase dietary protein hydrolysis and thus enabling improved nitrogen utilization and reduce nitrogen excretion in the manure. A total of 800 Ross 308 birds were randomly allotted to 5 treatments (10 replicates, 15 birds/replicate); standard con-

trol diet (STD), STD-0.75% (0.75% reduced CP), STD-1% (1% reduced CP), STD-0.75% with DGP, STD-1% with DGP. DM digestibility of DigeGrain Pro 6 supplementation treatments were higher ($p < 0.05$) than those of non-supplementation treatments, CP digestibility was the highest ($p < 0.05$) in STD-0.75% with DigeGrain Pro 6 treatment. Dietary DigeGrain Pro 6 supplementation increased ($p < 0.05$) lysine digestibility as compared between the same CP level diets treatments. Villus height of duodenum and jejunum were the greatest ($p < 0.05$) as DigeGrain Pro 6 supplemented to the STD-0.75% diet. Gene expression of IGF1, IGF2, GH in liver and MYOD1 and MYOG in the breast muscles increased ($P < 0.05$) when compared to control diet. Thus, DGP supplementation in the diet improved the growth performance of broilers via an increase in the expression of growth-related genes within liver and muscle tissue.

Key Words: DigeGrain Pro 6, growth related genes, DigeGrain Pro-6

P315 A determination of standardized ileal digestible lysine requirement of Arbor Acres broiler in the pre-starter phase from 0 to 10 days Thanaphat Siriphongsathat^{*1}, Ratchasak Thommasaroeh¹, Apidech Buangam¹, Suthasinee Udchachon¹, Nisaphat Wuttipapit¹, Kanitnat Taraka¹, J Pimpech¹, Patrapan Rungcharoen¹, Nickki Tillman², Roselina Angel³ ¹Thaifoods Group Public Company Limited, ²Nutritional Statistics LLC, ³Department of Animal and Avian Sciences, University of Maryland

Feeding to a nutrient requirement which optimizes performance, as well as economic return under commercial conditions, requires precise formulation and an understanding of nutrient needs. The objective of this study was to determine the standardized ileal digestible lysine (SID Lys)

requirement of male Arbor Acres broilers from d 0 to 10d by feeding varying SID Lys levels. Two corn-SBM diets were formulated to SID Lys levels of 0.85% (Low, Base) and 1.60% (High, Summit). The Low diet was created by the removal of added L-Lysine HCl from the High diet. As such, Key essential added amino acids: DL-Methionine, L-Threonine, L-Valine, L-Isoleucine, L-Arginine, Glycine and L-Tryptophan, were maintained in order to eliminate any confounding factors and to maintain CP level as similarly as possible. All diets were isocaloric based upon AMEN content. Four (4) intermediate treatments (Trts) were created by blending the Low and High Trts appropriately. The result was to create a total of 6 Trts with a SID Lys increment of 0.15% points. All diets were pelleted and crumbled. A total of 1,680 one-day-old male Arbor Acres broilers were allocated to 42 pens with 7 pens per Trt, containing 40 birds per pen. Linear and Quadratic contrasts were used to determine if regression analysis could be utilized. When the quadratic contrast showed a significant p-value ($p < 0.05$), a break-point determination using the Quadratic Broken-Line (QBL) regression model was used as it is a better analysis for a biological function. The SID Lys level which maximized BW, BWG and minimized FCR was determined at the break-point / plateau from the generated QBL model. BW and BWG were maximized when broilers were fed SID Lys at 1.39% with an R-square of 0.98, while FCR was minimized at SID Lys of 1.28% with a R-square of 0.78. Based on these results, the determined SID Lys which maximized BW and BWG is higher, while the determined SID Lys for FCR is lower, than the current breeder recommendation (1.32%). Overall, from this trial, the average SID Lys of 1.34% is slightly higher than the current breeder recommendation.

Key Words: Broiler, Lysine, Requirement, Quadratic broken-line model

Metabolism and Nutrition: Vitamins and Minerals

P316 Effects of different dietary sources and levels of zinc on bone mineralization and mineral digestibility of broiler chickens Mohammad Pilevar^{*}, Oluyinka Olukosi *Department of Poultry Science, University of Georgia*

The objective of this study was to evaluate bone characteristics and mineral digestibility for broilers fed Zn from four different sources (source) at two dietary concentrations (level) for each Zn source. A 4 × 2 factorial plus a basal diet was used. Factors were four Zn sources, namely Zn sulfate (ZS), Zn hydroxy chloride (ZH), Zn glycinate (ZG), and Zn lysine-glutamate (ZL), each supplemented at 25 or 100 ppm. The basal diet was a corn-soybean meal diet without supplemental Zn. On day zero, chicks with the same body weights ($\pm 4g$) were allocated into 9 treatments, each with 7 replicates and 6 birds per cage. The left tibia and toes were collected from two birds per cage on day 20, and ash percentage and Zn concentration were measured in the bones. Also, the ileal digesta were collected from the same birds and oven-dried to measure mineral apparent ileal digestibility using the index method. There was no significant source × level interaction for toe ash, tibia ash, and tibia Zn concentration. Toe ash was greater ($P < 0.05$) for birds receiving 100 ppm (12.7%) compared to 25 ppm (12.3%). The toe ash in birds receiving the basal diet (13.2%) was greater ($P < 0.05$) compared to the Zn-supplemented diets. There was a significant source × level interaction for ileal DM digestibility. This resulted in no change in DM digestibility by increasing Zn levels in ZS and ZH diets, but a decrease in DM digestibility in birds receiving higher supplemental levels of ZG and an increase ($P < 0.01$) in DM digestibility in birds receiving a higher supplemental level of ZL was observed. Zn sources significantly affected the digestibility of P, Cu, and marker-corrected concentration of Cu. Birds receiving ZS diets had lower P digestibility than those fed ZH, ZG, and ZL-supplemented diets. Birds fed the ZL diet had higher Cu digestibility ($P < 0.05$) and lower marker-corrected Cu concentration ($P < 0.05$) than birds receiving the other Zn sources. Higher dietary Zn level tended to show a lower Cu digestibility.

A significant main effect of Zn level was observed for marker-corrected concentration of Zn, with the ileal Zn concentration being higher in birds receiving 100 ppm diet. In conclusion, Zn source, rather than level, had a greater influence on responses reported herein.

Key Words: Zinc sources, Zn level, bone mineralization, minerals digestibility, broiler chickens

P317 A comparison of two methodological approaches for evaluating the effect of vitamin A supplementation in broiler chickens Pinar Sacakli¹, Muhammad Shazaib Ramay¹, Josoa Andre Harijaona¹, Yauheni Shastak², Wolf Pelletier², Alvaro Gordillo³, Kevin Matter^{*4}, Ali Calik¹ ¹Ankara University, ²BASF SE, ³BASF Espanola S.L., ⁴BASF Corp.

The most reliable measure of retinoid nutritional status is the concentration of total retinol in the liver. The present study aimed to compare two approaches for evaluating the effect of vitamin A (VA) acetate supplementation in broilers, focusing on the levels of retinol in the hepatic tissue. An ethoxyquin-free VA 1000 source, Lutavit® A 1000 NXT, was supplemented at incremental levels of 2,000, 4,000, or 8,000 IU per kg of feed. For the first approach (A1), 336 day-old Ross 308 chicks were distributed among 28 floor pens for a 23-day grow-out study. After a 15-day depletion period with no dietary VA, a VA unsupplemented control diet, or VA-supplemented diets based on wheat and soybean meal were fed for 7 days (repletion period) to 7 replicates per treatment, with 12 birds per pen. Simultaneously, for the second approach (A2), 336 day-old Ross 308 chicks were distributed among 28 floor pens for a similar 23-day grow-out study in the same poultry house. In contrast to the feeding method in A1, the same experimental wheat-soybean meal-based diets (with 0, 2,000, 4,000 or 8,000 IU of VA/kg feed) were fed from d 1 to 23 post-hatch with no depletion period (7 replicates per treatment, with 12 birds per pen). On day 23, three chicks representing the average body weight of birds in each pen were euthanized, and their liver samples were pooled per pen for

retinol analysis. Data were analyzed using the ANOVA procedure of the SPSS software. One-way ANOVA was used to determine the effects of VA supplementation, and significant means were separated using the Tukey test. Birds fed VA-supplemented diets had higher ($P < 0.05$) hepatic retinol levels than broilers fed VA-free diets in both approaches (A1 and A2). Birds fed 8,000 IU VA/kg feed had higher ($P < 0.05$) liver retinol levels compared to birds fed 4,000 or 2,000 IU/kg in A1 and A2. Furthermore, broilers supplemented with 4,000 IU of VA showed a significantly higher ($P < 0.05$) liver retinol concentration than those supplemented with 2,000 IU in A2 but not in A1. In conclusion, based on liver retinol concentrations, the second approach (A2) appears to be more sensitive in evaluating the effect of vitamin A supplementation due to the provision of a more sensitive ranking between single supplementation levels of VA.

Key Words: Vitamin A acetate, Evaluation, Broiler, Ethoxyquin-free

P318 Improvement of Zinc absorption and productive performance in Broilers through the supplementation of Glycinate of Zn (Glymet 40%). Organic vs inorganic sources. Javier López-Paredes*¹, Monica Puyalto¹, Karan Singh¹, Dayaram Suryawanshi², Juan Jose Mallo¹ ¹NOREL SA, ²OMEGA LABORATORIES

The present study was conducted to compare the effects of adding Zinc (Zn) in inorganic forms (ZnO and ZnSO₄) and organic form (Zn Glycinate, Glymet 40%) on the potential absorption of Zn and the productive performance of broilers from birth to 6th week. Firstly, Zn absorption was estimated according to Zhang et al. (2018), and Zn content was quantified in tibia, muscle, and serum expressed in milligrams of Zn per kilogram of tissue. Secondly, productivity parameters were assessed, including average daily gain (ADG, g/d), feed conversion ratio (FCR, kg of feed per increase in body weight in kg), and economic performance evaluated through the European Efficiency index of Fattening (EEF). In this trial, 500 birds were grouped by four treatments: T₁, which received the control basal diet; T₂, basal diet + 100 ppm of Zn from ZnSO₄; T₃, basal diet + 100 ppm of Zn from ZnO; and T₄, basal diet + 100 ppm of Zn from Glymet Zn 40%. Statistical differences between the treatments and the control were assessed using the Dunnett test, and differences between treatments were estimated through the Tukey test, establishing significance at $p < 0.05$. The results revealed a substantial increase in Zn retention for the organic source comparing to the basal diet (from -40 mg in T₁ to 289 mg in T₄) and with the diets supplemented with inorganic sources, signifying a 12% and 13% enhancement from T₂ and T₃ values, respectively ($p < 0.05$). Additionally, T₄ demonstrated an increase in Zn content compared to T₁, T₂, and T₃, in serum (115%, 49%, and 51%, $p < 0.05$), tibia (115%, 35%, and 36%, $p < 0.05$), and muscular tissue (128%, 18%, and 25%, $p < 0.05$). Productivity parameters consistently favoured T₄, with higher ADG values in 10.9, 7.5, and 7.9 g/d ($p < 0.05$) and a value of FCR of 1.54 kg/kg, reducing in 11.4%, 7.6%, and 7.9% ($p < 0.05$), comparing with T₁, T₂, and T₃, respectively. Furthermore, T₄ exhibited greater EEF, with values from 13% to 20% higher than T₁, T₂, and T₃ ($p < 0.05$). These results suggested that Glymet 40% serves as a more suitable source of Zn than inorganic sources, offering a potential economic advantage due to improvements in productivity, enhanced feed efficiency, and Zn absorption.

Key Words: organic trace minerals, Zinc, Productivity, economic performance, bioavailability

P319 The supplementation with Glycinate of Zn (Glymet 40) improves the intestinal development, the immunological status and reduces the generation of free radical in Broilers. Javier López-Paredes*¹, Monica Puyalto¹, Karan Singh¹, Dayaram Suryawanshi², Juan Jose Mallo¹ ¹NOREL SA, ²Omega Laboratories

The aim of this study was to evaluate the impact of the inclusion of an organic Zinc supplement (Glymet 40%) on intestinal development (villus width and length of the duodenum, ileum, and jejunum), the immune system (antibodies against Newcastle Disease (ND), Infectious Bursal

Disease (IBD), immunoglobulins (IgA, IgG, and IgM)), and potential redox status (T-SOD and Zn-SOD), in comparison with inorganic sources of zinc such as ZnO and ZnSO₄. The experiment was conducted by Omega Laboratories (Lonand, Tal-Khandala, India), where 500 birds were divided into four treatment groups: T₁, the Control basal diet; T₂, Basal diet + 100 ppm of Zn (ZnSO₄); T₃, Basal diet + 100 ppm of Zn (ZnO); and T₄, Basal diet + 100 ppm of Zn (Glymet Zn 40%). Ten animals per group were sampled for analysis of the proposed parameters. Statistical analyses using the Dunnett test and Tukey test were performed to assess differences between the groups. Intestinal development was significantly greater in T₄, with the most pronounced improvement was observed in the duodenum, where the width was a 25 to 44% and the length a 10 to 19% higher compared to the other treatments ($P < 0.05$). Additionally, the jejunum and ileum exhibited superior performance in T₄, with increased values for length (11 to 23% higher) and width (13 to 39% higher), indicating enhanced intestinal development in birds supplemented with more bioavailable sources of Zinc. Moreover, there were enhanced values of immune responses in T₄, with ND antibodies showing 3.7 times increase compared to the basal diet response ($P < 0.05$) and 8 times increase for IBD antibodies ($P < 0.05$). Higher values were also observed for IgG, IgA, and IgM. Additionally, the antioxidant status was superior to that of inorganic sources, implying a better adaptation to stressful conditions. These results emphasize the advantages of using organic sources of Zinc over inorganic sources and their superior potential in coping with stressful situations. In conclusion, the results demonstrate the benefits of using Glymet Zn to promote optimal immune status and improved intestinal health.

Key Words: organic trace minerals, Zinc, immunological system, health, poultry

P320 Evaluation of Zn, Cu and Mn Methionine-Hydroxy-Analogue Chelate associated with Benzoic acid and essential oils on performance, incidence of footpad lesions and carcass quality in broilers Liris Kindlein*¹, Tainá Simonetti¹, André Favero², Sergio Vieira¹, Kelen Zavarize³, Alessandro Lima³, Gabriela Cardoso³, David Torres³ ¹UFRGS, ²Santa Livia Farm, ³Novus do Brasil

The objective of this study was to evaluate the supplementation of Zn, Cu and Mn Methionine-Hydroxy-Analogue Chelate (MHA) in combination with Benzoic acid and Protect Thymol and Carvacrol on performance, incidence of footpad lesions and carcass quality in broilers. A total of 1,440 d-old Ross male chicks were used in this study and randomly distributed into 2 treatments with 20 replicates. The pens are built inside of a commercial house populated with broilers placed at the same day. The treatments were: T1: Sulfates 100 ppm Zn, 125 ppm Cu and 80 ppm Mn (sulfates), and T2: Methionine-Hydroxy-Analogue Chelate 40 ppm Zn, 30 ppm Cu and 40 ppm Mn + 500g Protect Benzoic acid + 15g Protect Thymol and Carvacrol 15g (OTM+eubiotics). The diets were formulated to meet nutritional requirements and be equivalent in amino acids value. Litter moisture was controlled over 45% to induce footpad lesions. At 42 days, feed intake (FI), body weight gain (BWG), feed conversion rate (FCR), footpad lesions (FPL) and carcass quality variables were measured. Data were analyzed using one-way ANOVA and means separated using T test ($p < 0.05$). Broilers fed organic minerals in combination with eubiotics presented better FCR (1.591 vs 1.621; $P < 0.05$). The supplementation of these additives also decreased severity of FPL in 38.7% (79.3 vs. 48.6%, $P < 0.05$). Broilers fed OTM+eubiotics presented lower incidence of high FPL (scores of 4 and 5; 5.9 and 6.6% respectively) than animals fed sulfates (26.9 and 36.9). Differences were also found for carcass quality variables. Birds fed OTM+eubiotics presented lower frequency of folliculitis (17.5 vs. 6.3%, $P < 0.001$); hock injury (22.9 vs. 6.6%, $P < 0.001$) and score 2 breast lesions (high level) (0.0% vs. 5.0, $P = 0.03$). Breast meat of OTM+eubiotics birds showed lower cooking loss than sulfate group (17.4 vs. 16.6, $P = 0.02$). No differences were found on breast myopathies incidence. The results indicate that supplementation 40 ppm Zn, 30 ppm Cu and 40 ppm Mn MHA-chelate in combination with Benzoic acid and

Protect Thymol and Carvacrol improved performance and foot pad dermatitis on broilers exposed to field conditions. Additionally, this nutritional strategy decreased skin lesions, folliculitis, hock injury and cooking loss in broilers.

Key Words: broilers, eubiotics, performance, meat quality, mineral supplementation

P321 Systematic review of composition of laying hen vitamin-mineral premixes used in the literature during the last 15 years AHMET PEKEL* *Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, 34320*

The aim of the study was to investigate the vitamin and mineral content of the premixes used in laying hen diets in peer-reviewed publications published in the last 15 years and to estimate premix composition and cost of premixes by using common vitamin and mineral sources to arrive at their dietary supplemented levels. The composition of 38 vitamin-mineral premixes and 22 separate vitamin and mineral premixes used in laying hen diets in published articles was studied. SPSS 21.0 statistical software was used for correlation and regression analysis. The average dietary inclusion levels for vitamin-mineral, vitamin, and mineral premixes were 0.42, 0.20, and 0.18%, respectively. The most commonly included vitamins among

the premixes were vitamin A, vitamin D, and vitamin B₃, which were present in all the premixes examined. Approximately 37%, 28%, 28%, 27%, and 27% of the premixes did not contain choline, vitamin B₆, vitamin B₇, vitamin B₁, and vitamin B₉, respectively. Manganese, Zn, and Cu were the most commonly included minerals and were used in 92%, 90%, and 85% of the premixes investigated, respectively. The lowest CV (6.5) was observed on Se levels among vitamins and minerals across premixes investigated. The highest CV in both vitamin premixes and vitamin-mineral premixes were for vitamin B₁₂ (311) and vitamin B₇ (249). Vitamin B₁₂, Mn, choline, and menadione supplementation levels were the ones most commonly lower than dietary supplemental levels recommended by Lohmann. Vitamin E, vitamin B₃, and vitamin A had the highest average estimated cost per vitamin in vitamin premixes investigated and three of them together represented approximately 50% of the total vitamin premix cost. The costliest minerals were Zn and Fe which accounted for 40 and 22% of the average estimated total cost of mineral premixes, respectively. The highest correlations between levels of vitamin-mineral per kg/diet and levels of vitamin-mineral per kg/premix were found for Se (0.99), vitamin B₁₂ (0.97), and vitamin B₇ (0.95). In conclusion, there is quite a high variation in the composition of vitamin and mineral premixes, and the premix compositions can be predicted from dietary supplemental levels

Key Words: laying hen, mineral, premix, survey, vitamin

Metabolism and Nutrition: General Nutrition

P322 Evaluation of the adaptation in the novel methodology to determine internal particle size of pellets Urias Castillo*^{UG}, Eva Guzman, Cistina Simões, Isadora Pegoraro, Beatriz Onishi, Erasmo Flores, Jose Vargas, Jose Hernandez, Joseph Gulizia, Leticia Orellana, Wilmer Pacheco *Department of Poultry Science, Auburn University*

Feed particle size (PS) influences broiler performance, organ development, and digestibility of nutrients. During the pelleting process, the PS of mash diets can be reduced by additional grinding, resulting in a lower PS in the pellets microstructure. Therefore, a novel methodology was developed to measure the PS in the microstructure of pellets of broiler diets. The methodology consists of three main steps: feed hydration, filtering, and drying. One step of the previous methodology consisted of recovering fine particles that passed through a 316 µm screen using paper filters inside aluminum pans. This step was modified by centrifuging the supernatant containing particles smaller than 316 µm for 5 min at 2,500 rpm. The objective of this study was to evaluate the consistency of the methodology after this adaptation. A total of 12 mash samples of corn-soybean meal-based diet were collected after mixing. Six samples were analyzed for PS distribution right after the sample collection (control mash) using the ASABE method S319.4, and the other six samples were submitted to the novel methodology (hydrated/dehydrated mash) with the modification and then submitted to PS analysis. Data were analyzed as one-way ANOVA using the GLM procedure of SAS, and the means were separated using the Tukey test. Significance was accepted at $P \leq 0.05$. A higher ($P < 0.05$) particle size was obtained in the hydrated/dehydrated mash samples in comparison to the control mash samples (747 vs. 699 µm). Samples analyzed with the novel methodology had a higher ($P < 0.05$) percentage of particles over sieves 420, 590, and 840 µm and a lower percentage over sieves with openings of 21, 149, 105, 74, and 53 µm in comparison with the control mash. This information can be used to improve the methodology further and, more precisely, measure the PS in the microstructure of pellets and calculate the degree of grinding during pelleting in future studies.

Key Words: Particle size, Methodology, Pellet microstructure

P323 Influence of conditioning temperature and retention time on pellet quality of corn soybean meal-based diets Eva Guzman Guzman*^{GS}, Beatriz Onishi, Cristina Simões, Erasmo Flores, Isadora Pegoraro, Jose Vargas, Jose Hernandez, Joseph Gulizia, Leticia Orellana, Urias Castillo, Wilmer Pacheco *Department of Poultry Science, Auburn University*

Pelleting improves broiler performance and flock uniformity as it reduces feed wastage, selective feeding, and nutrient segregation. Factors such as feed formulation, feed production rate, particle size, conditioning process, pellet die specifications, and cooling conditions can influence pellet quality. Steam added during the conditioning process increases moisture and temperature of the feed, increasing physiochemical reactions of starch and protein and activating their binding properties. This study consisted of two experiments aimed to assess the effect of retention time and conditioning temperature on pellet durability index (PDI). A corn-soybean meal-based diet was mixed in a twin-shaft ribbon mixer for 180 seconds and pelleted using a 4 mm die. During the first experiment, four batches of feed were conditioned at 85°C and subjected to two retention times (45 and 90 seconds). For each retention time, eight samples of pellets were collected in three-minute intervals directly after the pellet mill and cooled for 12 minutes. The second experiment evaluated two conditioning temperatures (85°C and 90°C) with a retention time of 45 seconds. Eight samples of pellets were collected after cooling. For both experiments, PDI was measured following the ASABE S269.5 tumbler and the Holmen durability (30 and 60 seconds) methods. Data were analyzed as a one-way ANOVA using the GLM procedure of SAS, and means were separated using a Tukey test. Significance was accepted at $P \leq 0.05$. Retention time during conditioning did not influence PDI ($P > 0.05$). However, diets conditioned at 90°C had higher ($P < 0.05$) PDI (95.46 %) compared with diets conditioned at 85°C (95.04 %) when analyzed with the tumbler method. In this study, increasing the retention time during conditioning from 45 to 90 seconds did not influence pellet durability. However, increasing conditioning temperatures from 85 to 90°C improved PDI slightly. Further studies are encouraged to evaluate different ranges of conditioning retention time and temperature and the effect of their interaction on pellet quality and animal performance.

Key Words: pellet quality, retention time, conditioning temperature, pellet durability index

P324 In vitro Dose-Response Effects of Fumonisin B1 and Deoxynivalenol on the HD-11 Macrophage Cell Line Following Lipopolysaccharide Challenge Laharika Kappari*^{1GS}, Passion Locklear², Joseph Dasireddy¹, Todd Applegate¹, Revathi Shanmugasundaram³
¹University of Georgia, ²University of North Carolina at Pembroke, ³Toxicology and Mycotoxin Research Unit, USDA

The high incidence of fusarium mycotoxins fumonisin B1 (FB1) and deoxynivalenol (DON) in cereal grains and silages can be a potential threat to feed safety and the poultry industry. The function of monocytes, which includes their differentiation into active macrophages, plays a pivotal role in the immune response. Employing cost-effective in vitro cell culture models proves efficient for the initial screening, assessment, and development of mitigation strategies for mycotoxins. To investigate the effect of FB1 and DON on chicken macrophage function, in vitro studies were conducted using the HD11 chicken macrophage cell line. This study focused on the effects of FB1 and DON on nitrite production and mRNA levels of interleukin (IL)-1 β , TNF- α , IL-10, and induced nitric oxide synthase (iNOS) post-lipopolysaccharide (LPS) challenge. The cells were pre-incubated for varying concentrations (500, 250, 100, and 50 mg/ml) of FB1, DON, or a combination of both for 48 h and treated with LPS. Each dose was tested in triplicate across three independent experiments ($n = 3$). The supernatants from the HD11 cells with FB1 at 500 and 250 mg/ml in the presence of LPS significantly decreased nitrite production by approximately 7-fold and 5-fold, respectively, compared to the LPS group ($P < 0.05$). The supernatants from the DON up to 100 mg/ml concentration significantly decreased the nitrite concentration ($P < 0.05$) in the presence of LPS compared to the LPS group by 10-fold, 7-fold, and 6-fold, respectively. Decreasing the DON concentration further to 50 mg/ml had no effect on the nitrite concentration. At 48 h, HD11 cells treated with FB1 at 100 mg/ml and stimulated with LPS had a lower level of iNOS mRNA compared to the LPS group. At 48 h post-LPS treatment, HD11 cells treated with either FB1 or DON and stimulated with LPS had significantly increased TNF- α ($p < 0.05$) and decreased IL-1 β and IL-10 mRNA compared to the group treated with LPS ($P < 0.05$). At 48 h post-LPS treatment, HD11 cells were treated with a combination of FB1+DON at 50 mg/ml, further decreasing mRNA transcription compared to the group treated with LPS ($P < 0.05$). In conclusion, FB1 and DON affected macrophages differently, and their combined effects were mainly additive in nature.

Key Words: Mycotoxin, Fumonisin, Deoxynivalenol

P325 Modeling of thermal inactivation of avian pathogenic Escherichia coli, E. coli O157:H7 and non-pathogenic E. coli in poultry feeds using a lab-based circulating water bath Michael Carroll*^{1GS}, Cassidy Morris¹, Charis Waters¹, Pratima Adhikari¹, Kelley Wamsley¹, Cangliang Shen², Timothy Boltz¹ ¹Mississippi State University, ²West Virginia University

Feed hygienics are of increasing concern for poultry producers in the effort to produce safe feed for poultry and ultimately safer products for the consumer. One disease of concern for broilers is colibacillosis which is caused by Avian Pathogenic *E. coli* (APEC). This disease is a persistent issue for poultry producers often resulting in decreased performance, production, and animal welfare. While a common vector for *Escherichia coli* (*E. coli*) transmission to poultry can be feed, proper feed manufacturing can help mitigate the prevalence of pathogens with thermal treatment during conditioning. This study aimed to model the thermal inactivation of non-pathogenic *E. coli*, APEC, and *E. coli* O157:H7 during thermal processing in a lab-based circulating water bath and to determine the suitability of this non-pathogenic *E. coli* as a surrogate for future research. Two-gram samples were inoculated with one of the three *E. coli* strains and submerged in a circulated water bath set at 75, 80, 85, 90, and 95°C, for 0 to 180 s. Feed samples were then spread-plated onto Tryptic Soy Agar (TSA) with 200 ppm Nalidixic acid (NaL) and incubated at 37°C for 24 h. United States Department of Agriculture (USDA) IPMP-Global fit software was used to

calculated D-values from Weibull models and z-values from Linear models for feed heated at the five temperatures. Weibull model D-values were 19.8, 12.8, 10.4, 8.6, and 8.06s for the APEC, 31.3, 17.6, 15.7, 11.4, and 9.74s for *E. coli* O157:H7, and 8.0, 8.55, 5.3, 5.96, and 4.77s for the non-pathogenic *E. coli* when heated at 75, 80, 85, 90, and 95°C, respectively. The z-value from linear models were 51.6, 43.6, and 56.8°C for APEC, *E. coli* O157:H7 and non-pathogenic *E. coli*, respectively. These data indicate that the non-pathogenic *E. coli* may not be an ideal candidate to be used as an *E. coli* surrogate due to its lower thermal resistance than APEC and *E. coli* O157:H7. Results from this study demonstrate that Weibull and linear models are appropriate for predicting thermal inactivation of *E. coli* in poultry feed. Future work from this study is to find a suitable non-pathogenic *E. coli* surrogate for feed hygiene research.

Key Words: Escherichia coli, Feed, Thermal Inactivation, Feed Microbiology

P326 Comparison of the nutritional value of soybean meal from five origins. Thiago Yabuta*¹, Joaquín Cabanas-Ojeda¹, Nicolas Mejía-Abaunza^{1,2}, Paula Lozano-Cruz^{1,2}, Valmiro Aragão-Netto¹, Edgar Oviedo-Rondón¹ ¹Prestage Department of Poultry Science, North Carolina State University, ²College of Veterinary Medicine and Zootenia, Universidad del Tolima, Colombia

Soybean meal (SBM) is the main source of protein and amino acids (AA) in diets for monogastrics. Still, its quality and nutritional value can fluctuate based on regional agronomic factors and processing. Consequently, this study evaluated the nutrient and energy composition of SBM from North Carolina (NC), Western and Eastern US Corn Belt, Brazil (BRA), and Argentina (ARG). Samples of solvent-extracted SBM produced in NC from soybeans grown in NC in 2020 ($n=35$) and 2021 ($n=45$) were collected and analyzed by NIRS with 15 replicates per sample. Proximate composition, total AA content, energy, and quality parameters were determined using AMINONIR® (Evonik) calibration curves. The nutrient and energy values of SBM produced in the Eastern ($n = 74$ and 94) and Western ($n = 150$ and 220) Corn Belt regions in the USA, BRA ($n = 2,874$ and 9,497), and ARG ($n = 87$ and 110) were obtained from the AMINONIR® 2020 and 2021 reports, respectively. Nutrient and energy data were analyzed in a one-way ANOVA and means separated using Tukey's HSD test. Significant differences between SBM of different origins were observed for all parameters evaluated ($P < 0.001$). The NC SBM had the highest ME values for poultry, between 29 and 218, and 57 to 167 Kcal/kg more for 2020 and 2021, respectively. Also for swine 170 Kcal/kg more in 2020 and 33 to 200 Kcal/kg more in 2021 were observed in NC SBM compared to all other sources. SBM produced in NC also had the highest crude protein content in both years, between 0.18 and 1.79% in 2020 and 0.72 and 1.65% in 2021 more than other SBM sources. NC SBM had slightly lower total AA content ($P < 0.001$) than BRA SBM. The AA of NC SBM were between 0.01 and 0.06% points lower than BRA SBM, but higher than ARG and Western US SBM and very similar to Eastern US SBM in lysine and TSAA. The arginine content was 0.05 and 0.11% points better in NC SBM than BRA SBM. Digestible AA for poultry and swine followed the same pattern since AMINONIR use similar AA digestibility coefficients per specie, independently of contents of other nutrients. In conclusion, the results of these analyses suggested that SBM derived from soybeans cultivated in NC during 2020 and 2021 exhibited superior nutrient and energy values, along with competitive AA content for poultry and swine feeds.

Key Words: soybean meal, origin, amino acids, metabolizable energy, NIRS

P327 Characterization, processing, and nutrition performance of dry extruded full-fat soybean meals from six different states in the U.S. ABDULLAH SCOTT*¹, Cole Umberson¹, Callie Selby¹, Jordan Weil¹, Nawin Suesuttajet¹, Ehsan Sheikhsamani¹, Diego Martinez¹, Mian Riaz², Craig Coon¹ ¹University of Arkansas, ²Texas A&M University, College station

A study was conducted to determine the nutritional composition of US soybeans from six different states processed through extrusion technology. Iowa, Illinois, Missouri, North Dakota, Ohio, and Pennsylvania soybeans were selected respectively.

Urease, KOH, Protein Dispersibility, and Trypsin Inhibitor were measured for over and under-processing of FFSBM. Afterward, the effects of FFSBM on nutrient profiles and energy content were determined in 1800 birds. Eighteen treatments of different FFSBM inclusion levels (4, 8, 12) were tested in 90 pens of 5 replicates. Birds from each treatment were placed in metabolic chambers to determine heat production. Separate birds were selected to determine body composition by DEXA. SID for AA, fat, P, starch, and total tract digestibility was utilized for determining ME. Data was collected from metabolic chambers and DEXA to determine the productive energy (PE) values for the six FFSBM samples.

The processing quality of the FFSBM samples was all within the standard range expected except the KOH value was 67.21, 63.03, and 66.14 for 3 sources of FFSBM. Iodine value ranged between 105-120, Thiobarbituric acid ranged between 1.2-1.7 mg/kg and Total Dietary Fiber ranged from 16.4-19.1% for the 6 sources of FFSBM. Proximate analyses revealed that the extrusion process increased DM, Ash, CP while reducing NDF and ADF compared to raw soybeans. Starch ranged between 0.57-1.31% and stachyose was between 3.7-4.2% after processing compared to the raw soybeans. Dry extrusion increased the mineral content and amino acid content (especially lysine 2.46-2.63%); however, fatty acid content showed a slight reduction in all FFSBM sources compared to the raw soybeans. Higher inclusion levels of FFSBM produced a higher BW at 28 and 42 days of age. Feed intake and FCR for chicks fed 12% inclusion level was significantly ($P=0.001$) lower compared to 8 and 4 percent, respectively. There were no significant nutrition-feeding effects of different FFSBM samples on broiler performance.

Overall, the use of high-shear dry extrusion to process U.S. soybeans was successful in producing a high-quality FFSBM ingredient that is suitable for poultry feeding. Additionally, increasing the inclusion level of FFSBM in the diets improved broiler performance.

Key Words: Full-fat soybean, productive energy, metabolic chambers, DEXA, Extrusion

P328 Understanding amino acid digestibility of locally (Tennessee) grown soybean cultivars for Broiler chickens, in vitro study Alexis McDade*¹, Virginia Sykes², Ali Taheri¹, Pramir Maharaja¹ ¹Tennessee State University, ²University of Tennessee

Feed nutritional labels mention the percentage of specific amino acids or crude protein; however, most do not specify the protein quality — based on the presence of the essential amino acids. Consumers need to have a clear understanding of the nutritional value of the feed. Thus, predicting the nutritional values of soybean cultivars and their digestibility using an adapted *in vitro* multi-enzyme bioassay is essential to predict the nutritional values of soybean cultivars locally grown in Tennessee (TN) and their digestibility profiles using an adapted *in vitro* multi-enzyme bioassay. This and allows for a more accurate diet formulation for the birds' diet formation and helps reduce feed costs and nitrogen output into the environment. Twelve soybean cultivars were obtained from a Variety Testing facility at the University of Tennessee. Three varieties were selected based on harvest year, yield, and maturity group levels. Tennessee's main soybean variety is categorized in the maturity group (MG) 4; however, others are noted to be grown

in the state, such as MG 3 and MG 5. Soybeans were dehulled, solvent extracted, then dried and ground to make soybean meal, which was further passed through a 2mm sieve for final usage. In the revised two-step *in vitro* assay, the conditions of the gastrointestinal tract of the bird's gastrointestinal tract were simulated in three steps, representing the crop, the stomach, and the small intestine. *In vitro*, rate of disappearance (RD) values were calculated, and the data was measured and analyzed by one-way ANOVA using R-Studio. One-way ANOVA was performed for differentiating significant means for *in vitro* factors. Means were considered significant with a P -value ≤ 0.05 . The results of this *in vitro* analysis study show that different soybean varieties exhibit varying amino acid digestibility profiles. The goal is to assist crop geneticists and agronomists in soybean breeding programs, in order to develop soybean varieties for poultry dietary formulation with the highest nutritional value and optimal digestibility values.

Key Words: amino acid digestibility, soybean varieties, Broilers, in vitro study

P329 Microbial dynamics in sorghum-based diets: impact of phase feeding and phytase superdosing vs. corn-based diets Santiago Sasia*¹, Elio Ortiz¹, Manuel Peña¹, Brett Lumpkins², Mireille Arguelles-Ramos¹ ¹Clemson University, ²Southern Poultry Feed and Research, Inc.

A better understanding of gut microbiome and the impact of diet composition could bring positive insight when seeking for new alternative ingredients. This study aimed to assess cecal microbiome of broilers fed sorghum-based diets with or without phytase superdosing compared to corn-based diets across feeding phases, employing 16S gene sequencing. A total of 1500 male broiler chicks (Ross 708) underwent a 42-day rearing period in a floor pen house utilizing built-up wood shavings as bedding and curtain sidewalls. The experiment was a complete randomized block design with 30 pens housing 50 birds each, with 10 replicates per treatment. The birds were subjected to three treatment diets: T1 – corn-based diet with 550 FTU/kg (standard), T2 – sorghum-based diet with 550 FTU/kg, and T3 – sorghum-based diet with 2000 FTU/kg (superdose). Birds followed a phase-feeding program (starter: 0-14; grower: 14-28; finisher: 28-42 d). At the conclusion of each feeding phase, 1 bird per pen was euthanized, and cecal content was collected for gene sequencing. Diversity analysis revealed differences among feed phases rather than between treatment diets. Alpha-diversity Shannon index and Simpson index were higher during the grower and finisher feeding phases than the starter ($P<0.05$). For beta-diversity assessment, Weighted UniFrac, complemented by standard multivariate statistical techniques, including principal coordinates analysis (PCoA), was employed. No significant differences were identified in beta-diversity between diets within each phase ($P>0.05$). Nevertheless, birds' gut microbiome during the starter were significantly different compared to the grower and finisher ($P<0.05$). A redundancy analysis showed that dietary Met+Cys, Ca, and crude fat levels explained the variance observed during the grower phase. Furthermore, indicator species analyses revealed that 61 taxa spanning 13 prokaryotic phyla could explain the variation observed in the starter, grower, and finisher phases, respectively. Therefore, through 16S sequencing, this study identified diverse microbes and environmental factors influencing changes in diet phases, while also demonstrating that the use of sorghum or corn, with or without phytase superdosing, did not induce alterations in the gut microbiota.

Key Words: broiler, microbiome, phytase superdosing, age, sorghum

P330 Dietary inclusion of Micelle Silymarin in Hy-Line brown laying hens ameliorates production performance, egg quality, and blood profile parameters. Sungbo Cho*, Sharif Uddin Khan, In Ho Kim *Dankook University, Department of Animal Resource and Science*

This study aims to investigate the effect of micelle silymarin (MS) on production performance, egg quality, egg water loss, and blood profile in Hy-line brown laying hens. For a period of 12 weeks, a total of 384,

28-week-old laying hens were randomly allocated into one of four treatment groups. The dietary treatments were control (CON) a basal diet and a basal diet supplemented with 0.02, 0.04, and 0.06% of MS. Basal diet was formulated according to the regulation of NRC (1994). The MS supplements contain 10.8% silybin, 16.3% silydianin, and 7% silychristin was obtained from a commercial company (Synergen Co.Ltd., Korea). All hens were housed in individual cages with 38 cm width × 50 cm length × 40 cm height. Growth performance was recorded at initial, week 6, and week 12. The egg production and feed conversion ratio (FCR) was recorded at the end of every week. The egg quality and water loss were measured at the end of weeks 4, 8, and 12. At initial and week 12, 5 ml of blood was drawn from the layers to analyze AST, ALT, ALP, triglycerides, albumin, and cholesterol levels. All data were analyzed using the GLM procedure of SAS. The polynomial orthogonal contrasts of increasing dietary MS supplementation were examined by linear and quadratic effects. Dietary MS supplement linearly ($P < 0.05$) improved the egg production performance and decreased FCR throughout the experiment, except for weeks 1, 3, and 5 in the case of FCR. MS-supplemented groups showed linear improvements in egg-shell thickness and strength during the entire experimental period, while egg-shell color and yolk color ($P < 0.05$) of MS groups were linearly increased at weeks 4 and 8, respectively. Egg water loss in the hens fed MS-included diet was linearly increased on the third day of incubation at weeks 4, 8, and 12. The level of albumin and cholesterol decreased ($P < 0.05$) linearly at the end of week 12. Based on the findings, we inferred that the inclusion of 0.04 to 0.06% of MS in the laying hen diet would be beneficial not only to increase production performance, egg quality, and egg water loss traits but also to laying hens' health.

Key Words: micelle silymarin, egg quality, Production performance, egg water loss, Hy-line brown layers

P331 Optimizing Protein Sources in Reduced-Protein Diets to Improve the Immune Responses During Coccidiosis in Broiler Chickens Revathi Shanmugasundaram^{*1}, Adeleye Ajao², Shana Fathima², Adelumola Oladeinde³, Ramesh Selvaraj², Todd Applegate², Oluyinka Olukosi² ¹Toxicology and Mycotoxin Research Unit, USDA-ARS, US National Poultry Research Center, Agricultural Research Service, ²Department of Poultry Science, University of Georgia, ³Egg and Poultry Production Safety Research Unit, USDA-ARS, US National Poultry Research Center, Agricultural Research Service

High dietary crude protein concentrations increase coccidiosis severity, and reduced-crude protein (RP) diets, supplemented with essential (EAA) and nonessential amino acids (NEAA), can improve the broiler's immune response, which may depend on the plant protein source. The objective of this study was to compare the effects of soybean meal (SBM), canola meal (CM), or corn-DDGS (cDDGS) inclusion in RP diets for broiler chickens during an *Eimeria* challenge. A total of 1120 broiler chicks were distributed in a 4 × 2 (four diets × with or without challenge) factorial arrangement until d28 in seven replications. The four dietary treatments, fed between 7 and 28d, were (i) a standard diet with 20% crude protein (SP); (ii) RP (16%) corn-SBM (RP-SBM); (iii) a RP diet in which 8% CM replaced 6% SBM (RP-CM); and (iv) a RP diet in which 10% cDDGS replaced 5% SBM (RP-cDDGS). On d15, birds were challenged with 12,500 oocysts of *E. maxima*, 12,500 oocysts of *E. tenella*, and 62,500 oocysts of *E. acervulina* (+E) oocysts. Samples were collected on d21, and the data were analyzed by a two-way ANOVA. There was a significant diet × *Eimeria* challenge interaction ($P < 0.05$) on bile anti-*Eimeria* IgG concentrations, splenocyte proliferation, macrophage nitric oxide (NO) production, and cecal tonsil IL-17 mRNA amounts. Birds in the RP-SBM +E group had higher ($P < 0.05$) bile anti-*Eimeria* IgG concentrations compared to the SP +E group. Though birds in the SP group had higher ($P < 0.05$) splenocyte proliferation than all other treatment groups, birds in the RP-SBM +E group had comparable splenocyte proliferation to the SP +E group. Within the E+ group, birds in the RP-SBM +E group had higher ($P < 0.05$) macrophage NO than the other groups. Birds in the RP-SBM +E

group had higher ($P < 0.05$) IL-17 mRNA amounts in the spleen and cecal tonsils compared to the other treatment groups. Birds in the RP-cDDGS +E group had a lower ($P < 0.05$) CD8⁺:CD4⁺ ratio compared to the RP-SBM+E group. It can be concluded that reducing the crude protein content of the grower diet by 4% (from 20% to 16%), achieved by reducing the SBM content of the diet, did not impair the host immune response to the *Eimeria* challenge. However, partial replacement of SBM with CM and cDDGS impaired the host immune response.

Key Words: reduced crude protein, Coccidiosis, Soybean meal, DDGs, Broilers

P332 Efficacy of hydrophilic and lipophilic clays against fumonisins in day old broilers chickens Carlos Mallmann^{*1}, Leandro Giacomin², Diego Sturza², Manuel Contreras^{1,2,3} ¹Federal University of Santa Maria, Laboratório de Análises Micotoxicológicas (LAMIC), Santa Maria, RS, Brazil, ²Laboratório de Soluções Analíticas, Microbiológicas e Tecnológicas (SAMITEC), ³Special Nutrients/Agrimprove

Hydrophilic clays are soluble in water and lipophilic can be diluted in oil. Both types of products have been used as mycotoxin binders for more than 20 years. The objective of this experiment was to demonstrate the efficacy against fumonisins (FUMs) of both types of clays produced in the US and commercialized globally by Agrimprove/Special Nutrients, USA. 1080-day-old male broiler chicks distributed in 9 treatments with 12 replicates of 10 birds each were used in a complete randomized design. The treatments were: (1) basal diet; (2) + 0.50% hydro clay; (3) + 100 ppm FUMs; (4) + 100 ppm FUMs + 0.15% hydro clay; (5) + 100 ppm FUMs + 0.25% hydro clay; (6) + 100 ppm FUMs + 0.50% hydro clay; (7) + 100 ppm FUMs + 0.05% organoclay; (8) + 100 ppm FUMs + 0.10% organoclay, and (9) + 100 ppm FUMs + 0.25% organoclay. At the conclusion of the trial, at 21 days of age, all parameters were measured and statistically analyzed ($\alpha = 0.05$). Compared to chickens fed the basal diet (T1), broilers receiving + 100 ppm FUMs (T3) displayed reduced feed intake ($P = 0.001$), lower body weight ($P < 0.001$), increased relative liver weight ($P < 0.001$), shorter jejunal villi ($P = 0.002$), deeper jejunal crypt depth ($P = 0.001$), lower jejunal villus height/crypt depth ratio ($P = 0.005$), and higher serum levels of sphinganine/sphingosine ratio ($P < 0.001$). The inclusion of hydro clay (0.15%, 0.25%, 0.50%) and lipophilic clay (0.25%) prevented the decline in feed intake ($P = 0.025$, $P = 0.036$, $P = 0.006$, and $P = 0.004$, respectively) and body weight ($P = 0.003$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively) caused by fumonisins. It also led to longer jejunum villi ($P = 0.009$, $P = 0.031$, $P = 0.012$, and $P < 0.001$, respectively), reduced relative liver weight ($P = 0.012$, $P < 0.001$, $P < 0.001$, and $P < 0.001$, respectively), and preserved the SA/SO ratio ($P < 0.001$, $P < 0.001$, $P < 0.001$, and $P = 0.024$, respectively). In conclusion, both clays demonstrated efficacy against FUMs, depending on the inclusion rate used.

Key Words: Fumonisin, clays, Bentonite, broiler, mycotoxins

P333 Changes in biological pathways of the liver and jejunum of broiler chickens associated with intestinal histology Luis Romero^{*1}, Laura Payling¹, Rami Dalloul² ¹Biofractal Lda., ²University of Georgia

Villi length to crypt depth ratio (V:C) is used as a metric of intestinal health in chickens, with a higher ratio indicative of good intestinal health. This study evaluated functional changes in gene expression in the jejunum and liver of chickens associated with differences in V:C. 64 Ross-708 broiler chickens were randomly sampled from 4 farms at 28 d of age. Jejunum samples were collected for histology and V:C was measured through microscopy. Jejunum and liver samples of the same chickens were collected for mRNA sequencing. Sequencing data was aligned to the chicken reference genome, normalized (DESeq2), and analyzed for differential gene expression (DESeq2) and pathway activation using a topology-based method (Biofractal). For pathway activation, a customized, tissue-specific pathway catalogue (GUT SAVVY™) was used. The statistical model included the effects of V:C, RNA Integrity, and Sex.

A threshold of $adjP < 0.05$ was used for statistical significance. Higher V:C resulted in an overall activation of nutrient metabolism and nutrient digestion and absorption in jejunum. Lipid metabolism was activated, whereas amino acid metabolism had diverse responses, with activation of tryptophan and phenylalanine metabolism, and inhibition of glutamate, glutamine, and choline metabolism, and methionine salvage. Cell turnover processes were clearly inhibited in birds with higher V:C, which also had activation of antioxidant systems, reduced production of free radicals from phagocytes, and activation of mTOR1 and PPAR signaling in jejunum. Immune function in jejunum showed inhibition of interferon related processes, and mixed effects in other areas of innate and adaptive immunity. In liver, birds with higher V:C showed an overall inhibition of immune functions except for IL-6 activation. Like in jejunum, lipid metabolism was activated in the liver of birds with higher V:C, which also showed inhibited cell turnover, synthesis and recycling of bile acids and salts, and glutathione metabolism. Overall, birds with increased V:C exhibited a functional profile that prioritized nutrient metabolism versus immune function, cell turnover, and oxidative stress processes compared to birds with lower V:C.

Key Words: broiler chicken, villi length, jejunum, liver, pathway activation

P334 Trends in mycotoxin contamination in 2023 United States corn
Chasity Pender*, Paige Gott, Shelby Corray *DSM Nutritional Products*

Historically, feed ingredients have been shown to be contaminated by mycotoxins, secondary fungal metabolites that may be detrimental to animal health. Classic signs such as reduced feed intake and oral and intestinal lesions often underestimate other costs of mycotoxicosis, including increased frequency and severity of disease, inflammation, and modulation of the gastrointestinal environment. The objective of this study was to evaluate mycotoxin prevalence and contamination levels in corn from US 2023 harvest and compare with prior years. Samples were analyzed utilizing liquid chromatography and tandem mass spectrometry (LC-MS/MS) for six major mycotoxin groups: aflatoxins (Afla), type A trichothecenes (A-Trich), type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN), and ochratoxin A (OTA). Statistical analyses were performed with JMP Pro 16 software using one-way ANOVA and means separated using Tukey's HSD with significance reported at $P \leq 0.05$. A limited number of samples are available thus far ($n = 37$) as harvest is still progressing, therefore the risk profile of this crop year is likely to change as the sample pool expands. Mean B-Trich, ZEN, FUM, OTA, and Afla contamination levels have remained consistent over the survey period with no statistical differences observed. To date, 100% of samples evaluated contained at least one mycotoxin, compared to 91% observed in 2022. Currently, FUM is numerically the most prevalent group (97 vs. 79, 64, 64, and 78%, 2022 through 2019, respectively). Prevalence of A-Trich was numerically increased in 2023 vs. 2022 (22 vs. 3%) but mean contamination for A-Trich was significantly lower ($P < 0.05$) in 2023 vs. 2019 (21 vs. 330 ppb) and did not differ from other years. Afla occurrence was numerically greater in 2023 compared to previous years (27 vs. 11, 7, 5, and 3%, 2022 through 2019, respectively). Prevalence of B-Trich in 2023 was numerically lower compared to 2022 (35 vs. 68%), while prevalence of ZEN has numerically increased (87 vs. 31%). As the mycotoxin risk of this harvest season is still coming into focus, preliminary results of the 2023 survey indicate a continued risk of contamination. Continued surveillance is advised to assess risk as new crop corn is fed out over the coming months.

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

P335 Productive energy (Arkansas Net Energy) value of diet and soybean meal for broilers as predicted by digestible nutrients Diego Martinez*, Nawin Suesuttajit, Cole Umberson, Craig Coon *University of Arkansas*

Productive energy (PE) predicts performance and economics better than N-corrected apparent metabolizable (AMEn) or classic net energy (CNE; $CNE = AME - \text{heat increment, HI}$). This study developed models to predict diet net energy for gain (NEg), CNE and PE, and soybean meal (SBM) PE from digestible (dig.) nutrients. 96 pens (8 blocks) with 20 chicks each were set to 12 dietary treatments: varying total dig. amino acids (TDAA; T1-T3) or dig. starch (dSt) and TDAA (T4-T6), or increasing (T7-T9; fat, TDAA) or decreasing (T10-T12; crude fiber) nutrient density. Every 7 days, one block started receiving treatment diets for one week; all were fed standard diets before. Feed was restricted to allow similar energy intake. Feed intake was recorded, body protein, fat and NEg were measured with dual-energy X-ray absorptiometry, and heat production (HP) at fasting (FHP = net energy for maintenance = NEm) and HI ($HI = \text{fed HP} - \text{FHP}$) in calorimetry chambers. PE was $NEg + NEm$. Diet AMEn, non-starch polysaccharides (total, tNSP; insoluble, iNSP; soluble, sNSP), dig. fat (dFat), dig. crude protein (dCP), dSt and TDAA were determined. Linear mixed models were fitted in JMP. A reference SBM was used to evaluate SBM PE models. Anabolic (ACPv; gain) and overall (OCPv; gain and maintenance) caloric-equivalent predictive values of dig. nutrients were calculated, defined as the number of calories for gain (NEg; ACPv) or gain and maintenance (NEg+NEm; CVO) that each g of dig. nutrient predicted. Models were validated for diet NEg (dCP, TDAA, dFat, sNSP; $adjR^2 > 0.86$), CNE (dFat, dSt, tNSP, iNSP, sNSP, dSt-to-tNSP or -sNSP ratios; $adjR^2 > 0.55$) and PE (dCP, TDAA, dFat, dSt, sNSP; $adjR^2 > 0.80$), and for SBM PE (dCP, TDAA, dFat, dCP- or TDAA-to-tNSP ratios; $adjR^2 > 0.57$). CNE was not influenced by dCP or TDAA ($P > 0.39$). SBM PE models showed high precision (deviation ≤ 72 kcal/kg) and accuracy (error $\leq 2.2\%$). dCP, TDAA, and dFat explained 92, 88 and 10% of diet NEg, 85, 81, and 12% of diet PE, and 96, 96, and 0.7% of SBM PE, and showed ACPv of 4.9, 5.3, and 1.8, and OCPv of 8.4, 9.2, and 4.3 kcal/kg, respectively. SBM contributed 44% of diet PE but 19% of AMEn. In conclusion, models for diet and SBM PE were validated. PE, not CNE, is sensitive to dCP and TDAA and credits the actual energy value of SBM.

Key Words: Productive energy, Arkansas Net Energy, net energy, soybean meal, broilers

P336 Productive energy: Exploring reasons for its superiority beyond apparent metabolizable energy and classic net energy Diego Martinez*, Nawin Suesuttajit, Cole Umberson, Craig Coon *University of Arkansas*

Productive energy (PE) predicts performance better than apparent metabolizable (AME), N-corrected AME (AMEn), and classic net energy (CNE; $CNE = AME - \text{heat increment, HI}$). This study assessed the relationship between body composition, fasting heat production (FHP = net energy for maintenance, -NEm), and economics, and explored why PE outstands. 1920 chicks in 96 pens (8 blocks) were assigned to 12 dietary treatments with changing levels of total digestible amino acids (TDAA; T1-T3), digestible starch (sSt) coupled with TDAA (T4-T6), nutrient density (T7-T9: fat, TDAA; T10-T12, fiber). During one week, each block was restrict-fed treatment diets allowing similar energy intake; every block started the experimental period every seven days. Before that, all received standard diets. For each block, performance was recorded, body protein, fat, and energy (net energy for gain, NEg) and processing weights (i.e., breast meat, leg quarters; breast-to-leg ratio, BLR) were determined with dual-energy X-ray absorptiometry. Heat production (HP) at fasting (FHP) and HI ($HI = \text{fed HP} - \text{FHP}$) were determined in calorimetry chambers, and PE ($NEg + NEm$) was calculated. Diet AMEn, non-starch polysaccharides fractions, and digestible nutrients (fat, crude protein -CP, starch, amino acids) were determined. Carcass market value (MKV) was calculated with market prices. Gross profit gain (GPG) was determined as

MKV – feeding cost. Linear mixed models were fitted in JMP to quantify empirical relationships, and a mechanistic model was developed. Models indicated positive linear relationships ($P < 0.001$) between dCP intake and NEm (kcal/bird/d; $\text{adj.R}^2 = 0.98$), body gain leanness with NEm ($\text{adj.R}^2 = 0.96$) and BLR ($\text{adj.R}^2 = 0.94$), BLR and MKV ($\text{adj.R}^2 = 0.93$), and MKV with GPG ($\text{adj.R}^2 = 0.99$). The models indicated that in contrast with PE, AME (and AMEn and CNE) (i) do not acknowledge ($P > 0.05$) changes in body composition, their influence on FHP and BLR, and its

effect on MKV and GPG, (ii) mislead the influence of CP on energetics by ignoring ($P > 0.05$) its effect on NEm, and (iii) assumes no variations in FCR ($P > 0.43$; other than digestion) and actual metabolism. In conclusion, PE is sensitive to changes associated with energy metabolism and can, therefore, influence performance and economics.

Key Words: Productive energy, Arkansas Net Energy, net energy, economics, broilers

Metabolism and Nutrition: Feed Additives

P337 Effect of dietary *Moringa oleifera* supplementation in ameliorating coccidial burden in broiler chickens Neketa Hughes^{*1UG}, Randy Domer¹, Colwayne Morris² ¹University of Guyana, ²Zinpro Corporation

Coccidiosis is the most economically significant disease affecting broilers and negatively impacting broiler production worldwide. With the growing concern of antimicrobial resistance caused by using antibiotic growth promoters (AGPs) in broiler diets, it is imperative that the poultry industry seek alternatives to reduce our dependence on these drugs. Over the years, research has indicated possible alternatives to antimicrobials, including phytochemicals such as *Moringa oleifera* (MO), Garlic, Turmeric and Noni. The MO plant contains a unique and diverse array of phytochemical substances with antimicrobial, anti-inflammatory and antioxidant properties. The objective of this study evaluated the anticoccidial effect of moringa leaf meal additive on broiler chickens fed 0.5% and 1% *Moringa oleifera* leaf meal (MOLM) added to the ration. A completely randomized design on 540 Cobb 500 broiler chickens was used to study the anticoccidial activity of MOLM over six weeks from hatch to slaughter. The number of oocysts shedded was determined using the McMaster method, and gross lesions were identified using methods highlighted by Johnson & Reid, 1970. Performance and oocyst count data were collected at d7, d14, d21, d28, d35 & d42 and gross lesions were evaluated at slaughter on d42. The study included four feed treatments viz; Control, 200 ppm amprolium, 0.5% and 1% MO leaf meal. The data was analyzed using a one-way ANOVA using STATISTIX 10 software. Overall 1% MOLM-treated group shedded less oocyst than the control group ($p < 0.005$), with no differences observed in the amprolium and 0.5% MOLM-treated groups. For performance, BW was higher in the control and 0.5% MOLM groups when compared to the birds treated with 1% MOLM at d7 & d21 ($p < 0.005$), while on d14 both amprolium and 1% MOLM treated groups BW was higher ($p < 0.005$). WG was higher in the birds treated with amprolium and 1% MOLM on d7 and d14 ($p < 0.005$). Like weight, feed consumption and FCR varied among treatments over the period. However, at d21 & d42, no differences were observed in FCR among treatments. In conclusion, the use of 1% MOLM added to broiler diets reduced oocyst count similarly to that of the conventional anticoccidial amprolium.

Key Words: *Moringa oleifera*, coccidiosis, oocyst, antiprotozoal, coccidiostat

P338 The effect of dietary essential oil of oregano on the lipid stability and fatty acid profile of eggs stored at room temperature. Ari Bragg^{*1UG}, Alexa Johnson¹, Jose Charre-Perales¹, Allie Todd², Ahmed Ali¹ ¹Clemson University, ²University of South Carolina

In many countries, eggs are stored at room temperature (about 20°C) without refrigeration. By providing laying hens with a feed additive, those effects can be seen in the egg. With high antioxidant properties, dietary oregano oil may be an option to change the fatty acid profile and storage life of eggs. This study explores the effects of dietary oregano oil (ORE) on the fatty acid profile and shelf life of eggs from laying hens. A total of 250 eggs were collected, and 50 were stored at 0, 10, 21, and 35 days. Yolks were analyzed for fatty acid profile and lipid peroxidation after

each storage period. Differences within measured parameters across time points and groups were assessed using GLMM with Tukey's Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. The main indicator of lipid peroxidation, malondialdehyde (MDA), was significantly higher in ORE eggs compared to CON eggs ($p = 0.001$). Storage time had a significant impact on MDA concentrations ($p = 0.023$), with the highest found after 35 days. Significant differences were found in individual fatty acids as well as summations of saturated (SFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA). All individual fatty acids were affected by treatment, with palmitic, stearic, oleic, linoleic, and arachidonic (AA) fatty acids significantly lower in ORE eggs compared to CON eggs across all storage times ($p < 0.05$). Palmitoleic acid ($p = 0.002$), linolenic acid ($p = 0.001$), and docosahexaenoic acid (DHA, $p = 0.001$) were significantly higher in ORE eggs compared to CON eggs. Total SFA, MUFA, n-6, and ratio of n-3 to n-6 (n-3:n-6) PUFAs were all significantly higher in eggs from CON treatment compared to eggs from the ORE treatment ($p < 0.05$), while the ratio of SFA to PUFA (SFA: PUFA, $p = 0.005$) and total n-3 PUFA ($p = 0.001$) were significantly higher in eggs from ORE birds. In conclusion, this study has shown a significant change in the lipid stability of eggs from hens-fed oregano oil, which may increase the shelf life of eggs. It also shows significant changes in fatty acid profile which may influence the human diet and the impact of eggs on the human body.

Key Words: laying hens, oregano oil, designer eggs, fatty acid profile, storage

P339 Effect of dietary betaine supplementation and post hatch delayed access to feed and water on performance, digestion of nutrients, and carcass characteristics of heat-stressed broiler chicks Jalon Gaines^{*UG}, Ala Abudabos *Department of Agriculture, School of Agriculture and Applied Sciences, Alcorn State University*

The experiment aimed at studying the effect of delayed access to feed and water post-hatch and dietary betaine (BET) supplement on performance, digestion of nutrients, and carcass characteristics of heat-stressed (HS) broiler chickens. A total of 576 one-day old broiler chicks were distributed into 6 treatments in two environmentally controlled rooms. The treatments for the starter period (1-20 d) were as follows: immediate access to feed and water after placement (IAFW); delayed access to feed and water for 48 h after placement (DAFW); IAFW + 0.1% BET/kg diet; DAFW + 0.1% BET/kg diet; IAFW + 0.2% BET/kg diet; DAFW + 0.2% BET/kg diet. A 3 x 2 factorial arrangement of treatments was followed in a randomized completely block design. During the finisher period (21-35 d), a 3 x 2 x 2 factorial arrangement of treatments was applied. The broiler chicks in room (A) were kept at a moderate temperature of 22.0 ± 1.0 °C (thermoneutral group; TN) while chicks in the room (B) were raised at a cyclic high temperature (35 ± 1 °C for 8 h/d) and represented the HS group. The results of the experiment revealed that DAFW affected the performance of broiler chicks at 0 to 20 days of age. The results indicated that there was a significant difference in body weight gain (BWG) ($P < 0.01$), feed conversion ratio (FCR), and feeding efficiency factor (FEF) ($P < 0.001$) between DAFW and IAFW. Moreover, BET improved ($P < 0.001$) BWG, FCR and in FEF. The room temperature also affected the FI, BWG, FCR and FEF ($P < 0.001$). The crude protein and fat digestibility

were higher for IAFW compared to DAFW ($P < 0.001$). The percentage of crude protein and crude fiber digestibility were higher for TN group ($P < 0.05$). There was significant effect of BET on the pH ($P < 0.01$) of the breast muscle and the color of the muscle, especially the degree of yellowness ($P < 0.05$). In conclusion, these results indicate that there were negative effects of DAFW and HS on the productive performance of broiler chicks. This study showed that BET supplementation had a positive effect on the performance of broiler chicks, Therefore, it is recommended to use 0.2% BET to improve the performance and meat characteristics when the feeding time is delayed for 48 hours after hatching, as well as during the exposure of birds to HS.

Key Words: Betaine, post-hatch chicks, cyclic heat stress, performance, meat characteristics

P340 Effect of post hatch delayed access to feed and water with dietary betaine supplementation on intestinal morphology, blood parameters, antioxidant status, and body temperature of broiler chickens reared under cyclic heat stress. Opeoluwa Judo*^{1GS}, Ali Al Sulaiman², Ala Abudabos³ ¹*Department of Agriculture, School of Agriculture and Applied Sciences, Alcorn State University*, ²*National Center for Environmental Technology, Institute of Biology and Environment, King Abdulaziz City for Science and Technology*, ³*Alcorn State University*

This experiment was conducted to investigate the effect of delayed access to feed and water post-hatch and dietary betaine (BET) supplementation on immune organ growth index, intestinal morphology, blood parameters, and antioxidant status of cyclic heat-stressed broilers. A total of 576 one-day old broiler chicks were randomly distributed into 6 treatments: immediate access to feed and water after placement (IAFW); delayed access to feed and water for 48 h after placement (DAFW); IAFW + 0.1% BET/kg diet; DAFW + 0.1% BET/kg diet; IAFW + 0.2% BET/kg diet; DAFW + 0.2% BET/kg diet. All birds were reared to 20 d of age under standard environmental conditions. From 21 to 35 d, birds in thermoneutral room (TN group) were reared at recommended ambient temperature, whereas broilers in heat stress room (HS group) were raised at a cyclic high temperature ($35 \pm 1^\circ\text{C}$ for 8 h/d). At 15 d of age, compared with birds in the IAFW group, birds in the DAFW group exhibited decreased ($P < 0.05$) relative weight of bursa, villus height (VH), villus surface area (VSA), and VH to crypt depth ratio (VH: CD). Also, there was an increase (linear, $P < 0.01$) in VH by increasing dietary BET level. At 35 d of age, compared with TN group, HS decreased ($P < 0.05$) relative weight of the lymphoid organs, VH, villus width (VW), VSA, and VH: CD ratio, and increased ($P < 0.05$) heterophil: lymphocyte ratio (H: L), crypt depth (CD), and cloacal temperature. Heat stress increased ($P < 0.05$) triglyceride (TG), and malondialdehyde (MDA) contents, and alanine aminotransferase (ALT) activity. The BET supplementation increased (linear, $P < 0.05$) jejunal VH, whereas decreased (linear, $P < 0.05$) serum MDA level of heat-stressed broilers. The concentration of serum T-AOC and PCV proportion were highest ($P < 0.05$), whereas the activity of AST was lowest in broilers fed 0.2% BET-supplemented diet respective of environmental temperatures. In conclusion, these results indicate that supplementation with BET was effective in partially ameliorating intestinal barrier function in broilers. In addition, the results demonstrated that supplementation with BET at 2% can improve the physiological performance of broilers by enhancing oxidative status and alleviating oxidative damage caused by HS.

Key Words: Betaine, post-hatch chicks, cyclic heat stress, intestinal morphology, antioxidant status

P341 Evaluation to select a novel probiotic for efficacy in the feed and bird Anna Kathryn Riggs*^{1UG}, Dalton Dennehy¹, Emily Myers¹, Brody Hamm¹, Jorge Urrutia¹, Samantha Plocher¹, Skylar White², Kelley Wamsley¹ ¹*Poultry Science Department, Mississippi State University*, ²*NCH Life Sciences*

Probiotics (PRO) have become a staple in many poultry diets due to consumer interest, but also their positive impact on gut health and bird performance. As technology advances, new PRO become available, and their efficacy must be verified in vivo. The initial objective of this experiment (EXP) was to evaluate the impact of novel PRO on 0-35 d male Cobb x Cobb 500 broiler performance. Eight treatments (TRT) were tested in an initial study, including a medicated control (TRT1), an unmedicated control (TRT2), and six TRT varying in PRO strain and inclusion (TRT3-8). Each TRT was assigned to one of 96 pens as a randomized complete block design, with 12 replications/TRT and 17 birds/pen; data were analyzed using SAS 9.4, with significance set at $P \leq 0.05$. Performance results from 0-14 d demonstrated that birds fed TRT3 had a significantly higher d 14 BW ($P = 0.0494$) as compared to all other TRT. For d 25 BW, birds fed TRT3 were similar to those fed TRT1 and were significantly greater than birds fed TRT4, 7, and 8; birds fed TRT2, 5, and 6 had intermediate BW, similar to all other TRT ($P = 0.0141$). From d 25-35, data were inconsistent. Recovery analysis of PRO supported observed performance, indicating PRO and chosen diluent (salt flour), may have interacted with other feed particles, preventing it to be evenly dispersed within the final feed. This led to further EXP to determine an optimum diluent for obtaining a consistent mix of PRO into the feed. For EXP 1 of the mixing test, there were 3 replicate batches were mixed; each batch was 113 kg and each of the 7 PRO TRT were mixed in a random order. Three samples were taken from each replicate. Despite vigorous cleaning of the mixer, recovery analysis of PRO demonstrated contamination from TRT7. This led to another EXP mix test, wherein batch size was reduced to 91 kg; there were 3 replicates per TRT and TRT were mixed randomly with the exception of TRT7, which was mixed last to prevent contamination with other TRT. Recovery analysis of PRO identified a diluent that evenly dispersed PRO throughout the feed. Future research will investigate the efficacy of the diluent of interest with the PRO TRT to conduct another live performance trial and determine best PRO candidate to maximize broiler performance and health.

Key Words: probiotics, broilers, performance, feed

P342 Early 1,25-dihydroxyvitamin D₃-glycosides supplementation: an efficient feeding strategy against bacterial chondronecrosis with osteomyelitis lameness in broilers assessed by aerosol transmission challenge model Anh Dang Trieu Do*^{1,2GS}, Andi Asnyanti^{1,2,3}, Khawla Alharbi^{1,2}, Ruvindu Perera^{1,2}, Layla Al-Mitib^{1,4}, Wesley Vaught^{1,4}, Ashley Ault^{1,4}, Abigail Fanous^{1,4}, Hannah McCarver^{1,4}, Kathrin Buehler⁵, Jan Dirk Van der Klis⁵, Javier Gonzalez⁶, Michael Kidd², Adnan Alrubaye^{1,2,4} ¹*University of Arkansas Cell and Molecular Biology*, ²*University of Arkansas Center of Excellence for Poultry Science*, ³*National Agency of Drug and Food Control*, ⁴*University of Arkansas Department of Biological Science*, ⁵*Herbonis Animal Health GmbH*, ⁶*Nuproxia*

Bacterial chondronecrosis with osteomyelitis (BCO) lameness, a leg disorder caused by multi-bacterial infection of microfractures in long bones of broilers resulting in necrosis, causes economic losses, food safety concerns, and animal welfare issues. As such, preventive measures against BCO lameness remain a constant and crucial goal for the poultry industry. The active metabolite of vitamin D₃, 1,25-dihydroxyvitamin D₃, is known to play essential roles in mineral homeostasis, bone health, and immunity, all of which are essential for protection against BCO. Thus, we hypothesized that *Solanum glaucophyllum*-derived 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃-glycosides) supplementation would be an effective measure to control lameness in broilers. Using an aerosol transmission experimental model, our first aim was the determination of an optimum concentration of 1,25(OH)₂D₃-glycosides supplementation in reducing

BCO incidence by comparing inclusion levels of 0.5, 1.0, and 2.0 $\mu\text{g}/\text{kg}$ of 1,25(OH)₂D₃-glycosides to a negative control diet. The second aim was to assess the timing for 1,25(OH)₂D₃-glycosides inclusion by comparing its applications of 1.0 $\mu\text{g}/\text{kg}$ for the entire 56 d, the first 28 d, or the last 28 d. Using General Linear Model analysis of cumulative lameness incidence, the application of 1.0 $\mu\text{g}/\text{kg}$ of 1,25(OH)₂D₃-glycosides resulted in a significant difference in reduction compared to 0.5 $\mu\text{g}/\text{kg}$ (-53.7% vs. -18.8%, respectively; $P < 0.001$), but no such difference was found compared to inclusion level of 2.0 $\mu\text{g}/\text{kg}$ (-53.7% vs. -51.7%, respectively; $P > 0.05$). Similarly, feeding the broilers with 1.0 $\mu\text{g}/\text{kg}$ of 1,25(OH)₂D₃-glycosides for the first 28 d significantly differed from the last 28 d in lameness incidence reduction (-53.0% vs. -9.4%; $P < 0.05$), but was comparable to supplementation for the entire 56 d (-53.0% vs. -53.7%; $P > 0.05$). Therefore, feeding 1.0 $\mu\text{g}/\text{kg}$ 1,25(OH)₂D₃-glycosides in the first four wks was the optimum 1,25(OH)₂D₃-glycosides administration and provided equal protection against lameness to the supplementation for the entire production cycle. This early feeding strategy would minimize feed additive costs without compromising bone health while promoting protection against BCO lameness.

Key Words: broiler, bone, lameness, chondronecrosis, 1,25-dihydroxyvitamin D₃-glycosides

P343 Dietary treatments of Microalgae and Organic Trace Minerals to reduce the incidence of Bacterial Chondronecrosis with Osteomyelitis in Broilers Ruvindu Perera^{*1GS}, Andi Asnayanti¹, Amer Hasan², Khawla Alharbi¹, Intisar Hassan¹, Wesley Vaught¹, Walter Bottje¹, Samuel Rochel³, Marco Rebollo⁴, Michael Kidd¹, Adnan Alrubaye¹ ¹University of Arkansas, ²University of Baghdad, ³Auburn university, ⁴Zinpro Corporation

Bacterial Chondronecrosis with Osteomyelitis (BCO) causes lameness resulting in bird welfare issues and integrator financial losses. BCO occurs due to necrotic degeneration of leg bones by bacterial leakage from the gut and airways into blood, and their colonization in bone growth plates. In consequence to dietary and in ovo antibiotic restriction, our aim was to improve intestinal barrier integrity, bone strength, immunity and bactericidal activity via dietary interventions to minimize BCO. In a previous study, Zinpro® Availa® ZMC (Zinc, Manganese, Copper) decreased lameness by 20% to 25%. Additionally, benefits of microalgae on growth performance, immunity, and antioxidant activity in broilers due to their nutritional and bioactive ingredients is published. However, the effect of microalgae on BCO lameness has not been investigated. Therefore, our objective was to investigate effects of supplementation of microalgae, Availa ZMC, and their combination on BCO lameness incidence. The BCO infection source was the natural airborne bacteria of birds raised on wire-floor, proven to develop lameness, due to their continuously stressed leg-bones developing microfractures thereby facilitating bacterial colonization and necrosis. The experimental (randomized block) design consisted of five treatment groups; T1 (wire-flooring pens with negative control diet), T2 (negative control diet), T3 (Availa ZMC at 1,500 mg/kg), T4 (5% microalgae), and T5 (5% microalgae + 1,500 mg/kg Availa ZMC) with T2-5 raised on litter-flooring. Cumulative lameness was recorded daily and BCO lesion severity was examined via necropsy. Dietary effects on lameness were determined via logistic regression using GLM and dietary interaction effects were evaluated via two-way ANOVA. Results portrayed a 50% decrease and 6% increase of lameness ($P < 0.05$) compared to the control, via Availa ZMC and microalgae supplementation in diet, respectively. T5 resulted significantly lower lameness than T2 & T4, but significantly more than T3 ($P < 0.05$). In conclusion, Availa ZMC significantly reduced the incidence of lameness in broilers, while microalgae slightly increased lameness. A potential cause for the latter scenario may be cyanotoxin contamination, which warrants further research for confirmation.

Key Words: Chondronecrosis, Osteomyelitis, Lameness, Microalgae, Trace Minerals

P344 Synbiotic improves growth performance and immunity against lipopolysaccharide challenge by regulating splenic tryptophan metabolism in heat-stressed chickens Yuechi Fu^{*1GS}, Heng-wei Cheng^{1,2} ¹Department of Animal Sciences, Purdue University, ²Livestock Behavior Research Unit, USDA-ARS

The objective of this study was to investigate the effects of supplementation with a synbiotic on growth performance and immune response of heat-stressed chickens under lipopolysaccharide (LPS) challenge. One hundred and eighty 1-d-old Ross 708 male broiler chicks were randomly assigned to 2 dietary treatments (6 pens per treatment): without (CON) or with synbiotic supplementation at 0.5 g/kg (SYN) for a 42-day trial. The synbiotic is composed of fructooligosaccharides as the prebiotic and 4 microbial strains of probiotic (*Bifidobacterium animalis*; *Enterococcus faecium*; *Lactobacillus reuteri*; and *Pediococcus acidilactici*). From d 15, birds were heat-stressed at 32°C for 9 h daily (0800-1700). At d 16, 18, and 21, broilers from CON and SYN groups were intraperitoneally injected with sterile saline or LPS (1.0 mg/kg body weight) and divided into three groups: 1) saline + CON (CS), 2) LPS + CON (CLPS), 3) LPS + synbiotic (SLPS). Statistical analysis was performed using the PROC Mixed procedure in SAS. Samples of ileal tissue and spleen were collected at d 28 and d 42. Compared with CLPS, synbiotic supplementation increased body weight at d 28, and feed intake at d 42 ($P < 0.05$), with a tendency for increased ratios of villus height and crypt depth ($P = 0.06$) of broilers subjected to heat stress. SLPS broilers had increased level of splenic interleukin (IL)-1 β ($P < 0.05$), with a tendency for decreased level of interferon- γ but higher concentration of IL-10 in the spleen ($P < 0.1$) compared to the CLPS broilers. Additionally, tryptophan concentration of ileal mucosa tended to be decreased in SLPS broilers at both d 28 and 42 ($P = 0.10$). Increased tryptophan metabolism in the spleen was also found in SLPS broilers, reflected by enhanced levels of tryptophan hydroxylase 1, kynurenine, and quinolinic acid, together with higher mRNA abundance of indoleamine 2,3-dioxygenase 2 at d 28 ($P < 0.05$). In summary, synbiotic supplementation could protect broilers from the bacteria endotoxin effect by suppressing inflammatory response and improving splenic tryptophan metabolism under the heat stress condition. The data suggests that the use of these products shows promising effects on poultry health.

Key Words: broiler, synbiotic, tryptophan metabolism, immunity

P345 Effect of 50% inclusion of U.S. tannin-free sorghum and canthaxanthin pigment on laying hens Santiago Sasia^{*GS}, Alexa Johnson, Gracie Anderson, William Bridges, Mireille Arguelles-Ramos, Ahmed Ali *Clemson University*

Sorghum could be a potential alternative to corn in poultry. Thus, this research aimed to assess the impact of 50% inclusion of American sorghum varieties, either with or without the inclusion of commercial canthaxanthin (CX), on laying hen performance, egg quality, bone health, and antioxidant capacity. A total of 900 Hy-line Brown chicks were divided into 30 pens (30 birds/pen). The experimental design was a randomized complete block with a 3x2 factorial arrangement and 5 replicates/treatment (TRT). The combination of the main factors, which included 3 types of grain: corn, high protein sorghum, U.S. No. 2 sorghum (CON, HS, N2) and 2 levels of CX (Carophyll®, DSM international) inclusion of 0 mg/kg or 35 mg/kg (“-” or “+”), resulted in 6 dietary TRTs (CON-, CON+, HS-, HS+, N2- and N2+). Sorghum TRTs were formulated by replacing 50% of corn with sorghum grain from a corn-soybean meal-based diet from 18 to 40 weeks of age. Average daily feed intake, feed conversion ratio hen-day egg production, egg external and internal quality, bone health, and antioxidant capacity were evaluated at 30 and 40 weeks of age. Differences within measured parameters across treatments and weeks of age were assessed using GLMM with Tukey’s Post hoc test applied to significant results (α set at 0.05) in R 3.3.1. Even though results showed similar performance and external egg quality parameters across TRTs at weeks 20 and 40 ($P > 0.05$), birds fed sorghum showed a better internal egg quality in terms of albumen weight and Haugh unit than CON ($P < 0.05$). Across

weeks, yolk color was similar between diets with CX ($P>0.05$) while HS- and N2- had a lower pigmented yolk than CON regardless of CX inclusion ($P<0.05$). In terms of bone health, sorghum TRTs showed greater bone mineral densities than corn diets ($P<0.05$). CX inclusion increased total antioxidant capacity levels at least by 50% approximately ($P<0.05$), and malondialdehyde levels were lower in the C+, N2+, and HS/HS+ groups compared to the C- and N2- ($P<0.05$). Blood protein levels were higher in sorghum diets compared to corn diets ($P<0.05$). Thus, the partial replacement of corn with sorghum with CX, did not adversely impact performance, and can enhance some features of egg quality, antioxidant capacity and bone health of laying hens.

Key Words: layers, sorghum, pigment, egg, antioxidant

P346 Zymbiotics reduce Salmonella Typhimurium colonization and improve performance in broiler chickens Davis Fenster^{*IGS}, Sudhir Yadav², Rasha Qudsieh², Jeng-Jie Wang², Rami Dalloul¹ ¹University of Georgia, ²BioResource International

Salmonella Typhimurium is known to cause human salmonellosis via the consumption of contaminated animal products. This study investigated the effects of the zymbiotic EnzaPro[®] on performance and bacterial colonization of broiler chickens challenged with nalidixic acid-resistant *S. Typhimurium* (STNR). Day (d) of hatch Ross 708 male chicks ($n=192$) were randomly divided into 3 treatment groups (8 replicate cages; 8 birds/cage) fed either a standard energy diet (PC); a 100 kcal/kg reduced energy diet (NC) compared to PC; or NC with EnzaPro[®] (NC+EP) for 28 d all in mash form. All birds were orally inoculated with 1×10^7 CFU/mL STNR on d 7. Bird and feed weights (per cage) were measured on d 0, 7, 17, and 28. On d 10, 17, and 28 (3-, 10- and 21-d post inoculation), 1 bird per cage (8 birds/treatment) was euthanized to collect the ceca for STNR enumeration and the liver for prevalence of STNR. Performance and STNR enumeration data were analyzed using one-way ANOVA and significance ($P \leq 0.05$) between groups was determined by LSD test. The prevalence data were calculated as percent of STNR-positive samples and statistically analyzed using Kruskal-Wallis test and Chi-Square approximation. The dietary inclusion of EnzaPro[®] improved growth performance, which was more pronounced during the d 7–17 post inoculation period. Compared to NC, NC+EP showed 5% greater body weight gain and 7-point reduction in FCR during d 7–17 ($P < 0.05$). Further, NC+EP had a 2.69 average log CFU/mL STNR on d 10 compared to 4.39 and 2.83 log CFU/mL for NC and PC, respectively. There were no differences ($P > 0.05$) in the percent of STNR-positive liver samples between groups at any of the time points. The addition of EnzaPro[®] to a low energy diet improved performance, possibly due to the action of its xylanase in increasing nutrient digestibility. Further, the direct fed microbial component in EnzaPro[®] likely contributed to the reduction of *S. Typhimurium* colonization in the ceca. These results could provide means in developing strategies to mitigate *Salmonella* colonization and infection in broiler flocks pre-harvest, thus reducing the incidence of salmonellosis outbreaks and subsequent infections in humans.

Key Words: Salmonella Typhimurium, xylanase, broiler, performance, zymbiotics

P347 The effects of a multi-carbohydrase enzyme complex and direct-fed microbial supplementation to energy-reduced diets on broiler performance and carcass traits Emily Jiral^{*IGS}, Jessica Rocha¹, Hector Leyva-Jimenez², Yemi Burden², Carlos Soto², Brian Dirks², Gregory Archer¹ ¹Texas A&M Agrilife Extension, ²United Animal Health

This study evaluated the effect of supplementing a multi-carbohydrase enzyme complex (MCE) and a direct-fed microbial (DFM) in the feed of broilers on the performance and carcass traits. A complete randomized design was used to distribute 5 dietary treatments, each consisting of 10 replicate floor pens and 25 male broilers/pen. The treatments were: Positive control (PC, no energy reduction), Negative control (NC, no DFM and -132 kcal/kg energy reduction), NC + MCE (Enspira[®]+, United Animal

Health, US; at 125 ppm) and DFM (Amnil[®], United Animal Health, US) at 300 ppm (A300), 400 ppm (A400), and 500 ppm (A500). Body weight (BW) and feed consumption (FC) from 0-35d of age were measured for each pen. Mortality corrected feed conversion ratio (FCR) was calculated as well as weight adjusted FCR (WTC FCR) to the PC. On d36, 5 birds/pen were processed and carcass, breast, and leg quarter weights and yields were determined. Data were analyzed using the GLM model of Minitab. Fisher's LSD was used for mean separation ($P < 0.05$). No differences were observed between treatments in FC, BW, or mortality during starter (d0-14), grower (d14-28), finisher (d28-35) phases or cumulatively (d0-35) ($P > 0.05$). No differences were observed between treatments in FCR during the starter or finisher phase ($P > 0.05$). However, during the grower phase, the PC had better ($P < 0.05$) FCR than NC and A500 treatments. A300 and A400 were similar in FCR to PC in the grower phase ($P > 0.05$). Cumulatively, d0-28 FCR was also lowest in PC and A400 compared to NC. NC had lower carcass, breast, and leg quarter weights than all other treatments ($P > 0.05$). PC and A400 had greater carcass yield than NC ($P < 0.05$) and the remaining treatments were intermediate. No differences between treatments were observed in breast or leg quarter yield ($P > 0.05$). It should be noted that during the finisher phase a natural summer heat stress occurred which may explain the loss of effect on FCR after d28. In conclusion, these results suggest that the combination of DFM and MCE inclusion from 300 to 500 ppm can maintain FCR in an energy reduced diet through d28 and can improve carcass and part weights. Further research is needed to better understand the interaction between the MCE and the different DFM inclusion levels.

Key Words: DFM, Broiler, performance, yield, multi-carbohydrase enzyme

P348 Effects of phytase, 25-hydroxyvitamin D3 and cocci vaccination on growth performance, body composition and gut integrity of broilers fed with a calcium and phosphorus-reduced diet under Eimeria spp. challenge Hanyi Shi^{*GS}, Deependra Paneru, Milan Sharma, Fatemeh Mahdavi, Venkata Choppa, Doyun Goo, Jihwan Lee, Hanseo Ko, Woo Kyun Kim *University of Georgia*

A study was conducted to evaluate effects of phytase, 25-hydroxyvitamin D₃ (25(OH)D₃), and cocci vaccination on broilers fed a reduced calcium (Ca) and available phosphorous (avP) diet under *Eimeria* challenge. A total of 840 one-day-old male broilers were allocated to a 2×5 factorial arrangement based on cocci vaccination and diets. Half birds were cocci vaccinated at day 1 and all birds were orally gavaged with *Eimeria* spp. at day 14. The diets included 1) a nutrient adequate diet (PC), 2) a reduced 0.2% of Ca and avP diet compared to PC (NC), 3) NC + 1,500 FTU/kg of phytase (NC+Phytase), 4) NC + 5,000 IU/kg of 25(OH)D₃ (NC+25(OH)D₃), and 5) NC+Phytase+25(OH)D₃. Data were analyzed by SAS. Growth performance was similar during pre-infection. However, vaccinated birds had higher BW and BWG ($P < 0.05$) from 1-6 day-post-inoculation (DPI). During 7-12 DPI, the NC diet decreased ($P < 0.01$) BWG of birds compared to PC birds, while supplementing with phytase, 25(OH)D₃, or both, restored it to PC levels. FCR was higher ($P < 0.01$) in NC birds during 7-12 DPI and overall (day 0-26), but supplementing with phytase, 25(OH)D₃, or both, improved it to PC levels. For body composition, vaccinated birds had higher bone mineral density (BMD) ($P = 0.0026$) at 6 DPI. At 12 DPI, interactions ($P < 0.05$) were observed that unvaccinated NC+25(OH)D₃ birds had lower ($P < 0.05$) fat percentage and fat mass compared to other unvaccinated groups, whereas vaccinated NC+25(OH)D₃ birds had higher fat percentage and fat mass. Cocci vaccination increased ($P < 0.05$) BMD, total tissue, and lean mass, but decreased ($P = 0.0029$) bone area of birds. Additionally, vaccinated birds had lower ($P < 0.05$) gut permeability at 5 DPI and at 6 DPI, an interaction ($P < 0.05$) effect was found on duodenal lesion score. Unvaccinated NC+25(OH)D₃ birds showed lower scores than other unvaccinated groups, whereas vaccinated NC, NC+Phytase, and NC+Phytase+25(OH)D₃ groups showed lower scores than the vaccinated PC. In conclusion, cocci vaccination improved BWG, gut perme-

ability, BMD, fat mass in birds under *Eimeria* infection. Reducing Ca and avP compromised BWG and FCR of broilers, while supplementing with phytase, 25(OH)D₃, or both, improved growth performance, without affecting body composition or gut integrity.

Key Words: phytase, 25(OH)D₃, *Eimeria*, broiler, growth and health

P349 Effects of Panaferd®, a natural carotenoid source from *Paracoccus carotinifaciens*, on the performance, egg quality, yolk pigmentation, and carotenoid composition of eggs in laying hens Deependra Paneru*¹, Dima White¹, Yuki Kawashima², Woo Kim¹
¹University of Georgia, ²ANCI Inc.

Egg yolk pigmentation is a critical aspect of egg quality and nutritional content that is of significant interest to both consumers and producers. This study aimed to investigate the impact of Panaferd®, a natural carotenoid derived from *Paracoccus carotinifaciens*, a bacterium renowned for its astaxanthin and ketocarotenoid production, on the performance, egg quality, yolk pigmentation, and carotenoid composition of eggs in laying hens. A total of 40 Hy-Line W36 laying hens (50-weeks-old) were randomly allocated to four dietary groups, each receiving varying levels of Panaferd®: 0, 0.75, 1.5, and 3 kg per tonne of feed. The experiment lasted for four weeks, with data collected at the second and fourth weeks. Data were subjected to one-way ANOVA with treatment as the main factor, and significance was set at 0.05. Results showed that Panaferd® supplementation at 0.75 kg/tonne improved the body weight gain ($P=0.0015$) at week 4 and improved feed efficiency and egg production at specific levels. In week 2, the highest feed efficiency (FCR/dozen eggs) and hen day egg production (HDEP) were observed in hens fed 1.5 kg/tonne of Panaferd® ($P<0.0001$). In week 4, the highest HDEP was observed in hens fed 0.75 kg/tonne of Panaferd® ($P<0.0001$), while the highest FCR/dozen eggs were observed in hens fed 1.5 kg/tonne of Panaferd® ($P<0.0001$). Furthermore, Panaferd® supplementation improved the yolk color, increasing the redness and decreasing the lightness and yellowness of the yolk. In both weeks, the control group exhibited the highest lightness (L) and yellowness (b) values, whereas the 3 kg/tonne Panaferd® group had the lowest L and b values ($P<0.0001$). The redness (a) value linearly increased with increasing Panaferd® supplementation in both weeks ($P<0.05$). The astaxanthin composition of the egg yolk linearly increased from 0 to 10.70 µg/g with increasing Panaferd® from 0 to 3 kg/tonne in feed ($P<0.0001$). In conclusion, this study demonstrated that Panaferd® serves as a suitable natural carotenoid source for enhancing the nutritional value and pigmentation of egg yolk in laying hens.

Key Words: Egg yolk pigmentation, Panaferd®, Laying hens, Yolk color, Carotenoid composition

P350 Effects of *Artemisia annua* supplementation of performance and gut health of laying hens infected with *Eimeria* spp. Milan Sharma*, Guanchen Liu, Hanyi Shi, Doyun Goo, Jihwan Lee, Hanseo Ko, Venkata Sessa Reddy Choppa, Deependra Paneru, Fatemeh Sadat Mahdavi, Hamid Reza Rafieian Naeini, Brett Marshall, Woo Kim *Department of Poultry Science, University of Georgia*

Coccidiosis, when infected in laying hens, can significantly drop egg production and cause a substantial economic loss to the egg industry. Supplementation of poultry diets with chemotherapeutic agents is limited due to antimicrobial resistance and residue to the poultry products. Therefore, alternative strategies to control coccidiosis are needed, and *Artemisia annua* leaves (AA) might have potential as an alternative to anticoccidial agents. This study aimed to investigate the effect of dietary supplementation of powdered AA on the performance and gut health of laying hens infected with *Eimeria* spp. A total of 225 Hy-Line W-36 laying hens aged 23 weeks of age were allocated into five treatment groups: (1) non-challenged control (NCC), (2) pair-fed control (PFC), (3) challenged control (CC), (4) CC with 0.5% AA (0.5AA) and (5) CC with 1% AA (1AA). The hens grouped in CC, 0.5AA, and 1AA were orally inoculated with

sporulated oocysts of *E. maxima* (12,500), *E. tenella* (12,500), and *E. acervulina* (62,500) at 25 weeks of age. The PFC hens received the same amount of feed consumed by CC from 0-14 days post-inoculation (DPI). Performance of the hens, including BW, FI, egg production (HDEP), and FCR, were measured from 0-21 DPI, whereas gut permeability and lesion scores were measured on 5 and 6 DPI, respectively. Data were analyzed using one-way ANOVA (BW, permeability), split-plot in time (FI, FCR, HDEP), and Kruskal-Wallis's test (lesion scores) at $P<0.05$ significance. At 6 and 14 DPI, laying hens challenged with *Eimeria* spp. had the lower BW compared to PFC and NCC ($P<0.0001$). Supplementation of 1% AA improved the HDEP by 8.1% compared to that of CC; however, it is still 15.4% lower than PFC ($P<0.0001$). The inclusion of 1% AA did not have any beneficial effect on FI; however, FCR was improved by 0.61 (2.46) than that of CC (3.07; $P<0.0001$). Although non-significant, 1% AA supplementation tended to reduce the gut permeability ($P>0.05$) and severity of the intestinal lesions ($P<0.05$). To conclude, supplementation of AA has shown a potential to replace the chemotherapeutic agents either alone or in combinations; however, further studies are needed to understand the mode of action and dosages better to improve gut health without negative impacts on performance.

Key Words: *Artemisia annua*, Laying hens, Coccidiosis, gut health, Pair-fed

P351 Enhancing Broiler Chicken Welfare: Mitigating Bacterial Chondronecrosis and Osteomyelitis Lameness with 1,25 Dihydroxycholecalciferol-Glycosides and Phytogetic Antioxidants khawla alharbi*^{1,2}, Andi Asnayanti^{1,2}, Amer Hasan^{2,3}, Wesley Vaught², Abigail Fanous³, Kathrin Buehler⁴, Jan Dirk Van der Klis⁴, Javier Gonzalez⁵, Adnan Arubaye^{1,2,3} ¹Department of Poultry Science, University of Arkansas, ²Cell and Molecular Biology Program, University of Arkansas, ³Department of Biological Sciences, University of Arkansas, ⁴Herbonis Animal Health GmbH, ⁵Nuprox

BCO lameness has been a major concern in the poultry industry causing financial losses and animal welfare issues. BCO lameness is primarily caused by bacterial infection in the leg bones. Pathogens enter the bloodstream through the respiratory and intestinal epithelium and eventually infect the tibia and femoral heads of the legs. Improving gut integrity, immune system, and

and calcium metabolism can reduce the incidence of BCO lameness. Panbonis® (Herbonis, Switzerland) is a commercial source of the active form of vitamin D₃ (1,25(OH)₂D₃-glycosides), which is crucial for regulating calcium and phosphorus homeostasis, the immune system, and bone growth. This study investigated the effects of high and low concentrations of 1,25(OH)₂D₃-glycosides and the combination of 1,25(OH)₂D₃-glycosides and several antioxidant polyherbal (PH) and thyme oil, a phytogetic antioxidant, on BCO. 1,560 day-old male chicks were randomly placed into the T1 group for BCO source on wire floors and 6 experimental groups with 4 replicates in litter floor pens: T2 (negative control), T3 (10 µg/kg G-1,25(OH)₂D₃- glycosides - Panbonis® high), T4 (1 µg G-1,25(OH)₂D₃/kg - Panbonis® low), T5 (1 µg/kg G-1,25(OH)₂D₃- glycosides plus 500 mg/kg of PH1, T6 (1 µg/kg G-1,25(OH)₂D₃- glycosides plus 750 mg/kg of PH2), and T7 (1 µg/kg G-1,25(OH)₂D₃- glycosides plus 500 mg/kg thyme oil). PH1 and PH2 were *Solanum xanthocarpum* plus *Piper longum* and *Curcuma longa* plus *Trachyspermum ammi*, respectively. Daily cumulative incidence of lameness was measured by necropsying and scoring tibial and femoral lesions of lame birds. T1 and T2 developed 84.0% and 80.5 % cumulative lameness incidences, respectively, ($P=0.64$) until d56. Supplementation of low 1,25(OH)₂D₃-glycosides at 1 µg/kg (T4) was more effective in reducing the cumulative lameness incidence than at 10 µg/kg (T3) ($P=0.033$). There were no significant differences between the 1,25(OH)₂D₃-glycosides low (T4) alone and its combination with phytogetic antioxidants (T5-T7). In conclusion, 1 µg/kg of 1,25(OH)₂D₃-glycosides provided better protection against BCO lameness compared to 10 µg/kg. Additionally, the combination feed additives did not result in any

further reduction of BCO lameness compared to the use of 1,25(OH)₂D₃-glycosides alone.

Key Words: Broiler, Chondronecrosis, Lameness, Osteomyelitis, 1,25-dihydroxyvitamin D₃-glycosides

P352 Formaldehyde and propionic acid reduce starch gelatinization within a laboratory setting and may indicate reduced pellet quality Kristina Bowen*, Lucas Knarr, Emily Estanich, Joseph Moritz, Sean Estep *West Virginia University*

Starch gelatinization acts as a pellet binder, thereby increasing pellet quality. An improvement in pellet quality has been shown to increase bird performance. The use of antimicrobial feed additives to pre-pelleted feed may decrease risk of salmonellosis to consumers. An experiment conducted by Boney et al., (2020) varied conditioning time and use of an antimicrobial primarily composed of formaldehyde and propionic acid. When the authors steam conditioned feed with the antimicrobial for 10 sec compared to 60 seconds, they discovered a decrease in pellet quality. We hypothesize that the product inhibited starch gelatinization. Therefore, the objective of this experiment was to determine the effect of formaldehyde and propionic acid on starch gelatinization via spectrophotometry. The control formulation was prepared by adding 0.45 ml of the glucose solution to the base reagents. Three additional formulations were prepared by adding formaldehyde, propionic acid, or the combination of the two to the control formulation. The absorbance of the 4 formulations were measured using spectrophotometry after being boiled for 3 minutes 20 seconds or 20 minutes. Six additional concentrations of the glucose solution were added to the base reagents and their absorbance were measured to create a glucose standard curve. All 4 formulations were analyzed using a multiple comparison. A two-way ANOVA was employed to explore the 4 (formulation) × 2 (boiling time) factorial arrangement of treatments. Four replications were performed per treatment. Formulations boiled for 3 minutes 20 seconds had a significantly lower absorbance, or starch gelatinization, relative to those boiled for 20 minutes ($P=0.0029$). When analyzing the 4 formulations independent of boiling time, it was discovered that the control and propionic acid formulations demonstrated more starch gelatinization relative to the formaldehyde and formaldehyde + propionic acid formulations ($P<0.05$). Formaldehyde and propionic acid may reduce starch gelatinization during boiling and may lead to a decrease in pellet quality during feed manufacture. If including an antimicrobial containing formaldehyde and propionic acid pre-pelleting, a longer conditioning time may be required to mitigate the decrease in pellet quality.

Key Words: starch, gelatinization, pellet quality, antimicrobial, spectrophotometry

P353 Evaluation of humic acid properties and potential adsorption capacity of aflatoxin B1 in a simulated poultry digestive model Jesus Maguey-Gonzalez*, Maria de Jesus Nava², Sergio Gomez³, Juan Latorre¹, Maria de Lourdes Angeles³, Bruno Solis⁴, Daniel Hernandez⁴, Juan de Dios Figueroa⁵, Alma Vazquez², Abraham Mendez², Guillermo Tellez¹, Billy Hargis¹ *¹Department of Poultry Science, University of Arkansas., ²UIM, L14: Alimentos, Micotoxinas, y Micotoxicosis, FES-C, UNAM, ³CENID-INIFAP, ⁴UIM, L5: LEDEFAR, FES-C, UNAM, ⁵CINVESTAV-Querétaro*

Previous studies have used various humic acid (HA) sources as mycotoxin binders, but the efficacy of purified HA from vermicompost in binding aflatoxin B1 (AFB1) remains untested. This study evaluated the efficacy of two types of purified humic acid (HA) from vermicompost, natural HA and sodium-free HA (SFHA), in binding AFB1 in an *in vitro* model that simulates the digestive system of poultry. In addition, a non-commercial zeolitic substance served as a reference material. The adsorbents were characterized by using several techniques, including attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR), energy-dispersive X-ray spectroscopy (EDS), zeta potential (ζ -potential), scanning electron microscopy (SEM), and point of zero charge (pH_{pzc}). The

adsorption capacity of the adsorbents (0.2%, w/w) was assessed by incorporating them into a diet contaminated with AFB₁ (100 μ g AFB₁/kg) and using an *in vitro* model that simulates the digestive system of poultry. The characterization results revealed that carboxyl and phenol groups were the predominant functional groups present in HA and SFHA. Additionally, the adsorbents exhibited a highly negative ζ -potential across three different pH levels, suggesting that electrostatic interactions and hydrogen bonding were likely the primary factors influencing AFB₁ adsorption. Adding sorbents to the diet significantly reduced AFB₁ bioavailability in the intestinal tract ($p<0.05$). HA and SFHA achieved AFB₁ adsorption percentages of 97.6% and 99.7%, respectively, while the zeolitic material also showed significant adsorption capabilities (81.5%). These findings highlight the potential of HA and SFHA from vermicompost as effective adsorbents for removing AFB₁ from contaminated animal feeds.

Key Words: Aflatoxin B1, Humic acids, Adsorption, In vitro model

P354 Assessment of dietary glucose oxidase supplementation on Salmonella Enteritidis infection in broiler chickens. A pilot study. Grecia Cardoso-Luna*¹, Rubén Merino-Guzmán¹, Guillermo Tellez-Isaías², Juan Latorre-Cardenas², Raúl Marcon², Latasha Gray², Makenly Coles², Billy Hargis², Lauren Laverty², Kristen Martin², Udochi Kemakolam², Ileana Ramirez-Loeza² *¹Universidad Nacional Autónoma de México, ²University of Arkansas*

The interest in alternate methods to prevent bacterial infections in chicken production has increased because of growing worries about antibiotic resistance and its possible effects on public health. In this study, the effectiveness of glucose oxidase (GOx) as an antibiotic-free alternative for reducing *Salmonella* Enteritidis infections in chicken is assessed. A total of 126 one-day-old broiler chickens were randomly assigned to three groups: a control group receiving a standard commercial diet, a positive control group which also received a standard commercial diet and was challenged with 10⁸ CFU *Salmonella* Enteritidis and a treatment group receiving the same diet supplemented with GOx (100 U/Kg). The experiment spanned over 21 days, during which all birds were raised in batteries under standard commercial conditions. At 20 days old, the positive control group and the GOx group were orally challenged with 10⁸ cfu of *Salmonella* Enteritidis. Cecum, spleen, and liver samples were collected post-mortem 24 hours later, to evaluate *Salmonella* colonization in the gastrointestinal tract and internal organs. The results demonstrated that the dietary supplementation of glucose oxidase did not reduce the prevalence and abundance of *Salmonella* Enteritidis in the GOx group compared to the control groups ($P>0.05$). Body weight, body weight gain, feed intake and feed conversion were also measured, in which no significant statistical difference was observed with respect to the control group. To assure the safety and effectiveness of glucose oxidase, more research is needed to probe into its mechanisms of action and improve its administration in poultry diets. Future projects could evaluate the effect of glucose oxidase at different doses to see if it has any effect on performance and reduction of *Salmonella* levels.

Key Words: Glucose oxidase, broiler chickens, Salmonella Enteritidis, performance

P355 Effect of dietary Almond Hull supplement on growth performance, nutrient retention, organ weight, caecum microbial and noxious gas emission in broilers Songbo Cho*, Golam Sagir Ahammad, In Ho Kim *Dankook University, Department of Animal Resource and Science*

This study aimed to investigate the effect of almond Hull (AH) supplementation on growth performance, nutrient retention, organ weight, caecum microbial, and noxious gas emission in broilers. Ross 308- broilers (n=540, one day old) with an initial average body weight of 47.91± 0.28 g were divided into three treatment groups in a complete randomized block design with 10 replicates of 18 birds per cage. The test treatments were

control (CON), a basal diet, and a basal diet supplemented with 1 and 2 % of AH. For 35 days, all birds were offered an experimental diet following three phases feeding: starter (days 0-7), grower (days 8-21), and finisher (days 22-35). Growth performance (body weight, feed intake (FI), and feed conversion ratio) was recorded at the end of each growth phase and overall trial period. The nutrient retention (dry matter, nitrogen, and gross energy), caecum microbial, and noxious gas emissions were measured at the end of d 35. 10 birds/ treatment were euthanized by cervical dislocation, and the breast muscle, liver, spleen, abdominal fat, bursa of Fabricius, and gizzard were carefully removed to measure the organ weight. Duncan Multiple range test was performed using the GLM procedure of SAS. The polynomial orthogonal contrasts of increasing dietary AH were examined by linear and quadratic effects. The cage served as an experimental unit for growth performance, nutrient retention, caecum microbial, and noxious gas emission, while individual birds served as an experimental unit for organ weight. The broilers fed AH supplement showed higher ($P<0.05$) body weight gain and a tendency to increase FI at the starter phase and overall trial period compared to those fed CON. In addition, the inclusion of graded levels of AH tends to increase ($P<0.10$) the nutrient digestibility of nitrogen and energy at the end of d 35. Compared to the CON group, broilers received 0.1 and 0.2% AH had linearly decreased ($P<0.05$) caecal *Salmonella* shedding and ammonia emission. However, no significant effects were found on the feed conversion ratio and organ weight. From this, we inferred that dietary supplementation of 0.2% AH could serve as a potential feed additive for broilers to enhance their growth performance and nutrient utilization and reduce ammonia emissions.

Key Words: almond hull, broilers, growth performance, gas emissions, nutrient retention

P356 Comparative effects of eubiotic feed additives used in shuttle or straight modes on performance, coccidia lesion scores and bacterial counts in environmentally challenged broilers Luis Gomez^{*1}, Vasil Stanev¹, Sandra Bonaspetti¹, Bruce Johnson¹, James McNaughton², Tim Buisker³ ¹Phibro Animal Health Corporation, ²AHPharma, Inc., ³Smart Data Science Solutions

This broiler study evaluated effects of eubiotic feed additives (FA) in improving intestinal health, which may lead to improved performance and reduced incidence of coccidia and intestinal bacterial counts. Eubiotic FA used were phytonics (QY-*Quillaja saponaria*+*Yucca schidigera*), direct-fed microbials (DFM-4-*Bacillus* strains) and monoglyceride (MG) blends. The same corn-soybean meal-based ration (in mash form) was used in all groups, with varying FA inclusion levels (as a step-down; STR or STRd) or in shuttle (SHT) mode. A clean control with new litter was used in each pen and no disease organisms were intentionally introduced. A severe disease challenge involved used litter known to contain *Eimeria acervulina*, *Eimeria maxima* oocysts, and spores of *Clostridium perfringens* (CP). Dividers used between pens limited cross contamination. This 42-day trial used a randomized complete block design with 5,616 mixed-sex Ross 708 broilers allocated to 9 treatment groups across 108 pens (12 pens/treatment; 52 broilers/pen). Nine treatments were used with 3 rations: T1-non-treated, unchallenged control; T2-non-treated, challenged control; T3-MG STRd mode; T4-T7-combinations of MG, QY, and DFM in tandem on stepdown mode; and T8-T9-MG, QY and DFM in SHT mode in a severe challenge model. Broilers were vaccinated for coccidiosis at the hatchery (day-of-age). Effects of disease challenge were evident. Control birds under challenge had higher lesion scores and fecal CP counts than control birds without imposed challenge. Performance was affected similarly. The MG STRd treatment (T4) and MG-QY tandem/stepdown (T5) had statistically significantly superior performance outcomes (including weight, uniformity, average daily gain and feed conversion rate), larger intestinal morphometric characteristics (ileal villi height and crypt depth), lower microbiological loads (CP, *E. coli*, average plate count), and superior performance in almost all processing measures (including pre- and post-chill carcass weights and yields). The treatment

in SHT modes had better performance than T2 but not better than STR or STRd. This trial helps shed light on what combinations or common usage practices can improve outcomes with current eubiotics. Future studies will include other pathogens of interest.

Key Words: Eubiotics, Phytonics, Monoglycerides, Broilers, Coccidia

P357 Efficacy of a low inclusion clay in preventing the deleterious effects caused by aflatoxins in broiler chickens Carlos Mallmann^{*1}, Leandro Giacomini¹, Diego Sturza², Manuel Contreras^{2,3} ¹Federal University of Santa Maria/Laboratório de Análises Micotoxológicas (LAMIC), ²Laboratório de Soluções Analíticas, Microbiológicas e Tecnológicas (SAMITEC), ³Special Nutrients/Agriprove

Scientific reports demonstrating the efficacy of clays against the toxicity caused by Aflatoxins (AFLs) have been published for decades with inclusion rates ranging from 0.25% to 0.50%. The objective of this experiment was to demonstrate that a sodium bentonite produced in the US and commercialized globally by Agriprove/Special Nutrients, USA showed efficacy at a low inclusion (0.15%). 360 one-day-old male broiler chicks distributed in 6 treatments with 6 replicates of 10 birds each were used in a complete randomized design. Treatments were: (1) control diet; (2) control + 0.50% clay; (3) control + AFL; (4) control + AFL + 0.15% clay; (5) control + AFL + 0.25% clay; and (6) control + AFL + 0.50% clay. Performance (FCR/body weight (BW)/feed intake (FI), relative liver weight (RLW), intestinal integrity/jejunum (villus height /VH, crypt depth/CD + VH/CD ratio), total plasmatic protein (TPP), and thiobarbituric acid relative substances (TAS) were measured. A two-tailed t-test compared the means of the two groups at a significance level of $\alpha=0.05$. At the end of the trial, 21 days of age, all birds were euthanized, and all parameters measured. Chickens exposed to AFLs (T3) alone exhibited toxic effects ($\alpha=0.05$), including reduced FI, lower BW, lower TPP levels, lower TAS, shorter VH, higher RLW ($P<0.001$), poorer FCR ($P=0.002$), and lower VH/CD ratio ($P=0.023$). However, the inclusion of clay provided protection. At 0.15% inclusion (T4), birds showed improved BW ($P<0.001$) and increased TPP ($P=0.006$). At higher inclusion rates (0.25%/T5 and 0.50%/T6), the protection was more significant, leading to higher BW, reduced RLW, higher VH ($P<0.001$), greater FI ($P=0.048$ and $P=0.030$), higher TPP levels ($P=0.002$ and $P<0.001$) and TAS ($P=0.023$ and $P=0.018$). Furthermore, the 0.50% inclusion resulted in an improved FCR ($P=0.019$). These results highlight the efficacy of the clay evaluated in mitigating the toxic effects induced by AFLs. Notably, the 0.15% inclusion rate provided partial protection, a significant finding given the high AFLs concentration used, a level of contamination not typically observed under commercial conditions. Higher clay concentrations (0.25% and 0.50%) demonstrated remarkable and statistically significant protection against AFLs' toxic effects.

Key Words: Aflatoxins, Mycotoxins, Bentonite, clay

P358 Supplementation of probiotics in drinking water improved the productive performance, gut health and tissue response of slow-growing pasture-raised broiler chickens Ayodeji Aderibigbe^{*} Florida A and M University

To address the unique nutritional challenges faced by pasture-poultry producers, previous research studies have explored several innovative solutions. Probiotics are common feed additives added for their beneficial effects on gut health and nutrient absorption. However, relevant data on the response of pasture-raised broiler chickens to probiotic supplementation (PS) are few or when available, produce inconsistent results. The goal of this study is to investigate the impact of PS via drinking water on growth performance, gut health, and tissue response of pasture-raised broiler chickens. On d 3 post hatch, 152 chicks (freedom ranger) were assigned to two dietary groups, control or PS, each with 4 replicates per treatment and 19 birds per replicate. On d 3 post hatch, probiotics was added via drink-

ing water for 24 h for the PS group. On d 18 post hatch, the PS treatment was repeated daily on d 18 – 23 post hatch. On d 24 post hatch, all birds were transferred onto pasture into separate schooners and reared until d 67 post hatch, however no birds received additional probiotics during this period. All birds were provided a corn-soybean-based organic commercial diet that contained a probiotics mixture as part of its formulation. All birds had *ad libitum* access to feed and drinking water throughout the study. Data obtained were analyzed as a randomized complete block design using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). Initial body weight (BW) was used as the blocking criterion. The BW gain (g/bird) and average daily gain (g/bird/day) at d 3 – 18 post hatch increased ($P < 0.001$) in PS birds relative to the control, but no difference was observed in the overall period (d 3 – 67). Final dressed weight (g) at d 67 post hatch and cecum length (cm) were higher ($P < 0.01$) in PS birds. There was a decrease ($P < 0.001$) in cecal pH and excreta egg count in PS birds. Cecal dry matter content and serum IgA were higher ($P < 0.01$) in PS birds. In conclusion, this study shows that additional probiotics via drinking water may improve productive performance and gut health of pasture-raised chickens. Response of birds to continuous PS via drinking water over the entire rearing cycle is unclear and requires further investigations.

Key Words: broilers, gut health, probiotics, outdoor

P359 Microencapsulation of phytogetic feed additive improves performance antioxidant status and egg quality in laying hens raised under tropical conditions Sureerat Thuekeaw*¹, Tonglian Buwjoom¹, Chackrit Nuengjamnong² ¹Faculty of Animal Science and Technology, Maejo University, ²Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University

Phytogetic feed additive (PEA) has antioxidant property. They can be used as feed additives to improve the gut health of poultry. However, antioxidants are low stability during feed pelleting, transportation as well as the gastrointestinal tract (GI). Microencapsulation is a technique to improve stability and controlled release of active ingredients at a target site. The present work aimed to evaluate the supplementation of PEA encapsulated in microcapsule (PEA-M) on growth performance, antioxidant status and egg quality in laying hens. A total of 300 twenty-week-old laying hens were randomly allocated into 2 groups (10 replicates of 15 birds). Dietary treatments were as follows: (i) basal diet (NC), (ii) basal diet with PEA-M at 500 ppm, respectively. PEA-M increased egg production (%) and feed efficiency ($P < 0.05$). Egg quality was not affected by dietary treatments in this experiment. However, the level of superoxide dismutase (SOD) was increased in the duodenal mucosa, whereas malondialdehyde (MDA) was decreased in PEA-M ($P < 0.05$). In conclusion, the PEA in microcapsules can be used as an antioxidant feed additive to improve egg production the antioxidant status laying hens.

Key Words: Essential oil, microencapsulation, gut integrity, intestinal targeted delivery, heat stress

P360 The potential of Zymbiotics® to replace coccidiostat in broiler production Basheer Nusairat*¹, Rasha Qudsieh^{1,2,3}, Jeng-Jie Wang^{1,3} ¹Jordan University of Science and Technology, ²BioResource International, Inc., ³BioResource International, Inc

This study was designed to investigate the effect of a Zymbiotic (Enza-Pro®), and monensin coccidiostat (Coban) on growth performance, gut lesions, and pathogens load in the digesta of broiler chickens (*E. tenella*, total aerobic count cells (APC), *E. coli*, and *C. perfringens*). A 2 x 2 factorial dietary treatment design was utilized to test 2 levels of the zymbiotic (0 and 100 g/MT), and 2 levels of Coban (0 and 90 g/MT) with 10 replicates per treatment and 52 birds per replicate, raised in floor pens for 42 days. Data were analyzed as two-way ANOVA, and means were separated by Tukey's. At 42 d, birds gained ($P < 0.007$) 39 g more compared to the control when fed the zymbiotic, and 57 g more ($P < 0.0002$) when fed the coccidiostat. Feeding both zymbiotic and coccidiostat increased

weight gain by 96 g compared to non-supplemented birds. Improvement in FCR was observed with approximately 2 points due to feeding zymbiotics, and 3 points due to feeding coccidiostat, while the combination of both products improved FCR by 4 points compared to non-supplemented birds. Lesion scores were reduced by zymbiotic supplementation at both 21 d ($P < 0.005$) and 42 d ($P < 0.001$). The same trend was also observed with coccidiostat supplementation at 21 d ($P < 0.002$) and 42 d ($P < 0.001$). There was a tendency ($P < 0.08$) at 42 d for a reduced gut lesion score due to feeding the combination of zymbiotic with coccidiostat compared to non-supplemented birds. Pathogens load in digesta was reduced ($P < 0.01$) when zymbiotic was supplemented, with similar improvement observed for coccidiostat ($P < 0.01$), and further reduction ($P < 0.03$) of *E. coli*, *Salmonella* and *C. perfringens* was achieved when birds were fed the combination of zymbiotic and coccidiostat. It can be concluded that a zymbiotics can be used as an alternative to coccidiostat for improving performance and reducing pathogens. Furthermore, feeding the combination of zymbiotics with coccidiostat has no negative impact on broiler performance, lesion scores, or pathogens load.

Key Words: Zymbiotics, Broiler, Coccidiostat, Lesion score

P361 Double-buffered sodium butyrate addition enables to maintain good performance in broilers fed low energy-protein diet Clemence Marecaille¹, Willem Smink², Julian Melo*³, Xavier Rouleau¹ ¹Dietaxion SAS, ²FEED INNOVATION SERVICES, ³Universidad Nacional de Luján

For economic and environmental reasons, it is important to optimize feed resources. The improvement of feed digestibility and the utilization of nutrients from the diets should be a priority. Previous trials have shown that supplementing 600ppm of double-buffered sodium butyrate (DBSB) led to better performance and intestinal health of broilers fed low energy and protein diets (LEP). The objective of this study was to evaluate if using a higher dose of DBSB in birds fed LEP could enable to reach the performance of birds fed a classic field diet. A total of 162 one day old Ross 308 female broilers were randomly allocated in 27 cages of 6 birds. Fifteen cages were for the control (CTRL) group and 12 for the LEP-DBSB group. CTRL birds fed classic field diets (Starter 1-10 days; Grower 11-28 days; Finisher 29-35 days). LEP diets were formulated to obtain a -3% reduction in energy, protein and digestible lysine, methionine and cysteine based on the CTRL diet. The LEP diets were supplemented with 0.1% of a DBSB (Butylin 54, Dietaxion SAS) from day 1 to 35. The parameters studied were feed intake (FI), body weight (BW), average daily gain (ADG) and feed conversion ratio (FCR) at every feed phase. Data were statistically analysed using t-test or Mann-Whitney test for non-parametric parameters, considering the cage as the experimental unit. There was an increase of the FI in the LEP-DBSB group in the grower and finisher phases compared to the CTRL group (+4% and +5% respectively; $P < 0.05$), without impacting FCR ($P > 0.05$). There were no significant differences in body weight or ADG between group, but ADG tended to be better in the grower phase and body weight tended to be higher at 35 days for the LEP-DBSB group ($P = 0.1$). The supplementation of 0.1% of DBSB in broilers fed LEP diets enables to get similar performance compared to birds fed a common field diet, which means that DBSB optimize better the feed and is then a good strategy to save resources and avoid excess of excretions in the environment.

Key Words: broiler, sodium butyrate, feed efficiency, nutrients savings

P362 Effect of saponins from Yucca and Quillaja, added alone or in combination on production performance and ammonia emissions in broilers Megan Koppen*, Rob Payne, Manuel Da Costa *Cargill Animal Nutrition*

Yucca schidgera (steroidal) and *Quillaja saponaria* (triterpenoid) are two saponins available for commercial feed use in the poultry industry. Though their targeted effects tend to overlap, notable differences in their chemical make-up, structural complexity and mechanistic action exist. While sapo-

nins are known to reduce ammonia emissions in livestock, direct saponin source comparisons are scarcely found in literature. The objective of this trial was to evaluate the impact of feeding Yucca (Y) and Quillaja (Q) alone or in combination (YQ) on Ross 308 male broilers reared through 42 d. Four dietary treatments were tested: 1) a 3-phase corn/soy basal diet (1-14d, 15-28d, 29-42d) Control (C), 2) C+250 g/MT Y, 3) C+125 g/MT Y and 125 g/MT Q (YQ), and 4) C+250 g/MT Q with 6 replicates/treatment. Birds were housed in 8 climate chambers comprised of 3 replicate floor pens with 12 broilers/pen with the same treatment to allow measurement of ammonia emissions. Therefore, ammonia measurements were based on two replicates/treatment. Feed intake, body weight gain, and feed conversion ratio were measured for each feeding phase. Ammonia emissions were determined by measuring the volume of air exchange in each chamber and analyzing online air samples per chamber taken every 13 minutes via an INNOVA photoacoustic gas detector (0-35d). Data were analyzed using ANOVA and means separated using Fisher's LSD at $p \leq 0.05$. All saponin supplemented diets numerically increased final body weight compared to C. Both YQ and Q significantly reduced FCR at 42d. Q resulted in a more pronounced effect on FCR reduction of 4.5 points improvement compared with the control ($P < 0.01$), followed by YQ. Birds fed Y were not statistically significantly different ($P > 0.05$) to C on FCR. Quillaja (Q & YQ) significantly reduced ammonia emission compared with C ($P < 0.05$), whereas the effect of Y was intermediate and not significantly different ($P > 0.05$) from C. The data in this trial demonstrate Q can significantly reduce ammonia emissions and improve FCR, whereas effects of Y were less pronounced and only numerically different from C, further validating differences between these two commercially available saponin sources.

Key Words: phytochemicals, saponin, broilers, performance, ammonia

P363 The calming effect of Melissa officinalis extract (Nor-Balm®) in association with magnesium to improve meat quality: an in-field trial Sekhou Cisse^{1,2}, Julia Laurain², Mohammed Benarbia^{*1,2} ¹Labcom FeedInTech, ²Nor-Feed SAS

Carcass and meat quality traits are important factors in broiler production, and can lead to significant economic losses when they are degraded. One of the main reasons for this degradation is stress generated during poultry farming. Indeed, stress has been documented to increase aggressiveness, leading to negative effects on carcass and meat quality traits. This phenomenon increases in some stressful events for birds, such as transportation and catching. Therefore, calming animals could reduce the economic losses associated with the carcass and meat quality traits. As lemon balm (*Melissa officinalis*) and magnesium are well known for their calming properties, this study aimed to evaluate their efficacy in combination, to improve carcass and meat quality after catching and transportation.

A total of 60,000 broiler chickens (Ross 308) were randomly assigned to two groups:

The control group (CTL, n=30,000) was fed the standard diet.

The Lemon balm/Magnesium group (LbM, n=30,000) was fed a standard diet and supplemented with a liquid combination of Lemon balm extract and Magnesium in the drinking water of broiler chickens (1 liter of LbM for 1,000 liters of drinking water), 3 days before catching and transportation to the slaughterhouse.

Carcass characteristics (hematomas on the wings, the breast area, the thighs, and injuries on the legs) were evaluated in 60,000 broiler chickens after slaughter. The quantity of grade A of meat (highest quality) was also measured. As the objective was not to measure zootechnical parameters, the birds were not divided into replicates for statistical analysis.

The results showed that birds in the LbM group had lower leg injuries (36%) compared to the CTL group (54%). In addition, the percentages of hematomas in the breast area and in the thighs were lower in the LbM group (16.6% and 7.6%, respectively) than in the CTL group (23.4% and

10.5% respectively). As a result, grade A of meat was 10.4% higher in the LbM group compared to the CTL group. The other parameters were similar between the 2 groups.

These results show that LbM supplementation can be effective in improving carcass and meat quality after catching and transportation, probably due to the calming effect of its active compounds.

Key Words: *Melissa officinalis*, Broiler chicken, transportation, carcass traits, welfare

P364 The effect of an organic, all-natural blend of yeast complex carbohydrates on broiler growth performance, carcass yield, and Salmonella prevalence of 0-to-42-day old broilers Theresia Lavergne*, Carl Jones, Charlie Elrod *Natural Biologics, Inc.*

Naverde (Natural Biologics, Newfield, NY) is an USDA organic certified, all-natural blend of yeast complex carbohydrates derived from *Saccharomyces cerevisiae*. This study was conducted to determine the effect of Naverde on broiler growth performance and *Salmonella* prevalence. One thousand two hundred male Ross 708 broilers were allotted to four dietary treatments: 1. Control (no feed additive), 2. Naverde (100 g/ton), 3. yeast culture (YC; 750 g/ton), or 4. hydrolyzed yeast + yeast culture (HY/YC; 100 g/ton). This was a randomized complete block design with 15 replicate pens per treatment and 20 birds per pen. Broilers were reared on used litter and were not administered a disease or pathogen challenge. Broilers were fed a standard starter, grower, and finisher diet, including the feed additives, from day 0 to 14, day 14 to 28, and day 28 to 42, respectively. Birds and feed were weighed on days 0, 14, 28, and 42 to calculate body weight gain (gain), feed intake (FI), and feed:gain (FG). On day 41, cloacal swabs were collected from five randomly selected birds per pen for determination of *Salmonella* prevalence and load. At the termination of the study, three randomly selected birds per pen were processed to determine percent carcass yield. From day 0 to 14, chicks fed Naverde had higher ($P < 0.01$) gain than chicks fed HY/YC, and better ($P < 0.01$) FG than chicks fed the control or HY/YC. Broilers fed Naverde tended to have higher ($P = 0.13$) gain than broilers fed HY/YC from day 14 to 28. During the finisher phase (day 28 to 42), broilers fed HY/YC had the lowest ($P < 0.05$) gain and FI. For the overall trial (day 0 to 42), gain and FI were higher ($P < 0.01$) for broilers fed Control, Naverde or YC than for broilers fed HY/YC. Although not statistically significant, broilers fed Naverde had two points better FG than broilers fed either YC or HY/YC from day 0 to 42. Percentage carcass yield was lowest ($P < 0.01$) for broilers fed HY/YC. *Salmonella* prevalence was numerically highest and *Salmonella* load (CFU) was highest ($P < 0.05$) for broilers fed HY/YC. Naverde can be utilized to improve growth performance, increase carcass yield, and reduce *Salmonella* prevalence and load in live broiler production.

Key Words: organic, complex carbohydrates, *Saccharomyces cerevisiae*, *Salmonella*, broilers

P365 Effect of feeding protected sodium butyrate on production performance and intestinal health of broilers: a meta-analysis Abdelhacib Kihal*, Monica Puyalto, Juan Jose Mallo *Norel S.A*

The objective of this meta-analysis was to evaluate the effect of feeding broiler chickens protected sodium butyrate (PSB) on production performance and intestinal health. A literature search was conducted to identify in vivo research studies on PSB supplementation in broiler chickens. Inclusion criteria were in vivo, broiler, PSB (Gustor®, Norel Nutrition S.A), and doses of PSB. Twenty-two experiments including 85 treatments and 16,774 broilers were selected from published papers and congress communications. Data extracted included the number of broilers in control and treatment groups and measures of variance of responses (standard error or standard deviation). The response variables for production performance were body weight (BW), average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR), and for intestinal health were villi height and crypts to villi height ratio. A random effect model

was used to examine the raw mean difference and standardized mean difference between PSB supplementation and the control group. The analysis was conducted using the metafor package of R. Protected sodium butyrate improved moderately the production performance and intestinal histomorphology responses of broilers with moderate ($30 < F < 60\%$, $P < 0.05$), to no evidence of heterogeneity ($I^2 = 0.0\%$, $P < 0.05$), respectively. Final BW and ADG were increased by 70 g and 1.23 g/day, respectively, or > 0.6 standard deviation units ($P < 0.05$). The FCR was ameliorated in response to PSB supplementation by 0.05 units or > 0.3 standard deviation units ($P < 0.05$), meanwhile, the ADFI of chickens was not affected by PSB supply ($P > 0.05$). The histomorphology profile inspection of the intestine showed increased villi high by 9% or by > 0.4 standard deviation units ($P < 0.05$), and villi: crypts ratio by 5% or by > 0.2 standard deviation units ($P > 0.05$). In conclusion, the meta-analysis results proved that PSB supplementation to broiler diet is effective in increasing production performances and promoting intestinal health and, yet, increasing production profits.

Key Words: protected sodium butyrate, broiler, production performance, intestinal health

P366 The effects of an anti-mycotoxin agent containing adsorbent material, curcumin and silymarin extracts and yeasts on the gut integrity of broiler chickens challenged by T-2 mycotoxin Insaf Riahi*¹, Meritxell Sadurni¹, Diego Sturza², Leandro Giacomini², Carlos Mallmann³
¹Technical Department, BIÓNTE Nutrition, ²Instituto SAMITEC, ³Laboratório de Análises Micotoxicológicas, Universidade Federal de Santa Maria

T-2 mycotoxin is mainly produced by *Fusarium* species and belongs to the trichothecenes family (Sokolovic et al., 2008). T-2 toxin is a common contaminant of cereals grains, specially of corn, wheat and oats that can be found in poultry feeds (Li et al., 2011; Riahi et al., 2021). In broiler chickens, the oral lesions are the most significant signs of T-2 toxicosis (Chi and Mirocha, 1977). Furthermore, previous authors observed intestinal morphometry changes such as shortened and atrophied villi, as well as deeper crypts (Ruan et al., 2019). Additionally, it seems that T-2 mycotoxin can develop interactions with intestinal microbiota (Zhan et al., 2022). The present study was conducted to investigate the effects of an anti-mycotoxins agent on the gut health of broilers challenged by T-2 toxin at 2000 ppb. The anti-mycotoxins agent contained adsorbent material, curcumin and silymarin extracts and a combination of yeasts due to their effects on mitigating the mycotoxins and improving the intestinal barrier (Solovyov et al., 2020; Feshangchi et al., 2022; Kpomasse et al., 2023). A total of 480 d 0-broiler chickens Cobb 500 were randomly distributed in four experimental treatments (n=12) for 21 days. Feed and water were administered *ad libitum*. At d 21 of age, the villus height/crypt depth ratio and absorption surface area were calculated to characterize the intestinal histomorphometry. In addition, digestive content was collected to analyze the total *Bacillus* and Enterobacteria population. Differences between treatments were evaluated using the T test. The broilers fed T-2 toxin had lower absorption surface ($p = 0.018$), confirming the toxic effects of T-2 mycotoxin damaging the cells in the digestive tract (Zhan et al., 2022). The use of the anti-mycotoxins agent increased the villus height/crypt depth ratio ($p = 0.051$) and the absorption surface ($p = 0.002$) of broilers under T-2 toxin challenge. On the other hand, the dietary supplementation with the anti-mycotoxin agent modulates ($p = 0.022$) the *Bacillus*/Enterobacteria ratio decreased by the T-2 toxin ($p = 0.070$). The results suggest that the combination of adsorbent material, curcumin and silymarin extracts and yeasts beneficially impacts on the intestinal integrity of broiler chickens challenged by T-2 toxin.

Key Words: T2-mycotoxin, broiler chickens, gut health, anti-mycotoxins agent, curcumin silymarin extracts

P367 Calibrin®-Z decreases effects of Aflatoxins, Fumonisin, and T-2 toxin in broilers San Ching*, LeAnn Johnston, Eduardo Baggio
Amlan International

In nature, multiple mycotoxins produced by fungus usually coexist in grains. Calibrin-Z® (Amlan International, Chicago, IL, available in select international markets) is a calcium montmorillonite that has been shown to bind toxins. A total of 240 one-day-old male Cobb 500 broiler chicks with an average weight of 44.67 grams was used in a 21-day experiment to evaluate the effect of Calibrin-Z and Product Y on chicks fed a diet contaminated with multiple mycotoxins. A completely randomized trial was conducted with 4 treatments in 6 pens per treatment and 10 birds in each pen. Statistical analysis was conducted using the Student T test in JMP software v15.0. Statistical significance was set at $P < 0.05$. The treatments were: 1) Unchallenged control; 2) Challenged control with 1.8 ppm Aflatoxins, 50 ppm Fumonisin and 1 ppm T-2 Toxin; 3) Challenged control with 0.5% Calibrin-Z; and 4) Challenged control with 0.5% Product Y. The diet was corn-soybean meal-based and met the requirements in the Cobb Broiler Management Guide. No major mycotoxins were detected when raw materials were screened. Birds fed multi-toxins with Calibrin-Z, but not Product Y had higher 21 d BW than birds fed diets with multi-toxins alone ($P < 0.05$), 575 g vs 512 g, while the unchallenged control had the highest body weight (748 g, $P < 0.05$). Feed intake at day 21 followed the same pattern as BW ($P < 0.05$). Feed to gain increased with the multi-toxins challenge from 1.537 to 1.699 ($P < 0.05$). Feeding Calibrin Z, but not Product Y, resulted in a F:G that was not different from the unchallenged control (1.638, $P > 0.05$). Relative liver weight was higher ($P < 0.05$) in the challenged control (5.01%) compared to the unchallenged control (2.84%), and Calibrin-Z (4.46%) but not Product Y (5.12%) ($P < 0.05$). Blood was collected on d 21 and serum was analyzed for malondialdehyde (MDA, mg/g protein) and total protein. Calibrin Z (4.81) and Product Y (4.37) groups decreased ($P < 0.05$) serum MDA vs. multi-toxins challenge group (7.31). For serum total protein, only Product Y show an improvement, 3.91 vs 2.19 ($P < 0.05$). In conclusion, adding Calibrin-Z but not product Y to a diet containing multi-toxins improved weight gain, feed intake, and relative liver weight compared to the challenged control.

Key Words: Mycotoxins, T-2 toxin, Fumonisin, Aflatoxin, Broilers

P368 In vitro efficacy of ammonium formate vs sodium formate against different bacteria of interest in poultry Laura Martínez-Villagrasa*¹, Monica Puyalto¹, Juan José Mallo¹, Aida Yuste Parareda², María Ángeles Calvo Torras² ¹NOREL, ²Universidad Autónoma de Barcelona

The Regulation (EU) No 1831/2003 on additives for use in animal nutrition, was updated in 2022, and will entry into force completely in 2024. One of the changes of this update, is the restriction in the use of ammonium formate in some animal species, like laying hens. In the process of developing products to accomplish this regulation, ammonium formate and sodium formate were tested to figure out if this change may affect the efficacy of some of these new products.

A study was performed by Norel and Universidad Autónoma de Barcelona. Sodium formate and ammonium formate were tested in triplicates against well-known poultry bacteria of interest: *Clostridium perfringens*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella gallinarum*, *Campylobacter jejuni*, *Escherichia coli* and *Staphylococcus aureus*.

Were performed Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) tests in triplicates, following the recommendations of document "Proceedings in clinical microbiology" of the Spanish Society of Infectious Diseases and Clinical Microbiology.

MIC and MBC had always the same result. MIC and MBC of ammonium formate were 50.000 ppm against *C. perfringens*, *S. enteritidis*, *S. typhimurium*, *S. gallinarum* and *S. aureus*. While, MIC and MBC of so-

dium formate were 33.333 ppm against *C. perfringens*, *S. enteritidis*, *S. typhimurium*, *S. gallinarum*, *C. jejuni*, *E. coli* and *S. aureus*.

On the other hand, MIC and MBC for ammonium formate were 25.000 ppm against *C. jejuni* and *E. coli*, while MIC and MBC of sodium formate were 50.000 ppm against *C. jejuni* and *E. coli*.

In conclusion, sodium formate is 66.7% more effective against *C. perfringens*, *S. enteritidis*, *S. typhimurium*, *S. gallinarum* and *S. aureus* than ammonium formate. And ammonium formate is two times more effective against *C. jejuni* and *E. coli*.

Key Words: Ammonium Formate, Sodium Formate, MIC, MBC, Bacteria

P369 Impact of Organic Acid-Phytogen Composite on intestinal health of Broiler Breeders using the ISI Sys system in Brazil Carolina Perry¹, Bruna Belote^{*2}, Igor Soares², Fernando Urganí³, Rafael Canonenco de Araujo¹ ¹GRASP, ²ISI Institute, ³State University of Maringá

Non-specific enteritis describes inflammatory processes in the small intestine whose cause has not been characterized and in breeders, the condition might impair their reproductive function. This study aimed to compare the effects of different doses of Activo Liquid® (GRASP), a liquid blend of essential oils and organic acids, on controlling non-specific enteritis in three commercial breeding barns in Brazil. Each positive pressure barn contained around 10,000 Ross breeders, with automatic drinkers and feeders. At 48 weeks (w) of the flocks age, 5 birds per barn were necropsied for macroscopic evaluation and the observed alterations were scored from 0 to 3 (0=no change; 3=severe) on the ISI Sys mobile app, which generated ISI Score for each bird. The app provides images and descriptions of each score to standardize evaluations. This indicator expresses the bird overall health based on the assigned scores and on fixed impact factors, where higher the ISI Score, worse the bird health. Right after the first necropsies, one barn remained untreated (NP=no product) and two barns received different doses of the liquid blend in the drinking water tank for four times a day. The doses of the liquid blend were (product/water): 500mL/1000L (AcLiq500) and 750mL/1000L (AcLiq750). After one week of treatment, 5 birds (now 49 w old) per barn were necropsied for the same macroscopic evaluation. Data were submitted to the analysis of variance and Kruskal-Wallis' test and only significant results (P<0.05) are described next. The treatment AcLiq500 reduced the ISI Score for presence of mucus and desquamation in the intestinal lumen (7.6 to 1.2) and the reduction in the Total ISI Score, which represent all the evaluated parameters, was of 73% (from 11.2 to 3.0). With the dose of AcLiq750, the ISI Scores for presence of mucus and desquamation in the intestinal lumen, and for mucosal hyperemia got the respective decreases (8.8 to 0.04) and (1.6 to 0.6). The Total ISI Score was reduced in 89% (13.8 to 1.4) with this same dose. Birds from the NP barn presented no significant difference between the evaluated ages. At both doses, the tested liquid blend was effective within 1 w of use in reducing intestinal alterations that usually compose episodes of non-specific enteritis.

Key Words: Non-specific enteritis, organic acids, essential oils, gut health, Scoring method

P370 The impact of a triple-strain Bacillus-based probiotic on gut microbiome modulation and egg contamination in laying hens challenged with Salmonella Typhimurium Antoine Meuter^{*1}, Samiullah Khan², Kay Russo³, Eric Sobotik³ ¹Chr. Hansen A/S, ²School of Animal and Veterinary Sciences, The University of Adelaide, ³Chr. Hansen Inc.

Reducing Salmonella challenges, leading to safer eggs and egg products is a priority for the poultry industry. Salmonella Typhimurium disturbs the enteric microbiota balance, and can result in continuous fecal shedding and contaminated eggs. This study evaluated the impact that a triple strain Bacillus-based probiotic had on maintaining the balance of the gut microbiota in the face of a Salmonella Typhimurium challenge. Day-

of-hatch female chicks were divided into six treatment groups (n=7 per group), raised in pens until 14-weeks and then transferred into individual cages. Treatment groups included a negative control (NC), a Salmonella challenge (PC), a continuous probiotic supplemented and Salmonella challenge (CPPC), a continuous probiotic supplemented control without challenge (CPNC), an intermittent probiotic supplemented and Salmonella challenge (IPPC) and an intermittent probiotic control without challenge (IPNC). At 18-weeks of age, pullets from the challenge groups were orally inoculated with Salmonella Typhimurium (106 CFU/ml). The Salmonella Typhimurium load data in feces (log₁₀MPN) and in organs (mean percent value) were analyzed in Statview software (Version 5.0.1.0). The level of significance was determined by Fisher's LSD at P<0.05. For microbial community profiling, the OTU table was analyzed in Calypso software using ANOVA. The Salmonella challenge negatively impacted the diversity and abundance of gut microbial genera involved in essential functions such as organic acid and vitamin production. The probiotic supplementation in CPPC and IPPC improved the gut microbiota by maintaining the initial abundance level of most of the genera seen in NC. Clearer effects were observed in CPPC that showed an increased concentration of butyrate in feces, a decreased load of Salmonella in feces and organs (ceca and shell gland; P<0.05). Supplementing laying hens with a triple-strain Bacillus-based probiotic helped maintain a diverse and balanced gut microbiota with a higher abundance of butyrate-producing bacteria despite the Salmonella Typhimurium challenge. The potential contribution of the probiotic to food safety was clearly substantiated by reduced fecal shedding and organ contamination of this zoonotic agent.

Key Words: Salmonella, Bacillus, Probiotic, Microbiome, Food Safety

P371 Enhancing the shell strength, intrinsic quality, and shelf-life extension of eggs during the late lay period through a commercial triple-strain Bacillus-based probiotic supplementation in layer diets Antoine Meuter^{*1}, Rogerio Frozza², Ibiara Paz³, Eric Sobotik⁴, Kay Russo⁴ ¹Chr. Hansen A/S, ²Chr. Hansen, ³Faculdade de Medicina Veterinária Zootecnia. UNESP, ⁴Chr. Hansen Inc.

The quality of table eggs is determined by a complex interplay of factors. These include the age of the laying flock, the genetic background of the birds, the rearing conditions, seasonal variations, the duration of the storage period, and the nutritional regime implemented. A well-balanced gut microbiota is essential for optimal nutrient absorption, which in turn significantly affects productivity and egg quality in laying hens. An investigation was conducted to determine the efficacy of a commercial triple-strain Bacillus-based probiotic on the shell strength, intrinsic quality, and shelf-life extension of eggs. Two treatments were tested, each containing 15,000 layer pullets from the same breeder flocks at 18-weeks-of-age. On top of a standard diet, the treatments were: 1) No supplement (Control, CON), and Probiotic (PRO, 1.6×10⁶ CFU/g of finished feed). Thiobarbituric reactive substances (TBARS), specific gravity (SG), and eggshell strength (ESS) of eggs were measured from each treatment group at 71-weeks-of-age. Eggs from each group were collected and stored at room temperature. The characteristics of egg quality were assessed at three intervals: immediately upon laying (d0), and subsequently at 15- (d15) and 30-days post-lay (d30), following a period of storage. Data were analyzed using Minitab (Version 21.3.1), where significant differences were considered when P<0.05. Tukey's HSD post-hoc test was used for mean separation. Compared with CON, SG of PRO was increased (P<0.05) at d0, d15 and d30, as well as cumulatively (1067.59 vs. 1963.15). Similarly, in the PRO group, ESS was increased (P<0.05) immediately at lay (d0), with sustained improvements observed at d15 and d30, and for the entire period (3.709 vs. 3.203kg/cm²). TBARS level in the PRO group were not significantly different (P>0.05) from the CON at d0 or 15-days post-lay. However, lower TBARS levels (P<0.05) in the PRO group were observed at d30 compared to CON (0.195 vs. 0.219 mg/kg). In conclusion, supplementation with the commercial triple-strain Bacillus-based probiotic at the onset of production markedly improves egg quality during the late

laying period. Furthermore, this intervention serves as a preventative measure against the adverse effects of storage on the shelf-life of eggs.

Key Words: Probiotic, Bacilli, Layers, shelf-life, shell strength

P372 The effects of essential oil from *Lippia organoides* and herbal betaine on performance, intestinal integrity, bone mineralization and meat quality in broiler chickens subjected to cyclic heat stress. Roberto Senas-Cuesta^{*1}, Billy Hargis¹, Andressa Stein¹, Juan Latorre¹, Clay Maynard¹, Xochitl Hernandez-Velasco², Victor Petrone-Garcia³, Elizabeth Greene¹, Makenly Coles¹, Latasha Gray¹, Lauren Laverty¹, Kristen Martin¹, Ileana Loeza¹, Alvaro Uribe⁴, Blanca Martinez⁴, Jaime Isaza⁴, Danielle Graham¹, Casey Owens¹, Guillermo Tellez-Isaias¹ ¹*Department of Poultry Science, University of Arkansas.*, ²*Universidad Nacional Autonoma de Mexico*, ³*Facultad de Estudios Superiores de Cautitlan*, ⁴*Promitec*

The purpose of this study was to evaluate the effect of essential oils (EO) on performance, intestinal integrity, bone mineralization, and meat quality in broiler chickens subjected to cyclic heat stress (HS). Day-of-hatch Cobb 500 male broiler chicks (n = 475) were randomly divided into four groups. Group 1: No heat stress (Thermoneutral) + control diets; Group 2: heat stress control + control diets; Group 3: heat stress + diets supplemented with thymol chemotype (45 ppm) and herbal betaine (150 ppm) formulation EO1; Group 4: heat stress + diets supplemented with phellandrene (45 ppm) and herbal betaine (150 ppm) formulation EO2. From day 10–42, the heat stress groups were exposed to cyclic HS at 35°C for 12 h (8:00–20:00). All data were subjected to ANOVA in a complete randomized design. Statistical significance was set up a $p < 0.05$. BW, BWG, FI, and FCRc were measured at d 0, 10, 28, and 42. Chickens were orally gavaged with FITC-d on days 10 (before heat stress) and 42. Morphometric analysis of duodenum and ileum samples and bone mineralization of tibias were done. Meat quality was assessed on day 43 from ten chickens per pen per treatment. Heat stress reduced BW by day 28 ($p < 0.05$) compared to thermoneutral chickens. At the end of the trial, chickens that received both formulations of EO1 and EO2 had significantly higher BW than HS control chickens. A similar trend was observed for BWG. FCRc was impaired by EO2 supplementation. There was a significant increase in total mortality in EO2 compared with EO1. EO1 chickens had lower FITC-d concentrations at day 42 than the HS control. In addition, EO1 treatment is not statistically different if compared to EO2 and thermoneutral. Control HS broilers had significantly lower tibia breaking strength and total ash at day 42 than HS chickens supplemented with EO1 and EO2. Heat stress affected intestinal morphology compared to thermoneutral chickens. EO1 and EO2 improved intestinal morphology in chickens under HS. Woody breast and white striping were more common in thermoneutral chickens than HS chickens. In conclusion, the EO-containing diet could improve broiler chicken growth during cyclic heat stress, becoming increasingly relevant in antibiotic-free production in harsh climates.

Key Words: betaine, chickens, essential oils, heat stress, performance

P373 Use of animal feed grade sodium bisulfate supplementation, alone or in combination with other feed additives, on broiler performance Bradley Turner^{*1}, Craig Coufal², Juan Suarez² ¹*Poultry Research Partners, LLC*, ²*Jones-Hamilton Co.*

Sodium bisulfate is commonly used to acidify poultry litter to control ammonia and as an acidifier for drinking water, animal feed and human food products. Research has shown that animal feed grade (AFG®) sodium bisulfate can improve gut health parameters and performance in broilers. However, use of AFG in combination with commonly used feed additives has not been previously investigated. Therefore, the objective of this study was to evaluate the impact of AFG supplementation, alone or in combination with feed additives, on broiler performance and intestinal health in birds subjected to a coccidiosis vaccine challenge. The 5 dietary treatments (Trt) were Trt A - 0.05% zoalene (Zoamix®), Trt B - 0.025% saponin supplement (Magni-Phi®), Trt C - 0.4% AFG, Trt D - 0.05% zo-

alene + 0.2% AFG, and Trt E - 0.025% saponin supplement + 0.2% AFG. All Trt groups received diets with feed additives during the starter (0-14 d) and grower (14-28 d) phases only. A common finisher diet without any additives was fed to all Trt groups for 28-42 d. All diets were formulated to be nutritionally equivalent and meet or exceed NRC guidelines. Two thousand male Cobb 500 by-product broiler chicks were randomly allocated to 50 pens (10 pens/Trt) with used litter covering a dirt floor. Prior to placement in the pens, all birds received a 2x dose of coccidiosis vaccine. Feed consumption and BW were recorded on d 0, 14, 28 and 42. On d 28, 2 birds per pen were euthanized and coccidiosis lesion scoring was performed. Data were analyzed by one-way ANOVA using the GLM procedure of SPSS with means deemed different at $P \leq 0.05$. No significant differences were found for BW, FCR or mortality on d 14 or 28 between any of the treatments. Trt D had lower intestinal lesion scores than Trt B on d 28, but Trt D was not different from the other 3 Trt. Similarly, Trt D had higher d 42 BW than Trt B and C, but was not different from Trt A and E. Trt D also had the lowest d 42 FCR compared to all other Trt, and Trt E had a lower FCR than Trt A, B and C which were similar. These results indicate that AFG alone (Trt C) performed similar to the commonly used feed additives (Trt A and B), but combining AFG with these feed additives (Trt D and E) resulted in improved broiler performance.

Key Words: sodium bisulfate, feed additives, broiler, intestinal lesions, coccidiosis

P374 Evaluation of organic acid Fortrol on the microbial loads of feed, the microbiota of broiler chickens, and broiler performance from d0-49 Callie Selby^{*1}, Enrique Montiel^{1,2}, Juan David Latorre², Guillermo Tellez², Billy Hargis², Raul Marcon², Roberto Senas-Cuesta², Makenly Coles² ¹*Anitox Corporation*, ²*Department of Poultry Science, University of Arkansas*

Organic acids are utilized in commercial poultry for both performance and food safety. Organic acids can serve as antimicrobials for animal feed and in the animal's gastrointestinal tract (GIT). Target organisms for organic acids can include *Salmonella*, *campylobacter*, or other microbes of poultry origin important for food safety. By reducing pathogenic bacteria within the poultry GIT, beneficial bacteria is favored, theoretically improving performance. In the present study, the organic acid Fortrol was utilized to reduce the microbial load of broiler starter, grower, and finisher diets. Post application, the microbial load of the feed, microbial shifts in the GIT of broiler chickens, and broiler performance were evaluated by dietary phase. All data was subjected to ANOVA via JMP Pro 17. Significant differences between means were determined by Student's T Test at $P < 0.05$ as the significance level. Birds fed a diet with the inclusion of Fortrol had significantly lower ($P < 0.05$) numbers of presumptive coliforms in both the starter and grower phase, while total aerobic bacteria were similar between treatments. No statistical differences in BWG or FCR were observed between treatments, however, strong numerical trends were observed. Data from this study indicates the organic acid Fortrol in broiler diets impacts the microbial populations in the GI tract of broilers, further impacting performance. More research is needed to evaluate the impacts of Fortrol on microbial populations in the GIT as well as broiler performance. Fortrol's impacts in other poultry species is yet to be investigated.

Key Words: broiler, organic acids, gut health

P375 ALTERNA® improved early lay performance when fed after onset of production in commercial cage free layers Ashley Wagner^{*1}, Olivia Martin¹, Jessica Suagee-Bedore², Bill Achor³, Ivan Girard¹ ¹*Probiotech International Inc.*, ²*Virginia Tech*, ³*York Ag Products*

Optimal gastrointestinal health begins with the establishment of a healthy gut microbiome; however, in some production systems, pullet rearing and nutrition may be managed by a third party. In those cases, management and nutritional control may only begin after the onset of lay; therefore, commercial based trials are warranted. The objective was to determine the

efficacy of Alterna® Poultry (Probiotech International, Inc.; Saint Hyacinthe, Quebec, Canada) at improving lay performance when fed after the onset of production in commercial cage free layers. Alterna® Poultry was fed to Alterna birds at ¼ lb/ton wk 23–40; ½ lb/ton wk 41–55, and 3 periods were assigned P1:23-30wk, P2:31-40wk, and P3:41-55 wk. Dekalb layers (20,000/house) in cage-free housing were assigned to either Alterna (A) or control (C). Commercial production parameters were assessed weekly, and week within period was used as the EU. As production rate is reported as a percentage and not normally distributed, data were corrected using Bland-Altman agreement analysis by comparing observed values to the breed standard (B; Observed-Expected). The difference between the observed and B was analyzed using ANOVA for the effects of period and treatment. FCR was also not normally distributed and was analyzed using a Poisson distribution. These analyses are suggested for commercial based trials with limited sample sizes (Schmid and Duan 2014). Statistical analysis was performed in SASv9.3, and significance was set at $P < 0.05$. The observed production rate was greater than the B for all periods ($P < 0.001$) and for A as compared to C ($P = 0.013$). The production rates were P1:A-96.5%, C-95.7%, B -93.8%; P2:A-98.8%, C-97.9%, B-94.7%; P3:A-98.6%, C-97.7%, B -90.8%. The difference in observed case weights from B was greater ($P = 0.004$) for A than C in P1. The observed number of eggs per hens housed was greater ($P = 0.001$) than the B for period 3 in A and as compared to the C ($P = 0.006$). There was no effect ($P > 0.05$) of treatment within period on FCR. This commercial investigation into the utilization of a gut health additive after the onset of production demonstrated potential for impacting production. Due to the limitations of this commercial based trial, additional investigations are warranted with more replications.

Key Words: gut health, layers, cage-free

P376 Effects of Peptasan supplementation on Growth performance, Gut health and Antioxidant enzyme in Broiler chickens challenged with Eimeria spp. Jihwan Lee*, Doyun Goo, Hanseo Ko, Woo Kim
University of Georgia

This study was conducted to investigate the effects of supplementation of peptasan on growth performance, gut permeability, intestinal lesion score, oocyst shedding count, tight junction, pro-inflammatory cytokine, and antioxidant enzyme in broiler chickens challenged with *Eimeria* spp. A total of 288 one-day-old Cobb 500 male broilers were randomly allocated with 8 replicates per treatment of 12 birds each cage for 28 days. Treatment were as follows: basal diet (nonchallenged control, NC), basal diet with *Eimeria* spp. inoculation (Challenge control, CC) and CC + 500 ppm peptasan (Pep). *Eimeria* spp. Broiler chickens in CC and Pep will be inoculated with 62,500 oocyst of *E. acervulina*, 12,500 oocyst of *E. maxima* and 12,500 oocyst of *E. tenella* on 14 days, and NC group orally inoculated with the same amount of phosphate buffered saline (PBS). *Eimeria* spp. challenge decreased ($P < 0.001$) BW on 20 and 28 days compared to other treatments. Moreover, *Eimeria* spp. challenge caused ($P < 0.0001$) high oocyst excretion of *E. acervulina*, *E. maxima* and *E. tenella*, high incidence of severe lesion in duodenum and ceca, poor gut integrity such as high fluorescein isothiocyanate-dextran (FITC-D) level in serum and lower villus height to crypt depth ratio (VH: CD) and reduced glutathione peroxidase (GPX) in jejunum compared to NC group. For gene expression, *Eimeria* spp. challenge increased ($P < 0.05$) the gene expression of claudin 1 (CLDN-1) and pro-inflammatory cytokine including interleukin 1 beta (IL-1 β), IL-6, tumor necrosis factor alpha (TNF- α) and interferon gamma (IFN- γ) in jejunum compared to NC group. On the other hands, supplementation of Pep alleviated ($P < 0.001$) poor performance caused by *Eimeria* spp. Furthermore, Pep supplementation decreased ($P < 0.0001$) oocyst excretion of *E. acervulina*, *E. maxima* and *E. tenella*, incidence of severe lesion in duodenum and ceca, the gene expression of CLDN-1, IL-1 β and TNF- α in jejunum while slightly increased ($P = 0.0079$) GPX in jejunum compared to CC group. In conclusion, peptasan showed positive effects on performance, lesion score, oocyst shedding, gut integrity and antioxidant capacity in this study, suggesting that pep supplementation

could be a dietary strategy to improve gut health in broilers challenged with coccidiosis.

Key Words: Broiler, Coccidiosis, *Eimeria* spp., Peptasan, Intestinal health

P377 Role of novel natural nutritional emulsifier 'BioEmulsin DS' in optimizing fat utilization and energy saving in poultry diets Shivi Maini*¹, Hitesh Pawar², Deepak Singhare³, Sanjay Suradkar³ ¹Indian Herbs Specialities, ²Verity Vet Laboratories, ³IPMT

The integration of nutritional emulsifiers in diets play a pivotal role in improving energy efficiency of fats & oils leading to reduced feed costs. Considering the significant role of fats and oils as high-energy sources in feed formulation for high-performing broilers, it is essential to ensure their optimal utilization for economic benefits. By using nutritional emulsifiers to enhance fat digestibility, the overall energy efficiency of these raw materials can be improved. This improvement in energy efficiency subsequently leads to reduced feed costs, benefiting the economic aspect of animal production.

An experiment was undertaken in VenCobb 430Y broiler model (0-6 weeks) to evaluate physiological effect of supplementing phyto-genic emulsifier on broiler performance. Birds of control and treatment are fed with low energy ration (-2.5% ME) (pre-starter, ME: 2929 Kcal, CP: 22.52%, Oil: 1.72%), [starter, ME: 3049 Kcal, CP: 21%, Oil: 3.26%) and (finisher, ME: 3175 Kcal, CP: 19.50 %, Oil: 4.75%). Treatment group birds were supplemented with BioEmulsin DS@250g/ton supplemented to treatment group 1 (T1) throughout lifecycle of 6 weeks. A significant ($P < 0.01$) improvement in zootechnical performance was recorded in BioEmulsin DS supplemented group. Mean Body weight (BW) at 6th week in BioEmulsin DS supplemented group was 2510g than control (2399g). In the treatment group BW at 6th week was higher by 111g, FCR & mortality were lowered by 1.9% & 1.04%. BioEmulsin DS significantly ($P < 0.01$) increased serum levels of bile acids and pancreatic lipase activity & allowed energy saving upto 60Kcal. BioEmulsin DS is a research-breakthrough product (M/S Indian Herbs Specialities) that provides benefits beyond emulsification and ensures optimized fat utilization therefore makes poultry flock more fat and energy efficient. BioEmulsin DS is designed in such a way that it trigger higher physiological production of bile and stimulates activity of pancreatic lipase enzyme. The use of nutritional emulsifier serves as an effective strategy for optimizing energy efficiency in poultry diets. This, in turn, not only reduces feed costs but also contributes to a more economical and sustainable model of poultry production, addressing the key concerns of the global poultry industry.

Key Words: nutritional emulsifier, fat digestibility, bile, lipase, energy saving

P378 BioEmulsin DS - Not just another emulsifier Shivi Maini*¹, Hitesh Pawar² ¹Indian Herbs, ²Verity Vet Laboratories

Bile acids are used for emulsification, digestion & absorption of dietary fat in chicken. Similarly, exogenous lipases potentiates chicken digestive system. Owing to potential of bile acids & lipases, their use has been increased in recent years, for better fat emulsification & growth performance in broilers. Bile acids strengthen defense mechanism against bacterial endotoxins & play a key role in lipid metabolism as signaling molecules. It has been demonstrated that bile acids & lipases improve feed efficiency by enhancing digestive enzyme activity, fat digestion and absorption. However, contradicting results have been reported by many scientists, which needs further investigations to elucidate various nutritional aspects of bile acids and lipase supplementation in broiler diet. A research study was undertaken on the mechanism of action of phyto-genic feed supplement (BioEmulsin DS) for its potential application to physiologically stimulate production of bile acids and lipases in broiler diets. An experiment was undertaken in VenCobb 430Y broilers(0-6 weeks) to evaluate genomic effect of supplementing phyto-genic emulsifier (BioEmulsin DS) on utili-

zation of dietary fats and added lipid / oils to feed. Birds of control & treatment are fed with ration as per standard requirements of energy & CP (pre-starter, 3005 Kcal,CP:22.52%,Oil:3%), [starter,3127 Kcal, CP:21%,Oil: 4.5%) and (finisher, 3255 Kcal,CP:19.50 %,Oil:6%). Treatment group birds were supplemented with BioEmulsin DS@250g/ton throughout lifecycle of 6 weeks. Data was analysed by two way ANOVA & using GraphPad Prism Software (Version 6.01) General Linear Model & the results were expressed as the mean \pm standard deviation. Differences were considered significant at $p \leq 0.05$. Serum levels of bile acid and pancreatic lipase was analysed using kit method. BioEmulsin DS supplementation significantly stimulated physiological production of bile acids (69 to 87%) from liver and lipase enzyme (45 to over 100%) from pancreas. On basis of trial findings it is inferred that supplementing BioEmulsin DS physiologically stimulates endogenous bile and lipase production in broilers and is a suitable & an alternate option to replace to exogenous bile and lipase in poultry flocks.

Key Words: exogenous bile supplementation, lipase, fat digestion, phyto-genic supplement, serum bile concentration

P379 Interplay of phyto-genics and genomics : Potential to boost performance in broilers by improving fat utilization Hitesh Pawar¹, Shivi Maini^{*2} ¹Variety laboratory, ²Indian Herbs

Nutrigenomics is one of the most rapidly developing scientific fields that holds great promise for improving performance of food-producing animals. Nutrigenomics begins a new era of working with nutrition & genetics, and gives us an insight into how nutrients interfere with an organism's genetics and the resulting phenotypic response. It focuses on elucidating effect of dietary nutrients on expression patterns of different genes & epigenetic modifications. Nutraceuticals & phyto-neutraceuticals in poultry diets can improve expression of different genes related to metabolism, health, growth, antioxidant capacity & immunity. An experiment was undertaken in VenCobb 430Y broilers(0-6 weeks) to evaluate genomic effect of supplementing phyto-genic emulsifier(BioEmulsin DS) on utilization of dietary fats and added lipid / oils to feed. Birds of control & treatment are fed with low energy ration(-2.5%ME) (pre-starter, ME:2929 Kcal, CP:22.52%,Oil:1.72%), [starter,ME:3049 Kcal,CP: 21%,Oil: 3.26%) and (finisher, ME:3175 Kcal,CP:19.50 %,Oil: 4.75%). Treatment group birds were supplemented with BioEmulsin DS@250g/ton supplemented to treatment group1(T1) throughout lifecycle of 6 weeks. Relative quantification assays were performed to evaluate relative expression of liver carnitine palmitoyl transferase (CPT-1) gene, pancreatic lipase (PL) gene and fatty acid transporter protein-4 (FATP-4) gene. Real time PCR & fold change analysis revealed that BioEmulsin DS supplementation in broilers upregulated gene expression of CPT-1 in liver. CPT-1 is vital for fat metabolism. CPT-1 is transporter of fatty acids into mitochondria, where they are oxidized & converted to energy. BioEmulsin DS upregulated gene expression of PL, PL plays most critical role in fat digestion that breakdown triglycerides into free fatty acids & glycerol. BioEmulsin DS upregulated gene expression of FATP-4 in small intestine, FATP4 expression on the intestinal brush-border enhanced fatty acid uptake by enterocytes, better absorption of fatty acids and lipids. In this study of advanced molecular sequencing technology of Nutrigenomics it is validated that phyto-genic feed supplement BioEmulsin DS significantly potentiates fat metabolism, digestibility, transport and absorption of dietary fat, lipids & oils.

Key Words: Nutrigenomics, phyto-neutraceuticals, Liver CPT-1, Pancreatic lipase, Fatty acid transport

- A**
- Abdelhamed, Hossam, P216
 Abdul-Careem, Mohamed, P264
 Ablanedo, Agustín, T180
 Abudabos, Ala, P339, P340
 Achor, Bill, P375
 Adam, Merghani, M45, P242
 Adams, Daniel, T145
 Adedokun, Sunday, M62
 Adeniyi, Desmond, M41
 Adeola, Olayiwola, M62, P304, T162
 Aderibigbe, Ayodeji, P358
 Adhikari, Pratima, M72, M94, P235, P238, P239, P325
 Adhikari, Roshan, M68, M72, P313
 Adhikari, Yagya, P227, P256
 Aggrey, Sammy, M128
 Aggrey, Samuel, M49
 Aguilera, Matias, T173
 Ahammad, Golam Sagir, P355
 Ahmad, Mueez, M36
 Ahmad, Naseem Saud, M113
 Ahmad, Sohail, M135, P280, T204
 Ahmed, Md Wadud, M1
 Aicher, Eric, M33
 Ajao, Adeleye, M69, P331
 Ajuwon, Kolapo, M11, P304
 Akintunde, Olaitan, M41
 AKUKWU, CHIOMA, P291
 Albores, Abraham, M86
 Alharbi, Khawla, P342, P343
 alharbi, khawla, P351
 Ali, Ahmed, M121, M122, M123, M134, P274, P338, P345
 Ali, Muhammad, M131, M19
 Allen, Tiffani, T159
 Al-Mitib, Layla, P342
 Al-Qudah, Omar, P299
 Alrubaye, Adnan, P342, P343
 Altevogt, Walter, M111, M64, M65
 Alvarado, Bet Wu, M22
 Alvarez, Rosa Mary Lopez, T194
 Alves, Rafael, P300
 Amalaradjou, Mary Anne, M82
 Amarin, Nadim, M87
 Amerah, A., M88
 An, Su Hyun, M63
 Anderson, Annalise, M68, P313
 Anderson, Gracie, M121, P345
 Anderson, Kenneth, M124, M125, M85, P217
 ANDERSON, Kenneth, P284
 Anderson, Mallory, M122, P274
 Andrews, Jayla, P223
 Angel, Benjamin, M67
 Angel, Roselina, M13, M14, P315, T201
- Ao, Tuoying, P302
 Apalowo, Oluwabunmi, M51
 Appel, Arthur, P267
 Applegate, Todd, P310, P324, P331
 Aragão-Netto, Valmiro, P326
 Arango, Marcela, T166, T167, T168
 Araujo, Cristiane, M92, P301
 Araujo, Lucio, M92, P301
 Araújo, Lúcio, T154
 Arbe, Xabier, M13, M14
 Archer, G.S., P306
 Archer, Gregory, P273, P298, P305, P347
 Argüelles-Ramos, Micille, M123
 Arguelles-Ramos, Mireille, M121, P274, P329, P345
 Arick II, Mark, P238
 Arick, Mark, M23
 Arsi, Komala, P223
 Arubaye, Adnan, P351
 Asnayanti, Andi, P342, P343, P351
 Assumpcao, Anna Luiza Facchetti V, P223
 Augspurger, Nathan, M41
 Augusto, Karolina von Zuben, T190
 Ault, Ashley, P342
 Avila, Luis, M142
 Ayala-Velasteguí, David, P254
 Ayora-Talavera, Guadalupe, P241
- B**
- Baek, Insuck, M22
 Baggio, Eduardo, P367
 Bailey, Cory, M5, M6
 Bailey, Matthew, M138, M60, P227, P256
 Bailey, Matthew A., M59
 Bak, Suyeong, P220
 Baker-Cook, Bethany, M28, P296
 Bannister, Grace, P253
 Bañuelos-Hernández, Bernardo, P241
 Barahona, Linda, P220, P224
 Barbalho, Ricardo, T154
 Barcelo, Colin, M9, P214
 Barducci, Robson, M50, P255
 Barri, Adriana, M78
 Baugh, Kathryn, M134
 Baumhardt, Patrice, T205
 Baxter, Jennie, M46, P246, P268, T170
 Becerra, Roel, M40, M47
 Beck, Chrysta, M15, M16
 Bedecarrats, Gregoy, M10
 Bedford, Michael, T156
 Bedford, Mike, M116, P304
- Bello, A., P306
 Belote, Bruna, P243, P369
 Benarbia, Mohammed, P363
 BENARBIA, Mohammed, T182
 BENARBIA, Mohammed el Amine, M35
 Bergam, Melanie, M119
 Berghaus, Roy, M46, T170
 Berghman, Luc, T172
 Bergman, Melanie, M120, P279
 Bernadino, Michele, M90
 Betiku, Eniola, P226
 Bhattraí, Shailes, M7, M79, P214
 Bianchi, Sophia, M105, M93
 Bible, Megan, T144
 Bist, Keshav, P270
 Bist, Ramesh, M117, M127, P270, P271
 Black, Micah, M22
 Black, Telah, P230
 Blanch, Alfred, T144
 Blue, Candice, P215, P263, T160
 Bobeck, Elizabeth, P250
 Bodempudi, Venkat Umesh Chnadra, M49
 Bodle, Brooke, M93
 Boerboom, Gavin, T198
 Boiago, Marcel, T179
 Bolek, Kevin, P250, P269
 Boltz, Tim, P216
 Boltz, Timothy, M94, P325
 Bonal, Nathalie, P236
 Bonaspetti, Sandra, P356
 Bonato, Melina, T154
 Boney, John, M100, M105, M4, M93
 Boontarue, Darby, M105, M4, M93
 Boot, Emmillie, M17
 Borchardt, Guilherme, T187
 Borst, Luke, M37, M38, P245
 Bortoluzzi, Cristiano, T184, T197
 Bottje, Walter, M16, P343
 Bourassa, Dianna, M138, M25, M26, M27, M28, M29, P227, P256
 Bowen, Kristina, M71, M95, M98, P311, P352
 Bowker, Brian, P233
 Bowker, Brian C., M19
 Bragg, Ari, M123, P338
 Breen, Vivian, M129
 Bridges, William, P345
 Brito, Alexandre, T181
 Broomhead, Jonathan, T176
 Brown, Chalise, M37, P245
 Brown, Jessica, M61
 Brown, Lindsay, P213
 Browning, Matthew, P245, P264
 Buangam, Apidech, P315
 Buehler, Kathrin, P351
- Bühler, Kathrin, P342
 Buhr, Jeff, P227, P256
 Buhr, Richard, M25, M26
 Buisker, Tim, P356
 Burden, Yemi, P298, P305, P347, T146
 Burin, Raquel, M12, M131, P219
 Burns, Robert, M136
 Bussiman, Fernando, M129
 Buwjoom, Tonglian, P359
 Byrd, Allen, P253
 Byrd, J, P240
- C**
- Cabanas-Ojeda, Joaquín, M102, P326
 Caceres, Carlos, M43
 Calik, Ali, P215, P317
 Camara, Ali, M41
 Campbell, Marc, M32
 Campbell, Yan, P225
 Canonenco de Araujo, Rafael, P369
 Cardoso, Gabriela, P320, T200
 Cardoso-Luna, G. Madeleine, P257
 Cardoso-Luna, Grecia, P354
 Carlisle, Sarah, M43
 Carlson, Anna, P252, P253
 Carlu, Claire, P236
 Carnaccini, Silvia, M43
 Carroll, Michael, M12, P325
 Cason, Emily, P252, P253, P254
 Castañeda, Claudia, P216, T154
 Castaneda, Maria, T202
 Castellanos-Huerta, Inkar, P241
 Castillo, Daniel, T173
 Castillo, Urias, P322, P323
 Cavero, David, M13, M14
 Chai, Lilong, M117, M127, P270, P271, P310
 Chan, Diane, M22
 Chance, Sophie, M124, M17, M18
 Chaney, William, P247
 Chao, Kevin, M22
 Charre-Perales, Jose, P338
 Chatman, Chamia, M81
 Chavatte, Dominique, T182
 Chen, Chongxiao, M104, M128, M131, M76, P212, P219
 Chen, Chongxiao (Sean), M19
 Chen, Wenzhen, M83
 Cheng, Heng-wei, P344
 Chew, Jo Ann, M112
 Ching, San, P367
 Cho, Inho, M63
 Cho, Songbo, P355
 Cho, Sungbo, P330
 Cho, Sungeun, M21, P220, P224
 Choi, Janghan, M31, M70, P233

- Choppa, Venkata, M142, P309, P348
 Choppa, Venkata Reddy, M108
 Choppa, Venkata Sessa Reddy, P350
 Christensen, Karen, P278, T206
 Cifuentes, Nicolás, T173
 Cifuentes, Pablo, T173
 Cisse, Sekhou, P363
 Clark, Alexis, M123
 Cline, Mark, M75
 Coles, Makenly, M107, M52, P354, P372, P374, T165
 Collier, Stephanie, P296
 Colom, Octavi, M76
 Comin, Antony, T179
 Contreras, Fanny, M27
 Contreras, Manuel, P332, P357
 Cookson, Kalen, P265
 Coon, Craig, M110, M114, M115, P327, P335, P336
 Corray, Shelby, M96, P334
 Cosby, Douglas, P231, P239
 Costa, Fernando Perazzo, T181
 Costa, Matheus, M67
 Cotter, Paul, M44, P234
 Coufal, Craig, M140, P373
 Crespo, Rocio, P211, P260, T158
 Cribillero, Nelly, M94
 Croom, Mazette, M118, P272
 Crowder, Stacie, T174
 Crozier, C., P231
 Cruz, Shaunie, P274
 Czarick, Michael, M126
- D**
 Da Costa, Manuel, P362, T175
 da Silva, Edney, M90
 da Silva, Kelvin, M39, T153
 Daigle, Courtney, P272
 Dalloul, Rami, P215, P261, P262, P263, P333, P346, T160, T161
 Daniels, Karrie, P240
 Dao, Thi Hiep, T199
 Dasireddy, Joseph, P324
 Davis, Adam, M103, M104, P303
 Davis, Jeremiah, M141
 de Bruin, Melchior, T155
 de Dios Figueroa, Juan, P353
 de Freitas, Dimitri, M39, T153
 de Jesus Nava, Maria, P353
 De Laet, Manu, T186
 de Lourdes Angeles, Maria, P353
 de Paula Dorigam, Juliano, P307, P312
 de Souza, Jacqueline, P300
 Decap, Sebastian, P232
 Dees, Jacob, P220
 Delago, Jodi, M36, P268, T151, T170
 Dennehy, Dalton, P341, T175
 Dersjant-Li, Y., P306
 De Voll, Josh, P231
 Devreese, Mathias, T152
 Dickson, John, P265
 Diez, David, T177
 Dilger, Ryan, M78, P307, P312
 Dillard, Reed, M103, M104, P303
 Dinh, Thu, M23
 Dirks, Brian, M33, M87, P298, P305, P347, T146
 DJOUVINOVA, Dimcho, T189
 Do, Anh Dang Trieu, P342
 Domer, Randy, P337
 Donaldson, Caroline, T186
 Dong, Bingqi, P261, P262
 Donoghue, Annie, P223
 Dorton, Kristy, P246, T193
 dos Santos, Elisangela, M39, T153
 dos Santos, Thiago Tedeschi, T181
 Doster, Jacob, P230
 Dowd, Steve, M66
 Dozier, William, M60, M62
 Dozier, William A., Jr., M59
 Drewry, Jessica, P288
 Du, Xiangwei, M86
 Dunham, Cierra, M54
- E**
 Edwards, Joan, M91, M97
 Elahi, Usman, M113
 Ellestad, Laura, M13, M14, M7, M79, M9, P214
 Elliot, Michael, M72
 Elliott, Katie, P244
 Elrod, Charlie, P364, T149, T163
 Ekenya, Ikenna, M51
 Engler, Paul, T182
 Enyetornye, Ben, M43
 Eppinger, Lindsey, P213
 Erasmus, Marisa, M132
 Erf, Gisela, M15, M16
 Escobar, Cesar, M138
 Estanich, Emily, M71, M98, P352
 Estep, Sean, P352
 Etherton, Joshua, M141
 Evans, Jeff, P239
 Evans, Jeffrey, P237, P238, P244
- F**
 Fairchild, Brian, M126
 Fanous, Abigail, P342, P351
 Fares, Abdelhamid, P264
 Faria, Douglas, M92, P301
 Farnell, Morgan, M50, P251, P255
 Farnell, Yuhua, M50, P251, P255
 Fascina, Vitor, P300
 Fasina, Yewande, M132, M51
 Fathima, Shahna, M8, P308
 Fathima, Shana, P331
 Faucher, Stéphane, T194
 Faust, Nikolas, M118
 Favero, André, M111, M64, M65, P320
 Feijo, Julmar, M64
 Feijó, Julmar, M111, M65
 Felici, Martina, T150
 Fenster, Davis, P346
 Ferguson-Noel, Naola, T171
 Ferket, Peter, M132, P302
 Fernandes, Jovanir, T200
 Fernandez-Juricic, Esteban, T205
 Ferrel, Jon, P311
 Fickler, Anna, M90
 Figueiró, Otto, P243
 Figueroa, Juan, M25, M28
 Fischer, Torey, M4
 Fletcher, Oscar, P211
 Flores, Erasmo, P322, P323
 Foley, Steven, M37
 Forga, Aaron, M107, T163, T165, T166, T176
 Fournis, Yann, T186
 Fraley, Greg, M11
 Fraley, Gregory, M119, M120, P218, P277, P278, P279, T205
 Franco, Monica, P293
 Freedman, Alysson, M81
 Freeland, Skye, P225
 Freitas, Dimitri, M65
 Frey, Drew, M120, P279
 Friesen, Walter, M99
 Fritzen, Cooper, M83
 Froebel, Laney, M78, P215, P307, P312
 Frozza, Rogerio, P371
 Fu, Yuechi, P344
 Fudge, Catherine, M131, M19, M76, P212
 Fugate, Hailey, M24
 Fuller, A., M33
 Fuller, Alberta, P261
 Fuller, Lorraine, M32
 Fulton, Janet, T161
- G**
 Gabarrou, Jean-Francois, P282
 Gaghan, Carissa, P211, P264
 Gaines, Jalon, P339
 Galindo, Leticia, P227
 Gallardo, Connie, T177
 Gallissot, Marie, T152
 Galvis, Jason, T158
 Gan, Hao, M133
 Gangaiah, Dharanesh, M41
 Ganske, Rachel, M61
 Gao, Maiuri, M82
 Gaonkar, Pankaj, P227, P256
 Garcia, Alejandra, M14
 Garcia-Gonzalo, Diego, P236
 Garcia-Orellana, Miriam, P269
 Garcia-Suarez, Maria, T160
 Garner, Laura, M20, M21, M22, P230
 Gay, Lindsey, M43
 Genovese, Kenneth, P240, P251
 Ghazi Al Hakeem, Walid, M8
 Giacomini, Leandro, P332, P357, P366
 Gibbs, Kirsty, T147, T148
 Gilbert, Elizabeth, M75
 Gilbert, Jeff, M37
 Gilbert, Robin, M55
 Gimeno, Isabel, P264
 Girard, Claire, P236
 Girard, Ivan, P263, P375
 Girgis, George, T178
 Godbout, Alexia, M97
 Gogal, Robert, P310
 Golz, D., M89
 Gomes, Gilson, T181
 Gomes, Gilson A., T189
 Gomez, Luis, P356
 Gomez, Sergio, M86, P353
 Gómez-Verduzco, Gabriela, P241
 Gonzalez, Javier, P342, P351
 Gonzalez-ortiz, Gemma, M116
 González-Ortiz, Gemma, T156
 Goo, Doyun, M108, M70, M80, P233, P348, P350, P376
 Goodwin, Chloe, M43
 Gordillo, Alvaro, P317
 Gore, Aaliyah, P260
 Gott, Paige, P334
 Gougoulias, Christos, T192
 Govindaswamy, Shekar, M41
 Graham, B., M107
 Graham, Danielle, P372, T163, T165, T166, T176
 Gray, Latasha, P354, P372
 Greene, Elizabeth, P372
 Grief, Alexandra, M120
 Grilli, Ester, T150
 Grimes, J., T195
 Grimes, Jesse, P302
 Grimes, M., P306
 Grimes, Melinda, P298, P305
 Groves, Delaney, M84
 Guardado, Carla, P267
 Guevara, Camila, T157
 Gulabrai, Bhavisha, M124, M17, M18
 Gulizia, Joseph, M106, M109, M60, P322, P323
 Gulizia, Joseph P., M59
 Guzman, Eva, M106, M109, M60, P322
 Guzman, Eva G., M59
 Guzman, Eva Guzman, P323

- Guzman, Luis, M20, M22, P221, P230
 Guzmán, Luis, M21
- H**
- Haag, Derek, T146
 Haetinger, Vitor Santos, M62
 Hakeem, Walid Al, P289
 Hakeem, Walid Ghazi Al, P308
 Hambrecht, Ellen, T155
 Hameed, Arif, M135, P280
 Hamm, Brody, P341
 Hamzat, Rasheed, P283
 Han, Jing, M37
 Hanlon, Charlene, M28
 Harbottle, Heather, M37
 Harding, Kari, M125, M17, P284
 Hargis, Billy, M107, M52, M86, P257, P353, P354, P372, P374, T165
 Harijaona, Josoa Andre, P317
 Harris, Caitlin, M19
 Harrison, Cerano, M122
 Harsh, Bailey, P307
 Hart, Jon, M66
 Hasan, Amer, P343, P351
 Hassan, Intisar, P343
 Hatmaker, Coleman, M104, P303
 Hauck, Rüdiger, M42
 Hauck, Ruediger, M56, M59, M60, P267, T157
 Hawkins, Shawn, M136
 Hayden, Michelle, P224
 He, Haiqi, P240
 He, Xi, M34
 Heinichen, Brendan, P302
 Helmes, Emmily, M41
 Hernandez, Daniel, P353
 Hernandez, Jose, M106, M109, M60, P322, P323
 Hernandez, Jose R., M59
 Hernandez-Velasco, Xochitl, P372
 Hidalgo, Jorge, M129
 Higuaita, James, T166, T176
 Hisasaga, Cirenio, M137
 HISASAGA, CIRENIO, P291
 Hite, Jessica, M61
 Hoa, Bui, M35
 Hoerr, Fred, M33, M55
 Hofacre, Charles, M33, M46, P246, P247, P268, T170, T185
 Holcombe, Nicole, P258, P266
 Horn, Raquel, M111, M64, M65
 House, A., M89
 House, Gabrielle, P273
 Hsu, Chuan-Yu, M23, P237, P238
 Huberman, Yosef Daniel, P248, P249
 Hughes, Jay, M46
- Hughes, Matthew, M26, M28, M29
 Hughes, Neketa, P337
 Humphrey, Brooke, P250, P269
 Hussain, Jibrán, T204
 Hutchens, Lilian, T158
 Hyda, J., T195
- I**
- Idan, Frank, P297
 Ines-Lima, Julia, M54
 Isah, Abubakar, P216
 Isaza, Jaime, P372
 Iwuozo, Odinaka, M51
- J**
- Jackson, Mark, M95
 Jacobs, Leonie, M130
 Jaihuni, Mustafa, M133, P281
 Jaramillo, Bibiana, M13, M14
 Jasek, Austin, M12, M131, P219
 Jaszczka, Klaudia, T208
 Javid, Arshad, M135, P280
 Jendza, Joshua, M61
 Jennings, Madalyn, M26, M27, M28
 Jeon, Yun Ji, M63
 Jeong, Jae Hark, T183
 Jespersen, Julianna, M78, P307, P312
 Jessup, Ainsley, P224
 Jesudhasan, Palmy, P223
 Jia, Linan, M138, P235, P237, P238
 Jiral, Emily, P347
 John, Alagbe, P283
 Johnson, Alexa, M121, M122, P274, P338, P345
 Johnson, Bruce, P356
 Johnson, Madison, M37
 Johnson, Timothy, M143, P247
 Johnston, LeAnn, P367
 Jones, Carl, P364, T149, T163
 Jones, Jeryl, M122
 Jones, Matthew, M33, M46, P247, P268, T170, T185
 Jones, Thomas, M104, P303
 Jordan, Aaron, P231
 Jordan, Brian, M33, T164
 Joseph, Jiddu, P235
 Judo, Opeoluwa, P340
- K**
- Kaczmarek, Sebastian, M91
 Kadardar, Houssein, M76
 Kalapala, Tanmaie, P223
 Kalia, Sahil, P250
 Kamruzzaman, Mohammed, M1
 Kang, Seong, T206
- Kanike, Eswari, M82
 Kappari, Laharika, P324
 Karcher, Darrin, T205
 Karnezos, T, M84
 Kasula,, T207
 Kasula,, Rajasekhar, M144
 Kawashima, Yuki, P349
 Kemakolam, Udochi, P354
 Kerros, Sylvain, P236
 Khadem, Alireza, T192
 Khalid, Zubair, M56
 Khaliduzzaman, Alin, M1
 Khan, Ehsaan, M87
 Khan, Junaid Ali, T204
 Khan, Muhammad Zeeshan Alam, M113
 Khan, Samiullah, P370
 Khan, Sharif Uddin, P330
 Kidd, Michael, M67, P342, P343
 Kiess, Aaron, M124, M18, P216, P217, P225, T154
 Kihal, Abdelhacib, P365
 Kilonzo-Nthenge, Agnes, P290
 Kim, In Ho, P330, P355, T190
 Kim, Moon, M22
 Kim, Sunhyung, P311
 Kim, Woo, M108, M142, M31, M70, P233, P309, P310, P349, P350, P376, T196, T203
 Kim, Woo Kyun, M80, P348
 Kimminau, Emily, M84
 Kindlein, Liris, P320
 Kindler, Joseph, M14
 Kitchens, Cassandra, T157
 Kitchens, Steven, M25, M42, P227, P256
 Klein, Sasha van der, T148
 Knarr, Lucas, M71, M95, M98, P311, P352
 Ko, Hanseo, M80, P348, P350, P376
 Koch, Joao, M50, P255
 Kogut, Michael, P240, P251
 Kong, Byungwhi, P233
 Kong, Changsu, M63
 Konsens, Hans, P232
 Koppen, Megan, P362, T175
 Kosuri, Venkata Praveen Raja, M82
 Koutsos, Elizabeth, T145
 Krafka, K., T195
 Krehling, James, P256
 Kriseldi, Ruben, M72
 Krishnan, Hari, P311
 Kulkarni, Raveendra, P264
 Kulkarni, Ravi, P211
 Kumar, Arvind, M41
 Kumar-Phillips, Geetha, P223
 Küpeli, Erdem, P229
 Kuterna, Leni, M91, M97
 Kuttappan, Vivek, P247
- L**
- Landinez, Martha Pulido -, T167
 Landreth, Mallory, P276
 Latorre, Juan, M107, M52, M86, P257, P353, P372
 Latorre, Juan David, P374
 Latorre-Cardenas, Juan, P354
 Laurain, Julia, P363
 Lavergne, Theresia, P364, T149, T163
 Laverty, Lauren, P354, P372
 Lavorgna, Mark, P246
 LaVorgna, Mark, T193
 Lawal, Abiola, P304
 Lee, Chae Won, M63
 Lee, Jason, M67, M68, M72, P313
 Lee, Jihwan, M31, M70, M80, P348, P350, P376
 Leeds, Payten, P230
 Leigh, Spencer, P237
 Leon, Luis, T173
 Leonard, Lindsay, T151
 Lerner, Steve, T178
 Létourneau-Montminy, Marie-Pierre, M97
 Leyva, Hector, M87
 Leyva-Jimenez, Hector, P298, P305, P347, T146
 Lgoue, Catherine, M48
 Li, Guoming, M127, M128, M49
 Li, Xiang, P289, P294, P295
 Liang, Yi, P288
 Liao, Yen-te, M24
 Lima, Adiel, T181
 Lima, Alessandro, P320
 Lima, Amanda, T181
 Lima, Julia, M48, M53
 Lima, Julia Ines, T169
 Lima, Matheus Ramalho, T181
 Lin, Yun-mei, M92, P301
 Linares, Leonardo, T201
 Linhoss, John, M141, P296
 Linnemann, Erich, M30, M57
 Liu, Dongmin, M75
 Liu, Guanchen, P309, P350
 Liu, Haijun, M83
 Liu, Jundi, M107, T166, T185
 Liu, Keshun, M74
 Lloyd, K., T195
 Locklear, Passion, P324
 Loeza, Ileana, M86, P372
 Logue, Catherine, M40, M53, M54, T169
 Logue, Catherine M., M47
 Lomónaco, Jorgelina, P249
 Longhini, Giovana, M92
 Lopes, Mario, M92
 Lopes, Taina, T157
 López-Paredes, Javier, P318, P319
 Lourenco, Daniela, M129
 Lowery, Justin, P217

- Lozano-Cruz, Paula, P326
 Lucas, Jeffrey, M119, P277, P278
 Lugo, Cecilia, T177
 Lumpkins, Brett, M32, P232, P329, T145
 Luo, Yi-Chen, T159
 Lynch, Elizabeth, M71
 Lyons, Alyssa, M66
 Lyte, Mark, P240
- M**
- Maboni, Grazieli, T159
 Machado, Gustavo, T158
 Mackey, Rebecca, T168
 MacKinnon, Nicholas, M22
 Macklin, K, M138
 Macklin, Ken, M12, M42
 Macklin, Kenneth, M23, P216, P227, P256, P267
 Magee, Christopher, P237, P239, P244, P285
 MAGEE, CHRISTOPHER, P296
 Maguey-Gonzalez, Jesus, M86, P353
 Maguregui, Ekaitz, M76
 Maharaja, Pramir, P328
 Maharjan, Pramir, M110, P290
 Mahdavi, Fatemeh, P348
 Mahdavi, Fatemeh Sadat, P350
 Mahmood, Absaar, M87
 Mahmoud, Kamel, P299
 Maia, Rosana, M90
 Maina, Teresia, P247
 Maini, Hitesh Pawar, Shivi, P379
 Maini, Shivi, P377, P378, T196, T203
 Majumder, Erica, M81
 MAKAGON, MAJA, P291
 Makagon, Maja M., M137
 Malheiros, Dimitri, M17, M85
 Malheiros, Ramon, M125, M17, M18, P284, P302
 Malik, Sara, M113
 Mallmann, Carlos, P332, P357, P366
 Mallo, Juan Jose, P318, P319, P365
 Mallo, Juan José, P368
 Manaig, Yron Joseph, T194
 Manginsay, Natalie, T168
 Manjankattil, Shijinaraj, M26
 Mante, Alberta, P297
 Manuja, Simmi, M41
 Maqueda, Maricela, M16
 Marcon, Raul, P374
 Marcon, Raúl, P354
 Marecaille, Clemence, P361, T180
 Maria, Douglas, M111, M64, M65
 Marquinez, Raimundo, T157
 Marshall, Brett, P350
 Marshall, Caleb, M101
 Martin, Cameron, T172
 Martin, Kristen, P354, P372
 Martin, Olivia, P375
 Martinez, Blanca, P372
 Martinez, Diego, M110, M114, M115, P327, P335, P336
 Martinez, Ingrid, T202
 Martinez, Marilyn, M37
 Martinez-Villagrasa, Laura, P368
 Masadeh, Mahmoud, P246, T193
 Mason, Hunter, M142
 Massei, Arthur, M39, T153
 Mathis, Greg, M32, T145
 Matté, Fabrizio, T179
 Matter, Kevin, P317
 Maynard, Clay, P372
 McBride, Hannah, T178
 McCafferty, Klint, M60, M94
 McCafferty, Klint W., M59
 McCarver, Hannah, P342
 McConnell, Abigail, M26, M27, M28
 McCormick, Katherine, T146
 McCrea, Brigid, M29
 McCullough, Kathryn, M30
 McDade, Alexis, P328
 McElroy, Audrey, M50, P255
 McIntyre, Michael, P231
 McKinley, Enid, M36, P268, T151, T170
 McMillan, Amy, M41
 McNaughton, James, P269, P356
 Médina, Bertrand, P263
 Mehmood, Shahid, M135, P280
 Mejia-Abaunza, Nicolas, M128, M131, M19
 Mejía-Abaunza, Nicolas, P326
 Melo, Julian, P361
 Melo, Julián, T180
 Mendez, Abraham, P353
 Méndez, Laura, P248
 Mendoza, Mary, P217
 Menezes, Raíssa, M111
 Merino-Guzmán, Rubén, P257, P354
 Mero, Reyna, P292
 Merritt, Jill, P277
 Messias, Rodrigo, M90
 Meuter, Antoine, P370, P371, T178, T187
 Meyer, Alicia, P247
 Middlebrooks, C., M89
 Milby-Blackledge, Allison, M50, P255
 Mills, Kelsey, T158
 Mishra, Prashant, T174
 Misztal, Ignacy, M129
 Montiel, Enrique, M52, P258, P266, P374
 Moon, Jonathan, M12, P288
 Moonen, Peter, T194
 Moore, Dan, P266, T183
 Morales, Arturo Garcia, M71
 Morey, Amit, M2, M20, M21, M22, M3, P221, P230
 Moritz, Joseph, M71, M95, M98, P311, P352
 Morris, Cassidy, M12, P325
 Morris, Colwayne, M12, M131, P219, P337
 Moss, Amy Fay, T199
 Mounicou, Sandra, T194
 Mountzouris, Konstantinos C., T192
 Munoz, Luis, M138, P227, P256
 Muttathukonam, Sulthana Humayoon, M82
 Myers, Emily, M96, P341
- N**
- Nabors, Haley, M138
 Nacer-Khodja, Elise, T160
 Naeini, Hamid Reza Rafieian, P212, P350
 Naeini, Hamidreza, M108
 Nankemann, Jannis, T157
 Naqvi, S., P247
 Nascimento, Carlos, T181
 Nascimento, Mariana, T163
 Nava, Maria de Jesus, M86
 Navarro, Rosa, P232
 Neeno-Eckwall, Eric, M61
 Nelson, Kenneth, M67
 Nemeth, Nicole, M43
 Neto, Manoel Garcia, M101
 Neto, Roberto Montanhini, T186
 Netto, Raimundo, P301
 Neupane, Nabin, M14, M7, P214
 Neves, Duarte, M12, M131, P219
 Nguyen, Hoang Duy, T199
 Nicholds, Jenny, M55, T159
 Nicola, Brenda, M111, M64, M65
 Niraula, Abhisek, T161
 Nolan, Lisa, T169
 Nolan, Sean, P259
 Noll, Sally, M71
 Novak, Richard, M120
 Nuengjamnong, Chackrit, P359
 Nungester, Eric, M41
 Nusairat, Basheer, P299, P360
- O**
- Obe, Tomi, M139, P223, P226
 Oelschlager, Maci, P312
 Ogundare, Tunde, M51
 Ogundipe, Tolulope Tiwa, M139, P226
 Ogunribido, Tobi, P304
 Oladeinde, Ade, P289
 Oladeinde, Adelumola, M49, P294, P295, P331
 Olanrewaju, Hammied, P286
 OLANREWAJU, HAMMED, P296
 Olinger, Christopher, M144, T207
 Oliveira, Núbia, M39, T153
 Oliveira, Paulo, T179
 Olson, Elena, M81
 Olukosi, Oluyinka, M116, M58, M69, M77, P316, P331
 Oluwagbenga, Esther, M11, P218
 Oluwayinka, Eniope, T171
 Oluwayinka, Olatunde, M57
 Omaliko, Paul, M132, M51
 Onishi, Beatriz, M109, P322, P323
 Orellana, Leticia, M106, M12, P322, P323
 Orimaye, Oluteru, M51
 Orłowski, Sara, M16
 Ortiz, Elio, P329
 Osho, Saheed, P269
 Oso, Oluwadamilola, M128
 Otott, Haley, M99
 Ovi, Fozol, P239
 Oviedo-Rondon, Edgar, M101
 Oviedo-Rondón, Edgar, M102, M73, P326
 Owens, Casey, P372
- P**
- Pachacco, Leticia, M50
 Pacheco, Leticia, P255
 Pacheco, Wilmer, M106, M109, M60, P322, P323
 Pacheco, Wilmer J., M59
 Pais, Vitor, P301
 Palma, Sabrina, T200
 Paneru, Bidur, M117, P270, P271
 Paneru, Deependra, M108, M70, P348, P349, P350
 Parareda, Aida Yuste, P368
 Park, Chan Sol, T183
 Parnin, Heidi, M120, P279
 Parsons, Benjamin, M74
 Parsons, Carl, M62, M74
 Pasayo, Ramón Alejandro González, P248, P249
 Pasquali, G., M88
 Paulk, Chad, M99
 Pavlidis, Hilary, T183
 Pawar, Hitesh, P377, P378
 Payling, Laura, P333
 Payne, Rob, P362, T175
 Paz, Ibiara, P371
 Pegoraro, Isadora, M109, P322, P323
 PEKEL, AHMET, P321
 Pelletier, Wolf, P317

- Peña, Manuel, P329
Pender, Chasity, P334
Penen, Florent, T194
Peng, Fang, M34
Peng, Xianfeng, M83
Perera, Ruvinidu, M15, P342, P343
Perez, Daniel, M43
Perry, Carolina, P369
Persia, M, M66, M84
Persia, Michael, M83
Petek, Eyuphan, P228, P229
Petek, Metin, P228, P229, P287
Peterson, Daniel, P238
Petrone-Garcia, Victor, P372
Petrone-García, Victor, P241
Pia, Eduardo Della, P300
Pieringer, Christian, T173
Pieringer, Hans, T173
Pierzchala-Koziec, Krystyna, T208
Pietruska, Andrea, M42, M56, T157
Pilevar, Mohammad, M58, P316
Pillai, Suresh, T172
Pimpech, J, P315
Pineda, Lane, T190
Piva, Andrea, T150
Plocher, Samantha, P341
Plue, Collette, M119, M120
Plumstead, Peter, T201
Poholsky, Courtney, M100, M105, M93
Pokhrel, Diksha, M23
Poudel, Sabin, M26, P235
Prado, Maria, M133
Price, Stuart, M25, M42, P227, P256
Prince, Jacqueline, P223
Proszkowiec-Weglarz, Monika, M79
Pulido-Landinez, Martha, T168
Pullin, Allison, M124
Punti, E. Vinyeta, P306
Purswell, J., P288
Purswell, Joseph, M141, P244, P285, P286, P296
Putarov, Thaila, M50, P255
Puyalto, Monica, P318, P319, P365, P368
- Q**
Qi, Hairong, M133
Qin, Jianwei, M22
Qin, Zonghua, M83
Qudsieh, Rasha, M92, P301, P346, P360
- R**
Rafieian-Naeini, Hamid reza, P212
Rajasekhar, T207
Ramachandran, Reshma, M23, P216, P235, P238
Ramay, Muhammad Shazaib, P317
Ramirez, Hugo, T167
Ramirez, Jose, P258
Ramírez-Loeza, Ileana, P354
Rana, Asif, M87
Rasheed, Shameer, P246, T193
Rasmussen, Simone, T144
Raut, Rabin, P290
Rawson, Ian, M26
Raza, Muhammad Asif, T204
Rebollo, Marco, P343
Reddyvari, Ragini, M82
Regmi, Prafulla, M14, M7, P214, P310
Rehman, Atif, T204
Reina, Marco, P254
Ren, Yuying, M82
Reznik, Gary, P246, T193
Riahi, Insaf, P366
Riaz, Mian, P327
Riaz, Muhammad Faisal, T204
Richards, Amber, P222
Richter, Jennifer, M129
Ricke, Steven, M61, M81
Riggs, Anna Kathryn, P341
Riggs, Montana, M26, M27, M28
Riley, K., M89
Ritter, Don, T191
Ritter, DON, T192
Robinson, Kelsy, P237, P238, P239
Robison, Cara, M134
Rocchi, Alessandro, T163
Rocha, Jessica, P298, P305, P347
Rochel, Samuel, P343
Rochell, Samuel, M60, M68, P313, T176
Rochell, Samuel J., M59
Roda, Dipak, P314
Rodehutsord, Markus, M62
Rodrick, Gene, M14
Rodrigues, Sandra, M92, P301
Rodriguez, Maria, T152
Roieski, Patrick, T179
Romero, Hugo, T199
Romero, Luis, P333
ROMERO-SANCHEZ, HUGO, T202
Rosalino-Marcon, Raul, M52
Rossi, Aldo, M46
Rosso, Diogo, P300
Rostagno, Marcos, T155
Rothrock, Michael, P289, P294, P295
- Roulleau, Xavier, P361, T180
Rousseau, Xavière, T181
ROUSSEAU, Xavière, T189
Rowland, Mitchell, T165, T166
Ruiz, Nelson, M74
Runacharoon, Klao, T169
Runcharoon, Klao, M48, M53, M54
Rungcharoen, Patrapan, P315
Russo, Kay, P370, P371, T178
Rutherford, Grace, P276
Rutjens, Sofie, T152
Rvans, Caitlin, M105
- S**
Sacakli, Pinar, P317
Saddoris-Clemons, Kari, P269
Sadurní, Meritxell, P366
Saacidifar, Mahtab, M127
Sandoval, Constanza, T173
Sands, Jason, P258
Sandvang, Dorthé, T178
Santamaria, Jossie, M15, M16
Santin, Elizabeth, P243
Santini, Matheus, M56
Santos, Anderson, M50, P255
Sasia, Santiago, P329, P345
Saud, Usman Bin, M113
Scanes, Colin, T208
Schaeffer, Jon, P265
Schaumlöffel, Dirk, T194
Schilling, Wes, M23
Schleifer, John, P246, T193
Schober, Jenna, M119, M120, P218, P277, P278, P279
Schwartz, Meghan, T174
SCOTT, ABDULLAH, P327
Sedaghat, Asghar, M8
Seibert, Brittany, M43
Selby, Callie, M52, P258, P266, P327, P374
Sellers, Holly, M30, M57
Selvaraj, Ramesh, M8, P308, P331
Senas, Roberto, M86
Senas-Cuesta, Roberto, P372, P374
Señas-Cuesta, Roberto, M52, P257
Senechal, Pascale, T194
Shaban, Nadir, M113
Shaffer, Cheryl, P258, P266
Shah, Sanjay, P284
Shah, Sanjay', M125
Shaji, Syamily, M8
Shanmugasundaram, Revathi, M108, P308, P324, P331
Shariat, Nikki, P222, P252, P253, P254, P259
- Sharma, Milan, M108, M70, P309, P310, P348, P350
Shastak, Yauheni, P317
Sheaffer, Abe, M81
Sheikhsamani, Ehsan, P327
Shen, Cangliang, P325
Sheth, Parimal, M8
Shi, Hanyi, M108, P348, P350
Shields, Amari, T167
Shields, Jacob, M41
Siceloff, Amy, P222, P259
Siddique, Aftab, P230
Siegel, Paul, M75
Siegford, Janice, M134, P287
Sierra, Katherine, M20, M21, M22, P230
Sigmon, Christina, P217, P225
Silva, Isabela, P301
Silveira, Samantha, M111, M64, M65
Silvestrim, Andrea, T190
Simões, Cistina, P322
Simoës, Cristina, M106
Simões, Cristina, M60, P323
Simões, Cristina T., M59
Simonetti, Tainá, P320
Sinclair-Black, Micaela, M13, M14
Singer, Randall, M143
Singh, Amit, M70
Singh, Karan, P318, P319
Singhare, Deepak, P377
Siriphongsathat, Thanaphat, P315
Skrobarczyk, Jill, P251
Skrobarczykj, Jill, T172
Smink, Willem, P361
Smith, Alexandra, M36, P268, T151, T170
Smith, Jordan, P275
Snow, Janet, T183
Soares, Igor, P243, P369
Sobotik, Eric, P370, P371, T178, T187
Sokale, A., M88, M89
Sokale, Adebayo, M78
Sokolov, Stanislov, M22
Solis, Bruno, P353
Solis, Fausto, M144, T207
Sommer, Kaitlyn, M78, P307, P312
Song, Zehe, M34
Soto, Carlos, M87, P298, P305, P347
Souza, Jason, M55
Souza, Cleverson, M39, T153
Souza, Fernando, T154
Souza, Paloma, T181
Spears, J., T195
Staggs, Keith, M33
Stanev, Vasil, P356
Stanton, James, M57

- Star, Laura, M112
 Stark, Charles, M99
 Starkey, Jessica, M141
 Stein, Addressa, P372
 Stephan, Aaron, P273
 Strickland, William, M126
 Sturza, Diego, P332, P357, P366
 Suagee-Bedore, Jessica, P375
 Suarez, Juan, P373
 Subedi, Sachin, P270, P271
 Subirana, Maria Angels, T194
 Suesuttajet, Nawin, P327
 Suesuttajit, Nawin, M110, M114, M115, P335, P336
 Sukumaran, Anuraj, M23, P216, P238
 Sulaiman, Ali Al, P340
 Sulaiman, Usman, M75
 Sung, Jung, M62, T162
 Suradkar, Sanjay, P377
 Suryawanshi, Dayaram, P318, P319
 Sutherland, Hayley, M132
 Suyemoto, M, M37, M38, P245
 Suyemoto, M., P268, T170
 Swaggerty, Christi, M50, P255
 Swaggerty, Christina, P240
 Sykes, Virginia, P328
- T**
- Tabish, Rana, M42
 Tabish, Rana W., M59
 Tabish, Rana Waqar, M60
 Tabler, Tom, M133, M136, P288
 Tacconi, Antonia, T176
 Taheri, Ali, P328
 Tan, Zhigang, M83
 Taniguchi, Masahiko, P225
 Tapia, Brenda Hernandez, P293
 Tapia, Victoria, P232
 Taraka, Kamitnat, P315
 Tarcin, Ashley, M61
 Tarrant, Katy, P292, P293
 Tashiguano, Vianca, M21, M22, P230
 Tawde, Snehal, T176
 Taylor, Isabella, M119
 Taylor, Peyton, M5, M6
 Taylor, Robert, Jr., T161
 Teague, Kyle, M107, T163
 Tejada, Oscar, P275, P276
 Tellez, Guillermo, M86
 Tellez, Guillermo, P353, P374
 Tellez-Isaias, Guillermo, M107, M52, P241, P372
 Tellez-Isaias, Guillermo, P354
 Téllez-Isaías, Guillermo, P257
 Terra-Long, Maria, M42
 Tesouro, Anna, T177
 Tetel, Victoria, P218
- Thachil, Anil, P211
 Thames, Hudson, M23
 Thommasaroch, Ratchasak, P315
 Thornton, Jay Kay, T167, T168
 Thornton, Tanner, M136, P281
 Thuekeaw, Sureerat, P359
 Tillman, N., M89
 Tillman, Nickki, P315
 Tiwari, Utsav, P314
 Todd, Allie, P338
 Tompkins, Yuguo, P309
 Toro, Haroldo, T157
 Torras, María Ángeles Calvo, P368
 Torres, Cibele, T201
 Torres, David, P320, T200
 Torres, David Sanchez, T202
 Troyer, Anne, P278
 Tsai, Yu Yang, M53
 Tsai, Yu-Yang, M48, T169
 Tugnoli, Benedetta, T150
 Turner, Bradley, P373
 Tzirin, Marvin, P253
- U**
- Udchachon, Suthasinee, P315
 Ulans, Alexandra, M130
 Ullah, Subhan, M30
 Ulloa, Soledad, T173
 Ulrey, MacKenzie, P277, P278
 Umberson, Cole, M114, M115, P327, P335, P336
 Umberson, Cole Umberson, M110
 Urbano, Tiago, T179
 Urgnani, Fernando, P369
 Uribe, Alvaro, P372
 Urrutia, Jorge, M140, P341, T175
- V**
- Valenta, Jaroslav, M21, P230
 Valido, Iris, T194
 van der Klein, Sasha, T147
 Van der Klis, Jan Dirk, P342, P351
 van Emous, Rick, T209
 Vargas, Jose, M106, M109, M60, P322, P323
 Vargas, Jose I., M59
 Vasefi, Fartash, M22
 Vaughan, Reagan, M75
 Vaught, Mitchell, M67
 Vaught, Wesley, P342, P343, P351
 Vazquez, Alma, P353
 Velázquez-Juárez, Gilberto, P241
 Veluri, Shrivani, M116, M77
 Venter, Kyle, T201
 Vestergaard, Gisle, T187
 Vidal, Arnau, T191
 Vides, Marvin, M118
- Vieira, Rita, M90
 Vieira, Sergio, M111, M64, M65, P320
 Villarino, Natalia, P248, P249
 Vinueza, Valeria Criollo, P211
 Vipham, Jessie, P253
 Voelker, Matias, T180
 Voy, Brynn, P213
 Vuong, Christine, T165
- W**
- Wagner, Ashley, P263, P375
 Walk, Carrie, T197
 Walker, Grayson, M37, M38, P245
 Walker, Lin, M18, P217
 Walker, Ryan, M5, M6
 Walsh, Kathryn, M130
 Walters, Hannah, P276
 Walzem, Rosemary, M118, P272
 Wamsley, Kelley, M140, M5, M6, M72, M94, M96, P325, P341, T175
 Wang, Jeng-Jie, P346, P360
 Wang, Jinqun, M131, M19, P219
 Waters, Charis, M72, P325
 Watkins, Susan, P290
 Watson, Mick, T184
 Watson, Parker, P273
 Weil, Jordan, M110, P327
 Wells, Jessica, M5, M6
 Wells-Crafton, Savannah, M67
 Wesley, Serena, M119
 Whelan, Rose, P312
 White, Cameron, M78, P307
 White, Dima, P349
 White, Shecoya, M23
 White, Skylar, P341
 Whitham, Stephanie, P237, P285, P296
 Wicker, D., M89
 Wiggins, Latoya, P231
 Williams, Chance, M67
 Wilson, Dan, P239
 Wilson, James, M40, M47
 Wilson, Jeanna, M142
 Wolfenden, Ross, M107, T166, T185
 Woodward, Adrienne, T146
 Wu, Vivian, M24
 Wu, Zhiyuan, P225
 Wuttipassit, Nisaphat, P315
 Wyatt, Craig, M105, M94
 Wysocky, Rebecca, M17, P217
 Wythe, Lindsey, M81, P251
- Y**
- Yabuta, Thiago, M101, M102, P326
- Yabuta, Thiago H., M73
 Yadav, Sudhir, M92, P301, P346
 Yan, Frances, T199, T202
 Yang, Xiao, M117, P270, P271
 Yap, Tze Yao, P277
 Yarbrough, Cory, T164
 Ye, Xin, P237
 Yoon, June Hyeok, M63
 Young, Kelsey, M57
- Z**
- Zavarize, Kelen, P320, T200
 zhai, hengxiao, T197
 Zhang, Haihan, M34
 Zhang, Li, M138, M139, M23, M24, M94, P216, P235, P237, P238, P239, T175
 Zhang, Qian, T197
 Zhang, Xue, P237
 Zhang, Yujie, M24
 zhao, yang, M133
 Zhao, Yang, M136, P281, P286
 Zhou, Shengyu, P281
 Zhuang, Hong, P233
 Ziezdold, Clara, M10
 Zuidhof, Martin, M112
 Zwirzitz, Benjamin, P289