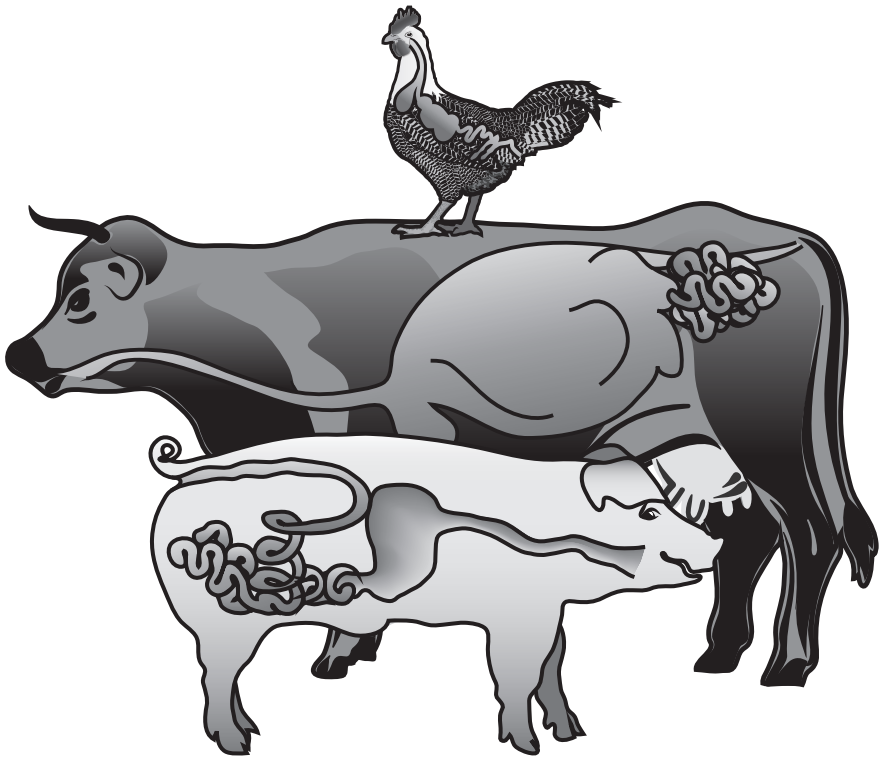
Symposium on Gut Health

in Production of Food Animals

October 29–November 1, 2023

St. Louis, Missouri



Program and Abstracts

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WELCOME

On behalf of the Organizing Committee for the 11th Symposium on Gut Health in Production of Food Animals, I welcome you back to St. Louis, Missouri! With the worst of the pandemic seemingly behand us, we are excited to return to St. Louis and to an in-person Symposium.

As always, the aim of the Symposium is to bring together a group of scientists from academia, government, and industry to discuss the role of gut health in animal production and the essential role that the gut plays in establishing and maintaining animal health. The overall aim of the conference is to promote the unifying concepts that the gut drives animal health and performance. Although the gastrointestinal tract is frequently described simply as "the gut," it is actually made up of (1) an epithelium; (2) a diverse and robust immune arm, which contains most of the immune cells in the body; and (3) the commensal bacteria, which contain more cells than are present in the entire host organism. Understanding of the crosstalk between *all* of these interrelated components of the gut is what cumulatively makes the gut the basis for the well-being of animals and the motor that drives their performance. The abstracts submitted to the Symposium explore the links and mechanisms that interconnect the three components of the gut and how each can be manipulated to improve animal health.

As in the past, this year we have invited three distinguished plenary speakers who will cover current research topics in avian, bovine, and porcine gut health. Please take advantage of the presence of these scientists to engage in productive talks and develop collaborations between different laboratories in order to further the science of gut health.

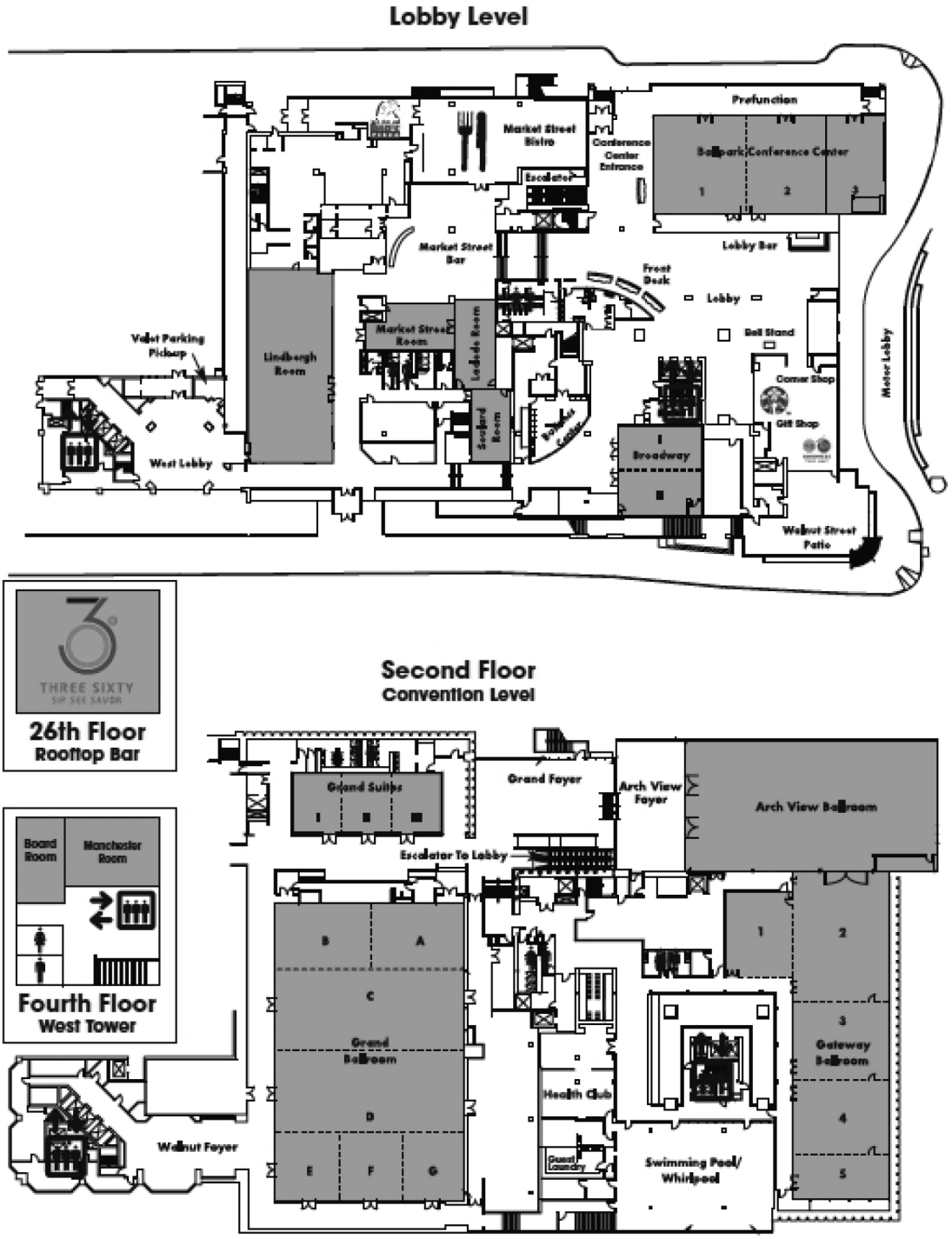
Finally, this year we will be organizing a mini-symposium on Monday afternoon, on the topic of Standardizing a Protocol for Microbiota Analysis in Poultry. One of the takeaways from last year's Symposium was a discussion to organize a small group of prominent scientists who are heavily involved in analyzing the composition of the microbiota of production animals. At present, with the number of protocols available, it is virtually impossible to make comparisons between results from studies from around the world. It was decided to organize a small action group at this year's Symposium to discuss and decide on a standard protocol for microbiota analysis, concentrating on poultry at this time. Everyone is invited to attend and participate in the discussion.

I encourage all of you to please take advantage of the informal nature of the Symposium; it was planned this way to encourage interaction between scientists. I again ask that senior researchers make special efforts to engage with the graduate students who are attending and presenting. Remember that, whatever your research specialty or food animal commodity, we are all working together to improve food quality for consumers.

Welcome again, and enjoy the Symposium and your stay in St. Louis!

Mike Kogut
Chair, Organizing Committee

Hilton St. Louis at the Ballpark



Program

Sunday, October 29

5:05 PM - 7:05 PM Registration: Grand Foyer

Monday, October 30

8:00 AM - 9:00 AM Breakfast: Arch View Ballroom

8:00 AM - 4:30 PM Registration: Grand Foyer

SESSION 1

Chair: Mike Kogut, USDA-ARS
Salons ABC

9:00 AM **Promoting health by managing the four "M's" of homeostasis.**
B. Aldridge*, *College of Veterinary Medicine and Health Innovation Professor, Carle Illinois College of Medicine, University of Illinois*, *Urbana-Champaign, IL, USA*.

10:00 AM **"Both/and" antibiotic alternatives: Beneficial feed additives that target the host immunometabolic interface.**
R. Arsenault*[1], F. Perry[1], and C Johnson[2], [1]*Department of Animal and Food Sciences, University of Delaware*, *Newark, DE, USA*, [2]*Feed and Food Safety Research Unit, Southern Plains Agricultural Research Center, USDA ARS*, *College Station, TX, USA*.

11:00 AM Coffee Break: Grand Foyer

11:30 AM **A botanicals-based microencapsulated feed additive protects weaning piglets during a challenge with *Escherichia coli* LPS.**
A. Bonetti*[1], B. Tugnoli[2], A. Piva[1,2], C. Stahl[3], and E. Grilli[1,4], [1]*DIMEVET, Department of Veterinary Medical Sciences, University of Bologna*, *Ozzano dell'Emilia, Bologna, Italy*, [2]*Vetagro S.p.A.*, *Reggio Emilia, Italy*, [3]*Department of Animal and Avian Sciences, University of Maryland*, *College Park, MD, USA*, [4]*Vetagro Inc.*, *Chicago, IL, USA*.

12:00 PM - 1:30 PM Lunch: Arch View Ballroom

Poster Session: Grand Foyer

100 **Genomic analysis and adhesion characteristics of enterotoxigenic *Escherichia coli* F4 and F18 strains on porcine intestinal epithelial cell lines.**
C. Li[1], D. Liu[1], N. Gallina[1], N. Horn*[2], and A. Bhunia[1], [1]*Purdue University*, *West Lafayette, IN, USA*, [2]*United Animal Health*, *Sheridan, IN, USA*.

101 **Effect of feeding a direct-fed microbial (DFM) supplemented diet on the microbiome of young turkeys.**
D. Ayala*[1], E. Kimminau[1,2], N. Evans[1,3], and T.P. Karnezos[1], [1]*Purina Animal Nutrition Center*, *Gray Summit, MO, USA*, [2]*Elanco, Bentonville, AR, USA*, [3]*Adisseo*, *Alpharetta, GA, USA*.

102 **Impact of microencapsulated fermentation extracts, essential oils, and organic acids on productivity and fecal microbiota of dairy cows.**
S. E. Izzo Crespo*[1,2], O. Villalobos[2], O. AlZahal[2], L. Lahaye[2], and M. Costa[1],

[1]*University of Montreal*, *Saint-Hyacinthe, QC, Canada*, [2]*Jefo Nutrition Inc.*, *Saint-Hyacinthe, QC, Canada*.

103 **Receptor-targeted next-generation probiotics ameliorate inflammation and promote gut health.**
N. Gallina* and A. Bhunia, *Purdue University*, *West Lafayette, IN, USA*.

104 **Microbiome characterization and identification of potential causative agents of tail necrosis in pigs.**
D. Grum*, D. Ayala, K. Bamesberger, B. Trible, D. McManus, and T. P. Karnezos, *Purina Animal Nutrition Center, Gray Summit, MO, USA*.

105 ***E. coli* pathotypes identified through 16S microbiome and bacterial isolation in pigs with post-weaning diarrhea (PWD).**
D. Ayala*, D. Grum, K. Bamesberger, B. Trible, D. McManus, and T. P. Karnezos, *Purina Animal Nutrition Center*, *Gray Summit, MO, USA*.

106 **Assessment of antimicrobial effect of potential methanogenic inhibitors against enterohemorrhagic *Escherichia coli* and *Salmonella* in bovine rumen contents.**
R. C. Anderson* and R. B. Harvey, *United States Department of Agriculture/ Agricultural Research Service, Southern Plains Agricultural Research Center*, *College Station, TX, USA*.

107 **Microencapsulated essential oils supplementation helps maintain growth performance and intestinal health during coccidiosis challenge in broilers.**
H. H. Salgado*, G. Tactacan, and L. Lahaye, *Jefo Nutrition Inc.*, *Saint-Hyacinthe, QC, Canada*.

108 **Effect of phytase supplementation on growth performance, nutrient retention, footpad lesion score, tibia bone mineralization, and meat quality in broilers.**
C. B. Lim*, V. Sampath, and I. H. Kim, *Dankook University*, *Department of Animal Resource and Science, No. 29 Anseodong, Cheonan, Choongnam, South Korea*.

109 **Inclusion of xylanase supplement to high- and low-density diets reveals a positive result on growth performance, nutrient digestibility, gas emission, and fecal microbiota in growing pigs.**
C. B. Lim*, S. T. Wahid, and I. H. Kim, *Department of Animal Resource and Science, Dankook University*, *Cheonan, South Korea*.

110 **Supplementation of a medium-chain fatty acid blend and a phytogenic feed additive improved performance of pigs challenged with *Escherichia coli*.**
J. C. González-Vega*, S. May, B. Smith, K. Moran, and E. Teddy, *Cargill Animal Nutrition*, *Lewisburg, OH, USA*.

111 **Chicken enteroid kinome responses to *Salmonella* infection and organic acid/ essential oil blend.**
J. Elango*[1], F. Perry[2], K. Sutton[3], J. Mitchell[3], L. Vervelde[3], E. Santin[4], L. Lahaye[4], and R. Arsenault[2], [1]*Department of Biological Sciences, University of Delaware*, *Newark, DE, USA*, [2]*Department of Animal and Food Sciences, University of Delaware*, *Newark, DE, USA*, [3]*Division of Immunology, The Roslin Institute, University of Edinburgh*, *Edinburgh, Scotland, UK*, [4]*Jefo Nutrition Inc.*, *Saint-Hyacinthe, QC, Canada*.

3:30 PM - 4:30 PM Standardizing a Protocol for Microbiota Analysis in Poultry: Salons ABC

4:30 PM - 6:00 PM Reception: Arch View Ballroom

Tuesday, October 31

7:00 AM - 8:00 AM Breakfast: Arch View Ballroom

7:00 AM - 5:00 PM Registration: Grand Foyer

SESSION 2

Chair: Mike Kogut, USDA-ARS
Salons ABC

8:00 AM **The impact of environmental conditions on the gut microbiome of broiler chickens.**
J. G. Kers*[1,2], H. Smidt[2], and F. C. Velkers[3], [1]*Laboratory of Microbiology, Wageningen University & Research, Wageningen, the Netherlands*, [2]*Department of Population Health Sciences, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, Netherlands*, [3]*Department of Population Health Sciences, Division of Farm Animal Health, Utrecht University, Utrecht, the Netherlands.*

9:00 AM **Microbiome mapping: Gut microbiome development of broilers from 0 to 42 days of age.**
T. Lavergne*[1], C. Elrod[1], A. Figueiredo[2], and M. Nascimento[3], [1]*Natural Biologics Inc.*, *Newfield, NY, USA*, [2]*Aleris Nutrition*, *Jundiai, Sao Paulo, Brazil*, [3]*Sapiens*, *Jundiai, Sao Paulo, Brazil*.

9:30 AM ***Ex vivo* assessment of the direct and indirect antimicrobial capacities of glycerides of lauric acid using gastrointestinal fluids.**
N. Vieco-Saiz[1], V. Michel[1], A. Mellouk[1], O. Lemâle[2], H. Yakout*[3], N. Evans[3], T. Goossens[4], and J. Consuegra[1], [1]*Adisseo France S.A.S., Center of Excellence and Research in Nutrition*, *Malicorne, France*, [2]*Adisseo NL*, *Raamsdonksveer, the Netherlands*, [3]*Adisseo USA Inc.*, *Alpharetta, GA, USA*, [4]*Adisseo Belgium*, *Sint-Niklaas, Belgium*.

10:00 AM Coffee Break: Grand Foyer
Sponsored by Jefo Nutrition Inc.

10:30 AM **Effects of maternal live yeast supplementation on sow milk proteomic profile, intestinal tight junction proteins, and inflammatory markers in the offspring.**
Y. Fu*, E. Li, T. Casey, O. Adeola, and K. Ajuwon, *Department of Animal Sciences*, *Purdue University, West Lafayette, IN, USA*.

11:00 AM **Effects of ruminal SCFA concentration and pH on intestinal digestibility and digesta pH of dairy calves.**
M. H. Paez Martins Narciso*, A. R. Wolfe, R. R. E. Uwiera, and A. H. Laarman, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

11:30 AM **Dietary tryptophan regulated performance, intestinal morphology and inflammation, and disease resistance of coccidia-challenged broiler chickens.**
R. A. Hernandez*, K. C. Klasing, and Y. Liu, *University of California, Davis*, *Davis, CA, USA*.

12:00 PM - 1:00 PM Lunch: Arch View Ballroom

SESSION 3

Chair: Mike Kogut, USDA-ARS
Salons ABC

1:00 PM **Understanding the influences of early life adversity on intestinal epithelial development and functions in pigs.**
Y. Li*, *University of Delaware*, *Newark, DE, USA*.

2:00 PM **Effects of second iron injection before weaning on growth performance, hematological parameters, fecal score, and microbiome of pigs fed nursery diets with different dietary iron levels under natural disease challenge.**
A. Johnson[1], B. Dittrich[1], A. Cole[1], M. Prodell[1], W. Lyons[2], J. Heisel[2], S. Fritz[3], W. Li[4], P. Fregulia[4], and Y. D. Jang*[1,5], [1]*University of Wisconsin–River Falls*, *River Falls, WI, USA*, [2]*Pharmacosmos Inc.*, *DeKalb, IL, USA*, [3]*Kansas State University*, *Manhattan, KS, USA*, [4]*US Dairy Forage Research Center, USDA-Agricultural Research Service*, *Madison, WI, USA*, [5]*University of Georgia*, *Athens, GA, USA*.

2:30 PM **Mapping critical gut homeostasis indices under diverse dietary inputs in broilers.**
K. Mountzouris*, *Agricultural University of Athens*, *Athens, Attika, Greece*.

3:00 PM Coffee Break: Grand Foyer

3:30 PM **COALMINERS—A novel system in studying chronic inflammation in chickens.**
A. Khadem[1,2] and C. Gougoulias*[1], [1]*INNOVAD NV*, *Antwerp, Belgium*, [2]*Lab of Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium*.

4:00 PM **Butyric glycerides act directly and indirectly on chicken enterocytes to enhance resistance to pathogen colonization.**
A. Mellouk[1], N. Vieco-Saiz[1], V. Michel[1], H. Yakout*[2], N. Evans[2], O. Lemâle[3], and T. Goossens[4], [1]*Adisseo France S.A.S., Department of R&I in Monogastric Animal Nutrition, Saint Fons, France*, [2]*Adisseo US*, *Raleigh, NC, USA*, [3]*Adisseo Belgium*, *Sint-Niklaas, Belgium*, [4]*Adisseo Netherlands B.V., Raamsdonksveer, the Netherlands*.

4:30 PM **Prevalence of *Enterococcus faecalis* and *Escherichia coli* in nonviable embryonated broiler eggs.**
J. Delago[1], M. Ahmad*[2], E. McKinley[1], and A. Smith[1], [1]*Arm & Hammer*, *Waukesha, WI, USA*, [2]*Arm & Hammer*, *Ewing, NJ, USA*.

5:00 PM **Phytogenic affects performance, egg quality, and expression of intestinal cytoprotective and inflammatory responses in laying hens.**
I. Brouklogiannis*[1], E. Anagnostopoulos[1], V. Paraskeuas[1], E. Griela[1], G. Kefalas[2], and K. Mountzouris[1], [1]*Agricultural University of Athens*, *Athens, Attica, Greece*, [2]*Nuevo SA*, *Schimatari, Viotia, Greece*.

5:30 PM - 7:00 PM Reception: Arch View Ballroom

Wednesday, November 1

7:30 AM - 8:30 AM Breakfast: Arch View Ballroom

7:30 AM - 10:00 AM Registration: Grand Foyer

SESSION 4

Chair: Mike Kogut, USDA-ARS
Salons ABC

9:00 AM **Extended influence of colostral cells on mucosal responses to routine health challenges in neonatal lambs.**
M. Donia*[1,2], J. Lowe[1,3], F. Zuckermann[1], C. Gaulke[1,4], and B. Aldridge[3,5], [1]*Department of Pathobiology, College of Veterinary Medicine, University of Illinois*, *Urbana-Champaign, IL, USA*, [2]*Department of Internal Medicine, College of Veterinary Medicine, Kafrelsheikh University*, *Kafrelsheikh, Egypt*, [3]*Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois*, *Urbana-Champaign, IL, USA*, [4]*Carle R. Woese Institute for Genomic Biology, University of Illinois Urbana Champaign*, *Urbana-Champaign, IL, USA*, [5]*Department of Biomedical and Translational Sciences, Carle Illinois College of Medicine, University of Illinois*, *Urbana-Champaign, IL, USA*.

9:30 AM **Glycerides of lauric acid supplementation in the chicken diet enhances the humoral and cellular immune response to infectious bronchitis virus.**
A. Mellouk[1], V. Michel[1], N. Vieco-Saiz[1], H. Yakout*[2], N. Evans[2], O. Lemâle[3], T. Goossens[4], and J. Consuegra[1], [1]*Adisseo France S.A.S., Department of R&I in Monogastric Animal Nutrition, Saint Fons, France*, [2]*Adisseo US*, *Raleigh, NC, USA,* [3]*Adisseo Belgium*, *Sint-Niklaas, Belgium*, [4]*Adisseo Netherlands B.V., Raamsdonksveer, the Netherlands*.

Session 1

1 Promoting health by managing the four "M's" of homeostasis. B. Aldridge*, *College of Veterinary Medicine and Health Innovation Professor, Carle Illinois College of Medicine, University of Illinois, Urbana-Champaign, IL, USA.*

Biological health is a complex topic of discussion and is often misunderstood as existing as a binary state in terms of the presence or absence of disease. In many ways it is useful to explain health at a population or system level as the existence of individuals in 1 of 3 states: those in a healthy condition, those with a pre-diseased status, and those in a diseased state. At a systems level, it is useful to understand that there is a dynamic, ever-changing flow of individuals and the population as a whole between these 3 states. We would contend that it is actually quite unusual for an individual to move directly between the healthy and diseased state, without some transition through the pre-diseased state. A useful perspective is to view each individual in a population as having a health "phenotype." The healthy, "normal" state is that in in which individuals are highly resilient to perturbation (disruptions to homeostasis). The second or intermediate state is termed the "pre-disease" state, in which individuals are unstable, sensitized to perturbation and existing at the very edge of normal function. We can view this as a reversible state and a great place to intervene to restore normal health. The "disease" state occurs when the unstable pre-disease state undergoes decline, or a downward transition to clinical deterioration. Importantly, individuals in this disease state are also somewhat stable, and can be considered as resilient and robust in their pathological state. As such this state is not readily reversible. So, what might induce or influence the transition of an individual between the healthy and pre-diseased state? We would suggest that to understand this, it is useful to appreciate the biology and dynamics of stress, or system perturbation, on the healthy state. We jokingly tell our students that "Stress is spelled C-H-A-N-G-E," and that to understand stress we need to appreciate how an individual or population resists or responds to change. In addition, to help us design interventions that can prevent the transition of an individual from the healthy to pre-disease state, or to promote restoration of an individual from the pre-disease to healthy state, we need to understand the nature and operation of the stressors, or the drivers of change, within our systems. There are 4 main channels of stress to the individual, the 4 "M's": mucosal, mental (neuroendocrine), microbial, and metabolic. The mucosal sites, such as the respiratory, gastrointestinal, urogenital, and skin surfaces are where the host directly interacts with its physicochemical and microbiological environment. Neuroendocrine stress works through special senses and is communicated and manifest through the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Metabolic stress is the response of the host to changes in water or nutrient supply, particularly in terms of energy and proteins, but also with respect to electrolytes, acid-base, vitamins, and minerals. Microbial stress involves invasion by a unique, virulent pathogen or, more commonly, emergence of a resident pathogen from perturbations of the mucosal microbiota. Now stressors, or drivers of change, can act at both the local or the systemic level. For instance, mucosal stressors act directly at the specific body surfaces described earlier, whereas neuroendocrine and metabolic stressors are, by definition, systemic. It follows that the impact of stress can be either local or systemic. For instance, physical damage of the nasal mucosa will act as a local stressor. But the involvement of the neuroendocrine and metabolic system means that the response to some stressors can involve every body system. For instance, imagine a prolonged and difficult transport journey. This would invoke neuroendocrinological responses through fear and new social interactions, mucosal responses through dehydration and microbial shifts, cardiovascular responses through dehydration, and metabolic changes through alterations in nutrient demand and availability So while the 4 major avenues of stress are distinct from each other, they are also but inter-related to, each other. Envision a thermoregulatory stressor—either heat or cold stress. This would challenge the health of an individual through all 3 portals: it could cause physicochemical or microbial changes in the respiratory tract, it could put pressure on metabolic resources of the body, and might invoke neuroendocrinological perturbation through changing physical environment. As described, the stressors to health are not only variable in nature and magnitude but are also dynamic and continuous in time. We should recognize that these challenges to health are a normal part of life, and so cannot be avoided completely. The drivers of change are all around us—they are physical, chemical, microbial, climatic, and social, so are often related to the nature and quality of the living environment. Because they are universal in life, the body is actually designed to recognize and counter these common stressors. In the normal state of health, change drivers are resisted, or countered, by normal anatomical-physiochemical processes, and so stress mechanisms and responses should not always be considered as pathological, but should be recognized as normal adaptations to normal events of change and uncertainty. The ability to resist and respond to these many drive changers are innate, but are influenced by both genetics and epigenetics in that they are both inherited and learned or developed. While it is understandable to think of stress as a single and it is important to appreciate that the effects of stress can be cumulative and formative. So, although the transition from health to the pre-disease state could arise from a sudden and severe incursion of change, it is as likely to develop in an individual that is exposed to repeated, low-grade, non-catastrophic physiological, neuroendocrinological, or metabolic perturbations. For instance, it has been shown that stressors actually determine the trajectory of organ development. Individuals that have experienced early-life adversity may have long-term, detrimental immune system disturbances. Maternal diets during pregnancy can impact future neurological development and cognitive abilities, and have long-term effects on metabolic progression and inclination. In view of the importance of change and of the stressors that drive and influence change, and our understanding that stress impacts the biological resilience and disease resistance of a host, it is important to appreciate that stress can and should be measured and influenced at both the level of input (i.e., the stressors themselves), and the level of response (i.e., the capacity of the host to resist or adapt to the stressor). This is especially important in defining our approach to managing and caring for individuals and populations, because, ultimately, resistance and adaptability to stressors determine health outcomes and therefore quality and productivity of life.

2 "Both/and" antibiotic alternatives: Beneficial feed additives that target the host immunometabolic interface. R. Arsenault*[1], F. Perry[1], and C. Johnson[2], *[1]Department of Animal and Food Sciences, University of Delaware, Newark, DE, USA, [2]Feed and Food Safety Research Unit, Southern Plains Agricultural Research Center, USDA ARS, College Station, TX, USA.*

Significant research and development has been committed to finding alternatives to antibiotics that are at least as effective as conventional antibiotics in preventing disease and promoting growth. It has been well known for 70 years that antibiotics have this dual disease/growth effect. However, it was only around the turn of the century that consideration of antibiotic host effects on growth and immunity were significantly studied. The sequence of events beginning with antibiotic restrictions in animal production, the advent of immunometabolism approaches in animal agriculture in the past 10 years, and the understood need for knowledge of mechanism of action for feed additives have led to an explosion in the study of antibiotic alternatives. Our laboratory approaches these studies with a functional proteomic approach and immunometabolic perspective. Given the disease resistance/growth promotion functions of antibiotics and the unseverable link between immunity and metabolism, it follows that the most effective antibiotic alternatives would affect host and microbe. Here, we describe the dual mechanism of action of 2 feed additives, butyrate and a postbiotic with fermentate. Unlike probiotics, postbiotics containing fermentate will include metabolites and immune stimulatory factors in a single product; these can impact numerous physiological points along the gut. The bioavailable metabolites feed the host metabolism while the microbial components stimulate immune activity. This dual nature of postbiotics is a closer analog to antibiotics than a feed additive targeting a single mechanism. With regard to butyrate, depending on the metabolic status of the host, the fate and effect of butyrate can be distinct. Depending on the immunometabolic context, butyrate can feed oxidative phosphorylation or modify gene expression by directly modifying histones. This dual nature may explain the often-conflicting findings in the literature regarding butyrate efficacy and function. The ultimate determinant of feed additive efficacy under diverse production conditions may be that it is both antimicrobial and host enhancing.

Key Words: Immunometabolism, feed additives, dual action

3 A botanicals-based microencapsulated feed additive protects weaning piglets during a challenge with *Escherichia coli* LPS. A. Bonetti*[1], B. Tugnoli[2], A. Piva[1,2], C. Stahl[3], and E. Grilli[1,4], *[1]DIMEVET, Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy, [2]Vetagro S.p.A., Reggio Emilia, Italy, [3]Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA, [4]Vetagro Inc., Chicago, IL, USA.*

At weaning, pigs develop significant stress with long-lasting effects on their performance and health. Botanicals include a wide variety of bioactive molecules able to control inflammation and oxidation. The aim of the study was to investigate the ability of a microencapsulated thymol-based blend of botanicals (BOT) to support piglets' performance and health during an LPS inflammatory challenge. To examine this, 72 weaning pigs were divided in 24 pens and assigned to 3 experimental groups: a negative control (CTR−), a positive control (CTR+), and a group treated with BOT (BOT+). After 14 d, CTR+ and BOT+ received the inflammatory challenge, consisting of two 30-µg/kg BW intraperitoneal *Escherichia coli* O55:B5 LPS injections, 48 h apart. One pig per pen was then sacrificed on d 21, and all the remaining on d 28, to collect samples for gene expression analysis. In the challenge week (d 14–21), BOT significantly improved BW ($P = 0.02$) and ADG ($P = 0.04$) compared with CTR+, with trends confirmed also for the final week (d 21–28) and across the last 2 weeks (d 14–28). BOT also increased ADG in the first 3 weeks of the study ($P = 0.04$). Moreover, during d 14–21 and 14–28, FCR was deeply impaired in CTR+ (+0.36 and +0.24 points vs. CTR−, respectively), but BOT re-established values in line with CTR− ($P \leq 0.05$). In the overall period, ADG and final BW tended to be increased by BOT at levels closer to CTR− ($P = 0.09$), with numerical improvements also for FCR. Gene expression analysis in liver showed that BOT lowered the inflammatory activation caused by the challenge at d 21 and 28, with reductions in TLR4, TNFα, and IL-6 ($P < 0.05$). Moreover, BOT modulated the expression of several antioxidant enzymes, such as GPX2, SOD1, SOD2, and CAT. To conclude, BOT helped weaning piglets to face an inflammatory challenge improving performance parameters and overall health. Its mechanism of action counteracted stress by controlling inflammation and enhancing the oxidative response. BOT can be proposed as a nutritional supplement to support pigs at weaning.

Key Words: Botanicals, inflammation, oxidative stress, liver, piglets

Session 2

4 The impact of environmental conditions on the gut microbiome of broiler chickens. J. G. Kers*[1,2], H. Smidt[2], and F. C. Velkers[3], *[1]Laboratory of Microbiology, Wageningen University & Research, Wageningen, the Netherlands, [2]Department of Population Health Sciences, Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, the Netherlands, [3]Department of Population Health Sciences, Division of Farm Animal Health, Utrecht University, Utrecht, the Netherlands.*

Knowledge of factors that influence the functioning of gut microbes is essential to improve health and reduce the use of antibiotics in poultry production. The environment, and more specifically housing conditions, can affect the gut microbiome of broiler chickens. The gut microbiome is defined as the collection of all the microorganisms and their "theatre of activity" in the gut environment. Previous research has shown that the development of the gut microbiome is hampered in animals raised in high-hygiene environments, such as isolators. It has also been reported that human individuals and animals who live together show less variation in the gut microbiome compared with a group of random individuals. In a Dutch human population, it has been shown that the gut microbiome was primarily shaped by environmental factors and cohabitation. Therefore, a proper understanding of how the composition and functioning of the gut microbiome in broiler chickens are affected by interactions between hosts, and hosts and their environment, is needed. To this end, the effect of environmental factors should be considered in the design, analyses, and interpretation of study outcomes. In addition, knowledge of the development of intestinal microbiome composition and function would be of great value for optimizing the resilience of broiler flocks that can help in reducing the need for therapeutic antibiotics. Although the mechanisms driving gut microbiome development are not fully understood, there is data suggesting that this development is major in the first week post-hatch, and that around 3 weeks post-hatch, cecal microbiota composition can be considered mature in a well-performing commercial broiler flock. It is important to further study mechanisms underlying gut microbiome development and function to develop better diagnostics and management tools to improve broiler health.

Key Words: Gut microbiome, environment, broiler chickens

5 Microbiome mapping: Gut microbiome development of broilers from 0 to 42 days of age. T. Lavergne*[1], C. Elrod[1], A. Figueiredo[2], and M. Nascimento[3], *[1]Natural Biologics Inc., Newfield, NY, USA, [2]Aleris Nutrition, Jundiai, Sao Paulo, Brazil, [3]Sapiens, Jundiai, Sao Paulo, Brazil.*

To produce resilient and healthy poultry, it is necessary to understand the effects of diet and dietary feed additives on the microbiome and its development. This trial was conducted to evaluate the microbiota of broilers 14, 28, and 42 d of age, while feeding them a combination of yeast postbiotics and fermentation extracts (Provillus 4Poultry). A total of 300 Ross 308 male chicks were allotted to 2 treatments: control or control + Provillus (1 lb/ton). Chicks were reared on used litter and fed diets formulated to meet nutrient specifications for Ross 308 broilers. Starter, grower, and finisher diets were fed from 0 to 14, 14 to 28, and 28 to 42 d of age, respectively. On d 14, 28, and 42, broilers and feed were weighed to calculate ADG, ADFI, and FCR. Also on d 14, 28, and 42, cloacal swabs were collected from 6 broilers per treatment and 16S DNA sequencing was performed by a commercial laboratory. These data were analyzed by an artificial intelligence platform (Sapiens) to identify and quantify the total number of genera present, positive and negative biomarkers, and microbiota robustness. There was no effect ($P > 0.05$) of dietary treatment on ADG, ADFI, or FCR. Broilers fed Provillus maintained their microbiota richness until 42 d of age, while control-fed broilers had decreased microbiota richness. Feeding Provillus resulted in a more robust microbiota with positive biomarkers present at 14 d of age. At 28 d of age, negative biomarkers that indicate dysbiosis due were present in both treatment groups. With the maturation of the microbiota at 42 d of age, reappearance of positive biomarkers occurred in both groups. The eubiosis state was more susceptible to environmental disturbances during early development than it was after maturation. Growth performance rate may have been disturbed due to the appearance of clusters of negative bacteria. The microbiota in earlier phases is considered more susceptible to disturbances, which is why it is important to choose an additive that maintains the robustness, diversity, and richness of the microbiota in poultry produced without the use of growth promoters.

Key Words: Microbiome, biomarkers, broilers, growth performance

6 *Ex vivo* assessment of the direct and indirect antimicrobial capacities of glycerides of lauric acid using gastrointestinal fluids. N. Vieco-Saiz[1], V. Michel[1], A. Mellouk[1], O. Lemâle[2], H. Yakout*[3], N. Evans[3], T. Goossens[4], and J. Consuegra[1], *[1]Adisseo France S.A.S, Center of Excellence and Research in Nutrition, Malicorne, France, [2]Adisseo NL, Raamsdonksveer, the Netherlands, [3]Adisseo USA Inc, Alpharetta, GA, USA, [4]Adisseo Belgium, Sint-Niklaas, Belgium.*

α-Monolaurin is an antimicrobial agent with a potent *in vitro* activity against gram-positive (G+) but less against gram-negative (G−) bacteria. Hence, glycerides of lauric acid (C12G) are used as a feed additive to prevent intestinal infections, thereby enhancing animal resilience against pathogen challenges, and improving animal performance. Here, we aimed to evaluate the *ex vivo* antimicrobial activity of C12G using gastrointestinal tract (GIT) fluids from animals receiving a C12G-supplemented feed. A total of 100 chicks were divided into 2 groups, both receiving the same standard diet supplemented or not with C12G. GIT fluids were extracted from crop, ileum, and ceca contents. Their antimicrobial capacity was assessed using minimal inhibitory concentration (MIC) assay against *Enterococcus faecalis* and *Escherichia coli* APEC. MIC analyses showed that GIT fluids from animals receiving C12G have an antimicrobial activity against G+ and G− bacteria. Specifically, *E. faecalis* growth was significantly reduced by the GIT fluids from all compartments. *Ex vivo* antimicrobial activity shows that C12G conserve their antimicrobial effect all along the GIT, which ensures animal protection against pathogen challenges. Interestingly, only cecal fluids presented an antimicrobial activity against *E. coli* APEC. Because C12G are less active in vitro against G− bacteria, we

hypothesize that this antimicrobial activity may be explained by a shift on cecal microbiota exerted by C12G toward a microbial profile of metabolite producers that may inhibit G− bacteria. To test this hypothesis, cecal microbiota was analyzed. Altogether, using GIT fluids and the *ex vivo* assessment of its antimicrobial activities, our study suggests that C12G have a dual mode of action to inhibit pathogenic bacteria. First, they can act directly against G+ bacteria all along the GIT. Second, they can indirectly inhibit G− in the ceca, possibly by inducing a microbiota shift toward the production of antimicrobial metabolites. More studies are needed to identify the molecules produced by the microbiota explaining this G− antimicrobial effect.

Key Words: Gastrointestinal fluids, glycerides of lauric acid, antimicrobial activity

7 Effects of maternal live yeast supplementation on sow milk proteomic profile, intestinal tight junction proteins, and inflammatory markers in the offspring. Y. Fu*, E. Li, T. Casey, O. Adeola, and K. Ajuwon, *Department of Animal Sciences, Purdue University, West Lafayette, IN, USA.*

Sow milk serves as a crucial source of nutritional, immunological, and growth-promoting components for the piglets. The objective of this study was to determine the effects of dietary supplementation with live yeast (LY) to sows during late gestation and lactation on milk proteomic profile in dams and the intestinal health of offspring. On d 77 of gestation, 40 sows were allocated to 2 dietary treatments: without (CON) or with LY supplementation at 0.05% of diet during gestation and 0.1% during lactation. Milk samples were collected on d 0, d 10, and d 18 postpartum (n = 6). On postnatal days (PND) 0, 10, and 18, and post-weaning days (PWD) 7 and 14, 1 piglet from 10 sows per treatment was selected for intestinal tissue collection (n = 10). With shotgun proteomics analysis, the milk of sows on LY treatment was found to be more abundant in proteins associated with immunity, including immunoglobulin and complement proteins (Ig-like and C1q domain-containing proteins) on d 0 and d 10. IGFBP5 was also more abundant in the d-0 milk of LY sows, whereas prostaglandin synthetase was greater in the d-10 and d-18 milk of CON sows. Correspondingly, maternal LY supplementation increased mRNA expression of interleukin (IL)-6 on PND 18 and IL-1β on PWD 14 in the ileal mucosa ($P < 0.05$), with a tendency for higher IL-10 on PND 18 ($P = 0.08$). Additionally, LY piglets had increased mRNA expression of superoxide dismutase 1 on PND 10 and 18 and PWD 14 ($P < 0.05$), and tended to have a higher mRNA abundance of catalase on PND 14 in the ileal mucosa ($P = 0.09$). Also, LY piglets had an increased abundance of adhesion protein E-cadherin on PND 0 and PWD 7 and 14, with a higher abundance of tight junction proteins, including occludin and claudin-4 in the jejunal mucosa on PWD 14 ($P < 0.05$). Taken together, results suggest that maternal LY supplementation could alter immune-associated proteins in milk that may affect the health and gut development of the offspring.

Key Words: Live yeast, milk proteome, intestinal inflammation, tight junctions

8 Effects of ruminal SCFA concentration and pH on intestinal digestibility and digesta pH of dairy calves. M. H. Paez Martins Narciso*, A. R. Wolfe, R. R. E. Uwiera, and A. H. Laarman, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Although the importance of pH and short-chain fatty acids on rumen development are well known, their impact on the small and large intestine are unclear. This study investigated the effects of ruminal short-chain fatty acid concentrations ([SCFA]) and pH on the rumen and intestine of calves. Holstein bull calves (n = 32) were individually housed and fed milk replacer (900 g/d) twice daily and calf starter and water *ad libitum*. At 10 ± 3 d of life, the rumens were fistulated and cannulated. At 21 d of life, calves were grouped by body weight and assigned in a 2 × 2 factorial arrangement of treatments: high or low [SCFA] (285 vs. 10 m*M*) and high or low pH (6.2 vs. 5.2), forming 4 treatment groups: high [SCFA], high pH (HH); high [SCFA], low pH (HL); low [SCFA], high pH (LH); and low [SCFA], low pH (LL). On wk 3, 5, and 7, feces were sampled for digestibility analysis, after which the rumen was evacuated and washed for 4 h with 1 of 4 treatment buffers. After completion of rumen wash on wk 7, calves were harvested, and the tissue weight and length, and digesta pH of the rumen, cecum, colon, and rectum were recorded, along with the digesta pH of duodenum, jejunum, and ileum. Data were analyzed with main factors as fixed effects and repeated measures for weekly measurements. Body weight and calf starter intake were unaffected by treatments but increased over time ($P < 0.01$). Digestibility was unaffected by treatments and weeks. High [SCFA] increased milk replacer intake by 255 g ($P < 0.05$). Fecal pH on wk 3 was lower than wk 7 ($P < 0.05$) and tended to be lower than wk 5 ($P = 0.09$). Rumen digesta pH on wk 3 was higher than on wk 5 and 7 ($P < 0.05$). Duodenum and jejunum digesta pH was higher for HH than LH ($P < 0.05$). Ileum digesta pH was higher for HH and LL than LH ($P < 0.01$). High [SCFA] increased colon and rectum digesta pH ($P < 0.01$) and tended to increase cecum digesta pH ($P = 0.06$). In summary, although ruminal [SCFA] and pH impact intestinal digesta pH within hours, the rumen digesta pH reduction did not decrease fecal pH, digestibility, and BW gain.

Key Words: Digestibility, rumen pH, rumen SCFA, digesta pH

9 Dietary tryptophan regulated performance, intestinal morphology and inflammation, and disease resistance of coccidia-challenged broiler chickens. R. A. Hernandez*, K. C. Klasing, and Y. Liu, *University of California, Davis, Davis, CA, USA.*

The objective of the study was to evaluate tryptophan's functionally essential role in regulating performance, intestinal inflammation, and disease resistance during a coccidia challenge. A total of 300 two-day-old Cobb 500 broiler chickens (54.29 ± 0.284 g body weight [BW]) were group housed (5 chickens/pen; 10 pens/treatment) in battery brooders and had *ad libitum* access to a basal diet and water. At 7 d of age, pens were randomly assigned to 1 of 6 levels of dietary tryptophan: 0.15%, 0.185%, 0.22%, 0.32%, 0.42%, or 0.52% of the total diet. Following a 3-d adaptation period, chickens received an inoculum in the feed, consisting of *Eimeria acervulina* (7.2×10^3), *Eimeria maxima* (1.7×10^3), and *Eimeria tenella* (2.8×10^3). Weight gain and feed intake were recorded at 5 and 10 d post-inoculation (DPI). On 5 and 10 DPI, feces were collected for oocyst enumeration by flow

cytometry, and jejunum was sampled from 1 bird per pen to analyze morphology and cytokine gene expression. Data were analyzed by one-way ANOVA, and orthogonal polynomial contrasts were used to evaluate the linear and quadratic effects of tryptophan on the dependent variables. Dietary tryptophan increased (linear and quadratic, $P < 0.01$) BW and feed intake and decreased (linear and quadratic, $P < 0.01$) feed conversion ratio during the entire experimental period. Chickens fed more tryptophan had increased (linear and quadratic, $P < 0.05$) shedding of *E. acervulina* at 5 and 10 DPI and *E. tenella* at 5 DPI. Supplemental tryptophan increased (linear and quadratic, $P < 0.01$) crypt depth at 5 and 10 DPI and decreased (linear and quadratic, $P < 0.01$) the ratio of villus height to crypt depth at 5 DPI. Tryptophan also decreased (linearly, $P = 0.03$) interferon-γ expression at 5 DPI and increased (quadratically, $P < 0.01$) interferon-γ, FOXP3, and interleukin-10 expression in the jejunum at 10 DPI. Current results suggest that dietary tryptophan levels above 0.22% improved growth performance, although concentrations between 0.22% and 0.52% may exacerbate intestinal inflammation and damage associated with coccidia infection.

Key Words: Tryptophan, *Eimeria*, broiler

Session 3

10 Understanding the influences of early life adversity on intestinal epithelial development and functions in pigs. Y. Li*, *University of Delaware, Newark, DE, USA.*

Developmental plasticity during the prenatal and early postnatal periods allows animals to adapt quickly to their environment and efficiently construct organ systems crucial for survival. These adaptations, which may be irreversible later in life, can lead animals to develop beneficial survival strategies or predispose them to chronic diseases. The gastrointestinal (GI) tract is a highly adaptive organ, contending with the continuous changes in the complex luminal environment. It senses and selectively absorbs nutrients while blocking harmful antigens and pathogens. Optimal intestinal functions largely depend on the renewal and maturation of epithelial cell populations, which are actively produced by intestinal epithelial stem cells (IESC). Considering the rapid turnover rate of epithelial cells (every 3 to 5 d), any long-lasting alterations in epithelial functions could potentially stem from intrinsic changes in IESC. During early development, the relatively high number and activity of stem cells enable efficient adaptation to environmental challenges. The impact of early life stress on IESC activities and epithelial cell populations remains largely unexplored. We used the unique Ussing chamber electrophysiological technique and cutting-edge enteroid culture technique to assess intestinal stem cell activities and epithelial cell composition, as well as intestinal barrier, nutrient sensing, and transport functions both immediately and long-term post-early weaning stress in pigs. Overall, our findings suggest that early weaning stress disrupts intestinal development and function, partly due to alterations in IESC proliferation and differentiation activities. The stress signals contributed to long-term changes in the number and function of epithelial absorptive and secretory cell populations, enhancing survival efficiency. Understanding IESC regulation during early life development can pave the way for identifying novel strategies to ensure optimal gut health and regeneration under stress.

Key Words: Pigs, stress, stem cell, nutrient transport, enteroids

11 Effects of second iron injection before weaning on growth performance, hematological parameters, fecal score, and microbiome of pigs fed nursery diets with different dietary iron levels under natural disease challenge. A. Johnson[1], B. Dittrich[1], A. Cole[1], M. Prodell[1], W. Lyons[2], J. Heisel[2], S. Fritz[3], W. Li[4], P. Fregulia[4], and Y. D. Jang*[1,5], *[1]University of Wisconsin–River Falls, River Falls, WI, USA, [2]Pharmacosmos Inc., DeKalb, IL, USA, [3]Kansas State University, Manhattan, KS, USA, [4]US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI, USA, [5]University of Georgia, Athens, GA, USA.*

This study was conducted to demonstrate the effects of second iron injection before weaning and iron levels in nursery diets on growth performance, hematological parameters, and fecal microbiome of pigs. A total of 70 newborn pigs from 7 sows were allotted to 4 treatments within litter, housed in farrowing crate without creep feed, and received the first dose (200 mg iron) at 2 to 3 d of age. At weaning (d 27–30 of age), all pigs were housed in nursery pens within their original treatments for a 27-d growth period and naturally challenged with *Escherichia coli* and rotavirus. Treatments were as follows: (1) NC: no additional iron injection and supplementation (100 ppm iron in the basal diets); (2) NC+IRON: second iron injection (200 mg) at least 5 d after first injection + NC diets; (3) PC: additional 100 ppm iron supplementation; and (4) PC+IRON: second iron injection (200 mg) + PC diets. A common iron-dextran product (UNIFERON®200, Pharmacosmos Inc.) was used for both i.m. injections. Fecal microbiome composition was analyzed using amplicon sequencing targeting the V1–V9 regions of the 16S rRNA gene. Second iron injection increased hemoglobin (HG; 9.92 vs. 11.45 g/dL), hematocrit (HCT), and serum iron ($P < 0.05$) levels until d 13 postweaning. Second iron injection increased body weight at d 27 postweaning ($P = 0.05$, 18.8 and 20.8 kg), overall growth rate ($P = 0.08$), and feed intake ($P < 0.05$) but reduced overall fecal score. The ANCOM showed a higher abundance of the bacterial taxon *Lactobacillales* in the 2-injection group than in the 1-injection group at weaning. The pigs fed the PC diets had greater HG and HCT levels ($P < 0.05$) at d 27 postweaning and lower fecal score ($P = 0.09$; tendency) from d 13 to 27 postweaning than the NC diets. The second iron injection to pigs before weaning could improve postweaning hematological parameters and growth performance, reduce fecal score, and affect fecal microbiome, whereas 100 ppm of dietary iron supplementation increased hematological parameters and reduced fecal score in the late nursery period.

Key Words: Iron, second injection, dietary iron level, fecal microbiome, pigs

12 Mapping critical gut homeostasis indices under diverse dietary inputs in broilers. K. Mountzouris*, *Agricultural University of Athens, Athens, Attika, Greece.*

A deeper understanding of critical bird homeostasis responses may augment poultry industry efforts for improved bird resilience and production sustainability. The aim of this work was to explore the expression range of critical genes in the broiler gut as responses to diverse dietary inputs. The gene transcripts studied were grouped based on their functional role under functional indices, termed detoxification (DTI), cytoprotection (CTI), and inflammation index (ITI), respectively. A central composite statistical design (CCD) was used to study the effects of dietary metabolizable energy (ME) content, crude protein (CP) content (90–100% of the Ross 308 recommended specifications), and phytogenic inclusion (0–2,000 mg/kg of diet), each at 3 levels, on the functional indices DTI, CTI, and ITI in the duodena and ceca of 42-d-old broilers. A total of 540 1-d-old male Ross broilers were allocated in 36 experimental runs for 42 d. At the end of the trial, 3 broilers per run (108 total) were analyzed for the relative expression of 25 genes, grouped under DTI (5 genes), CTI (8 genes), and ITI (12 genes) per each intestinal segment. Response surface methodology (RSM) was used to assess dietary factor interactions, and empirical models describing the DTI, CTI, and ITI were fitted. The fitted models were significant ($P < 0.001$) with no lack of fit ($P > 0.05$) and explained most of the variance in the functional indices, having R^2 values of 0.63 and 0.90 for DTI, 0.59 and 0.66 for CTI, and 0.92 and 0.91 for ITI in the duodena and ceca, respectively. The functional indices were significantly

($P < 0.05$) modulated by dietary ME and CP contents, irrespective of phytogenic inclusion. Reducing dietary ME and CP from 100% to 90% of recommended specifications increased DTI and ITI but decreased CTI. Phytogenic inclusion strongly ($P < 0.001$) modulated all functional indices, increased CTI, and reduced DTI and ITI levels, resulting in higher cytoprotective and lower detoxification and inflammatory physiological response levels. Defining critical gut homeostasis response levels and how these can be modulated via diet may hold opportunities for better gut function and ultimately sustainable poultry production.

Key Words: Dietary specifications, statistical modeling, gene transcripts

13 COALMINERS—A novel system in studying chronic inflammation in chickens. A. Khadem[1,2] and C. Gougoulias*[1], *[1]INNOVAD NV, Antwerp, Belgium, [2]Lab of Nutrition, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

Modern poultry broiler production strives for the maximum live weight, which can contribute to chronic intestinal inflammation and broader metabolic syndromes. There is thus a need to establish realistic models that help the industry. Several challenge models have been proposed by the scientific community and these include biological agents or pathogens, chemicals, reused litter, and, more recently, different non-starch polysaccharides. However, they all lack the realism of true live production. Here, we propose a novel approach termed COALMINERS (ChrOnic intestinAl infLamMatIoN modEl under real faRming conditionS), in which mini-wired pens (high level of replication) are placed inside a commercial facility so that the birds under study have the same cumulative environmental production exposure. A gradient of intestinal challenges can be created by varying degrees of exogenous nutritionally induced stressors. In one experiment, the mean bacterial enteritis (BE) score, as defined in the literature, was statistically higher in the COALMINERS group (when corn was replaced with wheat) versus the actual farm diet and a "clean" experimental setting (the latter 2 groups, standard corn-soy diets) at d 21 and d 28 (3.75, 2.13, and 2.25, and 3.25, 1.88, 1.00, respectively; n = 8; $P < 0.01$). More importantly, the BE scores were reduced proportionally (~13%) over time only in the COALMINERS and real-farm environments, whereas the complete recovery in the experimental setting may reflect the lack of "cumulative production pressure" under such controlled conditions. In another COALMINERS iteration, aiming to study related mechanisms, the drop from a "high" (d 21–28) to a "low-intensity" (d 29–35) natural heat stress (mean temperatures: ~10°C and ~5°C higher than breeder standards, respectively) resulted in significant reduction in intestinal oxidative stress expressed as MDA (~25%; $P < 0.01$). However, 2 inflammatory (IFN-γ) and immune (sIgA) markers increased significantly (~36% and 40%, respectively, n = 8; $P < 0.01$). The COALMINERS approach offers a novel system for studying chronic inflammation in chickens and addresses the needs of modern poultry production.

Key Words: Novel, model, chronic, intestinal, inflammation

14 Butyric glycerides act directly and indirectly on chicken enterocytes to enhance resistance to pathogen colonization. A. Mellouk[1], N. Vieco-Saiz[1], V. Michel[1], H. Yakout*[2], N. Evans[2], O. Lemâle[3], and T. Goossens[4], *[1]Adisseo France S.A.S., Department of R&I in Monogastric Animal Nutrition, Saint Fons, France, [2]Adisseo US, Raleigh, NC, USA, [3]Adisseo Belgium, Sint-Niklaas, Belgium, [4]Adisseo Netherlands B.V., Raamsdonksveer, the Netherlands.*

Butyrate supplementation to feed is mainly employed for its advantageous effects on chickens' gut health. As a butyrate source, mono-, di-, and triglycerides of butyric acid provide a better distribution of butyrate in the entire gastrointestinal tract. Here, we aim to study both direct and indirect effects of butyric glyceride mixtures on avian enterocyte resistance to pathogen colonization. The immortalized chicken enterocytes Chic-clone-8E11 (8E11) were used to conduct in vitro assays. First, using Cell-ELISA, we assessed their resistance to *Campylobacter jejuni* and *Salmonella* Typhimurium adhesion after preincubation with sodium butyrate (SB) or butyric glyceride mix (BG). At 2 m*M*, BG significantly reduced *C. jejuni* and *Salmonella* Typhimurium adhesion to 8E11 cells by 84% and 60%, respectively. SB reduced *Salmonella* Typhimurium adhesion by only 20% (n = 3, $P < 0.001$). BG also preserved 8E11 cells from the toxicity of *Clostridium perfringens* α-toxin, measured by Resazurin survival test. BG maintained 8E11 survival levels at 80% versus 15% and 20% in the control and SB groups, respectively (n = 3, $P < 0.001$). Next, using Seahorse®, we evaluated the effects of the digested and non-digested BG treatments on the mitochondrial activity in 8E11 cells. Only SB and butyrate released by lipase significantly increased the basal and maximal oxygen consumption rates (n = 8, $P < 0.001$). In mammals, this metabolic response is known to induce an intestinal ecology shift to an anoxic profile, favoring anaerobic microbiota and disfavoring aerobic pathogens. We confirmed these findings in chickens by analyzing cecal microbiota from broilers fed BG-supplemented diets. In summary, our findings show how a butyric glyceride mix confers a triple antimicrobial effect to enhance animal resilience against pathogens. Besides the described effect of α-monoglycerides, we demonstrate that butyric glycerides act directly on enterocytes, making them more resistant to pathogen adhesion and toxicity. Moreover, when released by lipolysis, butyrate acts as a source of energy for avian enterocytes, which indirectly drives a microbial shift toward a resistant profile to pathogen invasions.

Key Words: Butyric glyceride, enterocyte, mode of action, bacterial pathogens

18 Prevalence of *Enterococcus faecalis* and *Escherichia coli* in nonviable embryonated broiler eggs. J. Delago[1], M. Ahmad*[2], E. McKinley[1], and A. Smith[1], *[1]Arm & Hammer, Waukesha, WI, USA, [2]Arm & Hammer, Ewing, NJ, USA.*

Enterococcus faecalis is considered a normal inhabitant of the poultry gastrointestinal tract. Although it has been known to be an opportunistic poultry pathogen, high levels of this organism can be recovered from the intestinal tract of normal, healthy birds with no known disease issues. More recently, an increasing number of poultry veterinarians and diagnostic laboratories have been reporting that *E. faecalis* is being isolated from yolks of unhatched embryonated eggs or from yolk sacs of morbid chicks shortly after hatch. *Escherichia coli*, which is known to be associated with embryonic and early chick mortality, has also been isolated from these cases. Recent studies have suggested that co-infection with *E. faecalis* and *E. coli* may enhance the virulence

of both organisms, resulting in decreased hatchability and poor chick quality. To better understand this issue and the potential role of co-infection of embryonated eggs with *E. faecalis* and *E. coli*, a survey was conducted to determine the prevalence of *E. faecalis* and *E. coli* in hatch residue. A total of 405 hatch residue samples that had evidence of early embryonic mortality were selected for sampling. Ten different samplings were taken from 6 hatcheries. Samples were collected by opening eggs aseptically and swabbing the yolk area. Standard microbiological methods were used to determine the presence of *E. faecalis* and *E. coli*. Of the 405 samples tested, 174 (43%) were found to be positive for both bacteria, whereas *E. faecalis* alone was recovered from 54 samples (13%) and *E. coli* alone was recovered from 53 samples (13%). Neither bacterial species was recovered from the remaining 124 samples (31%), possibly indicating that these embryonic mortalities may not have been a result of infection with pathogenic bacteria. The results indicated that *E. faecalis* and *E. coli* were frequently isolated from cases of early embryonic mortality and that co-infection was far more common than either organism isolated alone. The results suggested that co-infection with *E. faecalis* and *E. coli* may enhance virulence of the bacteria, leading to early embryonic mortality, which has not been previously reported.

Key Words: *Enterococcus*, *E. coli*, broiler

15 Phytogenic affects performance, egg quality, and expression of intestinal cytoprotective and inflammatory responses in laying hens. I. Brouklogiannis*[1], E. Anagnostopoulos[1], V. Paraskeuas[1], E. Griela[1], G. Kefalas[2], and K. Mountzouris[1], *[1]Agricultural University of Athens, Athens, Attica, Greece, [2]Nuevo SA, Schimatari, Viotia, Greece.*

This study was conducted to investigate the inclusion level effects of a natural phytogenic blend (PB) on production performance, egg quality, and underlying detoxification (aryl hydrocarbon receptor; AhR), antioxidant (nuclear factor erythroid 2-related factor 2; Nrf2) and inflammatory (nuclear factor-kappa B; NF-κB) responses in layers' duodena and ceca. Depending on PB inclusion level (0, 250, 750, 1,000, or 1,500 mg/kg) in the basal diet, 21-wk-old Hy-Line Brown laying hens (n = 385) were assigned into 5 treatments: CON, PB250, PB750, PB1000, or PB1500, with 7 replicates of 11 hens each. The PB consisted of selected Mediterranean plants having olive oil polyphenols, carvacrol, and thymol among its main bioactive components (NuPhoria®; Nuevo SA, Greece). Performance and egg quality parameters were determined weekly for a 12-wk period (i.e., wk 33 of layers' age) and reported as overall. Intestinal samples from the duodena and ceca of 33-wk-old layers were collected for qPCR analysis. Data were analyzed by ANOVA, and statistical significance was determined at $P < 0.05$. Linear and quadratic patterns of biological responses to PB inclusion levels were studied via polynomial contrast analysis. Inclusion of PB improved ($P < 0.05$) laying rate, egg mass, and feed conversion ratio, compared with CON, and peaked at PB750. Incremental levels of PB quadratically increased ($P \leq 0.01$) albumen height and Haugh unit. Increasing dietary PB inclusion level downregulated ($P < 0.05$) the AhR pathway related genes in both intestinal segments. Additionally, most of the genes related to the NF-κB pathway were downregulated ($P < 0.05$) with increasing PB inclusion level, in both the duodenum and the cecum, respectively. However, most of the antioxidant genes implicated in Nrf2 pathway were upregulated ($P < 0.05$) with increasing PB inclusion level in both intestinal sites. Overall, phytogenic inclusion upregulated cytoprotective genes, downregulated inflammation related ones, and documented further production and egg quality improvements in layers, with PB750 displaying the optimal benefits.

Key Words: Phytogenic, nutrigenomic, laying hen, performance, gut cytoprotection

Session 4

16 Extended influence of colostral cells on mucosal responses to routine health challenges in neonatal lambs. M. Donia*[1,2], J. Lowe[1,3], F. Zuckermann[1], C. Gaulke[1,4], and B. Aldridge[3,5], *[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, USA, [2]Department of Internal Medicine, College of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, [3]Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, USA, [4]Carle R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign, IL, USA, [5]Department of Biomedical and Translational Sciences, Carle Illinois College of Medicine, University of Illinois, Urbana-Champaign, IL, USA.*

Ruminant neonates rely on colostral immunoglobulin and nutrients to support health, welfare, and performance, by protecting against infectious disease and providing early life resilience. Recent evidence suggests that transferred colostral cells also contribute to neonatal immunity under controlled experimental conditions. This study provides evidence that colostral cells enhance neonatal resilience to routine, early-life, and mucosal health challenges in commercial lambs. A total of 78 lambs were tube-fed cell-rich (CRC, n = 39) or cell-free (CFC, n = 39) colostrum in 3 feedings within 12 h of birth. Lamb health was evaluated using body weight gain and natural disease incidence over time. Validated, observational scoring systems and thermal imaging were used to quantify peri-scrotal tissue swelling, skin temperature, and wound healing in male CRC (n = 13) and CFC (n = 16) lambs for 7 weeks post-castration. Fecal coccidial oocyst shedding was monitored in all lambs over 60 d of age, for 3 consecutive weeks. Statistical analysis employed a mixed linear model for intergroup effect, a repeated measure ANOVA for time effect, and Levene's test for homogeneity and variability. The results showed that colostral cells significantly (P = 0.02) enhanced protection against coccidial infection in 63- to 70-d-old lambs and promoted a balanced inflammatory and healing response during the post-castration period, with CRC lambs exhibiting a significantly higher post-castration healing score at d 7 (P = 0.001), 35 (P = 0.002), and 42 (P = 0.03) and a lower post-castration swelling score at d 7 (P = 0.001), 28 (P = 0.04), and 35 (P = 0.017) compared with CFC lambs. This study shows that transferred colostral cells play an extended homeostatic role in intestinal mucosal health and in post-castration tissue inflammatory and healing responses. Although further research is needed to characterize the functional basis for these findings, the notion that colostral cells provide important benefits to neonatal health and welfare has significant implications for colostral management practices in livestock production systems.

Key Words: Colostrum, mucosal immunity, lambs, coccidia, healing

17 Glycerides of lauric acid supplementation in the chicken diet enhances the humoral and cellular immune response to infectious bronchitis virus. A. Mellouk[1], V. Michel[1], N. Vieco-Saiz[1], H. Yakout*[2], N. Evans[2], O. Lemâle[3], T. Goossens[4], and J. Consuegra[1], *[1]Adisseo France S.A.S., Department of R&I in Monogastric Animal Nutrition, Saint Fons, France, [2]Adisseo US, Raleigh, NC, USA, [3]Adisseo Belgium, Sint-Niklaas, Belgium, [4]Adisseo Netherlands B.V., Raamsdonksveer, the Netherlands.*

With the increase of pathogen infection risks and the ambition to reduce medication in the poultry industry, various approaches are used to complement the vaccination strategies and enhance the immune response. Glycerides of lauric acid are candidates of interest, possessing antimicrobial effects and abilities to modulate T and B cells' responses. This study aims to explore the effects of glycerides of lauric acid (GLA) supplementation in chickens' diets on the levels of humoral and cellular responses to viral infections, employing an infectious bronchitis virus (IBV) vaccine model. One-day-old Ross 308 broilers were administered live attenuated IBV via eye-nose drops and provided with diets supplemented or not with GLA at 3 kg/t. Broilers fed the GLA-supplemented diet exhibited significantly higher sera levels of specific anti-IBV on d 7 (P < 0.05), indicative of an enhanced primary immune response. Basal cytokine secretions of pan (IL-2 and IL-16), Th1 (IFN-γ), Th17 (IL-21), and regulatory (IL-10) T cell responses were similar in the spleens of both vaccinated groups. In contrast, splenocytes from broilers fed with GLA displayed heightened activation and effector abilities, as demonstrated by IFN-γ ELISpot quantification after 24 h of exposure to IBV antigens or an antigen-independent mitogen (Con A). The GLA group's splenocytes exhibited a 2-fold increase in spot numbers and a 3-fold increase in spot surfaces (P < 0.01) in response to the N 261–280 peptide, as well as a 2-fold increase in spot surfaces and numbers (P < 0.01) upon Con A stimulation. In summary, 2 significant findings emerged from the study. First, GLA supplementation in the feed enhances the intensity of the primary humoral immune response in broilers. Second, GLA elevates global and specific cellular immune responses, mediated by Th1 and Cytotoxic T lymphocytes. In conclusion, in vivo results indicate that supplementation with glycerides of lauric acid in the diet strengthens chicken resilience against pathogenic challenges by fortifying their immune responses.

Key Words: Glycerides of lauric acid, infectious bronchitis virus, humoral and cellular immune response

Poster Session

100 Genomic analysis and adhesion characteristics of enterotoxigenic *Escherichia coli* F4 and F18 strains on porcine intestinal epithelial cell lines. C. Li[1], D. Liu[1], N. Gallina[1], N. Horn*[2], and A. Bhunia[1], *[1]Purdue University, West Lafayette, IN, USA, [2]United Animal Health, Sheridan, IN, USA.*

Background: Enterotoxigenic *Escherichia coli* (ETEC) strains are the primary perpetrators of colibacillosis in piglets, resulting in mortality and agrobusiness economic woes. ETEC expressing fimbrial antigens, F4 and F18, are the predominant contributors to colibacillosis. F4 is most prevalent in neonatal diarrhea. F18 is more common in post-weaning colibacillosis. Intestinal epithelial interaction and colonization are crucial steps toward colibacillosis. Understanding the genotypic virulence properties and their interaction with swine intestinal cells can help develop mitigation strategies. Methods: We investigated 3 colibacillosis-causing F18 strains (3EC1, 27EC1, and 3247EC) isolated from swine in Iowa and Maryland and a single human ETEC isolate (O78:H11). We compared their whole genome sequence (WGS), virulence gene profiles, and adhesion characteristics to swine intestinal IPEC-1 and IPEC-J2 cell lines. Results: All F18 isolates were β-hemolytic and positive for the fimbrial gene, *fedA.* F4 was positive for *k88c* analyzed by PCR. WGS of 3 F18 strains and F4 revealed sequence variation associated with flagellin, fimbriae, and lipopolysaccharide (LPS) without any differences in toxin gene profiles (EAST1, OrtT, RatA, etc.). No F18 strains possessed genes that encode heat-labile (LT), heat-stable (STa, STb), or Shiga toxins (Stx1, Stx2, or Stx2e). Adhesion of F18 to IPEC-1 and IPEC-J2 cell lines showed variable results after 30 min without statistical significance. Likewise, the F4 strain showed similar adhesion to both cell lines. The lactate dehydrogenase (LDH)-based cytotoxicity assay and microscopic examination showed no cell damage during the 30-min ETEC exposure. Significance: Despite a significant genotypic difference between F18 and F4 strains, all showed strong interaction with pig intestinal epithelial cell lines, IPEC-1 and IPEC-J2. The results offer insights into developing more effective mitigation measures using these cell models.

Key Words: ETEC, genomics, swine, adhesion

101 Effect of feeding a direct-fed microbial (DFM)-supplemented diet on the microbiome of young turkeys. D. Ayala*[1], E. Kimminau[1,2], N. Evans[1,3], and T. P. Karnezos[1], *[1]Purina Animal Nutrition Center, Gray Summit, MO, USA, [2]Elanco, Bentonville, AR, USA, [3]Adisseo, Alpharetta, GA, USA.*

Cellulitis is one of the leading causes of morbidity and mortality in commercial turkeys in the United States. It is also one of the major causes of carcass condemnation at slaughter, with significant economic losses for turkey producers. The supplementation of diets with direct-fed microbials (DFM) confers health benefits to the host by reducing pathogenic load and increasing performance. The aim of this study was to characterize the microbial community of 5-week-old turkeys fed a customized DFM product, GDP600, targeting the 2 major bacterial pathogens associated with cellulitis, *Clostridium septicum* and *Clostridium perfringens*, and to compare it to the microbial community of 5-week-old turkeys on a non-DFM diet. Ileum samples from 10 turkeys fed a (1) DFM-supplemented diet (DFM group) and (2) non-DFM-supplemented diet (Control group) were collected when turkeys were between 35 and 48 d of age. The microbial profile of all samples was characterized by 16S metagenomics. The ileal microbiome of DFM group was found dissimilar to Control group ($P < 0.05$); the microbiome of turkeys fed GDP600 was dominated by *Lactobacillus* spp. with *Lactobacillus aviarius* being the most abundant, with 27.65% versus 7.83% in the Control group. The concentrations of the targeted pathogenic microorganisms *C. septicum* and *C. perfringens* were found reduced in the DFM group, with 0.06% and 0%, respectively, compared with 1.17 and 0.99% in the non-DFM group. *Escherichia coli* was also found in reduced concentrations in the DFM group with a 2.59% relative abundance compared with 6.20% in the non-DFM group. The most commonly isolated bacteria were *Lactobacillus plantarum* and *Lactobacillus johnsonii* from DFM group and *E. coli* and *C. perfringens* from the non-DFM group. Results from this study highlight important differences in the microbiome of young turkeys fed a DFM-supplemented diet versus turkeys in a Control group. Targeted *Clostridium* bacteria, previously identified as the causative agents of bacterial cellulitis, were found at lower concentrations in the DFM group, potentially reducing the risk of cellulitis infection later in life.

Key Words: Cellulitis, gut health, DFM supplementation, pathogen reduction

102 Impact of microencapsulated fermentation extracts, essential oils, and organic acids on productivity and fecal microbiota of dairy cows. S. E. Izzo Crespo*[1,2], O. Villalobos[2], O. AlZahal[2], L. Lahaye[2], and M. Costa[1], *[1]University of Montreal, Saint-Hyacinthe, QC, Canada, [2]Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada.*

The objective of this study was to investigate the effects of a novel blend of fermentation extracts, essential oils, and organic acids P(F+EO+OA) on markers of gut health and productivity of multiparous Holstein cows. The blend was rumen-protected by microencapsulation and engineered for synchronized release and optimal synergy of its active ingredients in the small intestine. Thirty close-up cows in a commercial herd were enrolled in the trial based on their anticipated calving dates using a crossover design. Cows received a corn silage and haylage-based diet. Control cows received the carrier only and subsequent test cows received 10 g/d of P(F+EO+OA) throughout the transition period (−21 to +21 d relative to calving). Milk yield, milk composition, and rumination time were recorded throughout the trial. Blood BHBA, BCS, and fecal samples were collected on d −21, −7, +7, and +21 relative to calving. Performance data were analyzed using Proc Mixed of SAS 9.4. The model included period (P[F+EO+OA vs. Control]) or test day (where applicable) as the fixed effect and cow as a repeated measurement. Bacterial 16S rRNA was sequenced using the Illumina platform. Cows that received P(F+EO+OA) had a greater ($P < 0.05$) milk yield (54.8 vs. 47.0 kg/d), protein content (3.42 vs. 3.26%) and yield, increased rumination time (+45 min/d) and ECM yield ($P = 0.07$) compared with control. Tested cows had greater BCS and lower blood BHBA (0.66 vs. 0.97 mmol/L) on d 7 ($P < 0.05$). AMOVA indicated a significant separation of fecal microbiota composition

according to day of sampling (d −21 from d −7 and d +7 but not d +21, $P < 0.05$). There was no difference in composition due to treatment, suggesting the product is acting at the duodenal and cecal level. Further taxonomic analyses are being conducted to investigate shifts in key bacterial groups across treatment. Our findings support that cows supplemented with P(F+EO+OA) had a greater nutrient output through their gastrointestinal tract due, in part, to improved overall nutrient degradability and utilization in the proximal digestive tract.

Key Words: Transition cow, gut health, fecal microbiome, milk yield

103 Receptor-targeted next-generation probiotics ameliorate inflammation and promote gut health. N. Gallina* and A. Bhunia, *Purdue University, West Lafayette, IN, USA.*

Background: Intestinal barrier dysfunction, inflammation, and elevated expression of heat shock protein 60 (Hsp60) are features of the dysbiotic gut. Probiotics can alleviate inflammation but are ineffective due to poor adhesion and adaptation to the inflamed bowel. We hypothesize that enhancing probiotic adhesion to intestinal cells may augment the immunomodulatory response, mucosal healing, and tight junction restoration. Earlier, we identified *Listeria* adhesion protein (LAP; 94-kDa) that aids *Listeria* attachment to the epithelial cells by interacting with Hsp60. Next-generation bioengineered *Lactobacillus casei* (Lbc) probiotics (BLP) expressing LAP from *L. innocua* showed strong interaction with epithelial Hsp60, high immunomodulatory response, and epithelial barrier integrity while reducing inflammation. Method: We fed BLP for 10 d to mice pretreated with dextran sulfate sodium (DSS, 2% for 7 d), as a chemically induced inflamed gut model. Results: BLP-fed DSS-treated mice gained 5% body weight compared with the DSS-treated mice that did not receive any probiotics during that period. BLP feeding conferred a 67% reduction in disease activity index compared with the control and 50% to LbcWT-treated mice. BLP treatment restored fecal consistency to Type 3–4 (Bristol scoring) within 9 d of feeding, while the control and LbcWT treatment groups failed. BLP-fed mice showed improved gut barrier; >50% reduction in FITC-labeled 4-kDa dextran (FD4) permeability compared with LbcWT or the control group. DSS-induced shortened colon length, abdominal adhesions, and mucus accumulation were substantially improved by BLP feeding relative to the control and LbcWT groups. Colon pathology, including neutrophil infiltration of the BLP-fed group, was 50% lower than the LbcWT group. BLP-fed mice also showed reduced Hsp60 expression compared with the control groups. Conclusions: BLP feeding ameliorated gut inflammation, thus offering a potential dietary supplement for reducing gut-associated inflammation and improving gut health in livestock.

Key Words: Next-generation probiotics, *Listeria* adhesion protein, inflammation

104 Microbiome characterization and identification of potential causative agents of tail necrosis in pigs. D. Grum*, D. Ayala, K. Bamesberger, B. Trible, D. McManus, and T. P. Karnezos, *Purina Animal Nutrition Center, Gray Summit, MO, USA.*

Tail docking of pigs is banned in many countries based on animal welfare concerns. An unintended consequence of the ban can result in the loss of tail integrity from bacterial pathogens causing inflammation, lesions, and necrosis which impacts pig welfare and results in significant monetary losses. Tail docking is used in many countries as a preventive measure; however, in these countries, an intact tail is a desirable trait in pigs and can increase value in certain markets. This study aimed to use 16S microbiome for the characterization of the microbial community of inflamed and necrotic tails and compare those to the microbial communities of healthy tails. A total of 60 rectal swabs and 60 tail (20 necrotic, 20 inflamed, 20 healthy) samples were used in this study for 16S microbiome analysis and bacterial isolation. A corresponding duplicate set of samples was taken for bacterial isolation. Linear discriminant analysis of the microbiome identified *Fusobacterium necrophorum*, *Streptococcus dysgalactiae*, and *Staphylococcus hyicus* as the potential causative agents of tail necrosis due to their increased relative abundance in sick versus healthy animals. These pathogens were found in trace amounts in fecal samples of the healthy pigs, and in significantly higher abundance in the samples from affected pigs. These results were confirmed by plating, with *Streptococcus dysgalactiae* and *Staphylococcus hyicus* being isolated exclusively from fecal samples, and samples from inflamed and necrotic tails from the sick animals group. A total of 20 proprietary direct-fed microbials (DFM) were evaluated for their antimicrobial properties against the isolated pathogens by using a combination of agar well-diffusion (AWD) and competitive exclusion (CE) screenings. The most inhibitory DFM strains were used to develop a customized DFM product that is being used at swine facilities with cases of tail inflammation and necrosis. The results of this study suggest a potential translocation of these pathogens from the gut to the dermis, resulting in inflammation and tail lesions.

Key Words: Tail necrosis, swine, gut health, translocation, DFMs

105 *E. coli* pathotypes identified through 16S microbiome and bacterial isolation in pigs with post-weaning diarrhea (PWD). D. Ayala*, D. Grum, K. Bamesberger, B. Trible, D. McManus, and T. P. Karnezos, *Purina Animal Nutrition Center, Gray Summit, MO, USA.*

Post-weaning diarrhea (PWD) is a serious health and welfare problem for the global swine industry. PWD is a multifactorial condition occurring the first 10–14 d after weaning; it is generally associated with the proliferation of pathogenic *Escherichia coli* groups, specifically enterotoxigenic *E. coli* (ETEC); however, PWD is also associated with several viral infections. Antibiotics are commonly used as a control strategy against *E. coli* infections; however, the increase of antimicrobial resistance and the push toward antibiotic-free pigs highlight the importance of identifying alternative interventions. The purpose of this study was to determine the underlying bacterial cause of PWD and a potential vertical transmission from sows to pre-weaned piglets, as well as to determine whether a customized direct-fed microbial (DFM)-based product could reduce the pathogens isolated from affected pigs. A total of 120 fecal samples from a farrow to finish farm were collected for 16S microbiome analysis and bacterial isolation. These included fecal samples from (1) sows, (2) pre-weaning piglets, (3) healthy piglets after weaning, (4) and piglets with PWD. Analysis of the 16S microbiome results showed an increase in the overall level of *E. coli* in piglets with PWD compared with healthy piglets. Linear discriminant

analysis showed that the bacterium most commonly associated with sick piglets was *E. coli.* These results were confirmed by plating, with *E. coli* isolates being recovered from all samples collected in this study. Three major groups of pathogenic *E. coli* were identified and confirmed by PCR, with ETEC being the most commonly isolated, followed by enteropathogenic *E. coli* (EPEC), and enterohemorrhagic *E. coli* (EHEC). A customized DFM solution was developed to target these pathotypes with a significant bacterial reduction by using 2 different inhibitory methods. Results from this study suggest the potential interaction of multiple *E. coli* pathotypes in the development of PWD; a customized solution could be used as an alternative intervention to reduce these pathogens and the incidence of PWD.

Key Words: Post-weaning diarrhea, pathogenic *E. coli*, antimicrobial effect, DFMs

106 Assessment of antimicrobial effect of potential methanogenic inhibitors against enterohemorrhagic *Escherichia coli* and *Salmonella* in bovine rumen contents. R. C. Anderson* and R. B. Harvey, *United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, USA.*

New technologies are needed to help livestock producers maintain optimal health and wellbeing in their animals while minimizing risks of propagating pathogenic bacteria to humans or other animals. Where possible, these interventions should contribute to the efficiency and profitability of animal production so as to avoid passing higher costs on to the consumer. Methane production in the rumen results in the loss of 2–12% of the gross energy consumed by the host, costing the US cattle feeding industry as much as $700,000/day. Rumen methanogenesis also contributes >20% of the US emission of this greenhouse gas. Results from *in vitro* incubations of freshly collected rumen fluid treated without or with potential methanogenesis inhibitors revealed that nitrocompound treatment inhibited methanogenesis ($P < 0.05$) by 43–98% after 24 h compared with untreated controls. Methane production was also decreased by 57% ($P < 0.05$) in incubations treated with 9 m*M* lipoic acid but not with treatments of 9 m*M* taurine or cysteine sulfinic acid. When tested against anaerobically-grown (in phosphate-buffered tryptic soy broth, pH 6.7) pure cultures of *Salmonella* Typhimurium DT104 and *Escherichia coli* O157:H7, the potent methane inhibitor ethyl nitroacetate (9 m*M*) decreased ($P < 0.05$) mean specific growth rates by 26 and 36%, respectively, compared with that of controls (0.481 ± 0.05 and 0.357 ± 0.02 h^{-1}, respectively). Ethyl nitroacetate was bacteriostatic rather than bactericidal, as evidenced by nearly equivalent ($P > 0.05$) maximum optical densities (0.40 ± 0.01 and 0.39 ± 0.03 at 600 nm, respectively) after 24-h incubation. The methane inhibitors nitroethane and 3-nitropropionate were ineffective in inhibiting *Salmonella* Typhimurium DT104 or *E. coli* O157:H7, although 9 m*M* lipoic acid decreased ($P < 0.05$) growth rates and maximal optical densities of *Salmonella* Typhimurium DT104 by 98 and 65% and of *E. coli* O157:H7 by 93% and 59%, respectively. Mechanistically, evidence indicates that ethyl nitroacetate disrupts hydrogen transfer reactions.

Key Words: Antibacterial, antimethanogenic, nitrocompound, *E. coli*, *Salmonella*

107 Microencapsulated essential oils supplementation helps maintain growth performance and intestinal health during coccidiosis challenge in broilers. H. H. Salgado*, G. Tactacan, and L. Lahaye, *Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada.*

Coccidiosis is an enteric disease that causes important economic loss to the poultry industry. Due to the presence of drug-resistant *Eimeria* strains and concerns about drug residues in the meat, microencapsulated essential oils are becoming of interest as new alternatives for coccidiosis control. Thus, the objective of this study was to test the anticoccidial effectiveness of a commercially available microencapsulated proprietary blend of essential oils P(EO) in broilers challenged with coccidiosis. A total of 720 male broiler chickens (Ross 308) were fed with the same basal diet and randomly allocated to one of the following treatments: (1) non-challenged control, (2) challenged control, and (3) challenged + P(EO) added to the basal diet at (600 g/T). The challenge was introduced through the feed inoculated with a solution of live *Eimeria* oocysts (*E. acervulina*, *E. maxima*, and *E. tenella*; 21 K/mL) at 1 d of age. At d 28, one bird per pen was slaughtered, and lesions in the gastrointestinal tract were evaluated by "I See Inside" scoring system methodology (ISI®). A portion of the jejunum was collected for villus length and crypt depth measurements. Data were submitted to ANOVA and non-parametric test by Kruskal Wallis. Regarding growth performance, broilers challenged with coccidiosis had significantly lower BW (1,806 vs. 1,864 g) and higher FCR (1.79 vs. 1.76) than the non-challenged birds at d 35. The presence of oocysts (3.10 vs. 0.66) and inflammatory cell infiltration in the lamina propria (2.98 vs. 1.96) was increased in the coccidiosis-challenged compared with non-challenged birds at d 28. The supplementation of P(EO) mitigated the negative impact of coccidiosis in both growth performance and measures of intestinal health, preventing BW (1,891 g) and FCR (1.75) losses, while keeping oocyst counts (0.84) and status of intestinal inflammation (2.37) at similar level as the non-coccidiosis-challenged birds. In conclusion, the supplementation of P(EO) helped mitigate performance losses, decreased *Eimeria* infection, and improved intestinal health in poultry challenged with coccidiosis.

Key Words: *Eimeria*, broilers, additives

108 Effect of phytase supplementation on growth performance, nutrient retention, footpad lesion score, tibia bone mineralization, and meat quality in broilers. C. B. Lim*, V. Sampath, and I. H. Kim, *Dankook University, Department of Animal Resource and Science, No. 29 Anseodong, Cheonan, Choongnam 330-714, South Korea.*

A total of 1,050 Ross 308 broilers were randomly assigned to 1 of 7 dietary treatments with 10 replications and 15 chicks/cage. The dietary treatments were positive control (PC), negative control (NC), and NC diet supplemented with 250, 500, 1,000, 1,500, and 3,000 U/kg of phytase. This trial lasted for 35 d through 3 stages: starter (d 1–7), grower (d 7–21), and finisher (d 21–35). Broilers fed NC diet supplements with graded levels of phytase linearly improved body weight gain and feed intake during the starter phase and overall trial period. Also, they showed a tendency to increase ($P < 0.1$) daily feed intake during the overall experimental

period. However, nutrient utilization of broilers showed neither increase nor decrease with PC and NC diet until d 35, but NC diet supplement with a graded dose of phytase showed linearly increased dry matter, gross energy, calcium, ash, and phosphorus digestibility, and tendency to increase nitrogen digestibility at the end of the starter phase. Unlike the starter phase, broilers fed the PC diet had significantly increased ash and Ca digestibility at the end of the grower and finisher phases compared with those fed the NC diet. Additionally, broilers fed the NC diet supplements with a graded level of phytase showed linearly increased ash, Ca, and P digestibility at the end of d 21 and 35. Furthermore, the nutrient digestibility of GE was linearly increased ($P < 0.05$) at the end of d 35. Moreover, the inclusion of graded levels of phytase in NC diet showed significantly increased (<0.0001) bone (ash, Ca, and P) mineralization in broilers. They also showed linearly increased ($P < 0.05$) gizzard weight, breast muscle color lightness, redness, pH, and decreased cooking loss and drip loss (at d 5 and d 7). We infer that feeding an NC diet supplemented with greater than 1,000 units (U)/kg concentrations of phytase could be beneficial to improve body weight gain, feed intake, and nutrient utilization, and to increase tibia bone mineralization and meat quality in Ross 308 broilers.

Key Words: Phytase, broilers, growth performance, nutrient digestibility

109 Inclusion of xylanase supplement to high- and low-density diets reveals a positive result on growth performance, nutrient digestibility, gas emission, and fecal microbiota in growing pigs. C. B. Lim*, S. T. Wahid, and I. H. Kim, *Department of Animal Resource and Science, Dankook University, Cheonan, South Korea.*

Corn-soybean meal-based diets are widely implemented in pigs' diets. However, corn and soybean meal contain 10% and 20% non-starch polysaccharides (NSP), respectively, and can adversely affect the nutritional value of such diets, as monogastrics do not produce xylanase. Therefore, this study aims to evaluate the effects of high- and low-density diets with xylanase supplementation on growth performance, nutrient digestibility, fecal microbiome, backfat thickness, and lean meat percentage in growing pigs. For a period of 6 weeks, a total 80 growing pigs ([Landrace × Yorkshire] × Duroc; 24.23 ± 2.43 kg) were randomly assigned to 1 of 4 treatments in a 2 × 2 factorial design with 2 different levels (high and low) of nutrient density diet with or without 0.01% xylanase supplement. At the end of wk 3, pigs fed diet supplements with 0.01% xylanase showed significantly increased body weight, average daily gain, and daily feed intake. Additionally, at the end of wk 6 and the overall experimental period, pigs fed high-density (HD) diet with 0.01% xylanase showed significantly increased body weight, average daily gain, and daily feed intake. The inclusion of an HD diet with 0.01% xylanase significantly increased the nutrient digestibility of dry matter, nitrogen, and energy compared with those fed low-density (LD) diet supplement with or without xylanase. In addition, pigs fed HD diet with 0.01% xylanase had significantly increased fecal *Lactobacillus* count at the end of wk 6. Furthermore, the inclusion of 0.01% xylanase to HD diet significantly decreased NH_3 and H_2S emissions compared with those fed LD diet supplements with or without xylanase. However, pigs fed neither HD nor LD diet supplement with or without xylanase showed no difference in their backfat thickness and lean meat percentage. Based on the results, we infer that dietary xylanase with HD diet would be beneficial to increase the growth performance, nutrient digestibility, and reduced gas emission in pigs.

Key Words: Growth performance, nutrient digestibility, backfat thickness, lean meat, xylanase

110 Supplementation of a medium-chain fatty acid blend and a phytogenic feed additive improved performance of pigs challenged with *Escherichia coli*. J. C. González-Vega*, S. May, B. Smith, K. Moran, and E. Teddy, *Cargill Animal Nutrition, Lewisburg, OH, USA.*

This study was conducted to investigate the effects of medium-chain fatty acid blend (MCFA) and a phytogenic feed additive (Fresta® Protect, Delacon, Austria) on growth performance and gut health of *Escherichia coli* (ETEC)-challenged pigs. A total of 200 weaned pigs with initial body weight of 5.52 ± 0.17 kg were allotted to 40 pens (5 pigs/pen) blocked by BW and randomly assigned to 5 treatments: (1) negative control (NC), (2) NC + 55 mg/kg carbadox (positive control; PC), (3) NC + 0.6% MCFA, (4) NC + 0.1% phytogenic, or (5) NC + 0.6% MCFA and 0.1% phytogenic. The study lasted 18 d, and on d 5 and 6, all pigs were orally inoculated with 5 mL of ETEC (blend of F4 and F18+) with a final titer of 10^{10} cfu/pig. Body weights and feed intake were recorded on d 0 and 18, with additional weight of pigs on d 4 and 11. Fecal scores were assessed daily, and a fecal swab was collected on d 12 to analyze ETEC shedding. Data were analyzed using the lme4 package of R 4.1.2, with treatment as fixed effect and BW block as random effect. Contrasts were used to compare individual treatments to NC. During the pre-challenge period, pigs fed MCFA had less ($P < 0.05$) BW and ADG compared with NC group. Whereas in the post-challenge period, pigs fed PC and MCFA and phytogenic combination had greater ($P < 0.05$) final BW and ADG compared with NC, as well as phytogenic, improved ($P < 0.05$) ADG compared with NC. As overall performance, pigs fed PC and MCFA and phytogenic combination had greater ($P < 0.05$) ADG and G:F compared with NC pigs, and pigs fed MCFA had greater ($P < 0.05$) G:F than NC pigs. Stool quality was improved ($P < 0.05$) in pigs fed PC, MCFA, and MCFA and phytogenic combinations compared with NC pigs. In addition, probability of positive ETEC fecal shedding was numerically increased in pigs fed PC, phytogenic, and MCFA and phytogenic combination, and this likely resulted in the tendency to reduce mortality of pigs (2.5%) compared with NC pigs (15%). In conclusion, under ETEC challenge, supplementation of phytogenic alone or in combination with MCFA resulted in improved performance and reduction in mortality.

Key Words: *Escherichia coli*, feed additives, phytogenic, pig

111 Chicken enteroid kinome responses to *Salmonella* infection and organic acid/essential oil blend. J. Elango*[1], F. Perry[2], K. Sutton[3], J. Mitchell[3], L. Vervelde[3], E. Santin[4], L. Lahaye[4], and R. Arsenault[2], *[1]Department of Biological Sciences, University of Delaware, Newark, DE, USA, [2]Department of Animal and Food Sciences, University of Delaware, Newark, DE, USA, [3]Division of Immunology, The Roslin Institute, University of Edinburgh, Edinburgh, Scotland, UK, [4]Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada.*

Enteroids provide a 3-dimensional microenvironment of the intestine, acting as a miniature model for understanding intestinal functions and immune responses. Hence, this shows a promising application in studying disease models and drug screening, among others. *Salmonella enterica* serovar Typhimurium is one of the food-borne pathogens affecting human health through production animals due to contaminated meat consumption. Research focus on discovering new antibiotic alternatives has increased to minimize the use of antibiotics in production animals, ostensibly preventing the development of antibiotic resistance in farm animals, humans, and pathogens. One of these antibiotic alternatives is a blend of organic acids and essential oils. Here, we use the enteroid model to understand host responses and disease pathogenesis toward *Salmonella enterica* infection and the antibiotic alternative from an immunometabolic perspective using the species-specific kinome array technique. The experimental groups include enteroids alone, + *Salmonella*, + 0.25 mg/mL product, + 0.5 mg/mL product, + 0.25 mg/mL product and *Salmonella*, and 0.5 mg/mL product and *Salmonella*. We performed statistical data analysis on the kinome array data and finalized 4 significant signal transduction pathways using a *P*-value and a false discovery rate of <0.05. These are insulin signaling pathway, T cell receptor pathway, PI3K-AKT signaling pathway, and FoxO signaling pathway. Results show an inclusion rate of 0.5 mg/mL of product, and the *Salmonella* inoculation results in an immune stimulatory response. The inclusion rate of 0.5 mg/mL with *Salmonella* and 0.25 mg/mL with and without *Salmonella* results in a balanced effector response downstream of the PI3K-Akt pathway. Our future work includes analyzing significant fold changes occurring in peptides of the respective signal transduction pathway and their relevance to the effect on the enteroids and the *Salmonella*-infected enteroids. This work will help us understand the ability of the enteroids to act as an alternative model of the chicken gut, providing a rapid way to screen antibiotic alternatives and their compatibility through the host responses against them.

Key Words: *Salmonella*, immunometabolism, enteroids

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