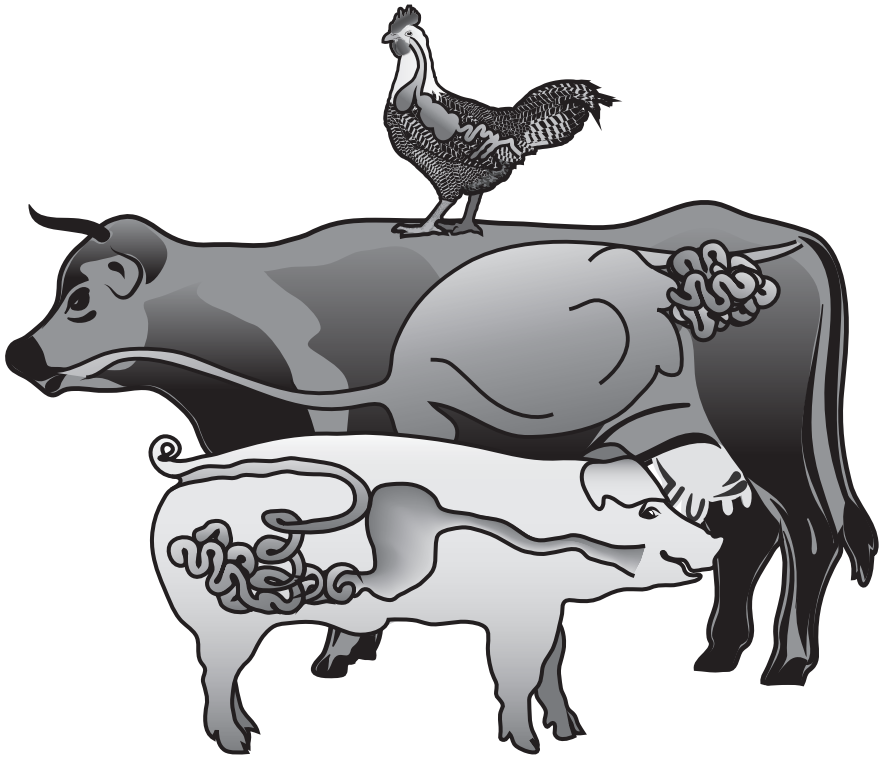
Symposium on Gut Health

in Production of Food Animals

September 8–11, 2026

Virtual Event



Program and Abstracts

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Program

Tuesday, September 8

INTESTINAL BIOLOGY AND ADDITIVES
10:00 AM–2:00 PM

10:00 AM **Defining intestinal homeostasis as a predictive and mechanistic framework for poultry performance.**
Dr. Fernández-Miyakawa.

11:00 AM 100 **The regulatory effect of tree fungi on barrier function in the porcine small intestine.**
B. U. Metzler-Zebeli*, S. Sharma, S. Koger, and L. Schwarz, *University of Veterinary Medicine Vienna, Vienna, Austria.*

11:30 AM 101 **A physiologically relevant in vitro simulation of the chicken gastrointestinal tract to advance gut health research and feed additive evaluation.**
S. Molck*, L. Bauer, and A. Hüser, *Evonik Operations GmbH, Halle-Künsebeck, Germany.*

11:45 AM 102 **Age-dependent stromal niche remodeling and butyrate responsiveness during early postnatal gut development in piglets.**
H. Ayansola, B. Girma, V. Brikshaus, A. Riesner, and Y. Jin*, *Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA.*

12:15 PM 103 **Differential oxidative stress responses and phytogenic antioxidant protection in apical-out chicken enteroids.**
Z. Zhu*, I. Ferraro, and Y. Li, *University of Delaware, Newark, DE, USA.*

12:30 PM **Break.**

WORKSHOP: WHY ARE BEST PRACTICE APPROACHES NEEDED IN FOOD ANIMAL MICROBIOME RESEARCH?
1:00 PM–2:00 PM

This workshop is open to all registrants. Best practice methodologies regarding poultry microbiome research experimental design and analysis will be reviewed. Implementation of best practice approaches across the microbiomes of other food animal species will be discussed. During the workshop, the floor will be open to other research groups (swine, ruminant, aquaculture) to discuss their challenges in microbiota experimental design and analysis as well as the possibility of best practice informed protocol development in other production animals.

1:00 PM **Workshop led by Dr. Monika Weglarz and Dr. Joshua Lyte.**

Wednesday, September 9

MICROBIAL ECOSYSTEMS
10:00 AM–2:00 PM

10:00 AM 104 **A tale of two ecosystems: The ruminal and hindgut microbial populations impact animal health, food safety, and cattle productivity.**
H. Perez, A. Osorio-Doblado, J. Louenco, F. Fluharty, and T. Callaway*, *Department of Animal and Dairy Science, University of Georgia, Athens, GA, USA.*

11:00 AM 105 **Effects of copper carbonate on growth performance and health of broilers challenged with necrotic enteritis.**
J. C. Gonzalez Vega*[1], J. Small[1], J. Bzura[1], A. Batal[2], and J. L. McNaughton[3], [1]*Old Bridge Minerals, Old Bridge, NJ, USA,* [2]*ABB Nutrition, Petal, MS, USA,* [3]*AHPharma Inc., Hebron, MD, USA.*

11:15 AM 106 **Early inoculation with a competitive exclusion product reshapes luminal and mucosal cecal microbiota in live-vaccine co-inoculated chicks.**
C. Achard*[1], A. Sacy[1], M. Bernardeau[1,2], and E. Chevaux[1], [1]*Lallemand SAS, Blagnac, France,* [2]*Université de Caen Normandie, Caen, France.*

11:45 AM 107 **In-feed cranberries enhance microbial resilience and restructure gut microbiota during necrotic enteritis in broiler chickens.**
S. Abdallah[1], D. Boney Jr.[1], A. Smith[1], P. Tamatey[2], D. Bryan[2], and A. Yitbarek*[1], [1]*University of Delaware, Newark, DE, USA,* [2]*The Pennsylvania State University, University Park, PA, USA.*

12:15 PM 108 **Size-dependent internalization and effects of micro- and nanoplastics on oxidative stress and barrier integrity in apical-out chicken enteroids.**
I. Ferraro*, Z. Zhu, A. Hadimundeen, and Y. Li, *University of Delaware, Newark, DE, USA.*

12:30 PM 109 **Field-applicable protocol for integrated analysis of microbiome function: A case study in nursery piglets.**
V. Blanvillain*[1], M. Alves da Costa[1], C. Paez Rodriguez[2], and G. Cordero[1], [1]*AB Vista Inc., Plantation, FL, USA,* [2]*Encipharm, La Reina, Chile.*

1:00 PM 110 **Dietary butyrate glycerides enhance piglet resilience to ETEC-induced postweaning syndrome.**
A. Mellouk*[1], N. Vieco-Saiz[1], O. Lemâle[1], V. Michel[1], A. M. Gutierrez-Montes[2], and J. Consuegra[1], [1]*Swine Innovation and Customer Success, Adisseo France SAS, European Laboratory of Innovation, Science and Expertise (ELISE), Lyon, France,* [2]*University of Murcia, Murcia, Spain.*

1:30 PM **Q&A: Open discussions.**

Thursday, September 10

NUTRITION-HOST-MICROBE INTERACTIONS AND INTESTINAL PHYSIOLOGY
10:00 AM–2:00 PM

10:00 AM **Host intestinal response to diets and microbial shifting: Implications for intestinal health and growth of nursery pigs.**
Dr. Sung Woo Kim.

11:00 AM 111 **Performance, diarrhea severity, hematology, and fecal microbiome responses to iron sources without pharmacological zinc oxide.**
L. Alves Rodrigues*[1], M. Ethier[2], K. Valiquette[2], A. Bussières[2], J. Richard[1], C. Bédard[3], and M. P. Létourneau Montminy[4], [1]*Zinpro Corporation, Eden Prairie, MN, USA,* [2]*Agri-Marché, Saint-Isidore, QC, Canada,* [3]*University of Montréal, Saint-Hyacinthe, QC, Canada,* [4]*Laval University, Québec, QC, Canada.*

11:30 AM 112 **Probiotic-mediated functional modulation of the intestine supports layer performance and reduces transovarian *Salmonella* transmission.**
M. A. Amalaradjou*, *University of Connecticut, Storrs, CT, USA.*

12:00 PM 113 **In ovo hyodeoxycholic acid enhances intestinal development and enteroendocrine function in broiler chickens.**
H. Abdallah*, Z. Zhaoyan, I. Ferraro, and Y. Li, *University of Delaware, Newark, DE, USA.*

12:15 PM 114 **Effects of maternal and postweaning dietary live yeast supplementation on growth performance, nutrient digestibility, intestinal health, and colonic microbiota composition of weanling pigs.**
Y. Fu*, A. S. Lawal, T. M. Casey, T. A. Johnson, O. Adeola, and K. M. Ajuwon, *Department of Animal Sciences, Purdue University, West Lafayette, IN, USA.*

12:30 PM **Break.**

POSTER SESSION: NUTRITION-HOST-MICROBE INTERACTIONS AND INTESTINAL PHYSIOLOGY
1:00 PM–2:00 PM

Poster sessions will include 5 minutes of oral presentation followed by 5 minutes of Q&A.

1:00 PM 115 **Effects of increasing heat stress challenge on intestinal immune response in chickens.**
K. Guirrati*, Z. Pardo, J. Gené, N. París, O. González-Rodríguez, M. Ballester, and J. Tarradas, *IRTA-Animal Nutrition, Mas Bové, Constantí, Catalonia, Spain.*

1:10 PM 116 **Rumen-protected methionine modulates fecal microbiota in dairy heifers.**
D. Franco da Silva*[1], I. Crespo[1], D. Valente[1,2], T. Bordeira Gaspar[4,5], L. Martim[3], R. Lino Neto Pereira[6], R. Cordeiro[3], F. Oliveira[3], A. Londo[1], J. Wyatt[1], L. Grilo[7,8], J. Teixeira[7,8], C. Egas[9], L. Montezinho[1,7], A. Gonella-Diaza[10], [1]*Vasco da Gama University School (EUVG), Vasco da Gama Research Centre (CIVG), Coimbra, Portugal,* [2]*Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, Quinta de Prados, Vila Real, Portugal,* [3]*Coimbra School of Agriculture (ESAC), Polytechnic University of Coimbra (IPC), Bencanta, Coimbra, Portugal,* [4]*Cancer Signalling and Metabolism Group, i3S, Institute for Research and Innovation in Health, Institute of Molecular Pathology and Immunology (IPATIMUP), University of Porto, Porto, Portugal,* [5]*Veterinary Clinical Laboratory (CEDIVET), Leça do Balio, Portugal,* [6]*National Institute for Agrarian and Veterinary Research (INIAV, IP), Quinta da Fonte Boa, Vale de Santarém, Portugal,* [7]*Centre for Neuroscience and Cell Biology (CNC/UC), University of Coimbra, Coimbra, Portugal,* [8]*Centre for Innovative Biomedicine and Biotechnology (CiBB), University of Coimbra, Coimbra, Portugal,* [9]*Next Generation Sequencing Unit, Biocant, Cantanhede, Portugal,* [10]*Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL, USA.*

1:20 PM 117 **Effect of heat stress on physiological responses and milk production in dairy cattle using field-based THI observations in District Nowshera and Mardan, Pakistan.**
L. Alam*, *Luqman Alam Veterinary, Peshawar, Khyber Pukhtunkhw, Pakistan.*

1:30 PM **Q&A: Open discussions.**

Fri**day, September 11**

IMMUNITY AND INTESTINAL RESILIENCE
10:00 AM–1:00 PM

10:00 AM **Neurophysiology as an emerging measure of poultry gut health: Understanding what gut neurochemistry can tell you regarding health and performance across poultry species.**
Dr. Joshua Lyte.

11:00 AM 118 **In vitro assessment of oleuropein-enriched olive leaf extract bioactivity against enterotoxigenic porcine *Escherichia coli*: Effects on oxidative stress, epithelial integrity, and cell viability.**
T. Hassan*[1,5], S. Bagatella[1], M. I. Malik[2,1], M. A. Arif[1], S. L. Bavaro[3], S. Mioletti[1], F. R. Anjum[4,5], B. Amato[5], S. Siomko[5], M. H. Kogut[6], and M. T. Capucchio[1,3], [1]*Department of Veterinary Sciences, University of Turin, Grugliasco, Italy,* [2]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA,* [3]*Institute of Science of Food Production, National Research Council, Grugliasco, Italy,* [4]*Animal and Plant Health Agency, New Haw, Addlestone, Surrey, UK,* [5]*Bristol Veterinary School, University of Bristol, Langford, North Somerset, UK,* [6]*VETANCO USA, St. Paul, MN, USA.*

11:15 AM 119 **In-feed freeze-dried cranberries improve performance, reduce intestinal lesions, and attenuate inflammatory responses in broiler chickens challenged with necrotic enteritis.**
A. Smith*[1], S. Abdallah[1], D. Boney Jr.[1], D. Bryan[2], and A. Yitbarek[1], [1]*University of Delaware, Newark, DE, USA,* [2]*The Pennsylvania State University, University Park, PA, USA.*

11:30 AM 120 **Neurochemical metabolic pathway expression is region-dependent in the intestinal tract of the turkey.**
S. S. Wickramasuriya[2], K. Matusik[1], D. Graham[1], and J. M. Lyte*[2], [1]*University of Arkansas, Fayetteville, AR, USA,* [2]*USDA-ARS Poultry Production and Product Safety, Fayetteville, AR, USA.*

11:45 AM **Open discussion and information on the next Symposium.**

Intestinal Biology and Additives

100 The regulatory effect of tree fungi on barrier function in the porcine small intestine. B. U. Metzler-Zebeli*, S. Sharma, S. Koger, and L. Schwarz, *University of Veterinary Medicine Vienna, Vienna, Austria*.
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Impaired barrier function is a common health problem in young pigs. Tree fungi have been used in traditional medicine but are less researched in farm animals. This study aimed to investigate the effect of tree fungi on the ex vivo permeability, ion flux, related gene expression, and muscle contractibility in the porcine small intestine. Decoctions were prepared from dried *Trametes versicolor*, *Fomitopsis betulina*, *Fomitopsis pinicola*, and *Fomes fomentarius*. Changes in short-circuit current (ISC) and transepithelial tissue conductivity (GT) as indicators for net ion flux and barrier function, respectively, were measured in midjejunal tissue (n = 4) from weaned pigs (n = 2/sex) in Ussing chambers. After completion, tissues were used for gene expression experiments using quantitative PCR. The organ bath system was used to measure jejunal muscle contractibility after precontraction with acetylcholine for 15 min. Data were analyzed using the Mixed procedure in SAS. All decoctions caused less negative ISC at the jejunal epithelium ($P < 0.05$), indicating reduced secretory action. Likewise, decoctions caused an immediate effect on tissue permeability and reduced the GT by ~45% compared with the basal GT ($P < 0.05$), suggesting an instantaneous closure of tight junctions. Results at the gene expression level after 40 min of exposure to the decoctions of *T. versicolor* and *F. betulina* but not to those of *F. pinicola* and *F. fomentarius* showed increased expression of *CLDN4* compared with the control ($P < 0.05$). Depending on the fungus, decoctions seemed to activate different signal transduction pathways. Decoctions of *F. betulina* and *F. pinicola* increased the expression of *TLR6* ($P < 0.10$), whereas that of *T. versicolor* decreased *NOD1* and *CLDN1* expression compared with the control ($P < 0.05$). Of note, the expression of *SOD1* was 16% higher with *F. betulina* than with *T. versicolor* ($P < 0.05$), corresponding to the greater antioxidant capacity of *F. betulina*. Overall, results show the potential of tree fungi to support intestinal barrier function and to mitigate increased secretory action at the porcine epithelium. The observed effects should be verified in vivo.

Key words: tree fungus, intestinal permeability, pig

101 A physiologically relevant in vitro simulation of the chicken gastrointestinal tract to advance gut health research and feed additive evaluation. S. Molck*, L. Bauer, and A. Hüser, *Evonik Operations GmbH, Halle-Künsebeck, Germany*.
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Understanding poultry gut health is complex due to dynamic interactions between feed composition and the intestinal microbiota. In vivo studies face variability and ethical constraints, whereas conventional in vitro systems oversimplify gastrointestinal physiology. To address this gap, a dynamic in vitro model of the chicken gastrointestinal tract, the Dynamic Avian Intestine in vitro System (DAISy), was developed. DAISy simulates the entire avian gastrointestinal tract (GIT) from crop to colon under physiologically relevant conditions, including temperature, pH gradients, retention times, oxygen levels, enzymatic digestion, bile acids, mucus, and microbial fermentation. It incorporates ileal and cecal microbiota and enables investigation of digestion, nutrient release, fermentation, and microbial interactions. Using DAISy, the germination and outgrowth of the probiotic *Bacillus* CECT 5940 (Ecobiol) were evaluated after introduction in spore form. For spore-based probiotics, rapid germination into vegetative cells within the GIT retention time is essential for efficacy across diverse types of feed formulations to represent variations in diets across regions and feeding phases (starter and finisher). DAISy showed that *Bacillus* CECT 5940 spores remain stable throughout the upper GIT, tolerating low pH and enzymatic digestion. Germination began in the nutrient-rich duodenum and jejunum, triggered by amino acids and sugars released from feed. Under simulated physiological conditions, germination occurred within ~60 minutes. In the ileum, the strain was predominantly detected as metabolically active vegetative cells. Across all tested feed formulations, *Bacillus* CECT 5940 displayed fast, consistent outgrowth, with quicker germination in nutrient-dense starter feeds than in finisher feeds. Compared with competing strains, it exhibited significantly faster outgrowth, whereas some competitors showed delayed or incomplete germination, potentially limiting in vivo efficacy. Overall, DAISy enables data-driven decisions on probiotic efficacy under more realistic and controlled conditions.

Key words: digestive, gut health, in vitro, probiotics, simulation

102 Age-dependent stromal niche remodeling and butyrate responsiveness during early postnatal gut development in piglets. H. Ayansola, B. Girma, V. Brikshaus, A. Riesner, and Y. Jin*, *Department of Animal and Avian Sciences, University of Maryland, College Park, MD, USA*.
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Early-life intestinal development is a major determinant of productivity in swine. In piglets, the intestine is immature at birth, creating a vulnerable period when impaired gut

maturation can contribute to postweaning intestinal dysfunction. However, stromal mechanisms coordinating epithelial growth and maturation remain poorly defined. We used neonatal piglets to determine how intestinal mesenchymal stromal cells (iMSCs) regulate early postnatal gut development and respond to microbiome-derived metabolites. Jejunal iMSCs isolated from piglets at days 0, 7, and 21 showed age-dependent effects in enteroid coculture: day-0 iMSCs supported predominantly large cystic enteroids, day-7 iMSCs increased multibudded morphology, and day-21 iMSCs generated mixed structures. These phenotypes aligned with stage-specific niche factor expression, with RSPO3 highest at day 0, BMP4 and PDGFRA peaking at day 7, and SFRP1 and GREM1 increasing by day 21. Bulk RNA sequencing identified 691 differentially expressed genes across ages and defined day 7 as a transitional stromal state enriched for differentiation, cytoskeletal remodeling, and calcium channel-associated pathways. To identify microbiome-derived compounds acting through the stromal niche, we developed an AI-based image analysis pipeline for high-throughput screening in intestinal organoid coculture. Among the candidates, sodium butyrate uniquely increased organoid size only in coculture, indicating a stromal-dependent effect, whereas other metabolites acted in both enteroid-only and coculture conditions. In iMSCs, butyrate induced transcriptional programs linked to fatty acid biosynthesis, cholesterol metabolism, and PPAR/AMPK signaling, together with increased intracellular lipid accumulation. These findings support a model in which age-dependent niche remodeling and microbial metabolite responsiveness jointly shape epithelial development in the pig intestine, providing a framework for feeding strategies to improve gut health in swine.

Key words: early postnatal intestinal development, intestinal mesenchymal stromal cell, stromal niche remodeling, butyrate, AI-based organoid screening

103 Differential oxidative stress responses and phytogenic antioxidant protection in apical-out chicken enteroids. Z. Zhu*, I. Ferraro, and Y. Li, *University of Delaware, Newark, DE, USA*.
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In modern broiler production, the intestinal epithelium is highly vulnerable to oxidative stress due to high metabolic activity, continuous luminal exposure, and environmental challenges. Phytogenic feed additives (PFAs) may mitigate intestinal oxidative damage, but their mechanisms remain incompletely defined in chicken intestinal models. Menadione (MD) induces intracellular oxidative stress, modeling mitochondrial and cytosolic reactive oxygen species (ROS) associated with high metabolic demand and heat stress, whereas tert-butyl hydroperoxide (tBHP) mimics exogenous organic hydroperoxide challenge, similar to dietary or microbial-derived oxidants. This study compared MD- and tBHP-induced oxidative stress and evaluated the protective effects of tea and rosemary extracts using apical-out chicken enteroids. Enteroids were pretreated with rosemary or tea extract (100 μg/mL) for 24 h before challenge with MD or tBHP at 100, 300, or 600 μ*M* for 1, 6, or 12 h. Cytosolic and mitochondrial ROS were quantified using CellROX and MitoSOX fluorescence. Barrier permeability was assessed by FITC-dextran 4 (FD4) influx. Expressions of antioxidant genes (*SOD1*, *SOD2*, *GPX4*) and caspase-3 were quantified by RT-qPCR, and DNA damage was evaluated by comet assay. MD induced rapid dose- and time-dependent ROS accumulation in both cytosol and mitochondria ($P < 0.05$) and downregulated *SOD1*, *SOD2*, and *GPX4*, suggesting impaired antioxidant defense. In contrast, tBHP rapidly upregulated *GPX4* at 1 h, followed by a time-dependent decline, whereas cytosolic ROS was not detectable until 12 h post-stress. Both oxidants increased DNA strand breaks and FD4 permeability, indicating cellular damage and epithelial barrier disruption ($P < 0.05$). Pretreatment with rosemary and tea extract attenuated ROS accumulation, restored antioxidant gene expression, and reduced caspase-3 expression and DNA damage ($P < 0.05$). These findings demonstrate that apical-out chicken enteroids respond distinctly to different oxidative stressors and provide a physiologically relevant platform for evaluating antioxidant mechanisms of PFAs.

Key words: phytogenic feed additive, oxidative stress, enteroid, gut health, chicken

Microbial Ecosystems

104 A tale of two ecosystems: The ruminal and hindgut microbial populations impact animal health, food safety, and cattle productivity. H. Perez, A. Osorio-Doblado, J. Louenco, F. Fluharty, and T. Callaway*, *Department of Animal and Dairy Science, University of Georgia*, *Athens, GA, USA*.
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It was the best of the gastrointestinal tract; it was the worst of the gastrointestinal tract. The ruminant animal has long been defined by the function of the rumen; however, research in the past few years has shed light on the fact that the hindgut of cattle can have profound impacts on animal health, feed efficiency, dietary energy harvest, and food safety. Although it was long understood that distinct microbial ecosystems are found in the rumen versus the hindgut, the impact of each ecosystem was not well understood. However, because of the rapid ruminal fermentation of feedstuffs, the rumen has traditionally been viewed as all that matters in terms of ruminant nutrition and animal health. However, work in the past decade has demonstrated that the rumen and hindgut operate as distinct yet related ecosystems. Both play important roles in energy harvest via fermentation and capture via absorption, but the rates differ. Some members of each of these ecosystems have been correlated with improved feed efficiency, in terms of the ratio of feed consumed to gain, at least in beef cattle. Further, the presence of some specific bacterial species (e.g., *Muribaculaceae*) have been linked with marbling, which is a critical component of carcass quality and profitability. The recto-anal junction (RAJ) in cattle has long been recognized as the primary location of *Escherichia coli* O157:H7 (and other Shiga toxin-producing *E. coli*) colonization by this critical foodborne pathogen. The recent accumulation of these evidences highlights the fact that our traditional dogma that the ruminant hindgut microbial ecosystem and fermentation are of only relatively minor importance is now incorrect. In this presentation, we will discuss the evidence for this dogmatic shift, and present thoughts on what this may mean for improving the precision of feeding cattle.

105 Effects of copper carbonate on growth performance and health of broilers challenged with necrotic enteritis. J. C. Gonzalez Vega*[1], J. Small[1], J. Bzura[1], A. Batal[2], and J. L. McNaughton[3], [1]*Old Bridge Minerals, Old Bridge, NJ, USA,* [2]*ABB Nutrition, Petal, MS, USA,* [3]*AH-Pharma Inc., Hebron, MD, USA*.
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Two experiments were conducted to determine the efficacy of Cu carbonate relative to other Cu sources at varying dietary inclusion levels in necrotic enteritis (NE)-challenged broilers. In experiment (Exp.) 1, a total of 2,560 mixed-sex Ross 708 broilers were randomly assigned to 1 of 8 treatments (trt; 8 pens/trt and 40 birds/pen). Diets without supplemental Cu served as the negative control (NC), whereas diets supplemented with bacitracin MD served as the positive control (PC). The other 6 diets contained 3 different levels of Cu (125, 180, and 250 ppm) from either Cu sulfate or Cu carbonate (Emerald-C). Birds were challenged with NE. Growth performance was evaluated and on d 49, crop samples were collected for bacteria count, and intestinal lesions were evaluated. Overall FCR improved (P <0.05) at 180 ppm of Cu sulfate and 250 ppm of Cu carbonate compared with NC and were not different from PC. Added Cu reduced (P <0.05) mortality and intestinal lesions scores, regardless of the level or source compared with NC. Additionally, dietary Cu concentrations greater than 180 ppm Cu reduced (P <0.05) crop bacteria counts regardless of the source compared to NC. In Exp. 2, a total of 3,360 mixed-sex Ross 708 broilers were randomly assigned to 1 of 7 dietary trt, (60 birds/pen, 7 pens/trt). Diet without supplemental Cu served as the NC. The remaining 6 diets consisted of 3 supplemented Cu levels (75, 125, or 175 ppm) from either tribasic Cu chloride (TBCC) or Cu carbonate (Emerald-C). Birds were challenged with NE. Performance was recorded, and on d 49, crop and intestinal samples were collected for *Escherichia coli* enumeration, and intestinal lesions were evaluated. The BW of birds fed 125 or 175 ppm supplemental Cu, regardless of Cu source, increased ($P < 0.05$) and crop *E. coli* counts decreased ($P <$ 0.05) compared with NC. All supplemental Cu treatments improved ($P < 0.05$) FCR and reduced ($P < 0.05$) intestinal lesion scores compared with the NC, regardless of Cu source. In conclusion, Cu carbonate is an effective Cu source for broilers, improving growth performance and bird health under NE challenge conditions, regardless of the level of supplemental Cu provided.

Key words: copper, level, source

106 Early inoculation with a competitive exclusion product reshapes luminal and mucosal cecal microbiota in live-vaccine co-inoculated chicks. C. Achard*[1], A. Sacy[1], M. Bernardeau[1,2], and E. Chevaux[1], [1]*Lallemand SAS, Blagnac, France,* [2]*Université de Caen Normandie, Caen, France*.
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In poultry, the absence of maternal microbial transfer under commercial hatchery conditions delays gut microbiota establishment and early immune maturation. Competitive exclusion (CE) products, administered after hatching, can help establish a protective microbiota, but their effects on mucosa-associated communities—critical for immune development—remain poorly characterized. Meanwhile,

live attenuated vaccines are routinely applied early in life and may also influence gut microbial composition. As CE products and vaccines are increasingly co-administered, understanding their interaction during this sensitive window is essential. This study evaluated the impact of a CE product (AviGuard, Lallemand SAS) co-administered with either a *Salmonella* Enteritidis vaccine (Cevac Salmovac, Ceva) or a coccidiosis vaccine (EVALON, HIPRA) on cecal luminal and mucosal microbiota in broiler chicks. Two independent trials were conducted in day-old ROSS 308 birds receiving vaccine alone or combined with CE. Cecal content and mucosal samples were collected at day 10 or day 5. Microbiota analyses were conducted using 16S rRNA gene sequencing, followed by DADA2 processing and statistical analyses with mixed models and ANOVA. With the *Salmonella* vaccine, low levels of *Salmonella* were detected, with greater abundance in the mucosal compartment. Across both trials, the CE product significantly modulated 16 shared genera (adjusted $P < 0.1$). *Escherichia* and *Klebsiella* decreased, whereas *Sutterella* increased. Enrichment in *Bacteroidia* genera, *Megamonas*, and *Phascolarctobacterium* (propionate producers involved in cross-feeding with *Bacteroides*) was observed. Mucosal analysis revealed enrichment of *Bacillaceae* genera, a potential immunostimulatory taxa, and reduced *Lactobacillus*. These findings indicate that CE products exert an early microbial imprint, even in the presence of live vaccines, particularly in the mucosal compartment. This may influence immune maturation and pathogen resistance, warranting further integration with host immune responses.

Key words: poultry, gut microbiota maturation, *Salmonella* vaccine, coccidiosis vaccine, pathogen resistance

107 In-feed cranberries enhance microbial resilience and restructure gut microbiota during necrotic enteritis in broiler chickens. S. Abdallah[1], D. Boney Jr.[1], A. Smith[1], P. Tamatey[2], D. Bryan[2], and A. Yitbarek*[1], [1]*University of Delaware, Newark, DE, USA,* [2]*The Pennsylvania State University, University Park, PA, USA*.
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Necrotic enteritis (NE) disrupts intestinal microbiota and compromises gut resilience in broiler chickens. This study evaluated whether cranberry supplementation modulates intestinal microbial ecology and predicted microbial function during NE challenge. A total of 1,232 Ross 308 broilers were assigned to seven treatments: nonchallenged control, NE challenge, NE + BMD (50 g/ton), or NE-challenged birds fed freeze-dried cranberries at 0.25%, 0.50%, 0.75%, or 1.0%. Birds were challenged with 108 CFU/mL *Clostridium perfringens* from d 17 to 20, and ileal mucosal swabs and cecal contents were collected on d 14 and d 21. Microbiota were profiled by 16S rRNA V4 sequencing and analyzed using DADA2, SILVA classification, α-diversity and Bray-Curtis β-diversity, LEfSe, co-occurrence networks, and PICRUSt2. Before challenge, cranberries had minimal effects on α- and β-diversity, whereas network analysis showed enhanced cooperative microbial interactions, especially at 0.25% and 0.50%, with interconnected fermentation- and gut homeostasis-associated genera, including *Blautia*, *Dorea*, *Anaerobutyricum*, and *Oscillospira*. After NE challenge, ileal Shannon diversity differed among treatments at d 21 ($P = 0.04$), and Bray-Curtis NMDS showed significant community restructuring ($P = 0.001$), with 0.75% cranberry separating from the challenged control. LEfSe identified enrichment of anaerobic taxa in cranberry-fed birds, including *Agathobaculum*, *Blautia*, *Negativibacillus*, and *Flavonifractor*, taxa linked to short-chain fatty acid production, polyphenol metabolism, and epithelial support. PICRUSt2 showed that NE suppressed predicted microbial pathways related to nucleotide salvage, urea cycling, L-serine biosynthesis, NAD salvage, peptidoglycan biosynthesis, and plasmalogen degradation. Cranberry at 0.5% partially restored these pathways, producing the functional profile closest to the nonchallenged control. Overall, cranberries mitigated NE-associated dysbiosis, preserved microbial complexity, promoted cooperative microbial networks, and restored NE-suppressed predicted functions, supporting improved intestinal resilience in broilers.

Key words: cranberry, necrotic enteritis, microbiota

108 Size-dependent internalization and effects of micro- and nanoplastics on oxidative stress and barrier integrity in apical-out chicken enteroids. I. Ferraro*, Z. Zhu, A. Hadimundeen, and Y. Li, *University of Delaware, Newark, DE, USA*.
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Micro- and nanoplastics (MNPs) are emerging environmental contaminants, yet their interactions with the intestinal epithelium, particularly in agricultural animals, remain poorly understood. This study used apical-out chicken enteroids to investigate whether MNPs at different sizes can be internalized by intestinal epithelial cells and to assess their subsequent impact on oxidative stress and barrier integrity. Fluorescently labeled polystyrene particles of different sizes (50 nm, 200 nm, and 2,000 nm) were introduced in enteroids for 2 hours under normal and stressed conditions induced by menadione or lipopolysaccharide (LPS). MNP uptake was evaluated by fluorescent measurement. In a separate experiment, nonlabeled polystyrene particles of the same sizes were exposed to enteroids for 24 h to determine reactive oxygen species (ROS) generation, as well as barrier leakage indicated by Lucifer Yellow (LY) influx. Data were analyzed using two-way ANOVA. Results revealed that MNP uptake into enteroids was highly size-

dependent and was modified by stress conditions. Both oxidative stress and LPS-induced inflammatory challenge significantly increased 50-nm NP uptake ($P < 0.05$). As for 200-nm NPs, inflammatory stress did not alter the uptake compared with unchallenged enteroids, whereas oxidative stress significantly increased uptake ($P < 0.05$). Uptake of the largest MPs tested, 2,000 nm, was not increased by oxidative stress and was conversely decreased under LPS challenge ($P < 0.05$). Following 24-h exposure, 50-nm and 500-nm NPs significantly increased ROS production and LY accumulation in enteroids, indicating oxidative stress and increased barrier permeability ($P < 0.05$). These findings demonstrate that MNP internalization by chicken intestinal epithelial cells is strongly size-dependent and can be altered by oxidative and inflammatory stress. Furthermore, acute MNP exposure also increased oxidative stress and compromised epithelial barrier integrity, highlighting potential risks of MNP contamination to poultry gut health.

Key words: micro- and nanoplastic, chicken enteroid, oxidative stress, barrier integrity, gut health

109 Field-applicable protocol for integrated analysis of microbiome function: A case study in nursery piglets. V. Blanvillain*[1], M. Alves da Costa[1], C. Paez Rodriguez[2], and G. Cordero[1], [1]*AB Vista Inc., Plantation, FL, USA,* [2]*Encipharm, La Reina, Chile*.
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A case study was performed to test a field-applicable protocol integrating microbial, metabolic, and immune biomarkers to standardize the assessment of the microbiome function of nursery piglets under commercial conditions. Selected biomarkers included lactic acid-producing bacteria, butyric acid-producing bacteria, volatile fatty acids (VFA), and lactic acid as indicators of fermentation activity, calprotectin as a marker of immune response, and total and enterotoxigenic *Escherichia coli* (ETEC) to assess pathogen load. Eight fresh fecal samples per group were collected from nursery piglets at 21 d after weaning across two sites with contrasting histories of diarrhea. All piglets received the same diet. Group effects were tested for analysis of variance, and the group effect on VFA profile was tested by regression analysis. Overall, the individual VFA concentrations were significantly lower in the site with recurring diarrhea (test site) than in the site with low historical incidence of diarrhea (control site), and the opposite was true for lactic acid ($P < 0.05$). However, the VFA profile was not affected by the group. Both lactic acid and butyric acid bacteria counts were significantly affected by the group. Pathogen load was significantly higher in the test site relative to the control site. Calprotectin concentration did not significantly differ between groups ($P = 0.1176$). The results indicated that the diarrhea observed on the test site was associated with a high load of ETEC. The greater accumulation of lactic acid in the test site may suggest reduced utilization of lactic acid substrate, explaining the lower concentration of total VFA in that group than in the control site. Overall, the similar VFA profiles observed in both groups indicate that diarrhea may have been associated with lower fermentation activity rather than with a shift in the fermentation pattern. The proposed biomarker panel enabled integrated characterization of microbiome function and may be further used to support in-field decisions.

Key words: piglet, gut health, biomarker, protocol, microbiome

110 Dietary butyrate glycerides enhance piglet resilience to ETEC-induced postweaning syndrome. A. Mellouk*[1], N. Vieco-Saiz[1], O. Lemâle[1], V. Michel[1], A. M. Gutierrez-Montes[2], and J. Consuegra[1], [1]*Swine Innovation and Customer Success, Adisseo France SAS, European Laboratory of Innovation, Science and Expertise (ELISE), Lyon, France,* [2]*University of Murcia, Murcia, Spain*.
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Postweaning syndrome (PWS), frequently triggered by enterotoxigenic *Escherichia coli* (ETEC), remains a major challenge in pig production, impairing growth performance, increasing mortality, and generating substantial economic losses. Butyrate glycerides (BG) are recognized for their beneficial effects on gut health and their antimicrobial properties. The present study aimed to assess whether dietary BG supplementation could enhance piglet resilience to ETEC-induced PWS. A total of 54 weaned male piglets (average BW: 6.3 kg), genetically susceptible to ETEC F18, were allocated to three experimental groups: control, challenged, and challenged supplemented with BG (2 kg/T). At 28 days of age, piglets in the challenged groups were orally inoculated with 108 CFU of ETEC F18 (O141:K94) per animal. Over a four-week period, growth performance, survival rate, and fecal scores were recorded. Salivary biomarkers associated with inflammation, stress, and immunity were also analyzed. ETEC challenge markedly impaired piglet performance, reducing survival to 88.9% and average daily gain to 68 g/d compared with 209 g/d in controls (–67.4%, $P < 0.01$) during the first week following the challenge. BG supplementation significantly mitigated these detrimental effects, restoring up to 70% of growth performance (168 g/d, $P < 0.05$) and 88.9% of feed efficiency (gain:feed ratio 0.53 vs. 0.21, $P < 0.05$). Additionally, BG reduced mortality to 5.6% and decreased diarrhea severity by 46% ($P < 0.05$). At the physiological level, supplemented piglets exhibited lower levels of inflammatory (S100A12), stress (α-amylase), and oxidative stress markers ($P < 0.05$), alongside higher IgG concentrations ($P < 0.05$), indicating enhanced humoral immunity. Dietary supplementation

with butyrate glycerides significantly improved piglet resilience to ETEC-induced PWS. These benefits were evidenced by reduced mortality and diarrhea severity, alleviated stress and inflammation, and partial restoration of growth performance. The results support BG as an effective nutritional strategy to enhance piglets' health and productivity.

Key words: glycerides of butyric acid, postweaning diarrhea, enterotoxigenic *Escherichia coli*, stress

Nutrition-Host-Microbe Interactions and Intestinal Physiology

111 Performance, diarrhea severity, hematology, and fecal microbiome responses to iron sources without pharmacological zinc oxide. L. Alves Rodrigues*[1], M. Ethier[2], K. Valiquette[2], A. Bussières[2], J. Richard[1], C. Bédard[3], and M. P. Létourneau Montminy[4], [1]*Zinpro Corporation, Eden Prairie, MN, USA,* [2]*Agri-Marché, Saint-Isidore, QC, Canada,* [3]*University of Montréal, Saint-Hyacinthe, QC, Canada,* [4]*Laval University, Québec, QC, Canada*.
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Iron deficiency anemia (IDA) and postweaning diarrhea (PWD) are major challenges in nursery pigs. Pharmacological zinc oxide (ZnO) controls PWD but may reduce liver Fe reserves, and feeding inorganic Fe (e.g., ferrous sulfate) has been linked with greater PWD than organic Fe. This study evaluated Fe sources without pharmacological ZnO on performance, diarrhea, blood indices, and fecal microbiome of nursery pigs. A total of 880 weaned pigs (6.0 ± 0.38 kg) were fed as follows for 33 d: (1) 100 mg/kg ferrous Fe as $FeSO_4$ with pharmacological ZnO ($FeSO_4$+; Zn 2,300, 465, and 0 mg/kg in phases, d 0–14, 14–26, and 26–33); or without pharmacological ZnO: (2) $FeSO_4$−, (3) 100 mg/kg ferrous Fe as Fe–amino acid complex (Fe2AA−), or (4) 100 mg/kg ferric Fe as Fe–amino acid complex (Fe3AA−). With ZnO (d 0–14), ADG was higher for $FeSO_4$+ vs. Fe3AA− (P = 0.05). Without ZnO (d 26–33), ADG was higher for Fe2AA− and Fe3AA− compared with $FeSO_4$− (P = 0.03), and overall feed conversion improved for Fe2AA− and Fe3AA− compared to $FeSO_4$− (P = 0.02). Diarrhea severity did not differ (P > 0.05). At d 14, hemoglobin was higher in $FeSO_4$− and Fe2AA− compared with Fe3AA− (P = 0.03), and cellular hemoglobin concentration mean was highest in Fe2AA− (P = 0.03). Plasma and fecal Zn were highest in $FeSO_4$+ (P < 0.01). Microbiome alpha diversity and overall community structure did not differ at d 0 or 14, but d-14 taxa shifts were treatment-specific, including increased *Lactobacillus* proportions in Fe2AA− and Fe3AA−. Overall, in diets without pharmacological ZnO, Fe–amino acid complexes improved late-nursery growth and overall feed efficiency versus $FeSO_4$− while maintaining similar diarrhea severity, with modest microbiome shifts consistent with altered gut ecology.

Key words: anemia, iron, microbiome, piglet, zinc oxide

112 Probiotic-mediated functional modulation of the intestine supports layer performance and reduces transovarian *Salmonella* transmission. M. A. Amalaradjou*, *University of Connecticut, Storrs, CT, USA*.
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The intestine regulates nutrient utilization, mucosal responses, and microbial colonization in poultry, making intestinal health central to productivity and egg safety. This study evaluated two novel probiotics, *Lactobacillus paracasei* DUP-13076 (LP) and *Lactobacillus rhamnosus* NRRL-B-442 (LR), for their ability to modulate intestinal responses associated with layer performance and *Salmonella* Enteritidis (SE) colonization. Lohmann Lite hens were fed LP, LR, or a probiotic cocktail (PR; ~9 log CFU/kg feed) from 19 to 48 weeks. Performance, jejunal histomorphology, gut-associated gene expression, immune response, cecal bacteria, SE colonization, and egg transmission were evaluated. Probiotics improved hen-day egg production during early lay, with PR increasing production by ~14%. LP improved feed conversion ratio (2.62 vs. 2.74) while reducing feed intake by ~4.5%. Probiotic-fed birds exhibited improved egg weight, shell thickness, Haugh unit, and yolk index. LP and LR increased jejunal villus height, crypt depth, and ratio of villus height to crypt depth. LP upregulated genes associated with nutrient transport and epithelial integrity, including *SLC15A1*, *SLC2A1*, *MUC13*, *TJP1*, *OCLN*, and *CLDN1*. Probiotics also increased serum IgA and cecal lactobacilli while reducing *Escherichia coli* and coliforms. In SE challenge studies, LP and LR reduced SE adhesion to cecal and oviduct tissues ex vivo by 58% to 74%. In vivo, probiotics reduced cecal SE colonization by ~0.8 log CFU/g and reduced oviduct recovery to below detection limits, indicating reduced translocation from the intestine to the reproductive tract. This translated to lower SE contamination of eggshell surfaces, inner shell membranes, and internal contents, with internal egg contamination reduced from >50% to ~10 to 15% in probiotic-fed birds. Collectively, these findings demonstrate that targeted probiotic modulation of intestinal responses can improve production efficiency while reducing horizontal and vertical egg-borne SE transmission.

Key words: gut function, *Lactobacillus*, layer performance, *Salmonella* Enteritidis, egg safety

113 In ovo hyodeoxycholic acid enhances intestinal development and enteroendocrine function in broiler chickens. H. Abdallah*, Z. Zhaoyan, I. Ferraro, and Y. Li, *University of Delaware, Newark, DE, USA*.
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Optimizing intestinal development early in life may establish a more robust gut that supports long-term health and productivity. In ovo delivery of bioactive compounds has emerged as a promising strategy to enhance gastrointestinal development and posthatching

performance in broiler chickens. Hyodeoxycholic acid (HDCA), a secondary bile acid, regulates genes involved in epithelial maturation, barrier integrity, and nutrient sensing through farnesoid X receptor (*FXR*) activation. We hypothesized that in ovo HDCA administration acts as a "fed-state" signal to the developing intestine, promoting epithelial maturation and enteroendocrine function during early and later posthatching stages. A single dose of 2.5 mg of HDCA was injected into Cobb500 broiler embryos at embryonic day 18, with intestinal development and gene expression evaluated at hatching and day 14 after hatching. The expressions of genes related to bile signaling (*FXR*, *OSTα/β*), enteroendocrine function (*Ngn3*, *NeuroD1*, *CCK*, *PYY*), barrier integrity (*CLDN1*), and nutrient transport (*FABP5*, *B0AT1*) were quantified by RT-qPCR. At hatching, the HDCA-treated group had increased relative gut length and gut weight with reduced yolk weight ($P < 0.05$), indicating enhanced intestinal development and yolk utilization. HDCA upregulated ileal *FXR* and *OSTα/β* expression, increased *NeuroD1* and *CCK*, and reduced *Ngn3* ($P < 0.05$), suggesting altered enteroendocrine maturation during the perihatching period. By day 14 after hatching, feed intake was reduced in HDCA-treated birds, whereas expression of satiety hormones *Ngn3*, *CCK*, and *PYY* was upregulated. Gene expression of *FABP5*, *B0AT1*, and *CLDN1* was also elevated ($P < 0.05$), indicating enhanced nutrient absorption and barrier integrity. These findings demonstrate that in ovo HDCA promotes intestinal development at hatching and supports enteroendocrine function, nutrient transport, and barrier maturation during posthatching growth, potentially improving broiler efficiency.

Key words: in ovo nutrition, HDCA, broiler, intestinal development, enteroendocrine function

114 Effects of maternal and postweaning dietary live yeast supplementation on growth performance, nutrient digestibility, intestinal health, and colonic microbiota composition of weanling pigs. Y. Fu*, A. S. Lawal, T. M. Casey, T. A. Johnson, O. Adeola, and K. M. Ajuwon, *Department of Animal Sciences, Purdue University, West Lafayette, IN, USA*.
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The objective of this study was to evaluate the effects of maternal and postweaning dietary live yeast (LY) supplementation on growth performance, nutrient digestibility, intestinal health, and colonic microbiota of weanling pigs. Forty sows were randomly allotted to 1 of 2 dietary treatments: a control diet (CON) or an LY-supplemented diet (0.05% during gestation and 0.1% during lactation). At weaning, 64 pigs from each maternal dietary group were assigned to CON or LY-supplemented diets (0.1%) for 5 wk. Feed intake and BW were recorded weekly, feces were collected on d 33 to 35 for nutrient digestibility analysis, and pigs were euthanized on d 35 for collection of serum, tissue, and colonic digesta. Maternal and nursery dietary LY supplementation did not affect BW, ADG, ADFI, or G:F ratio. However, maternal dietary LY supplementation increased apparent total-tract digestibility (ATTD) of nitrogen and decreased blood urea nitrogen concentration ($P < 0.05$). Postweaning dietary LY supplementation also increased ATTD of nitrogen, jejunal villi height, and mucosal IL-10 concentration ($P < 0.05$). Pigs from LY-supplemented sows had greater jejunal TNF-α and ileal IL-10 concentrations, with elevated mRNA abundance of *IL-10*, *IL-1β*, and *GPX2* in the jejunal mucosa compared with pigs from CON sows ($P < 0.05$). Nursery dietary LY supplementation reduced hepatic mRNA abundance of *TNF-α*, *CAT*, and *SOD1* ($P < 0.05$). Maternal dietary LY supplementation increased Chao1 richness ($P < 0.05$) and enriched ASVs belonging to genera such as *Veillonella*, *Prevotella*, and *Roseburia.* Correlation analysis revealed that *Rikenellaceae_RC9_gut_group* was positively correlated with ileal TNF-α concentration, whereas *Prevotellaceae_NK3B31_group* was negatively correlated with nitrogen digestibility. In conclusion, maternal and nursery dietary LY supplementation improved nitrogen digestibility and modulated gut immunity and colonic microbiota, suggesting potential benefits for nitrogen metabolism and gut health in weanling pigs.

Key words: intestinal health, live yeast, nutrient digestibility, weanling pig

Poster Session: Nutrition-Host-Microbe Interactions and Intestinal Physiology

115 Effects of increasing heat stress challenge on intestinal immune response in chickens. K. Guirrati*, Z. Pardo, J. Gené, N. París, O. González-Rodríguez, M. Ballester, and J. Tarradas, *IRTA-Animal Nutrition, Mas Bové, Constantí, Catalonia, Spain*.
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Heat stress (HS) affects performance, physiology, metabolism, and gut health in broilers, increasing peripheral blood circulation at the expense of gut irrigation, leading to intestinal hypoxia. This compromises the epithelial barrier, triggers inflammatory responses, and promotes the translocation of pathogenic bacteria and other external insults. The current study characterizes the effects of different simulated heat wave scenarios on intestinal gene expression. A total of 1,260 broilers were distributed into 3 rooms (12 floor pens each) under commercial conditions. From 21 to 35 d, the different HS challenges were applied daily as follows: T1, control (no HS); T2, moderate HS (28°C for 4 h); and T3, severe HS (32°C for 6 h). At 35 d, the jejunal mucosa of 12 animals/treatment was sampled for gene expression analysis. The Biomark X platform, with a panel of 48 genes related to intestinal integrity, metabolism, immunity, nutrient transport, and oxidative stress, was used. In the T2 group, *MUC2* and *MUC5ac* were downregulated, suggesting an impairment of mucus-producing cells and a potential reduction in epithelial barrier protection. Upregulation of *TLR2* and *TLR4* expression was observed in all HS groups compared with T1, which could contribute to the initiation of an immune response, as suggested by the increased expression of *IL1β*, *HSPA4*, and *RAG2* genes. Expression of *TGFβ* was also increased, which may reflect an anti-inflammatory response aimed at modulating the inflammatory process. In parallel, the genes *AKT* and *RPS6Kb1*, involved in the regulation of protein synthesis, cell growth, and cell proliferation, were upregulated, probably to repair the epithelial barrier damaged by HS challenge. However, in T3 the expression of *AKT*, *RPS6Kb1*, and *HSPA4* was numerically lower than in T2, and not different from T1, suggesting reduced activation protein synthesis, cell growth, and cellular immune pathways. In conclusion, birds appear to counteract the negative effects of moderate HS, inducing a strong immune response and promoting epithelial repair; however, this response appears to be diminished under severe HS conditions.

Key words: poultry, heat stress, gut health, gene expression, immunomodulation

116 Rumen-protected methionine modulates fecal microbiota in dairy heifers. D. Franco da Silva*[1], I. Crespo[1], D. Valente[1,2], T. Bordeira Gaspar[4,5], L. Martim[3], R. Lino Neto Pereira[6], R. Cordeiro[3], F. Oliveira[3], A. Londo[1], J. Wyatt[1], L. Grilo[7,8], J. Teixeira[7,8], C. Egas[9], L. Montezinho[1,7], A. Gonella-Diaza[10], [1]*Vasco da Gama University School (EUVG), Vasco da Gama Research Centre (CIVG), Coimbra, Portugal,* [2]*Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes and Alto Douro, Quinta de Prados, Vila Real, Portugal,* [3]*Coimbra School of Agriculture (ESAC), Polytechnic University of Coimbra (IPC), Bencanta, Coimbra, Portugal,* [4]*Cancer Signalling and Metabolism Group, i3S, Institute for Research and Innovation in Health, Institute of Molecular Pathology and Immunology (IPATIMUP), University of Porto, Porto, Portugal,* [5]*Veterinary Clinical Laboratory (CEDIVET), Leça do Balio, Portugal,* [6]*National Institute for Agrarian and Veterinary Research (INIAV, IP), Quinta da Fonte Boa, Vale de Santarém, Portugal,* [7]*Centre for Neuroscience and Cell Biology (CNC/UC), University of Coimbra, Coimbra, Portugal,* [8]*Centre for Innovative Biomedicine and Biotechnology (CiBB), University of Coimbra, Coimbra, Portugal,* [9]*Next Generation Sequencing Unit, Biocant, Cantanhede, Portugal,* [10]*Institute of Food and Agricultural Sciences (IFAS), University of Florida, Gainesville, FL, USA*.
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Gut microbiota modulation has emerged as a promising strategy to enhance metabolic efficiency, resilience, and physiological adaptation in dairy cattle, particularly during the prebreeding period, when nutritional interventions may affect future performance. This pilot study evaluated the effects of rumen-protected methionine (RPM) supplementation on the fecal microbiota of Holstein-Friesian heifers during the transition from summer to autumn. Twelve clinically healthy heifers (12–15 months old) were assigned to a control group (n = 6) or an RPM group (n = 6; 20 g/d) for 33 days. Fecal samples collected on day 33 were analyzed by 16S rRNA gene sequencing (V3–V4 region, Illumina platform). Microbial diversity and community structure were assessed using standard bioinformatic and statistical approaches. Alpha-diversity metrics revealed consistent, although nonsignificant, differences between groups. RPM-supplemented heifers showed greater species richness and higher Shannon diversity, indicating increased ecological complexity and representation of low-abundance taxa. Beta-diversity analysis demonstrated substantial overlap between groups, with no clear separation in Bray-Curtis principal coordinate analysis (PERMANOVA: $R^2 = 0.0985$, $P = 0.1878$). Across all samples, the microbiota was dominated by *Bacillota* (48.9%), *Bacteroidota* (41.7%), and *Verrucomicrobiota* (4.0%). Predominant genera included *Bacteroides*,

Alistipes, *Akkermansia*, *Rikenellaceae* RC9 gut group, and members of the *Prevotellaceae* family, reflecting a highly conserved microbial profile associated with carbohydrate fermentation, short-chain fatty acid production, and intestinal homeostasis. Venn analysis identified a shared core microbiome of 185 genera, whereas only a limited number of low-abundance genera were unique to the control (15) or RPM (33) groups. These findings suggest that RPM supplementation may subtly enhance microbial diversity while preserving core microbiome stability during a critical period of physiological and environmental adaptation. This study provides novel evidence linking RPM supplementation with fecal microbiota dynamics in prebreeding dairy heifers.

Key words: methionine, microbiome, adaptation, heifer

117 Effect of heat stress on physiological responses and milk production in dairy cattle using field-based THI observations in District Nowshera and Mardan, Pakistan. L. Alam*, *Luqman Alam Veterinary, Peshawar, Khyber Pukhtunkhw, Pakistan*.
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Heat stress is a major problem for dairy cattle in Pakistan during the summer season. It negatively affects animal health, feed intake, and milk production. The temperature-humidity index (THI) is commonly used to measure environmental heat load in livestock. The objective of this study was to evaluate the effects of heat stress on physiological responses and milk production in dairy cattle under field conditions. This field study was conducted on 850 lactating dairy cattle across 75 farms in District Nowshera and Mardan, Pakistan. The study animals included Holstein Friesian, Jersey, and crossbred cattle. I personally collected all field data during the study period. Environmental temperature and humidity were recorded to calculate THI for assessment of heat stress. Rectal temperature and respiratory rate were measured as physiological indicators. Milk yield and feed intake were recorded to assess production performance. Higher THI values were associated with increased rectal temperature and higher respiratory rate in dairy cattle. Animals exposed to heat stress showed reduced feed intake and a clear drop in milk production. Crossbred animals performed better under heat stress conditions compared with Holstein Friesian and Jersey cattle. Overall, increased environmental heat load had a clear negative impact on both physiological responses and milk production. Heat stress, measured through THI, significantly affected physiological stability and milk production in dairy cattle under field conditions. Rectal temperature and respiratory rate are simple and useful field indicators of heat stress. The findings highlight the importance of proper management practices to reduce heat stress and improve dairy performance during hot weather.

Key words: heat stress, THI, dairy cattle, field study, rectal temperature

Immunity and Intestinal Resilience

118 In vitro assessment of oleuropein-enriched olive leaf extract bioactivity against enterotoxigenic porcine *Escherichia coli*: Effects on oxidative stress, epithelial integrity, and cell viability. T. Hassan*[1,5], S. Bagatella[1], M. I. Malik[2,1], M. A. Arif[1], S. L. Bavaro[3], S. Mioletti[1], F. R. Anjum[4,5], B. Amato[5], S. Siomko[5], M. H. Kogut[6], and M. T. Capucchio[1,3], [1]*Department of Veterinary Sciences, University of Turin, Grugliasco, Italy,* [2]*College of Veterinary Medicine, University of Tennessee, Knoxville, TN, USA,* [3]*Institute of Science of Food Production, National Research Council, Grugliasco, Italy,* [4]*Animal and Plant Health Agency, New Haw, Addlestone, Surrey, UK,* [5]*Bristol Veterinary School, University of Bristol, Langford, North Somerset, UK,* [6]*VETANCO USA, St. Paul, MN, USA*.
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Olive leaf extract (OLE) is a polyphenol-rich gut modulator of potential interest for veterinary use, but its in vitro safety, antioxidant capacity, effect on gut barrier integrity, and effectivity against porcine enterotoxigenic *Escherichia coli* (ETEC) remain unclear. A standardized OLE (containing 20% oleuropein) was digested in a static in vitro model, and its antioxidant capacity was tested using DPPH. To evaluate its safety and biological effects on intestinal cells, cytotoxicity (MTT) and ROS (DCFDA) were assessed in Caco-2 and IPEC-J2 cell lines. Barrier integrity was assessed through transwell assays (TEER). Bactericidal and antiadhesive activity against porcine ETEC field isolates K88 and K99 (MOI = 10), and barrier damage under infection, were evaluated on the same cells. Digested OLE retained strong radical scavenging (>79% w.r.t. control at 30–90 min) and was noncytotoxic (≥85% viability ≤400 µg/mL). ROS remained below the positive control at 100 to 200 µg/mL but exceeded it at ≥400 µg/mL. TEER was preserved at 400 µg/mL (Caco-2, $P = 0.514$; IPEC-J2, $P = 0.563$). Growth inhibition results revealed that OLE was effective against both bacterial strains between 12.5 and 50 µg/mL. OLE did not affect K88 ($P = 0.159$) but killed K99 at 6.25 µg/mL ($\Delta\log_{10} = -0.66$; $P = 0.0096$). OLE reduced adhesion of K99 at 25 µg/mL ($\Delta\log_{10} = -0.39$; $P = 0.043$) in IPEC-J2 and, on Caco-2, of K88 at 3.125 to 12.5 µg/mL (6.3–8.4-fold; $P = 0.023$–0.044) and of K99 at 12.5 µg/mL ($\Delta\log_{10} \approx -0.63$; $P = 0.013$). Upon infection, the barrier was not compromised (K88 ~61%–66%; K99 ~16%–53% retention; $P \geq 0.186$). In conclusion, OLE proved to be a noncytotoxic antioxidant, ROS-neutral at lower doses (<400 µg/mL) and selectively bactericidal/antiadhesive against K99 (6.25–25 µg/mL) in IPEC-J2 and against both K88 (3.125–12.5 µg/mL) and K99 (12.5 µg/mL) in Caco-2.

Key words: oleuropein, in vitro gastrointestinal digestion, bioavailability, enterotoxigenic *Escherichia coli*, intestinal epithelial barrier

119 In-feed freeze-dried cranberries improve performance, reduce intestinal lesions, and attenuate inflammatory responses in broiler chickens challenged with necrotic enteritis. A. Smith*[1], S. Abdallah[1], D. Boney Jr.[1], D. Bryan[2], and A. Yitbarek[1], [1]*University of Delaware, Newark, DE, USA,* [2]*The Pennsylvania State University, University Park, PA, USA*.
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Necrotic enteritis (NE) remains a major challenge to antibiotic-free broiler production, partly because intestinal damage reduces nutrient utilization and amplifies mucosal inflammation. This study evaluated whether dietary cranberry supplementation improves performance, intestinal pathology, and immune regulation during NE challenge. A total of 1,232 male Ross 308 broilers were randomly allocated to floor pens (n = 8) and fed corn-soybean meal-based diets containing no antibiotic growth promoters, except for the bacitracin methylene disalicylate (BMD) control group. Treatments included nonchallenged control, NE-challenged control, BMD+NE-challenge, and freeze-dried cranberries at 0.25%, 0.50%, 0.75%, or 1.0% supplemented to NE-challenged chickens. Birds were challenged with 1 mL of 10^8 CFU/mL *Clostridium perfringens* from d 17 to 20. Growth performance was measured biweekly through d 42, intestinal lesions were scored on d 21, and ileal immune and barrier gene expression was quantified by qRT-PCR. NE challenge impaired grower-phase performance, increased lesion severity, and induced marked IL-1β upregulation. Cranberry supplementation preserved growth and feed efficiency comparable to BMD, reduced lesion progression, and significantly suppressed IL-1β expression. Notably, the 0.25% and 0.50% cranberry groups had ~88% lesion-free birds, comparable to BMD, with no advanced necrosis observed in cranberry-fed birds. Cranberry treatments also increased IL-10, IL-22, and ZO-1 expression, suggesting a shift toward immune regulation and epithelial recovery. Overall, freeze-dried cranberries improved broiler enteric health during NE challenge by maintaining performance, limiting intestinal damage, and modulating mucosal inflammatory responses, with 0.25% to 0.50% providing the most consistent protection for potential use in antibiotic-free broiler production.

Key words: necrotic enteritis, cranberry, mucosal immunity

120 Neurochemical metabolic pathway expression is region-dependent in the intestinal tract of the turkey. S. S. Wickramasuriya[2], K. Matusik[1], D. Graham[1], and J. M. Lyte*[2], [1]*University of Arkansas, Fayetteville, AR, USA,* [2]*USDA-ARS Poultry Production and Product Safety, Fayetteville, AR, USA*.
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The intestinal tract is a major source of monoamine neurochemicals that play multifaceted roles in poultry performance, health, and food safety. Little is known regarding the metabolic pathways involved in the synthesis, transport, and catabolism of monoamine neurochemicals in the intestinal tract of the turkey. As such, we sought to determine the quantitative distribution of enzymes and transporters involved in catecholamine and indolamine metabolism within the turkey intestinal tract. Turkeys were raised under age-appropriate conditions and with ad libitum access to feed and water. At 35 days of age, ileal, cecal, and colon tissues were collected. Tissue protein was extracted and then quantified using the Bradford assay. Quantitative protein-level expression was performed using the Jess capillary immunoassay platform. Targets quantified in the turkey ileum, cecum, and colon were monoamine oxidase A (MAO-A), monoamine oxidase B (MAO-B), serotonin and norepinephrine transporters (SERT, NET), DOPA decarboxylase (DDC), dopamine beta-hydroxylase (DBH), tyrosine hydroxylase (TH), and catechol-O-methyltransferase (COMT). Data were analyzed using ANOVA with Tukey post hoc test. Although all measured enzymes and transporters were detectable within the ileum, cecum, and colon, region-specific differences in expression profiles were observed. MAO-A, MAO-B, and DDC levels were significantly higher ($P < 0.05$) in the ileum compared with the cecum and colon. Among COMT isoforms, membrane-bound COMT was significantly more abundant than soluble COMT ($P < 0.05$) among all intestinal regions. Higher DBH expression ($P < 0.05$) was observed in the cecum compared with other regions, whereas no regional expression differences were observed for TH. NET expression was higher ($P < 0.05$) in the cecum compared with the colon, whereas SERT expression was observed in the ileum compared with the cecum. Together, our data establish the presence of monoaminergic systems in the turkey intestinal tract. These findings are expected to enable the development of strategies that seek to control enteric and systemic neurochemical concentrations in turkeys.

Key words: turkey, gut, neurochemical, enzyme, neuroendocrine

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