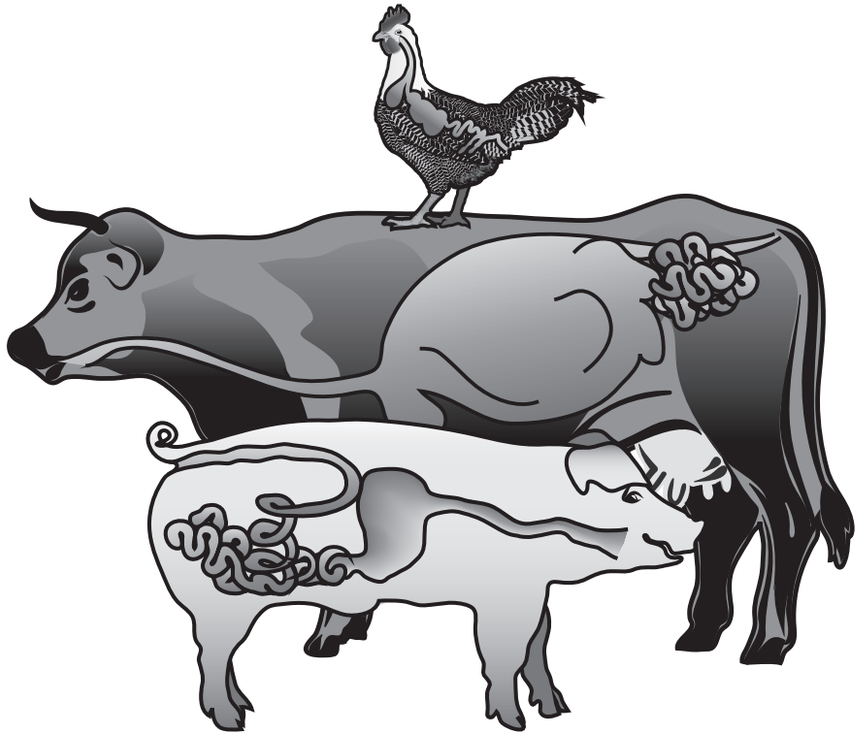
Symposium on Gut Health

in Production of Food Animals

November 10–13, 2024

St. Louis, Missouri



Program and Abstracts

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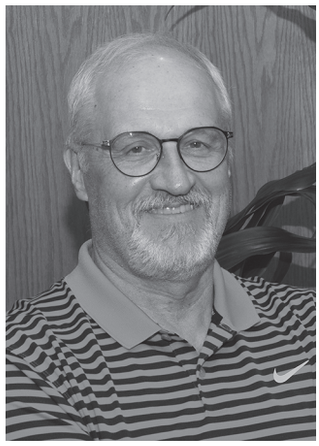
WELCOME



On behalf of the Organizing Committee for the Symposium on Gut Health in Production of Food Animals, I welcome you back to St. Louis, Missouri!

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; and (3) the commensal bacteria. 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, as well as the motor that drives their performance. The abstracts submitted to the Symposium are defining these links and mechanisms that interconnect the three components of the gut and how each can be manipulated to improve animal health.

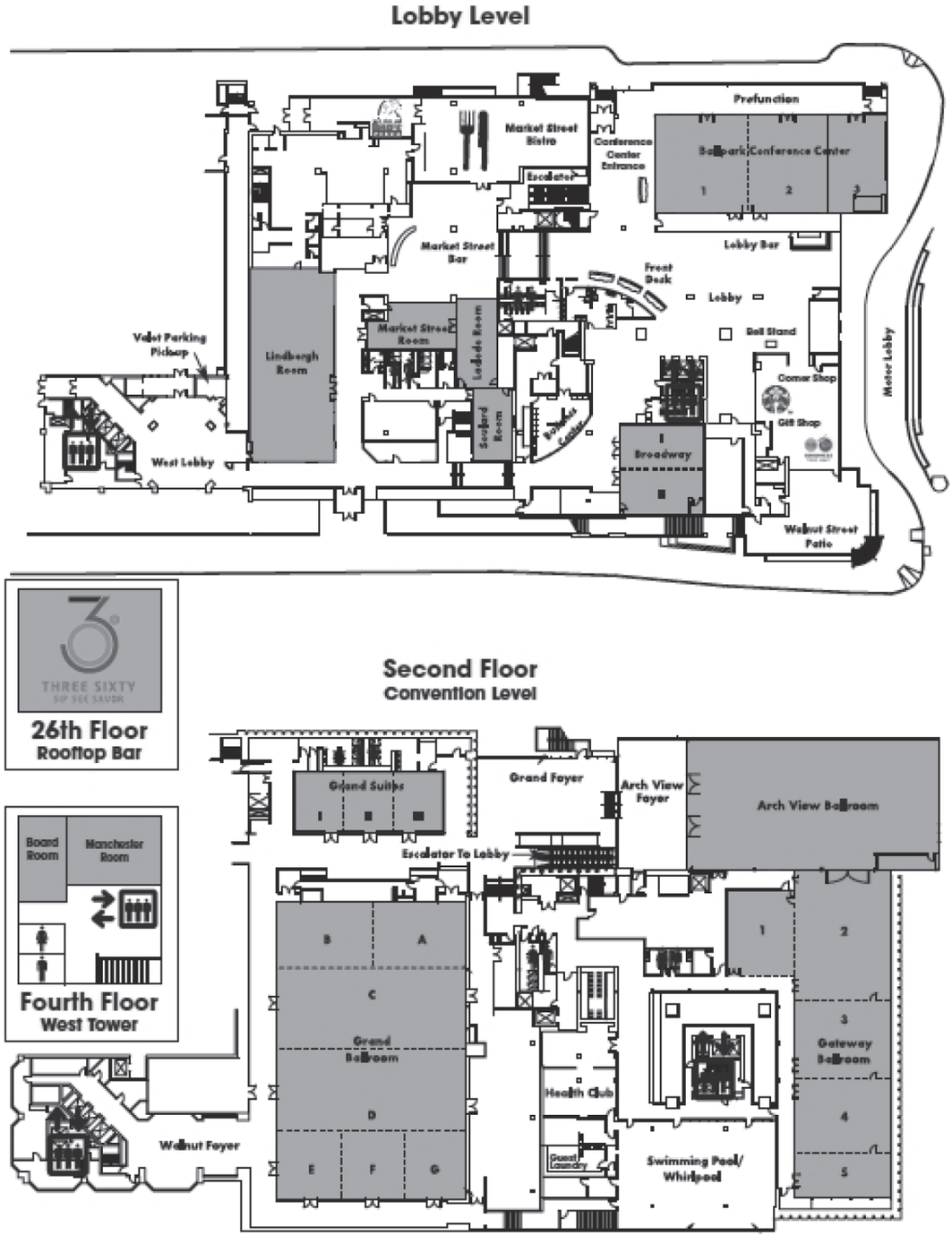
Like last year, we will be organizing a mini symposium on Monday afternoon which will discuss the Standardization of a Protocol for Microbiota Analysis in Poultry. This working group of poultry microbiota specialists has spent the last year attempting to standardize a protocol that can be used by all for microbiota analysis. This mini-symposium will highlight these discussions, featuring a summary presentation. Everyone is free 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 a special effort to engage with the graduate students who are attending and presenting. Remember, whatever your research, specialty or food animal commodity, we are all working together to improve food quality for the consumer.

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, November 10

5:00 PM–7:00 PM Registration: Ballpark Registration Desk

Monday, November 11

8:00 AM–9:00 AM Breakfast: Lindberg Room

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

SESSION 1

Chair: Brian Aldridge, Clemson University
Ballpark I and II

9:00 AM **Late weaning is associated with decreased abundance of *Enterobacteriaceae* and antimicrobial resistance transfer in the cecal contents of pigs.**
C. Anderson*[1], J. Wiarda[2], and C. Loving[1], [1]*Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture*, *Ames, IA, USA*, [2]*Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture*, *Ames, IA, USA*.

9:30 AM **Characterization of bacterial microbiomes according to the fecal phenotype in piglets before and after weaning.**
C. Vaggi*[1,2], J. C. Vötterl[1,2], F. Lerch[1,2], F. Yosi[2,3], S. Ricci[2,4], D. Verhovsek[5], and B. U. Metzler-Zebeli[1,2], [1]*Centre for Veterinary Systems Transformation and Sustainability, University of Veterinary Medicine Vienna*, *Vienna, Austria*, [2]*Christian Doppler Laboratory for Lab Innovative Gut Health Concepts of Livestock, University of Veterinary Medicine Vienna*, *Vienna, Austria*, [3]*Department of Animal Science, Faculty of Agriculture, University of Sriwijaya*, *Palembang, South Sumatra, Indonesia*, [4]*Centre for Animal Nutrition and Welfare, University of Veterinary Medicine Vienna*, *Vienna, Austria*, [5]*Clinical Centre for Population medicine in Fish, Pig and Poultry, University of Veterinary Medicine Vienna*, *Vienna, Austria*.

10:00 AM **Characterization of regional differences in neonatal calf gut-associated lymphoid tissues using high-resolution snRNA-sequencing.**
M. Donia*[1,2], B. Aldridge[3], J. Lowe[1], and W. Li[4], [1]*Department of Pathobiology, College of Veterinary Medicine, University of Illinois*, *Urbana-Champaign, IL, USA*, [2]*Department of Internal Medicine, Kafrelsheikh University*, *Kafrelsheikh, Egypt*, [3]*Department of Veterinary Medical Sciences, College of Veterinary Medicine, Clemson University*, *Clemson, SC, USA*, [4]*US Dairy Forage Research Center, USDA-Agricultural Research Service*, *Madison, WI, USA*.

10:30 AM Coffee Break: Ballpark Foyer
Sponsored by Innovad

11:00 AM **Functional and structural characterization of the enteric nervous system in broiler chickens: The inner intestinal barrier of defense against foodborne pathogens.**
V. Caputi*, A. M. Donoghue, and J. M. Lyte, *Poultry Production and Product Safety Research, USDA-ARS*, *Fayetteville, AR, USA*.

12:00 PM–1:30 PM Lunch: Lindberg Room

Poster Session

92720 **Effect of *Salmonella enteritidis* challenge on the microbiome of 54- to 56-week-old layers.**
T. Lavergne*[1], C. Elrod[1], and M. Nascimento[2], [1]*Natural Biologics, Inc.*, *Newfield, NY, USA*, [2]*Sapiens*, *Jundiai, Sao Paulo, Brazil*.

92713 **Deficiency in nitro-detoxification may limit *Salmonella* survivability in macrophages.**
A. Ingram[1], C. Johnson[2], N. Mast[1], R. Anderson*[2], and R. Arsenault[2], [1]*Department of Animal Science and Veterinary Technology, Texas A&M University-Kingsville*, *Kingsville, TX, USA*, [2]*United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit*, *College Station, TX, USA*.

92733 **Impact of heat stress on intestinal barrier integrity in broilers: A comparison of low- and high-water efficient lines.**
M. F. Cuadrado*[1], B. Roach[2], E. S. Greene[1], K. R. Lassiter[1], W. G. Bottje[1], S. K. Orlowski-Workman[1], and S. Dridi[1], [1]*University of Arkansas, Poultry Science*, *Fayetteville, AR, USA*, [2]*Har-Ber High School*, *Springdale, AR, USA*.

92704 **Standardization of methodologies in poultry microbiome research: One year of progress.**
J. Lyte*[1], D. Ayala[2], J. De Oliveira[3], S. Dridi[4], D. Grum[2], T. Johnson[5], J. Kers[6], M. Kogut[7], J. Rehberger[8], M. Seyoum[4], A. Smith[8], G. Zhang[9], and M. Proskowiec-Weglarz[10], [1]*USDA-ARS*, *Fayetteville, AR, USA*, [2]*Purina Land O'Lakes*, *Gray Summit, MO, USA*, [3]*Cargill*, *Vilvoorde, Belgium*, [4]*University of Arkansas*, *Fayetteville, AR, USA*, [5]*University of Minnesota*, *Saint Paul, MN, USA*, [6]*Utrecht University*, *Utretcht, the Netherlands*, [7]*USDA-ARS*, *College Station, TX, USA*, [8]*Arm & Hammer Animal Nutrition*, *Waukesha, WI, USA*, [9]*Oklahoma State University*, *Stillwater, OK, USA*, [10]*USDA-ARS*, *Beltsville, MD, USA*.

92716 **Effects of a stimbiotic on nutrient digestibility, oocyst shedding, blood profiles, intestinal gene expression of tight junction protein, and cecal microbiota in necrotic enteritis-challenged broilers.**
H. Kim*, S. Chang, D. Song, K. Jeon, and J. Cho, *Chungbuk National University*, *Cheong ju, Chungcheongbuk-do, Korea*.

92738 **Subclinical exposure to multiple mycotoxins causes cecal microbiota dysbiosis in broiler chickens.**
L. Kappari[1], J. Lourenco[2], T. Applegate[1], A.Glenn[3], and R. Shanmugasundaram*[3], [1]*Department of Poultry Science, University of Georgia*, *Athens, GA, USA*, [2]*Department of Animal and Dairy Science, University of Georgia*, *Athens, GA, USA*, [3]*Toxicology and Mycotoxin Research Unit, US National Poultry Research Center, Agricultural Research Service, US Department of Agriculture*, *Athens, GA, USA*.

92728 **Effect of yeast fermentate on rumen microbiota and gas emissions in an in vitro model.**
E. G. Olson*[1], A. Rodrigues[1], I. Ferreira[1], A. Scheaffer[2], and S. C. Ricke[1], [1]*University of Wisconsin–Madison*, *Madison, WI, USA*, [2]*SweetPro*, *Wahalla, ND, USA*.

92731 **Effects of feeding cashew nutshell liquid on rumen fermentation characteristics and methane emission in Japanese Black beef cattle (Wagyu).**
Y. Uyeno*[1], Y. Sakai[2], T. Abe[3], K. Takahashi[4], T. Kuramoto[5], and H. Iwata[5], [1]*Shinshu University*, *Minamiminowa, Nagano, Japan*, [2]*Japan Cattle Industry Cooperative*, *Tokyo, Japan*, [3]*National Livestock Breeding Center*, *Nishigo, Fukushima, Japan*,

[4]*SDS Biotech K.K.*, *Tokyo, Japan*, [5]*Tokyo University of Agriculture*, *Atsugi, Kanagawa, Japan*.

3:00 PM–4:30 PM **STANDARDIZING A PROTOCOL FOR POULTRY MICROBIOME ANALYSIS/ OTHER PRODUCTION ANIMALS?**

Chair: Monika Proszkowiec-Weglarz, Glenn Zhang, Josh Lyte
Ballpark I and II

This session is open to all registrants, as the committee will discuss their progress in standardizing the protocol of measuring poultry microbiota and the steps they have taken over the course of the past year. The obvious reason behind this effort is to be able to make comparisons across published research on the topic. During this session, the floor will be open to other research groups (academic and industry) to discuss the possibility of standardizing the protocol for microbiota analysis in other production animals.

4:30 PM–5:30 PM All Attendee Reception: Lindberg Room

Tuesday, November 12

8:00 AM–9:00 AM Breakfast: Lindberg Room

8:00 AM–4:30 PM Registration: Ballpark Registration Desk

SESSION 3

Chair: Brian Aldridge, Clemson University
Ballpark I and II

9:00 AM **The effects of heat stress and depressed feed intake on the cecal and ileal microbiota of broiler chickens.**
P. M. Campos*[1], M. Proszkowiec-Weglarz[1], and S. Dridi[2], [1]*USDA-ARS Animal Biosciences and Biotechnology Laboratory*, *Beltsville, MD, USA*, [2]*Center of Excellence for Poultry Science, University of Arkansas*, *Fayetteville, AR, USA*.

9:30 AM **Effect of heat stress on jejunal metabolic profile in broiler chickens.**
E. S. Greene*[1], H. F. Castro[2], C. J. Christopher[2], S. R. Campagna[2], and S. Dridi[1], [1]*University of Arkansas*, *Fayetteville, AR, USA*, [2]*University of Tennessee*, *Knoxville, TN, USA*.

10:00 AM **A curcumin derivative protease inhibitor mitigates *Brachyspira hampsonii*-induced swine dysentery.**
R. Puentes, R. Hannawayya, N. Mirzadzare, L. Perez-Perez, K. Tiedje, and E. R. Cobo*, *University of Calgary*, *Calgary, Alberta, Canada*.

10:30 AM Coffee Break: Ballpark Foyer
Sponsored by Innovad

11:00 AM **Effects of dietary fats on diversity and function of cecal microbiota in broiler chickens.**
V. V. Jadhav*, Y. O. Fasina, P. C. Omaliko, J. Han, and S. H. Harrison, *North Carolina Agricultural and Technical State University*, *Greensboro, NC, USA*.

12:00 PM–1:30 PM Lunch: Lindberg Room

SESSION 4

Chair: Allen Byrd, USDA-ARS, College Station, Texas
Ballpark I and II

1:30 PM **A commensal bacterium protects chickens against necrotic enteritis.**
I. Tobin*[1], J. Liu[1], M. Whitmore[1], M. Kim[1], Z. Zhao[1], J. Guo[1], J. Scaria[2], and G. Zhang[1], [1]*Department of Animal and Food Sciences, Oklahoma State University*, *Stillwater, OK, USA*, [2]*Department of Veterinary Pathobiology, Oklahoma State University*, *Stillwater, OK, USA*.

2:00 PM **A meta-analysis of necrotic enteritis in the microbiome.**
D. Varela and A. Ballou*, *SIWA, Iluma Alliance*, *Durham, NC, USA*.

2:30 PM Coffee Break: Ballpark Foyer

3:00 PM **Intestinal microbiome confers strong colonization resistance against necrotic enteritis in chickens.**
J. Liu[1], M. Whitmore[1], I. Tobin[1], M. Kaiser[2], S. Lamont[2], and G. Zhang*[1], [1]*Oklahoma State University*, *Stillwater, OK, USA*, [2]*Iowa State University*, *Ames, IA, USA*.

3:30 PM **Pathological and microbiological investigations of enteric disorders in postweaning pigs: Association between pathogen co-infections and enteritis pattern.**
T. Hassan[1], S. Bagatella[1], M. I. Malik[1], A. Bellato[1], D. Ippolito[2], S. Zoppi[3], M. B. Boniotti[4], G. L. Alboralis[4], and M. T. Capucchio*[1], [1]*University of Turin, Department of Veterinary Sciences*, *Grugliasco (Torino), Italy*, [2]*National Institute of Health, Department of Food Safety, Nutrition and Veterinary Public Health*, *Rome, Italy*, [3]*Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta*, *Torino, Italy*, [4]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna*, *Brescia, Italy*.

4:00 PM–5:00 PM All Attendee Reception: Lindberg Room

Wednesday, November 13

8:00 AM–9:00 AM Breakfast: Lindberg Room

8:00 AM–10:00 AM Registration: Ballpark Registration Desk

SESSION 5

Chair: Brian Aldridge, Clemson University
Ballpark I and II

9:00 AM **Calycosin from Astragali Radix as a potential phytogenic feed additive in weaning piglets: In vivo and in vitro studies.**
J. Wang*[1,2], [1]*Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences*, *Beijing, China*, [2]*Sino-US Joint Laboratory of Animal Science, Beijing Academy of Agriculture and Forestry Sciences*, *Beijing, China*.

9:30 AM **Can the mixture of *Hermetia illucens* and *Tenebrio molitor* meals influence the gut health of broiler chickens?**
T. Hassan*[1], M. I. Malik[1], C. Sferra[1], M. Gariglio[1], I. Ferrocino[2], I. Biasato[2], L. Gasco[2], and M. T. Capucchio[1], [1]*University of Turin, Department of Veterinary Sciences*, *Grugliasco (Torino), Italy*, [2]*University of Turin, Department of Agricultural, Forest and Food Sciences*, *Grugliasco (Torino), Italy*.

10:00 AM **Identifying and isolating primary degraders of resistant potato starch from the swine gastrointestinal tract.**

H. R. Watkins*[1,2], C. L. Loving[1], and C. L. Anderson[1], [1]*USDA-ARS-NADC*, *Ames, IA, USA*, [2]*Iowa State University*, *Ames, IA, USA*.

92741 Late weaning is associated with decreased abundance of *Enterobacteriaceae* and antimicrobial resistance transfer in the cecal contents of pigs. C. Anderson*[1], J. Wiarda[2], and C. Loving[1], [1]*Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA,* [2]*Virus and Prion Research Unit, National Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, Ames, IA, USA.*

Weaning age plays a crucial role in piglet health and gut microbiome development. Pigs are typically weaned at ~21 d of age in the United States, while pigs are weaned no earlier than 28 d of age in the European Union. Later weaning age can enhance gut barrier integrity, microbial diversity, and overall health, thereby mitigating postweaning stress and disease risk. However, the impact of weaning age on the composition and transfer of antimicrobial resistance (AMR) is unknown. Complex ecological factors govern the transfer of AMR via plasmids, but an increase in the abundance of donor and recipient bacterial populations typically increases the rate of plasmid transfer. Here, we investigated the abundance of *Enterobacteriaceae*, bacteria that readily transfer plasmid-borne AMR to *Salmonella*, in pigs weaned at 21 and 28 d of age. At 8 weeks of age, the abundance of *Enterobacteriaceae*, as determined by plating on CHROMagar Enterobacteria Agar, was significantly higher in the cecum of pigs weaned at 21 d compared with pigs weaned at 28 d of age on the same diet. We spiked cecal contents with *Salmonella enterica* Serovar Typhimurium strain X4232 and investigated AMR transfer from the donor microbiota to *Salmonella* in vitro. A higher proportion of *Salmonella* acquired ampicillin resistance in cecal samples from pigs weaned at 21 d of age, in line with these samples having a higher abundance of donor *Enterobacteriaceae*. Further, a targeted metagenome of cecal *Enterobacteriaceae* indicated that *Enterobacteriaceae* from pigs weaned at 21 d harbored more diverse plasmid-borne AMR genes. Together, these findings suggest that weaning age may have a lasting impact on the abundance of cecal *Enterobacteriaceae*, which influences AMR composition and transfer in the swine hindgut. Strategies to decrease the abundance of *Enterobacteriaceae* after weaning may be effective in reducing preharvest AMR transfer to foodborne pathogens.

Key Words: swine, weaning, antimicrobial resistance

92744 Effect of heat stress on jejunal metabolic profile in broiler chickens. E. Greene*[1], H. Castro[2], C. Christopher[2], S. Campagna[2], and S. Dridi[1], [1]*University of Arkansas, Fayetteville, AR, USA,* [2]*University of Tennessee, Knoxville, TN, USA.*

With climate change fueling increased global temperatures, poultry production must adapt to remain sustainable. Broilers are especially susceptible to heat stress (HS), with known reductions in feed intake (FI) and growth, as well as decreased gut integrity and health. Here, we examined the jejunal metabolome of broiler chickens during HS, as well as a pair-fed regimen to distinguish the direct HS effects from the indirect effects related to FI reduction. Day-of-hatch Cobb 500 male broilers (n = 672) were randomly allocated to 12 environmentally controlled chambers (two pens/chamber, 28 birds/pen) and maintained under standard conditions for 28 d. On d 29, birds were assigned to one of the five treatments from d 29 to 42: thermoneutral (TN, 23°C), cyclic HS (CHS, 35°C, 8 h/d), pair-fed (PF, 23°C, but fed the same amount of feed as the CHS group), pre-HS (PHS, subject to CHS but sampled in the cool period), and acute HS (AHS, 35°, 3 h on d 42). On d 42, eight birds per treatment were sampled, and jejunum samples were snap frozen in liquid nitrogen. Metabolomic analysis was performed using ultra-performance liquid chromatography–high-resolution mass spectrometry, and metabolite abundances were normalized based on the exact mass of the tissue extracted before statistical analysis in MetaboAnalyst 5.0. Partial least squares discriminant analysis (PLS-DA) was used to identify global metabolome alterations, and metabolites with a VIP score > 1 were used for pathway analysis to identify the pathways affected in each experimental group. In the three-dimensional PLS-DA plot, TN, PHS, and PF all overlapped, with AHS and CHS completely separated from all other groups, indicating a shift in the metabolic profile due to HS. Interestingly, VIP scores for metabolites in the PF group were mostly intermediate between those of the TN and HS groups. Differentially affected pathways included aminoacyl-tRNA biosynthesis; purine metabolism; valine, leucine, and isoleucine biosynthesis; glutathione metabolism; and pantothenate and CoA biosynthesis. Together, these metabolic alterations suggest a HS-associated oxidative stress response and impacts on the translation process that are distinct from the metabolomic changes with reduced FI alone.

Key Words: metabolome, jejunum, broiler, heat stress, pair-fed

92718 Functional and structural characterization of the enteric nervous system in broiler chickens: The inner intestinal barrier of defense against foodborne pathogens. V. Caputi*, A. M. Donoghue, and J. M. Lyte, *Poultry Production and Product Safety Research, USDA-ARS, Fayetteville, AR, USA.*

In the gastrointestinal tract, the mucosal epithelium serves as a defensive barrier against bacterial translocation

and its function is finely modulated by the immune system. Submucosa and smooth muscle layers, located underneath the intestinal epithelium, are innervated by the enteric nervous system (ENS), a complex network of neurons capable of functioning independently from brain signaling. Recently, it was discovered in mammals that enteric neuronal populations can produce proinflammatory cytokines that stimulate intestinal immune cells to release antimicrobial peptides against *Salmonella* infection. Hence, neuroimmune interactions modulated by the ENS may strengthen the canonical epithelial barrier against pathogenic infections such as *Salmonella*. Enteric neuronal structures and their relationship with the immune system have been widely explored in mammals and humans; however, limited information is available on ENS structure and function in the poultry intestinal tract. Therefore, in our study we characterized for the first time the different neuronal populations and structures within the three-dimensional environment of the chicken ENS by using tissue clearing and three-dimensional imaging without tissue sectioning. Intestinal whole mount preparations of ileum, cecum, and colon from 4-week-old chickens were fixed in 4% paraformaldehyde and stained for HuC/D, the cell body marker of all enteric neurons, and nNOS, the marker for inhibitory neurotransmission. Tissues were cleared to optimally visualize enteric neuronal cells and fibers in both the myenteric and submucosal plexuses of different intestinal regions with confocal microscopy. Our findings demonstrate a novel methodology of staining that preserves the structural integrity of all the neuronal units across the chicken intestinal wall and can be leveraged by poultry researchers to study the role of the ENS in protecting the integrity of the inner intestinal barriers of defense against infection and foodborne pathogen colonization during the preharvest phases of poultry production.

Key Words: enteric nervous system, tissue clearing, confocal imaging, broiler chicken

92719 Characterization of regional differences in neonatal calf gut-associated lymphoid tissues using high-resolution snRNA-sequencing. M. Donia*[1,2], B. Aldridge[3], J. Lowe[1], and W. Li[4], *[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, IL, USA, [2]Department of Internal Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt, [3]Department of Veterinary Medical Sciences, College of Veterinary Medicine, Clemson University, Clemson, SC, USA, [4]US Dairy Forage Research Center, USDA-Agricultural Research Service, Madison, WI, USA.*

The intestinal tract is a critical portal through which animal management practices modulate host physiological and pathophysiological responses. The compartmentalization of digestion along the intestine is well characterized, but variation of immunological structure and function across intestinal segments is often overlooked. In this study, we used high-resolution single-nuclear RNA sequencing to explore regional differences in immune cell structure and function in the neonatal bovine intestinal tract. A healthy, 3-d-old calf was euthanized, and tissues collected from ileal (PPi) and jejunal (PPj) Peyer's patches and from ileal (MLNi) and jejunal (MLNj) mesenteric and pre-femoral (PF) lymph nodes. Single nucleus RNA (snRNA) was extracted and sequenced with a depth of 60,000 reads per cell. Reads were mapped to the bovine genome using the Cell Ranger pipeline. Gene counts, cell types, and differential expression were analyzed in R using the Seurat package. Immune pathway enrichment was performed using EnrichR online tool, and cell-cell communication was analyzed by the CellChat package. The immune cell composition and function of equivalent lymphoid tissues varied by location. The PPi contained the highest proportion of B cells, and B cells had the highest proliferative activity. Distinct, compartment-specific immune cell gene expression profiles were identified, with each intestinal region displaying a unique, immune pathway-specific transcriptomic signature. Intercellular communication analysis demonstrated attenuated signaling activity of B cells and T cells in PPi, MLNi, and PPj compared with MLNj and PF. This pilot study demonstrates the utility of high-resolution snRNA sequencing in characterizing regional differences in immune cell dynamics along the intestinal tract of the bovine neonate. The study findings underscore the future potential of this powerful molecular tool in evaluating the impact of different management strategies and therapeutic interventions on the development of the gut-associated lymphoid tissue and its associated responses to heath challenges.

Key Words: snRNA-sequencing, neonatal calf, gut-associated lymphoid tissue, mucosal immune development.

92707 Characterization of bacterial microbiomes according to the fecal phenotype in piglets before and after weaning. C. Vaggi*[1,2], J. C. Vötterl[1,2], F. Lerch[1,2], F. Yosi[2,3], S. Ricci[2,4], D. Verhovsek[5], and B. U. Metzler-Zebeli[1,2], *[1]Centre for Veterinary Systems Transformation and Sustainability, University of Veterinary Medicine Vienna, Vienna, Austria, [2]Christian Doppler Laboratory for Innovative Gut Health Concepts of Livestock, University of Veterinary Medicine Vienna, Vienna, Austria, [3]Department of Animal Science, Faculty of Agriculture, University of Sriwijaya, Palembang, South Sumatra, Indonesia, [4]Centre for Animal Nutrition and Welfare, University of Veterinary Medicine Vienna, Vienna, Austria, [5]Clinical Centre for Population Medicine in Fish, Pig and Poultry, University of Veterinary Medicine Vienna, Vienna, Austria.*

Feed and osmolytes in digesta may change the consistency and color of feces in piglets irrespective of the abundance of pathobionts. Little research has been published relating

phenotypic aspects of piglets' feces to their microbiome and using them as a proxy for gut health. This study aimed to monitor piglet's fecal phenotype and its associated microbiota, assuming that fecal color, consistency, and different sample types resemble a specific microbiota before and after weaning. From day of life (d) 28 to 36, fecal consistency was scored daily (1–5; 1 = watery feces; 5 = very hard feces) from 192 piglets in two replicate batches (48 piglets/sex/replicate batch). Fecal samples from each piglet were collected on d 28 (weaning day) and d 33. Samples were collected by rectal stimulation with a sterile swab and classified by sample type (swab/feces), color (brown/yellow), and consistency. DNA was extracted for 16S rRNA gene amplification and subsequent bioinformatics using QIIME2. Relative bacterial abundances and fecal scores were analyzed using MIXED PROC in SAS. Daily monitoring showed a change in fecal consistency, from firm feces on d 28–32 to moister feces on d 33–36 (from average score 3.5 to 3; $P < 0.001$). Swab samples had more *Escherichia* and *Bacteroides* on d 28 but not on d 33, whereas they contained more *Prevotella* on d 33 compared with feces ($P < 0.05$). Yellow feces were characterized by higher abundances of *Escherichia* on d 28 and *Prevotella* and *Alloprevotella* on d 33 compared with brown feces, while *Lactobacillus* abundance was higher in brown feces on d 33 ($P < 0.05$). Firmer feces were characterized by lower abundance of *Escherichia* compared with moister feces on d 28 but not on d 33, whereas *Prevotella* and *Lactobacillus* were more abundant in firmer feces on d 33 ($P < 0.05$). Results confirmed that the fecal phenotype has a distinctive microbiome composition. Associations between taxa and fecal characteristics differed for late suckling and early postweaning phase, reflecting changes in gut homeostasis. Data indicated that swab samples had very little resemblance to the other fecal phenotypes and should not be regarded as being equal in microbiome studies.

Key Words: fecal phenotype, microbiome, piglet

92725 The effects of heat stress and depressed feed intake on the cecal and ileal microbiota of broiler chickens. P. M. Campos*[1], M. Proszkowiec-Weglarz[1], and S. Dridi[2], *[1]USDA-ARS Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, USA, [2]Center of Excellence for Poultry Science, University of Arkansas, Fayetteville, AR, USA.*

Selection for high growth rate in modern broiler chickens has led to greater susceptibility to heat stress (HS). HS affects gastrointestinal tract barrier integrity and leads to reduced feed intake, which may alter the gut microbiota and further affect gut health. This study aimed to evaluate the effects of chronic and acute HS on the cecal and ileal microbiota of broiler chickens and a pair-fed (PF) group under ambient temperature conditions matching the feed intake of chronic HS birds to distinguish between the effect of HS per se and the effect of depressed feed intake on the microbiota. Broiler chicks were subjected to a thermoneutral (TN, 23°C) or chronic cyclic HS (CHS, 8 h/d, 35°C) condition from d 29 to 42. Additionally, another group was raised under TN conditions and exposed to 35°C for 2 h (acute HS, AHS) before sampling on d 42. CHS affected α diversity, resulting in significantly lower richness (observed amplicon sequence variants) compared with TN ($P = 0.02$) and AHS ($P = 0.02$) groups in the cecal microbiota, and the PF group also had lower richness compared with AHS ($P = 0.03$). In β diversity analyses, CHS, AHS, and PF all had distinct cecal microbiota compared with TN based on bacterial abundances (weighted UniFrac, $P < 0.05$), showing that both HS and feed intake could alter the microbiota. Alternatively, robust Aitchison principal component analysis also supported that CHS was distinct from TN ($P = 0.01$), but PF was not distinct from TN, suggesting microbiota could be altered more under CHS than under decreased feed intake only. In the ileum, CHS and TN microbiota were distinct based on presence and absence of taxa (unweighted UniFrac, $P = 0.04$), with PF being distinct from TN ($P = 0.02$) and CHS ($P < 0.01$); however, α diversity was not significantly affected. Predicted functional abundances showed that the PF treatment may influence different metabolic pathways compared with CHS or AHS. Understanding both the direct effects of HS and indirect effects from feed intake decrease will be of importance in devising nutritional strategies to maintain gut microbial balance and limit negative effects to broiler chickens' health.

Key Words: microbiota, broiler chicken, heat stress, feed intake, 16S

92734 Pathological and microbiological investigations of enteric disorders in postweaning pigs: Association between pathogen co-infections and enteritis pattern. T. Hassan[1], S. Bagatella[1], M. I. Malik[1], A. Bellato[1], D. Ippolito[2], S. Zoppi[3], M. B. Boniotti[4], G. L. Alboralis[4], and M. T. Capucchio*[1], *[1]University of Turin, Department of Veterinary Sciences, Grugliasco (Torino), Italy, [2]National Institute of Health, Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy, [3]Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d'Aosta, Torino, Italy, [4]Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy.*

Enteritis due to viral or bacterial infections is common among postweaning pigs, leading to high on-farm mortality rates. Mixed infections occur frequently, yet corresponding pathological data are underreported, rendering early differential diagnoses challenging. To this end, we investigated the prevalence and co-infection of porcine enteropathogens and their correlation to gross

pathology and histopathology in 62 necropsied pigs from four farms in Northern Italy. Enteritis was grossly evident in 54% of pigs, 3% showed enterotyphlocolitis, and 28% showed gastritis. Gastritis was positively associated with acute moderate to severe segmental enteritis ($P < 0.05$). Preliminary histological examination showed that the predominant microscopic lesions consisted of a moderate, chronic-active, mixed (lymphoplasmacytic and eosinophilic) enteritis involving all small intestinal tracts (20/35 cases examined thus far). The enteropathogens detected (and their prevalence) included *Clostridium perfringens* (CP, 47%); pathogenic *Escherichia coli* (34%); *Salmonella* sp. (S, 64%); and Rotavirus-A (37%), -B (29%), -C (64%), and -H (6%). Neither *Clostridioides difficile* nor porcine Coronavirus were detected. Co-infections with two or more pathogens occurred in 89% of pigs, while a single agent or no enteropathogens were detected in 3% and 8% of cases, respectively. A positive correlation was observed for CP and S co-infection ($P < 0.01$). Infections with Rotaviruses, CP, and S were significantly associated with grossly evident acute segmental enteritis ($P < 0.05$) rather than diffuse enteritis or enterotyphlocolitis. Overall, our data indicate that CP/S co-infections occur frequently in postweaning pigs and that CP and S co-infection or Rotavirus infection should be included in the differential diagnoses when a pattern of acute segmental enteritis is macroscopically evident at postmortem examination.

Key Words: enteritis, piglet, gross pathology, co-infection, enteropathogen

92727 Intestinal microbiome confers strong colonization resistance against necrotic enteritis in chickens. J. Liu[1], M. Whitmore[1], I. Tobin[1], M. Kaiser[2], S. Lamont[2], and G. Zhang*[1], *[1]Oklahoma State University, Stillwater, OK, USA, [2]Iowa State University, Ames, IA, USA.*

Necrotic enteritis (NE) poses a significant economic challenge in poultry production, leading to intestinal lesions, severe dysbiosis, growth retardation, and even mortality. Manipulating intestinal microbiota offers potential for enhancing disease resistance. We first evaluated the relative resistance to NE in two highly inbred chicken lines, Fayoumi M5.1 (FM5.1) and Leghorn Ghs6, alongside Cobb broilers. FM5.1 chickens demonstrated the highest resistance, with no mortality or small intestinal lesions, whereas Ghs6 and Cobb chickens exhibited mortality rates of approximately 8% and 20%, respectively. To further test the hypothesis that NE-resistant FM5.1 chickens harbor unique intestinal microbiota conferring enhanced resistance, cecal microbiota from d 35 FM5.1, Ghs6, and Cobb chickens were transplanted into newly hatched Cobb chickens. Survival rates, intestinal lesions, weight gain, and ileal and cecal microbiota were analyzed. Cecal microbiota from FM5.1 chickens provided 100% protection against NE in recipient Cobb chickens, which otherwise had a 35% mortality rate. Surprisingly, Ghs6 and Cobb chicken cecal microbiota also offered significant protection against NE. Intestinal microbiota differed markedly among the three breeds under both healthy and NE conditions. FM5.1 chickens had significantly higher levels of *Bifidobacterium*, *Lactobacillus*, and *Ligilactobacillus salivarius* in their ileum and cecum, suggesting their potential role in NE resistance. These findings underscore the crucial role of intestinal microbiota in conferring NE resistance and highlight the potential of exploiting commensal bacteria for NE mitigation in poultry.

Key Words: Fayoumi chicken, cecal microbiota transplantation, necrotic enteritis, microbiome

92721 A commensal bacterium protects chickens against necrotic enteritis. I. Tobin*[1], J. Liu[1], M. Whitmore[1], M. Kim[1], Z. Zhao[1], J. Guo[1], J. Scaria[2], and G. Zhang[1], *[1]Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA, [2]Department of Veterinary Pathobiology, Oklahoma State University, Stillwater, OK, USA.*

Necrotic enteritis (NE), caused by *Clostridium perfringens,* is a major economically significant disease, costing the global poultry industry $6 billion annually. With the US withdrawal of in-feed antibiotics since 2017, NE has become more prevalent, highlighting the need for more effective mitigation strategies. This study investigated the potential of microbiome-mediated colonization resistance to protect chickens against NE. Twelve representative bacterial isolates from the cecum of healthy feral chickens were screened for their ability to inhibit *C. perfringens* growth using an in vitro competition assay. Seven isolates demonstrated inhibitory effects, with the most potent showing 62% inhibition. Additionally, the culture supernatants of these bacteria were screened for their ability to induce the synthesis of host defense peptides (HDPs) using a high-throughput assay with a chicken macrophage cell line expressing the luciferase reporter gene driven by a HDP gene promoter. The most effective bacterium in inhibiting *C. perfringens* also significantly induced HDP gene expression. Furthermore, a chicken model of NE was employed to confirm the protective efficacy of this bacterium with dual *C. perfringens*-inhibiting and HDP-inducing activities. Oral administration of the bacterium significantly improved the survival rate from 48% to 98% and further reduced intestinal lesion severity. Collectively, these results highlight the potential of a beneficial commensal bacterium to mitigate NE through direct competition with *C. perfringens* and augmentation of host innate immunity.

Key Words: necrotic enteritis, poultry, colonization resistance

92726 A meta-analysis of necrotic enteritis in the microbiome. D. Varela and A. Ballou*, *SIWA, Iluma Alliance, Durham, NC, USA.*

Several experimental models have been developed for the study of necrotic enteritis (NE), but reported disease severity and recovery vary between these models. Further, there is increasing recognition of the importance of understanding microbial factors in subclinical NE. Molecular tools allow for deeper characterization of clinical and subclinical NE (genetic sources of pathogenicity, co-factors, etc.), but much remains unknown about how different microbial profiles may predispose or protect animals from NE, or how the impact of the disease on gastrointestinal microbial composition may factor into recovery. We hypothesized that by using appropriate data from a variety of publicly available sources, we could ask and answer questions about the impact of different experimental NE models, incorporating evaluations of different challenge conditions, diets, and choices of sample collection and frequency. Strict criteria were used to identify nine publicly available and four internal data sets related to experimental NE. Data from each study were treated to a comprehensive analytical process intended to correct for batch effects and allow for meta-analysis of the effects on NE on gastrointestinal microbial populations, as well as effects on other physiological traits evaluated in each trial. Among other findings, this meta-analysis suggests that even though a significant portion of studies focus on cecal populations, the ileum is the site of more severe and more consistent disease-related microbiome changes. We identified several consistently affected microbiome biomarkers and will explore the role of disease severity in these patterns. A feature of the response in both the ileum and cecum was a suppression of microbial diversity; while levels of *C. perfringens* were typically elevated, most of the consistent effects were in reductions of normal microflora. This undertaking was intended to explore the microbial traits that appear to be shared across the largest number of NE models, their relation to other measures of disease severity and recovery, and the study design considerations that follow from these observations.

Key Words: necrotic enteritis, microbiome, poultry, meta-analysis

92745 A curcumin derivative protease inhibitor mitigates *Brachyspira hampsonii*-induced swine dysentery. R. Puentes, R. Hannawayya, N. Mirzadzare, L. Perez-Perez, K. Tiedje, and E. R. Cobo*, *University of Calgary, Calgary, Alberta, Canada.*

The weaning phase in the pork industry is challenging because piglets can experience diarrhea, death, or impaired growth. Swine dysentery caused by *Brachyspira* spp. is a critical muco-hemorrhagic colitis in grower-finisher pigs. Intestinal protein-cleaving proteases from the mucosa or the spirochetes may trigger inflammation and contribute to the "leaky" gut syndrome, which evolves into diarrhea and allows the invasion of intestinal bacteria/toxins. Curcumin, the active component of turmeric, has shown diverse antibacterial and anti-inflammatory roles related to its anti-protease effects. We investigated the impact of a curcumin derivative (CMC 2.24) on alleviating the clinical-pathological postweaning swine dysentery caused by *B. hampsonii* infection. Weaned pigs were challenged with *B. hampsonii* or sham and orally given CMC 2.24 or inert solution thrice every other day after the challenge (four to six pigs per group). Pigs that were challenged excreted *B. hampsonii* between d 7 and 9, regardless of treatment. However, while most of the pigs challenged with *B. hampsonii* (83%) showed watery, mucoid, or bloody diarrhea between 10 and 13 d post challenge, fewer *B. hampsonii*-challenged pigs treated with CMC 2.24 (16%) reached that grade of diarrhea. Microscopically, pigs infected with *B. hampsonii* had more infiltrating neutrophils in the colonic lamina propia. The mucosal thickness associated with hyperplasia increased in the pigs challenged with *B. hampsonii* but not in the challenged pigs treated with CMC 2.24. The total protease level in feces was increased in *B. hampsonii*-challenged pigs, but it was comparatively lower in those treated with CMC 2.24 on d 9 after the challenge until euthanasia (d 11–20 post challenge). This study shows that the curcumin derivative CMC 2.24 can reduce the diarrheic colitis induced by *B. hampsonii*. This anti-inflammatory effect is likely due to fewer infiltrating neutrophils and reduced synthesis of proteases in the colon. This proof-of-concept study demonstrates that CMC 2.24 can be a novel antibiotic-free therapeutic alternative in swine health management.

Key Words: swine dysentery, *Brachyspira*, colitis, curcumin derivative, protease

92735 Can the mixture of *Hermetia illucens* and *Tenebrio molitor* meals influence the gut health of broiler chickens? T. Hassan*[1], M. I. Malik[1], C. Sferra[1], M. Gariglio[1], I. Ferrocino[2], I. Biasato[2], L. Gasco[2], and M. T. Capucchio[1], *[1]University of Turin, Department of Veterinary Sciences, Grugliasco (Torino), Italy, [2]University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco (Torino), Italy.*

To address feed shortages, the feed industry is exploring insect meal (*Hermetia illucens*, BSF; *Tenebrio molitor*, TM) as a sustainable alternative for poultry. Although BSF and TM are commonly used individually, their combined effect on broiler health and production remains unknown. A total of 420 day-old male broiler chicks were divided into seven treatments: control, BSF or TM at 5% and 10% (BSF5, BSF10, TM5, and TM10), and their mixture (1:1) at 5% and 10% (MIX5 and MIX10). At 38 d, 12 birds/

treatment (two birds/pen) were slaughtered to evaluate gut histo-morphometry; liver, spleen, and bursa of Fabricius histology; and cecal microbiota. Intestinal measurements were similar for all groups, except the duodenal villi from TM5 and MIX10 birds, which were longer than those from TM10-fed broilers (2.082 and 2.100 mm vs. 1.863 mm; $P < 0.05$). The BSF10 birds had shorter jejunal villi than control birds (1.172 vs. 1.421 mm; $P < 0.05$). Dietary insect meal inclusion did not influence the development or the severity of the observed findings in either the intestine or the main organs ($P > 0.05$). Regarding microbiota composition, a common microbiota was observed, but the fraction of minor ASVs was differentially associated with the dietary treatments ($P < 0.05$). Some bacteria that produce short-chain fatty acids (i.e., *Alistipes* and *Ruminococcaceae*) significantly increased in the low percentages of inclusion and in MIX10 broilers. In conclusion, except for the BSF10 group, insect meal had no overall negative effects on intestinal histo-morphometry or animal health. Bacteria with potential positive effects have been observed in chickens fed insects. Overall, the low inclusion rates, as well as the 10% mixture, showed positive effects on gut microbiota. More in-depth studies are needed to establish the best inclusion rates of the two meal combinations.

Key Words: insect meal mixture, poultry, histomorphometry, microbiota

92737 Effects of dietary fats on diversity and function of cecal microbiota in broiler chickens. V. V. Jadhav*, Y. O. Fasina, P. C. Omaliko, J. Han, and S. H. Harrison, *North Carolina Agricultural and Technical State University, Greensboro, NC, USA.*

Diet has been reported to affect the diversity and function of gut microbiota. Our study investigated the effect of dietary fat types on cecal microbial composition and predicted function in broiler chickens at d 41 and 55 of age. Shotgun metagenomic sequencing was used to evaluate effects of dietary fat sources on cecal microbiota. Four dietary fat sources were evaluated and compared with a control dietary fat source of poultry fat: two diets rich in omega-3 polyunsaturated fatty acids (PUFA) from fish oil and flaxseed oil; a diet rich in long-chain saturated fatty acids (SFA) from lard; and a diet rich in medium-chain SFA from coconut oil. At d 55, broilers fed a PUFA-rich flaxseed oil diet exhibited significantly increased cecal microbial diversity and richness compared with the SFA-rich lard and coconut oil diets. More specifically, PUFA intake was associated with elevated levels of bacterial family *Lachnospiraceae* compared with SFA intake. Members of the *Lachnospiraceae* family promote gut health by producing short-chain fatty acids, which support gut barrier integrity, provide energy, and have anti-inflammatory effects. The fish oil diet, in comparison with the control, had increased levels of *Bacteroides finegoldii*, *Bacteroides faecium*, and *Bacteroides luhongzhouii*, all of which are involved in carbohydrate breakdown, immune modulation, and short-chain fatty acid production. Predicted pathway and gene ontology term abundances found for cecal microbiota showed a greater range of specific association with those chickens fed SFA-rich diets (lard and coconut oil) than for those chickens fed PUFA-rich diets (fish and flaxseed oil) compared with the control, suggesting a functionally distinct impact of SFA on the gut environment. Our findings overall highlight the contrasting effect of SFA and PUFA fat type on cecal microbiota and indicate a general potential of dietary fats for modulating host health and phenotype.

Key Words: broiler chicken, chicken microbiome, dietary fat, metagenomics, polyunsaturated fat

92464 Calycosin from Astragali Radix as a potential phytogenic feed additive in weaning piglets: In vivo and in vitro studies. J. Wang*[1,2], [1]*Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China,* [2]*Sino-US Joint Laboratory of Animal Science, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China.*

Astragali Radix is one of the most commonly used herbs in traditional Chinese medicine. The main active ingredient in Astragali Radix is calycosin (CA), an isoflavonoid. We have previously discovered that CA triggers host defense peptide (HDP) production in porcine IPEC-J2 cells. The purpose of this study was to explore the alleviation effects of CA on lipopolysaccharide (LPS)-induced cell stress and the supplementation effect of CA on the growth performance, intestinal immunity, and the microbiota in weaned piglets. In LPS-stimulated IPEC-J2 cells, CA pretreatment diminished the levels of reactive oxygen species, malondialdehyde, 8-hydroxy-2′-deoxyguanosine, and carbonyl, indicating its antioxidant activity. Pretreatment with CA rescued LPS-induced changes in antioxidant enzyme activity and associated gene expression. Mechanistically, CA activated the nuclear erythroid 2-related factor 2 (Nrf2) pathway, and upon *Nrf2* silencing, CA failed to rescue LPS-induced cell damage and changes in redox homeostasis. Further, CA pretreatment significantly alleviated changes in *IL-1β*, *TNF-α*, and *TGF-β* mRNA levels, while the NF-κB p65 phosphorylation was lowered, indicating the regulation of NF-κB signaling pathway. Supplementation with CA in weaning piglets enhanced average daily growth and feed intake and reduced the diarrhea rate. It also significantly increased serum IgA and IgG levels and intestinal goblet cell numbers compared with the control, alleviated intestinal immune dysfunction by downregulating inflammatory cytokine expression, and enhanced HDP expression. Furthermore, it regulated jejunal microbial diversity and composition, with certain differential bacterial genera showing high correlation with serum

cytokine and Ig levels, indicating the contribution of the intestinal microbiota to host immunity. Our results provide insights into future applications of CA as a potential feed additive in livestock production.

Key Words: calycosin, weaned piglet, cell damage, growth performance, intestinal health

92736 Identifying and isolating primary degraders of resistant potato starch from the swine gastrointestinal tract. H. R. Watkins*[1,2], C. L. Loving[1], and C. L. Anderson[1], *[1]USDA-ARS-NADC, Ames, IA, USA, [2]Iowa State University, Ames, IA, USA.*

Resistant starch (RS) is a fraction of dietary fiber that is not degraded by host amylases and undergoes microbial fermentation in the large intestine. RS fermentation has recognized prebiotic effects, including increased short-chain fatty acid production, changes to host physiology, and promotion of colonization resistance against enteric pathogens. However, only select members of the hindgut microbiota can adhere to RS granules and initiate hydrolysis. These bacteria act as primary degraders that render a substrate that secondary degraders can use and thus continue the network of RS metabolism. *Ruminococcus bromii* and *Bifidobacterium* sp. are well-characterized primary degraders of RS in humans; however, primary degraders from swine have not been isolated. Metagenomic sequencing of cecal samples collected from ~42-d-old pigs fed a diet amended with 5% resistant potato starch (RPS) indicated the abundances of *Bifidobacterium* and *Ruminococcus* peaked after 28 d of prebiotic administration. Further, *B. thermophilum* and *R. bromii* were detected at greater relative abundances in pigs that shed less *Salmonella*. We then used fecal enrichments with RPS and a quantitative reducing sugar assay to isolate and characterize pig-specific primary degraders of RPS. 16S rRNA gene amplicon sequencing demonstrated that the relative abundance of *Bifidobacterium* increased from 0.010% to 12% over a 30-h incubation with RPS. Our efforts to identify and describe pig-origin primary degraders of RPS will aid in addressing the variable nature of RS in feeding trials. We hypothesize that the disparate effects of RS on colonization resistance can be attributed to the absence of primary degraders in the gut microbiota of nonresponders. Moving forward, we aim to develop strategies that increase the abundance of RS primary degraders as well as other bacteria involved in metabolizing RPS in the swine hindgut.

Key Words: microbiome, swine, resistant starch, prebiotic, colonization resistance

Poster Session

92720 Effect of *Salmonella enteritidis* challenge on the microbiome of 54- to 56-week-old layers. T. Lavergne*[1], C. Elrod[1], and M. Nascimento[2], *[1]Natural Biologics, Inc., Newfield, NY, USA, [2]Sapiens, Jundiai, Sao Paulo, Brazil.*

This trial was conducted to evaluate the microbiota of 54- to 56-week-old layers under a *Salmonella enteritidis* (SE) challenge. Seventy-two layers were placed in 36 cages, two layers per cage and nine replicates per treatment. The four treatments were (1) nonchallenged control (NCC), (2) challenged control (CC), (3) challenged plus Cascade (hydrolyzed yeast + yeast culture; 100 g/ton), and (4) challenged plus Naverde (hydrolyzed yeast + hydrolyzed yeast cell wall; 100 g/ton). Layers were fed a corn-soybean meal mash diet formulated to meet nutrient requirements. Following a 4-week adaptation period, layers in the challenged groups were orally administered 1 mL of inoculum with 2×10^9 cfu/mL of nalidixic acid-resistant SE. The NCC group was given a sham inoculation. On d 14 post challenge, a cloacal swab was collected from one hen in each of replicates 1 to 6. A commercial laboratory performed 16S DNA sequencing on the swabs and the data were analyzed by an artificial intelligence platform, Sapiens, to identify and quantify the total number of genera present (richness), positive and negative biomarkers, and microbiota robustness (diversity and number of negative biomarkers). All groups had similar *Prevotella* to *Bacteroides* ratios, indicating that the hens had mature microbiota that were not affected by the SE challenge. Richness was higher for the NCC and Naverde groups than for the CC and Cascade groups. Diversity was higher for the Naverde, NCC, and Cascade groups than for the CC group. Robustness was better for the NCC and Cascade groups than for the CC and Naverde groups. Microbiota diversity and richness may not be sufficient to understand the entire scenario. The Naverde group had excellent diversity and richness; however, its robustness was low, indicating that the SE challenge increased negative biomarkers. The Cascade group had a higher ratio of positive to negative biomarkers than the NCC or CC groups. Thus, even with the mature microbiota of these hens, the SE challenge negatively affected their microbiota richness, diversity, and robustness, and pre- and postbiotics shifted the microbiota toward a more favorable microbiome.

Key Words: microbiome, layer, *Salmonella*, prebiotic, postbiotic

92713 Deficiency in nitro-detoxification may limit *Salmonella* survivability in macrophages. A. Ingram[1], C. Johnson[2], N. Mast[1], R. Anderson*[2], and R. Arsenault[2], *[1]Department of Animal Science and Veterinary Technology, Texas A&M University-Kingsville, Kingsville, TX, USA, [2]United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX, USA.*

Salmonella in lymph nodes can escape postharvest interventions. Removal of infected lymph nodes during processing is burdensome, and thus, interventions that get rid of infectious *Salmonella* would benefit food safety. Given that invasion and survival of *Salmonella* within host macrophages depends at least partly on their ability to detoxify reactive nitrogen radicals, we hypothesized that *Salmonella* deficient in this activity may be less competitive within the host's macrophage. To test this hypothesis, we infected wells containing 10^5 HD11 cells (a macrophage cell line) with 10^5 colony forming units (cfu) of *Salmonella* serovars Dublin and Newport and an uncharacterized *Salmonella* designated K290 that was isolated from beef lymph nodes. Each strain was manipulated via serial culture without or with 5 mM chlorate to generate populations that were either proficient or deficient in uptake of molybdenum (Mo), a potential cofactor requirement for nitro-radical detoxification. Intramacrophage *Salmonella* were enumerated on Brilliant Green agar. ANOVA (three wells/population) revealed a phenotype effect ($P < 0.05$) on intracellular invasiveness of Dublin and K290 against the HD11 cells at 1 h post infection, with Mo-deficient populations being up to 126-fold lower than Mo-proficient populations (0.3 to 1.8×10^3 cfu/well, respectively). Intracellular survivability of Dublin and Newport measured 24 h post infection was > 7-fold lower ($P < 0.05$) in Mo-deficient than in Mo-proficient populations (1 to 3×10^2 cfu/well, respectively). Survivability of K290 did not differ ($P > 0.10$) phenotypically. Nitrite accumulations 24 h post infection, a proxy for nitric oxide, were not observed in Dublin populations. However, they were 67% lower in Mo-deficient K290 populations, which did not phenotypically differ in survivability, and 91% higher in Mo-deficient Newport populations, which were less survivable intracellularly, than their respective nitro-degrading proficient populations (achieving 11.1 ± 0.9 and 3.5 ± 1.6 nmol/mL, respectively). These results suggest that deficiency in nitro-degradation can limit the intracellular survivability of certain *Salmonella*.

Key Words: macrophage, *Salmonella*, survivability

92733 Impact of heat stress on intestinal barrier integrity in broilers: A comparison of low- and high-water efficient lines. M. Cuadrado*[1], B. Roach[2], E. Greene[1], K. Lassiter[1], W. Bottje[1], S. Orlowski-Workman[1], and S. Dridi[1], *[1]University of Arkansas, Poultry Science, Fayetteville, AR, USA, [2]Har-Ber High School, Springdale, AR, USA.*

Water scarcity and heat stress (HS) are critical challenges facing the poultry industry, as they both affect the health, productivity, and welfare of birds. Intestinal integrity is of particular importance under challenging conditions to maintain nutrient absorption, health, and growth. Recently, we divergently selected birds for low- and high-water efficiency (LWE/HWE), and here we explore the impact of HS on gut integrity in these two lines. Day-old chicks from both lines were placed in 12 environmentally controlled chambers (two pens/chamber, 20 birds/pen, 240 birds/line). On d 29, daily cyclic HS (36°C, 9 h/d) was applied to half of the chambers; the rest remained under thermoneutral conditions (25°C). On d 49, samples were collected for measures of gut integrity and ileal gene and protein expression. LWE birds exhibited higher levels of gut permeability, regardless of temperature, as measured by fluorescein isothiocyanate–dextran (FITC-D), compared with HWE birds (P = 0.0009). Cadherin-1 (*Cdh1*) expression was lower in HWE birds exposed to HS (P = 0.0394), while gap junction protein α 1 (*Gja1*) was upregulated in HWE birds (P = 0.0258). Both claudins *Cldn1* (P = 0.0179) and *Cldn4* (P = 0.008) were upregulated in HWE birds, with the latter also induced by HS (P = 0.014). Claudin 5 (*Cldn5*) displayed different responses between the two lines: It was upregulated by HS in LWE birds (P = 0.0475); however it was downregulated in HWE birds. Lipocalin (*Lcn2*) was higher in HS-LWE birds (P = 0.0058), while no significant change in was observed in HWE birds. These results suggest that HWE birds had better gut integrity compared with their LWE counterparts, and they were more resilient to HS.

Key Words: broiler, heat stress, water efficiency, gut integrity

92704 Standardization of methodologies in poultry microbiome research: One year of progress. J. Lyte*[1], D. Ayala[2], J. De Oliveira[3], S. Dridi[4], D. Grum[2], T. Johnson[5], J. Kers[6], M. Kogut[7], J. Rehberger[8], M. Seyoum[4], A. Smith[8], G. Zhang[9], and M. Proskowiec-Weglarz[10], *[1]USDA-ARS, Fayetteville, AR, USA, [2]Purina Land O'Lakes, Gray Summit, MO, USA, [3]Cargill, Vilvoorde, Belgium, [4]University of Arkansas, Fayetteville, AR, USA, [5]University of Minnesota, Saint Paul, MN, USA, [6]Utrecht University, Utretcht, The Netherlands, [7]USDA-ARS, College Station, TX, USA, [8]Arm & Hammer Animal Nutrition, Waukesha, WI, USA, [9]Oklahoma State University, Stillwater, OK, USA, [10]USDA-ARS, Beltsville, MD, USA.*

The gastrointestinal microbiome has been implicated in virtually every aspect of poultry physiology and performance. Although 16S rRNA gene sequencing continues to be the most popular method to characterize the poultry microbiota, methodological variation often complicates data interpretation, negatively affects reproducibility, and prevents meaningful inter-laboratory comparison of 16S rRNA amplicon sequencing results. As such, a need exists for a set of guidelines that establish a standardized framework in poultry microbiota experimental design. At the 2023 Symposium on Gut Health in Production of Food Animals, a committee of experts from academia, industry, and government was created and tasked with developing an evidence-based, best-practices approach that would help eliminate errors or biases that arise from methodological inconsistencies at each stage of a poultry microbiota study. This abstract marks the one-year anniversary since the formation of the committee and presents an update on committee activities. A 16S rRNA amplicon sequencing study involves multiple stages that may be broadly generalized into sample collection, DNA extraction, library preparation, sequencing, bioinformatic analysis, and data deposition. Poultry researchers must consider unique species considerations and challenges that are not present in mammals, such as bifurcated ceca. Committee discussions have yielded a draft poultry-focused methodological framework that engages each of these experimental stages that will be submitted to Poultry Science for publication in 2025. In addition, this manuscript includes supporting data generated by committee members that demonstrate the choice of 16S primers and the taxonomic database selection have significant (P < 0.05) effects on poultry microbiota composition and classification results. Committee members are actively engaged in the planning stage of implementing a robust and comprehensive testing pipeline to empirically evaluate a standardized poultry microbiota study protocol, and develop specific reagents for poultry microbiota research.

Key Words: microbiome, standard, control, methodology, poultry

92716 Effects of a stimbiotic on nutrient digestibility, oocyst shedding, blood profiles, intestinal gene expression of tight junction protein, and cecal microbiota in necrotic enteritis-challenged broilers. H. Kim*, S. Chang, D. Song, K. Jeon, and J. Cho, *Chungbuk National University, Cheong ju, Chungcheongbuk-do, Korea.*

This experiment was conducted to investigate the effect of a stimbiotic (STB) on nutrient digestibility and gut health in broilers with necrotic enteritis (NE). A total of 160 day-old Arbor Acres (initial body weight of 44.03 ± 0.28 g) were used in this 28-d experiment. All broilers were randomly allocated into four treatments, and each experimental group had 10 replicate cages with four broilers per cage. The experiment was conducted in a 2 × 2 factorial design consisting of two levels of challenge (challenge and nonchallenge) and two levels of STB (0% and 0.05%). All broilers in challenged groups were orally challenged by overdosing with coccidia vaccines (10 times the recommended doses; Livacox® Q). The NE challenge significantly decreased (P < 0.05) nutrient digestibility,

interferon-γ, heterophil levels in blood, and villus height to crypt depth ratio (VH:CD) compared with the nonchallenge group. In addition, the NE challenge groups had significantly lower ($P < 0.05$) ZO-1 and higher MUC2 gene expression than the nonchallenge group. Supplementation of 0.05% STB with the NE challenge significantly increased ($P < 0.05$) gross energy digestibility and decreased ($P < 0.05$) the number of oocysts per gram of feces compared with the NE-challenged group. Supplementation of 0.05% STB significantly increased ($P < 0.05$) the VH:CD in ileum compared with the nonsupplemented group. Supplementation of 0.05% STB also significantly lowered ($P < 0.05$) MUC2 and TLR4 gene expression in the ileum compared with the nonsupplemented group. At the genus level, the supplementation of 0.05% STB with the NE challenge significantly decreased ($P < 0.05$) the abundance of *Muribaculaceae* compared with the NE-challenged group on d 21. In conclusion, dietary supplementation of 0.05% STB could positively regulate the cecal microflora and gene expression of tight junction protein and alleviate the decline in nutrient digestibility caused by NE.

Key Words: broiler, necrotic enteritis, tight junction, cecal microbiota

92738 Subclinical exposure to multiple mycotoxins causes cecal microbiota dysbiosis in broiler chickens. L. Kappari[1], J. Lourenco[2], T. Applegate[1], A. Glenn[3], and R. Shanmugasundaram*[3], *[1]Department of Poultry Science, University of Georgia, Athens, GA, USA, [2]Department of Animal and Dairy Science, University of Georgia, Athens, GA, USA, [3]Toxicology and Mycotoxin Research Unit, U.S. National Poultry Research Center, Agricultural Research Service, U.S. Department of Agriculture, Athens, GA, USA.*

Fusarium mycotoxins contaminate poultry feed ingredients, and their presence causes dysregulation of intestinal homeostasis, increased gut permeability, and inflammation. Hence, this study aimed to identify the effects of fumonisin B1 (FB1), deoxynivalenol (DON), and zearalenone (ZEA) on the cecal microbiota composition, diversity, and short-chain fatty acid (SCFA) profile in broilers. A total of 960 day-old broilers were distributed into eight dietary treatments, including T1 (control) and those with mycotoxins added (mg/kg diet): T2 (21.0 FB1 + 3.0 DON + 1.0 ZEA), T3 (17.0 FB1 + 1.0 DON + 0.2 ZEA), T4 (9.0 FB1 + 3.5 DON + 0.7 ZEA), T5 (5.0 FB1 + 0.4 DON + 0.1 ZEA), T6 (2.0 FB1 + 2.5 DON + 0.9 ZEA), T7 (0.6 FB1 + 1.0 DON + 0.3 ZEA), and T8 (0.8 FB1 + 0.5 DON + 0.1 ZEA). Cecal contents were collected on d 21 and 35, and bacterial compositions were analyzed by sequencing the V3–V4 region of the 16S rRNA gene. On d 21, there was a significant increase ($P < 0.05$) in relative abundance on *Lachnospiraceae* by 6.84% and *Ruminococcaceae* by 7.29%, while *Lactobacillaceae* and *Bacteroidaceae* decreased by 12.42% and 25.29% in T2 groups compared with the control groups. On d 35, *Lachnospiraceae* abundance significantly increased ($P < 0.05$) by 24.78%, while *Ruminococcaceae*, *Lactobacillaceae*, and *Bacteroidaceae* decreased by 20.95%, 8.69%, and 10.68% in T2 groups compared with the control group ($P < 0.05$). Microbial evenness and diversity significantly decreased in T2, T4, and T6 groups on days both d21 and d35 ($P < 0.05$) compared with the control group. On d 21, acetate concentrations in cecal contents were 17.5% and 15.5% higher in T5 and T6, and total SCFAs were lower in T7 and T8 groups (20.0% to 74.1%) compared with the control group ($P < 0.0001$). On d 35, cecal acetate and total SCFA concentrations were increased in T4 groups by 43.5% and 44.4%, and decreased in T5, T6, T7, and T8 groups (59.5%–69.1%) compared with the control ($P < 0.05$), respectively. The present findings show that multiple mycotoxin concentrations in the diet, even at subclinical levels, alter the bacterial microbiota composition and SCFA profile in the cecal content of the birds.

Key Words: mycotoxin, broiler, microbial dysbiosis

92728 Effect of yeast fermentate on rumen microbiota and gas emissions in an in vitro model. E. G. Olson*[1], A. Rodrigues[1], I. Ferreira[1], A. Scheaffer[2], and S. C. Ricke[1], *[1]University of Wisconsin–Madison, Madison, WI, USA, [2]SweetPro, Wahalla, ND, USA.*

Feed additives such as Probiotein® use yeast fermentate biotechnology to enhance animal health and performance. This study used an in vitro rumen model, supplemented with Probiotein Shape Flax (PSF) and Probiotein Walhalla Flax (PWF), to assess their impact on rumen microbiota compared with control. Volatile fatty acid (VFA) composition at 24 h and gas emissions (methane, hydrogen, and carbon dioxide) at 6 and 24 h were analyzed. The experimental setup involved adding 225 mg of total mixed rations and 75 mg of feed additive to 30 mL of rumen fluid, which was incubated at 39°C for 24 h. Gas composition was measured via gas chromatography, and statistical significance was determined using *t*-tests ($P < 0.05$). Spearman's correlation analysis ($P < 0.05$) was conducted on significant VFAs. Results showed a significantly higher total VFA concentration in the PSF group ($P < 0.05$), particularly increasing acetic acid, isovaleric acid, and valeric acid. Isobutyric acid levels were significantly lower in both PSF and PWF groups ($P < 0.05$). Spearman's analysis revealed a moderate negative correlation between acetic acid and both isobutyric and isovaleric acids, while valeric acid showed a weak positive correlation with acetic acid in the control. Isobutyric and valeric acids showed a moderately positive correlation. The treatments showed a moderately negative correlation between isobutyric acid and acetic and isovaleric acids, with a strong positive correlation between acetic, isovaleric, and valeric acids.

Despite a trend toward reduced methane in the PSF group at 6 h, the difference was not statistically significant ($P > 0.05$). No significant differences across treatments were observed in methane, hydrogen, or carbon dioxide concentrations. These findings suggest that Probiotein additives may promote fiber digestion and alter amino acid fermentation, likely due to shifts in microbial populations.

Key Words: feed additive, rumen microbiota, volatile fatty acid

92731 Effects of feeding cashew nutshell liquid on rumen fermentation characteristics and methane emission in Japanese Black beef cattle (Wagyu). Y. Uyeno*[1], Y. Sakai[2], T. Abe[3], K. Takahashi[4], T. Kuramoto[5], and H. Iwata[5], *[1]Shinshu University, Minamiminowa, Nagano, Japan, [2]Japan Cattle Industry Cooperative, Tokyo, Japan, [3]National Livestock Breeding Center, Nishigo, Fukushima, Japan, [4]SDS Biotech K.K., Tokyo, Japan, [5]Tokyo University of Agriculture, Atsugi, Kanagawa, Japan.*

The Japanese Black breed (Wagyu, JB), known for its superior beef quality, requires specific feeding regimens to maintain its premium status. To address concerns about greenhouse gas emissions, evaluating methane production from JB cattle and exploring practical feeding interventions to mitigate emissions are urgent needs. This study aimed to quantify methane emissions from JB cattle rumen and verify cashew nutshell liquid (CNSL) supplementation regarding methane emissions and rumen fermentation dynamics. Twenty-seven JB steers in the National Livestock Breeding Center were divided into two age groups: young ones at 15 months (n = 18) and aged steers at 22 months (n = 9). Each age group was subdivided into three groups that received different levels of CNSL (0, 100, and 200 g/d). The cattle were fed a commercial concentrate according to the Japanese feeding standards, with ad libitum access to rice straw as forage. Methane measurements were conducted monthly at baseline for 3 months using a headbox gas monitoring system. Furthermore, the rumen fluid was analyzed for volatile fatty acids using HPLC, and bacterial community structures were assessed using 16S rRNA gene sequencing. A factorial analyses included herds, CNSL levels, and treatment duration. No significant differences in methane production were observed between the treatment groups. In the young herd, feeding CNSL significantly reduced dry matter intake ($P = 0.04$) and concentrate intake ($P = 0.01$), although forage intake remained unchanged. Correlation analysis indicated that methane production was negatively correlated with animal age ($P < 0.05$) and duration of CNSL feeding. Bacterial families from the phyla Bacteroidota (e.g., Family_F082) and Firmicutes (*Selenomonadaceae*) increased in the CNSL-fed groups, with a trend toward reduced ruminal acetate levels. These results suggest that CNSL may influence the rumen environment and contribute to a reduction in methane emissions; however, this effect may be secondary to the natural reduction associated with the growth of JB cattle.

Key Words: greenhouse gas, cashew nutshell liquid, Japanese Black breed

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