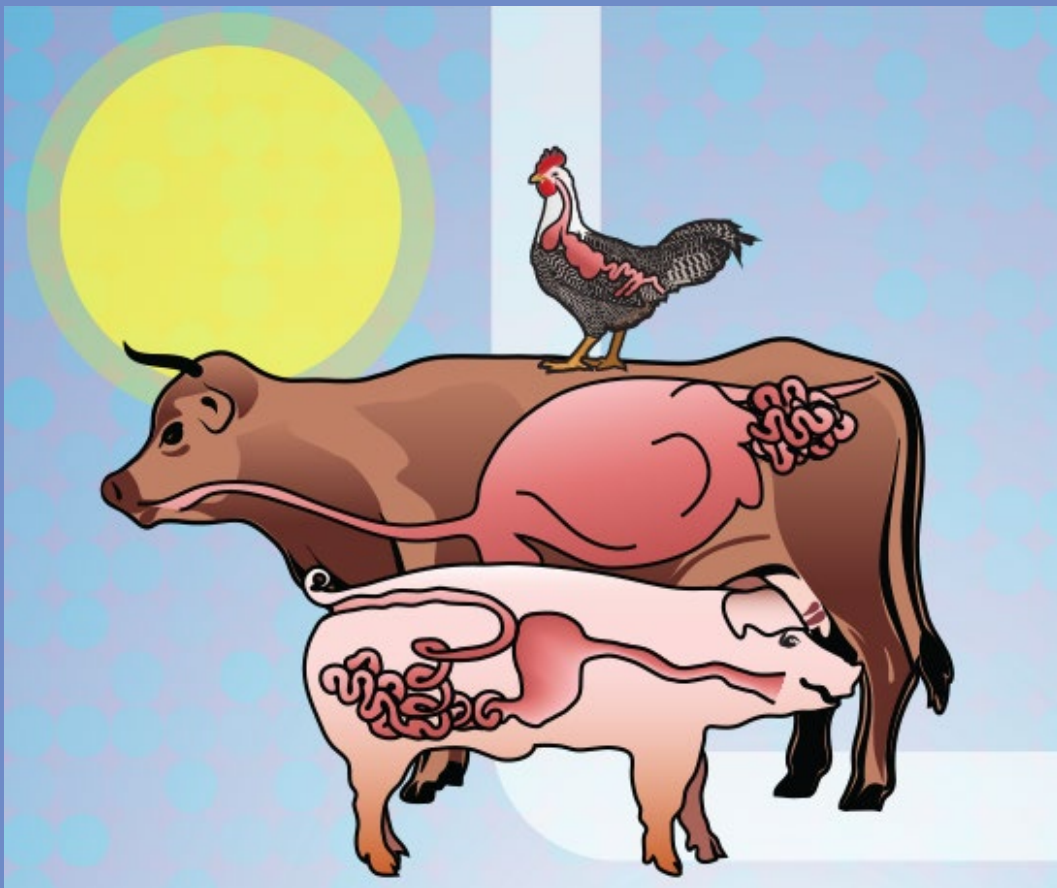


Symposium on Gut Health in Production of Food Animals

November 4–6, 2019, St. Louis, Missouri



Program and Abstracts
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WELCOME

On behalf of the Organizing Committee for the 8th Symposium on Gut Health in Production of Food Animals, I welcome you back to St. Louis, Missouri! After a very successful 7th Symposium here in 2018, we decided a return trip was in order. I look forward to another scientifically and socially rewarding meeting in 2019.

The aim of the symposium is to bring together 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 often described simply as “the gut,” it is actually made up of (1) an epithelium; (2) a diverse and robust immune arm, which contains most of the immune cells in the body; and (3) the commensal bacteria—more cells than are present in the entire host organism. Understanding the crosstalk between ALL of these interrelated components of the gut is what cumulatively makes the gut the basis for the well-being of animals and the motor that drives their performance. The abstracts submitted to the symposium define these links and mechanisms that inter-connect the three components of the gut and describe how each can be manipulated to improve animal health.



This year, as in the past, we have invited two distinguished plenary speakers, who will cover current research topics in avian and porcine gut health. Please take advantage of the presence of these scientists to engage in productive talks and develop collaborations between laboratories and research groups to further the science of gut health. I have organized a mini-symposium within the main Symposium. On Monday afternoon, we will have a series of presentations on the oxidative status of the gut and its role in overall gut health.

Likewise, I encourage all of you to 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 that, whatever your research specialty or food animal commodity, we are all working together to improve food quality for the consumer.

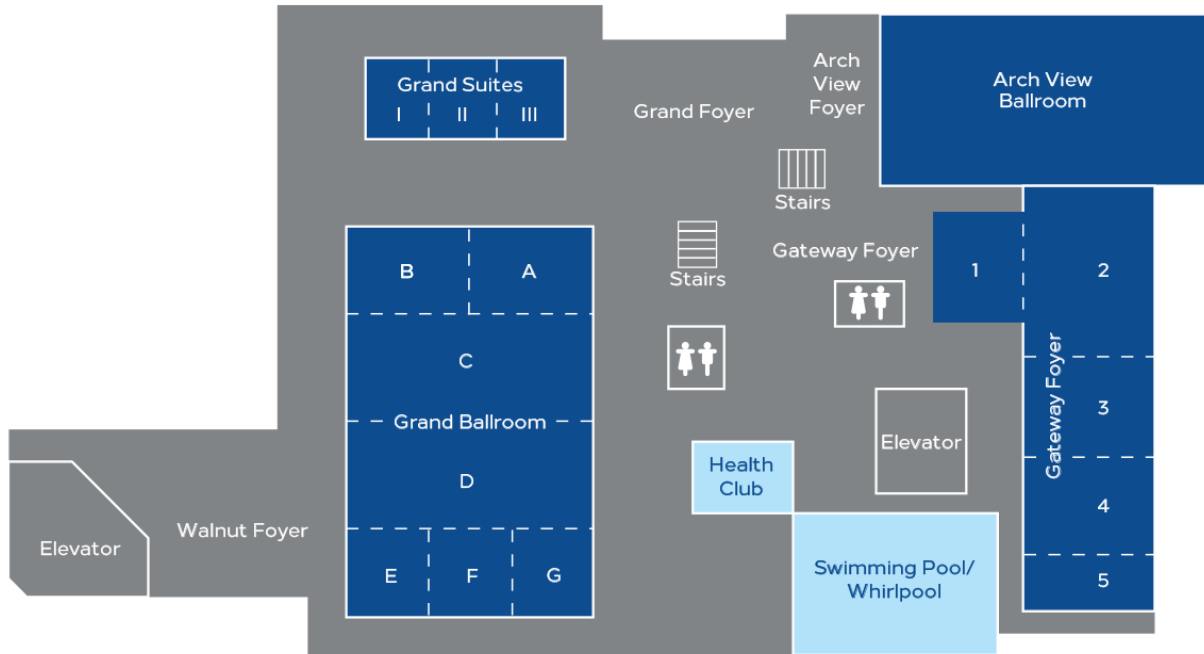
Welcome again and enjoy the Symposium and your stay in St. Louis!

Mike Kogut
Chair, Organizing Committee

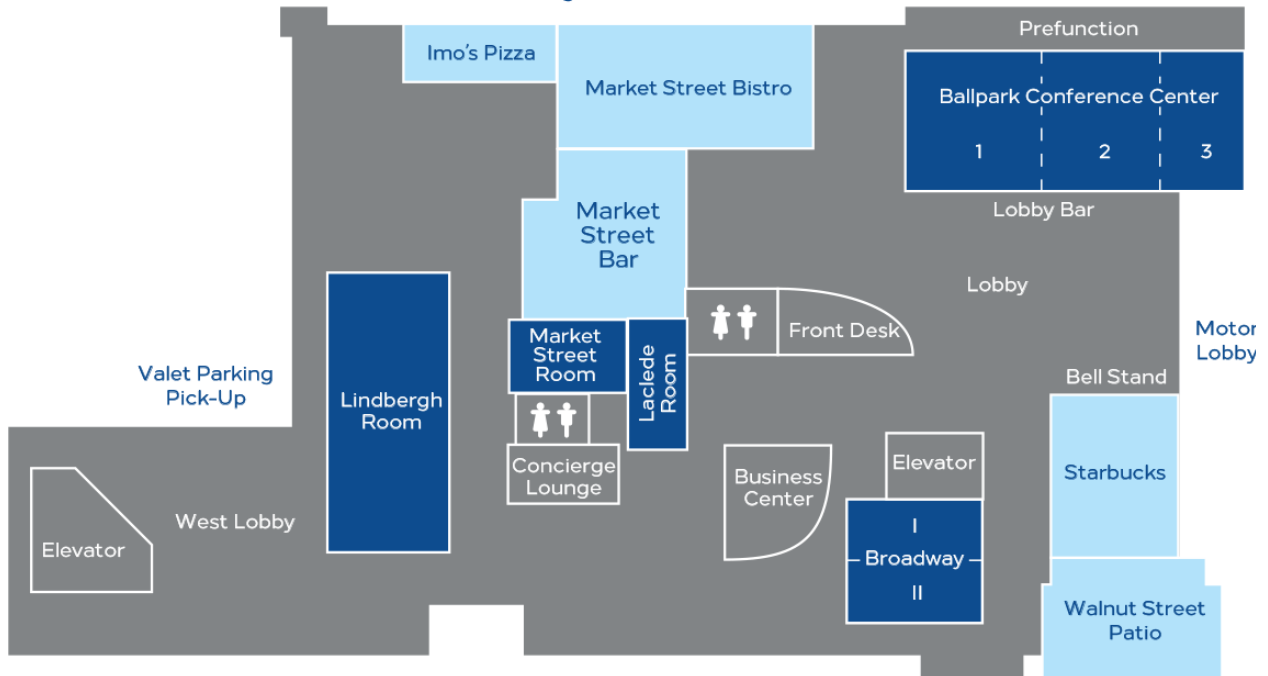


Hilton St. Louis at the Ballpark

Second Floor Convention Level



Lobby Level





Program

Sunday, November 3

5:00 pm – 7:00 pm Registration: Grand Foyer

Monday, November 4

7:00 am – 8:00 am Breakfast: Arch View Ballroom

SESSION 1: AGE-DEPENDENT DEVELOPMENT OF MICROBIOME

Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D

8:00 am **Invited presentation: The gut mycobiome: Implications in piglet health?**
(Abstract 100)
K. Summers*¹, J. F. Frey¹, T. Ramsay¹, and A. Arfken^{1,2}, ¹USDA, Beltsville, MD, USA, ²ORISE, Oak Ridge, TN, USA.

9:00 am **Chicken intestinal mycobiome: Biogeography, succession, and its response to bacitracin methylene disalicylate.** (Abstract 101)
K. Robinson*¹, T. J. Johnson², and G. Zhang¹, ¹Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA, ²Department of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota, USA.

9:30 am **Development of the piglet fecal bacteriome and mycobiome from birth through weaning.** (Abstract 102)
A. Arfken*, J. Frey, and K. Summers, USDA-ARS, Beltsville, MD, USA.

10:00 am Coffee Break: Grand Foyer
Sponsored by SilvaTeam

10:30 am **Comparison of gut microbiota between slow- and fast-growing broiler chickens.** (Abstract 103)
M. Proszkowiec-Weglarz*, L. Schreier, K. Miska, S. Kahl, Y. Qu, B. Russell, and T. Elsasser, USDA, ARS, Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, USA.

11:00 am **Trends involved in bacterial transfer for day-of-hatch chick microbiome.**
(Abstract 104)
J. Gruber*, E. Kim, M. Perry, and A. Thomas, DuPont Animal Nutrition, Wilmington, DE, USA.

11:30 am – 1:00 pm Lunch: Arch View Ballroom



SESSION 2: OXIDATIVE STATE AND GUT HEALTH

Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D

- 3:00 pm **Oxidative stress–gut health–animal performance.** (Abstract 106)
J. Ward*, *Camlin Fine Sciences, Urbandale, IA, USA.*
- 3:15 pm **Oxidative stress and animal health.** (Abstract 107)
T. Gaydos*, *Gaydos Technical Services LLC, Dallas, TX, USA.*
- 3:45 pm **Profiling phylogenetic inclusion level effects on the intestinal antioxidant capacity and the expression of protective genes against oxidation, stress, and inflammation in broilers.** (Abstract 108)
K. C. Mountzouris*, V. V. Paraskeuas, and K. Fegeros, *Agricultural University of Athens, Department of Nutritional Physiology and Feeding, Athens, Attika, Greece.*
- 4:15 pm **Intestinal homeostasis: The impact of the gut microbiota and its interaction with the host's epithelial cells.** (Abstract 109)
A. Byrd* and M. Kogut, *USDA, College Station, TX, USA.*
- 4:45 pm **Dietary inclusion of various antioxidant, antimicrobial, or immunostimulatory compounds as prophylaxis against blackhead.** (Abstract 110)
T. L. Barros¹, L. C. Beer¹, J. D. Latorre¹, X. Sun¹, S. J. Rochell¹, G. Tellez-Isaias¹, A. L. Fuller², B. M. Hargis¹, and C. N. Vuong*¹, ¹*University of Arkansas, Fayetteville, AR, USA,* ²*University of Georgia, Athens, GA, USA.*
- 5:15 pm **Multi-omics case study: Combining microbiome, gene copy number, and metabolic pathway in the analysis.** (Abstract 111)
B. Shannon*¹, J. Johnson², and G. Weinstock², ¹*BioRankings, St Louis, MO, USA,* ²*Jackson Lab for Genomic Medicine, Farmington, CT, USA.*
- 6:30 pm – 8:30 pm Reception: Arch View Ballroom
Sponsored by Elanco

Tuesday, November 5

7:00 am – 8:00 am Breakfast: Arch View Ballroom

SESSION 3: FEED SUPPLEMENTS AND GUT HEALTH

Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D

- 8:00 am **Invited presentation: Optimizing feed additives in poultry using microbiome analyses.** (Abstract 112)
S. Ricke*, *University of Arkansas, Fayetteville, AR, USA.*
- 9:00 am **Effects of supplemental butyrate on gene expression in the intestinal tissues of broiler chickens during *Eimeria maxima* infection.** (Abstract 113)
V. L. Hansen*¹, M. Proszkowiec-Weglarz¹, S. Ramos¹, S. Vaessen², and K. B. Miska¹, ¹*United States Department of Agriculture, Agricultural Research Services, NEA, Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, USA,* ²*Perstorp Waspik BV, Waspik, the Netherlands.*



- 9:30 am **Impact of oral prophylactics on gut microbiome and disease resistance in chickens.** (Abstract 114)
M. Mellata* and G. Redweik, *Iowa State University, Ames, IA, USA.*
- 10:00 am Coffee Break: Grand Foyer
Sponsored by Phytobiotics North America LLC
- SESSION 4: MECHANISMS OF BENEFICIAL MICROBES**
Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D
- 10:30 am **A new probiotic technology that boosts the benefit of *Bacillus*-based probiotics.** (Abstract 115)
G. Everett*, J. Church, C. Zetena, and C. Greenwald, *NCH Life Sciences, Irving, TX, USA.*
- 10:50 am **Assessment of a *Bacillus* spp. direct-fed microbial based on enzyme production to improve gut health integrity, immunological status, performance, and ability to counteract the toxic effects of aflatoxin B₁ in broiler chickens.** (Abstract 116)
G. Tellez-Isaias*¹, J. D. Latorre¹, D. Hernandez-Patlan², B. Solis-Crus², C. N. Young¹, B. D. Graham¹, C. A. M. Selby¹, T. L. de Barros¹, and M. Arreguin-Nava³,
¹University of Arkansas, Fayetteville, AR, USA, ²Euxxis Bioscience, Fayetteville, AR, USA, ³National Autonomous University of Mexico, Mexico City, Mexico.
- 11:10 am ***Bacillus subtilis* 29784 significantly improved the growth performance of broilers, likely through beneficial effects on microbiota and host.** (Abstract 117)
D. Preveraud*¹, P. Choi⁶, J. Barton², J. Brackenridge², A. Nelson³, K. Mann³, P. Thiery¹, L. Rhayat⁴, E. Eckhardt⁴, K. Sidelmann Brinch⁵, F. Van Immerseel⁶, and E. Devillard⁴, ¹Adisseo France SAS, Antony, France, ²Adisseo USA Inc., Alpharetta, GA, USA, ³Novozymes North America Inc., Durham, NC, USA, ⁴Adisseo CERN, Commentry, France, ⁵Novozymes A/S, Lyngby, Denmark, ⁶Department of Pathology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium.
- 11:30 am **Are all probiotics the same when it comes to microbial profile of ceca?** (Abstract 118)
S. Ramirez*¹, D. Rodrigues², W. Briggs², C. Pender¹, R. Murugesan¹, and L. Bielke²,
¹BIOMIN America, Overland Park, KS, USA, ²The Ohio State University, Wooster, OH, USA.
- 11:50 am – 1:00 pm Lunch: Grand Foyer
- SESSION 5: HOST–MICROBE INTERACTIONS, IMMUNOBIOLOGY**
Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D
- 1:00 pm **Delayed access to feed affects goblet cell distribution in chickens.** (Abstract 119)
K. Liu and E. A. Wong*, *Virginia Tech, Blacksburg, VA, USA.*
- 1:30 pm ***Clostridium perfringens* toxins affecting the gut-brain axis as mechanism of colonization.** (Abstract 120)
L. Redondo¹, J. Diaz-Carrasco^{1,2}, N. Casanova¹, A. Cangelosi², P. Geoghegan², J. Goldstein^{3,4}, and M. Fernandez-Miyakawa*^{1,4}, ¹Instituto de Patobiología Veterinaria, CICVyA-INTA, Buenos Aires, Argentina, ²Centro Nacional de Control de Calidad de



Biológicos, ANLIS "Dr. Carlos G. Malbran," Buenos Aires, Argentina, ³Departamento de Fisiología, Facultad de Medicina, UBA, Buenos Aires, Argentina, ⁴CONICET, Buenos Aires, Argentina.

- 2:00 pm **The immunometabolic responses of heritage and modern broilers to immune challenge: Learning from the past to inform the future.** (Abstract 121)
R. Arsenault¹, B. Aylward¹, C. Johnson¹, F. Perry¹, and R. Whelan², ¹University of Delaware, Newark, DE, USA, ²Evonik Nutrition & Care, Hanau-Wolfgang, Germany.
- 2:30 pm **Segmented filamentous bacteria-based treatment improves intestinal health in layer chicks.** (Abstract 122)
G. Redweik* and M. Mellata, Iowa State University, Ames, IA, USA.
- 3:00 pm Coffee Break: Grand Foyer
Sponsored by Camlin Fine Sciences
- 3:30 pm **Early intestinal microbiota as a driver of poultry immunity.** (Abstract 123)
D. R. Rodrigues*, W. Briggs, K. Chasser, A. Duff, J. Bielke, K. Wilson, and L. Bielke, Department of Animal Sciences, The Ohio State University, Columbus, OH, USA.
- 4:00 pm **High-throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics.** (Abstract 124)
W. Lyu, Z. Deng, L. T. Sunkara, and G. Zhang*, Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA.
- 4:30 pm **Effects of dietary protein source and litter condition on mitotically active cell and macrophage densities in the small intestine of broilers.** (Abstract 125)
A. J. Keel*, A. J. Calderon, O. J. Tejada, J. D. Starkey, and C. W. Starkey, Auburn University, Auburn, AL, USA.
- 5:00 pm **Changes in the host transcriptome and microbial meta-transcriptome of the ileum of dairy calves subjected to artificial rumen contents dosing.** (Abstract 126)
W. Li¹, A. Edwards², M. Cox², J. Skarlupka², S. Raabis², A. Steinberger², B. Murphy², A. Larsen², and G. Suen², ¹US Dairy Forage Research Center, Madison, WI, USA, ²University of Wisconsin-Madison, Madison, WI, USA.
- SECTION 6: NUTRITION AND GUT HEALTH**
Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D
- 5:30 pm ***Tenebrio molitor* meal inclusion in broiler chickens diet: A multidisciplinary approach to gut health.** (Abstract 127)
E. Colombino*¹, I. Biasato², I. Ferrocino², S. Dabbou¹, J. Nery¹, D. Soglia¹, L. Gasco², L. S. Cocolin², M. T. Capucchio¹, and A. Schiavone¹, ¹Department of Veterinary Sciences, University of Turin, Grugliasco (Torino), Italy, ²Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco (Torino), Italy.
- 5:50 pm ***Hermetia illucens* meal inclusion in piglets: Effects on gut health.** (Abstract 128)
I. Biasato¹, E. Colombino², I. Ferrocino¹, S. Dabbou², V. Vincenti², A. Imarisio², A. Schiavone², L. S. Cocolin¹, L. Gasco¹, and M. T. Capucchio*², ¹Department of Agricultural, Forest and Food Sciences, University of Turin, Grugliasco, Italy, ²Department of Veterinary Sciences, University of Turin, Grugliasco, Italy.
- 7:00 pm – 9:00 pm Reception: Arch View Ballroom



Wednesday, November 6

7:00 am – 8:00 am Breakfast: Arch View Ballroom

SESSION 7: NUTRITION AND GUT HEALTH (CONTINUED)

Chair: Mike Kogut, USDA-ARS
Salons A, B, C, D

- 8:00 am **Natural multifunctional solutions (Alquermix) in broilers.** (Abstract 129)
J. Pié*¹, D. Díez¹, C. Domenech¹, C. Gallardo², and M. E. Rosemberg², ¹*Biovet, Constanti, Spain*, ²*Universidad Científica del Sur, Lima, Peru*.
- 8:20 am **Interaction effects of feeding *Bacillus subtilis* DSM 32315 and dietary protein on intestinal barrier function, microbial profiles, and growth performance in weaned piglets.** (Abstract 130)
W. Tang¹, J. C. González-Vega², J. Gao³, A. Menconi⁴, A. Sokale⁴, and K. Doranalli*², ¹*Animal Nutrition Institute, Sichuan Agricultural University, and Key Laboratory of Animal Disease-Resistance Nutrition, Ministry of Education of China, Chengdu, Sichuan, People's Republic of China*, ²*Evonik Nutrition and Care GmbH, Hanau-Wolfgang, Germany*, ³*Evonik Degussa (China) Co. Ltd., Beijing, People's Republic of China*, ⁴*Evonik Corporation, Kennesaw, GA, USA*.
- 8:40 am **Expression of genes encoding proteins associated with nutrient uptake at either the brush border or basolateral surface of the intestine in six strains of chickens with different growth potential.** (Abstract 131)
K. Miska*, L. Schreier, S. Kahl, B. Russell, and M. Proszkowiec-Weglarz, *USDA/ARS, Beltsville, MD, USA*.
- 9:10 am **A unique hydrolyzed yeast product can prevent postweaning diarrhea in *Escherichia coli*-susceptible piglets.** (Abstract 132)
S. Hasan*, E. Valkonen, H. Kettunen, and J. Vuorenmaa, *Hankkija Oy, Hyvinkää, Finland*.
- 9:40 am Coffee Break: Grand Foyer
Sponsored by Delacon Biotechnik GmbH
- 10:10 am **Effects of zinc hydroxychloride on inflammation and intestinal integrity during feed restriction.** (Abstract 133)
E. A. Horst*¹, E. J. Mayorga¹, M. Al-Qaisi¹, S. Rodriguez-Jimenez¹, B. M. Goetz¹, M. A. Abeyta¹, R. S. Fry², S. K. Kvidera², and L. H. Baumgard¹, ¹*Iowa State University, Ames, IA, USA*, ²*Micronutrients USA LLC, Indianapolis, IN, USA*.
- 10:30 am **A high rumen degradable starch diet modulates jejunum microbiota and alters enterohepatic circulation of bile acids in dairy goats.** (Abstract 134)
J. Shen¹, J. Yao¹, and Y. Cao*^{1,2}, ¹*Northwest A&F University, Yangling, Shaanxi, China*, ²*Harvard Medical School, Boston, MA, USA*.
- 10:50 am **Thymol modulates chemo-sensing receptors and inflammation markers in the gut of weaning pigs.** (Abstract 135)
A. Toschi*¹, B. Tugnoli², B. Rossi², A. Piva^{1,2}, and E. Grilli^{1,3}, ¹*University of Bologna, DIMEVET, Ozzano Emilia, Bologna, Italy*, ²*Vetagro SpA, Reggio Emilia, Italy*, ³*Vetagro Inc., Chicago, IL, USA*.



Poster Session: Grand Foyer

- P100 **Evaluation of feeding spray-dried plasma during heat stress on broiler growth, intestinal permeability, and bone mineralization.**
J. Ruff^{*1}, T. Barros¹, J. Cambell², R. Gonzalez-Esquerro², D. Graham¹, C. Selby¹, C. Young¹, S. Dridi¹, E. Greene¹, B. Hargis¹, and G. Tellez-Isaias¹, ¹University of Arkansas, Fayetteville, AR, USA, ²APC, Ankeny, IA, USA.
- P101 **Is the regulation of intestinal inflammation defective in high breast yield strain?**
G. Cardoso Dal Pont^{*1}, M. Kogut², B. Mallmann³, K. Feye³, C. M. Owens³, S. Ricke³, and C. N. Coon³, ¹Texas A&M, College Station, TX, USA, ²USDA-ARS, College Station, TX, USA, ³University of Arkansas, Fayetteville, AR, USA.
- P102 **Investigating intestinal barrier integrity in heat-stressed modern broilers and their ancestor Jungle Fowl.**
T. Tabler^{*}, E. Greene, S. Orłowski, J. Hiltz, N. B. Anthony, and S. Dridi, University of Arkansas, Fayetteville, AR, USA.
- P103 **Clostridia variation over time within a dairy cattle herd in southeastern Wisconsin.**
R. F. Teal^{*}, A. LeMarche, V. G. Bretl, K. Van Zanten, J. S. Thompson, and T. G. Rehberger, Arm & Hammer Specialty Products Division, Waukesha, WI, USA.
- P104 **Enumeration and identification of *Clostridium* along the gastrointestinal tract of dairy cows.**
M. N. Griffin^{*1}, J. S. Thompson¹, F. F. Cardoso², F. Cardoso², R. I. Mackie², A. H. Smith¹, and T. G. Rehberger¹, ¹Arm and Hammer, Waukesha, WI, USA, ²University of Illinois, Urbana, IL, USA.
- P105 **Feeding chestnut tannins stimulates pro-inflammatory immune response in broiler chicks.**
A. Lee^{*1}, G. Cardoso dal Pont¹, M. Battaglia², and M. Kogut³, ¹Texas A&M University, College Station, TX, USA, ²Silvateam/Indunor S.A, Buenos Aires, Argentina, ³USDA-ARS, College Station, TX, USA.
- P106 **Fermented cottonseed meal reduces fat deposition in white-feather broilers through cecum bacteria-host metabolic cross-talk.**
J. Niu^{*1}, J. Zhang², L. Wei¹, X. Ma², W. Zhang¹, and C. Nie^{1,2}, ¹College of Animal Science & Technology, Shihezi University, Shihezi, Xinjiang, China, ²State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.
- P107 **Effect of varying dietary crude protein concentration on performance and gut health in a subclinical necrotic enteritis challenge model.**
A. M. Villegas^{*1}, A. Menconi², A. O. Sokale², J. D. Liu¹, and T. J. Applegate¹, ¹Department of Poultry Science, University of Georgia, Athens, GA, USA, ²Evonik Corporation, Kennesaw, GA, USA.
- P108 **Effects of rumen-protected niacin on dry matter intake, milk yield, feed digestibility, and fecal microbiota in early-lactation dairy cows.**
N. Gaowa, G. Liu, S. Li, Z. Cao, and Y. Wang^{*}, College of Animal Science and Technology, China Agricultural University, Beijing, China.
- P109 **Effect of a protected blend of organic acid + essential oil on growth performance of broiler chickens undergoing several *Eimeria* challenge models.**
M. Pujol, R. Scott-Delaunay, and E. Santin^{*}, Jefe Nutrition Inc., Saint-Hyacinthe, QC, Canada.



- P110 **Effect of yeast extract on early intestinal tract development of broilers.**
R. Raspoet*¹, M. T. Brufau², M. Castells-Valero², D. Moral-Anter², A. M. Perez-Vendrell³, E. Auclair¹, B. Vila³, J. Brufau³, R. Ferrer², and R. Martin-Venegas²,
¹*Phileo by Lesaffre, Marcq-en-Baroeul, France*, ²*Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, Barcelona, Spain*, ³*Institut de Recerca i Tecnologia Agroalimentàries (IRTA-Centre Mas de Bover), Constantí, Spain*.
- P111 **Comparative analysis of microbiota from commercial broiler farms of two integration companies using AGP or chestnut/quebracho polyphenol extracts.**
J. Diaz-Carrasco^{1,2}, L. Redondo*¹, N. Casanova¹, and M. Fernandez-Miyakawa^{1,2},
¹*Instituto de Patobiología Veterinaria, CICVyA – INTA, Buenos Aires, Argentina*,
²*CONICET, Buenos Aires, Argentina*.
- P112 **Effect of a blend of protected organic acids + essential oils on growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge.**
M. de Souza Vieira*¹, M. L. Moraes¹, J. C. Bodin¹, E. Santin¹, C. B. Adam², and C. Stefanello²,
¹*Jefo Nutrition Inc., Saint-Hyacinthe, QC, Canada*, ²*Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil*.
- P113 **Effect of feeding glycerol esters of butyric and valeric acid on broiler performance.**
S. Vaessen¹, J. M. Ros Felip¹, J. N. Broomhead*², and M. I. Gracia³,
¹*Perstorp, Waspik BV, the Netherlands*, ²*Perstorp, Toledo, OH, USA*, ³*Imasde Agroalimentaria, Madrid, Spain*.
- P114 **Holofood: A holo'omic solution towards sustainable animal food production.**
J. Tarradas*¹, S. Marcos², D. Sandvang³, M. Limborg⁴, J. Zentek⁵, D. Jozefiak⁶,
E. Johansen⁴, A. Estonba², N. Tous¹, E. Esteve-Garcia¹, A. Alberdi², and M. T. P. Gilbert⁴,
¹*Institute for Food and Agricultural Research and Technology (IRTA), Constantí, Spain*, ²*Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain*, ³*Chr Hansen A/S, Hoersholm, Denmark*, ⁴*Section for Evolutionary Genomics, the GLOBE Institute, Faculty of Health and Medical Sciences, Copenhagen, Denmark*,
⁵*Department of Veterinary Medicine Free University of Berlin (FUB), Berlin, Germany*, ⁶*Piast Pasze Sp.z o.o. (Piast Group llc), Lewkowiec, Poland*.
- P115 **Biomarkers to evaluate gut integrity in different models to induce intestinal inflammation in broiler chickens.**
G. Tellez-Isaias*, C. N. Voung, B. D. Graham, C. A. M. Selby, T. L. de Barros, and B. M. Hargis,
University of Arkansas, Fayetteville, AR, USA.
- P116 **Analysis of rumen fermentation and microbial composition of cattle, dzo, and yak under grazing conditions.**
C. Zhao¹, Y. Li¹, F. Zhang¹, J. Yao¹, and Y. Cao*^{1,2},
¹*Northwest A&F University, Yangling, Shaanxi, China*, ²*Harvard Medical School, Boston, MA, USA*.
- P117 **Changes in growth and motility of *Campylobacter jejuni* in response to serotonin.**
J. Lyte*¹, S. Shrestha¹, M. Lyte², and A. Donoghue¹,
¹*USDA-ARS Poultry Production and Product Safety Unit, Fayetteville, AR, USA*, ²*Iowa State University, Ames, IA, USA*.



Session 1: Age-Dependent Development of Microbiome

100 The gut mycobiome: Implications in piglet health? K. Summers*¹, J. F. Frey¹, T. Ramsay¹, and A. Arfken^{1,2}, ¹USDA, Beltsville, MD, USA, ²ORISE, Oak Ridge, TN, USA.

Interactions between bacteria and fungi in the gut microbiome can result in altered nutrition, pathogenicity of infection, and host development, making them a crucial component in host health. Associations between the mycobiome and bacteriome in the piglet gut remain unknown. Weaning is a time of significant stress, dietary changes, microbial alterations, and a predisposition to infection. The loss of animal health and growth makes potential microbial interventions of interest to industry. Recent studies have demonstrated the diversity of the microbiome in the gastrointestinal tract of piglets during weaning. Despite these advances, the piglet mycobiota and its contribution to microbiome development remains poorly understood. In this presentation we will review the piglet mycobiome and its interactions with the microbiome and host gastrointestinal (GI) tract. We will highlight our recent data investigating the bacteriome and the mycobiome after weaning in the GI tract organs and feces from 35-d old piglets. The α -diversity and amplicon sequence variants (ASV) counts of the bacteriome increased, proximally to distally, from the stomach to the feces along the GI tract, while the mycobiome α -diversity and ASV counts were highest in the porcine stomach. β -Diversity analyses show distinct clusters based on organ type in the bacteriome and mycobiome, but dispersion remained constant in the mycobiome between organ/fecal sites. *Bacteroidetes*, *Firmicutes*, and *Epsilonbacteraeota* were the most abundant bacterial phyla present and *Ascomycota* and *Basidiomycota* were the dominant fungal phyla based on mean taxonomic composition. Potential interactions were found in the lower GI bacteriome and mycobiome with positive correlations between the fungus, *Kazachstania*, and several bacterial species, including *Lactobacillus*. *Aspergillus* demonstrated negative correlations with the short chain fatty acid-producing bacteria *Butyricoccus*, *Subdoligranulum*, and *Fusicatenibacter*. This presentation highlights the distinct colonization dynamics between fungi and bacteria in the GI tract and feces of piglets directly following weaning and the interactions of these microbes in the porcine gut ecosystem.

Key Words: mycobiome, microbiome, weaning, piglet

101 Chicken intestinal mycobiome: Biogeography, succession, and its response to bacitracin methylene disalicylate. K. Robinson*¹, T. J. Johnson², and G. Zhang¹, ¹Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA, ²Department

of Veterinary and Biomedical Sciences, University of Minnesota, St. Paul, Minnesota, USA.

The gastrointestinal (GI) tract harbors a diverse population of microbes consisting of not only bacteria, but also fungi, viruses, and protozoa. While much work has been focused on the characterization of intestinal bacterial community, very little is known about the fungal community, or mycobiota, in different animal species and chickens in particular. Here we characterized the biogeography and maturation of the mycobiota in the GI tract of broiler chicks and further examined its possible shift in response to bacitracin methylene disalicylate (BMD), a commonly used in-feed antibiotic, through Illumina sequencing of the internal transcribed spacer 2 (ITS2) region of fungal rRNA genes. We revealed an obvious biogeographic difference in the mycobiota composition along the GI tract, with the crop, gizzard, and duodenum being the most diverse than the jejunum, ileum, cecum, and colon. The intestinal mycobiome was dominated by *Ascomycota* and *Basidiomycota*, with 3 genera including *Microascus*, *Trichosporon*, and *Aspergillus* accounting for over 83% of the total fungal population in any given segment, except for the duodenum, which harbored the most diverse fungal community. We also observed an obvious age-dependent shift of the cecal mycobiota. Dietary supplementation of BMD at a subtherapeutic level of 55 mg/kg resulted in a significant decrease in the cecal fungal diversity. Taken together, we provided a comprehensive biogeographic view and maturation of the chicken intestinal mycobiota and its influence by an in-feed antibiotic. A better understanding of intestinal mycobiota may lead to development of novel strategies to improve poultry health and productivity.

Key Words: mycobiome, microbiome, gut health, poultry

102 Development of the piglet fecal bacteriome and mycobiome from birth through weaning. A. Arfken*, J. Frey, and K. Summers, USDA-ARS, Beltsville, MD, USA.

The microbiota of the animal gastrointestinal tract is a critical component in host health, performance, and nutrition. Recently, the fungal microbiota (mycobiome) has been identified as a significant member of the microbiome. However, due to a limited number of culture-independent studies on fungal microbiota in animal hosts, relatively little is known about the mycobiome in swine. In piglets, weaning is a period of stress, dietary changes, and a predisposition to infections, making it a time point of interest to industry. In this study, we characterized and compared the development of the bacteriome and the mycobiome in piglet feces from birth through the critical weaning transition (d 1–35 post-birth). Bacterial diversity



increased over the experimental timeline, transitioning from an *Enterobacteriaceae* and *Bacteroidaceae* dominated population pre-wean (d 1–21) to fiber-degrading and short chain acid producing families *Prevotellaceae* and *Ruminococcaceae* post-wean (d 24–35). In comparison, fungal diversity peaked during the weaning transition (d 21–24) and decreased by d 35. The development of the mycobiome was characterized by an increased stable presence of dominant yeast families *Debaryomycetaceae* or *Saccharomycetaceae* post-wean (d 24–35) depending on litter. Based on mycobiome profiles of environmental sources, *Saccharomycetaceae* in the piglet gut likely originated from sow feces while *Debaryomycetaceae* may have originated from nursery feed. A co-occurrence network analysis of piglet feces from d 35 showed a strong association between fungal genera *Kazachstania* and *Hyphopichia* with the butyrate-producing and xylan-degrading bacterial genus, *Eubacterium rumantium*, while *Aspergillus* demonstrated a negative association. This study provides insights into the early development and post-wean establishment of the fecal bacteriome and mycobiome in healthy piglets. Future studies will investigate the effect of the mycobiome on piglet growth and health during the weaning transition, including its role in fast- versus slow-growing piglets.

Key Words: pig, development, microbiome, mycobiome, bacteriome

103 Comparison of gut microbiota between slow- and fast-growing broiler chickens. M. Proszkowiec-Weglarczyk*, L. Schreier, K. Miska, S. Kahl, Y. Qu, B. Russell, and T. Elsasser, *USDA, ARS, Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, USA.*

Gastrointestinal tract (GIT) microbiome plays an important role in the metabolism, immune competence, and growth performance of broiler chickens. It has been shown that interaction between the microbial population and host genetics affects the nutritional, immunological, and physiological status of the host. The aim of this study was to compare ileal and cecal microbiome between slow- (SGB) and fast-growing (FGB) broiler chickens. Three SGB (Athens Canadian Random Breed, ACRB; Longenecker Hatchery Heritage breed, LHR; Red-bro, RB) and 3 FGB (Ross 708, Cobb500 and Hubbard H1, HH1) broiler breeds were raised from hatch to d 35 post-hatch (PH) in a floor-pen setting with *ad libitum* access to the same feed and water. Ileal and cecal digesta and epithelial scrapings were collected –2, 0, 7, 14, 21, 28, and 35 d PH for microbiome analysis. Microbiota was determined by sequencing of the V3-V4 region of bacterial 16S rRNA and analyzed using Qiime2. Body composition of birds were determined by DEXA. Significant differences ($P < 0.05$) in body weight and feed intake were observed between breeds.

Cobb500 was characterized by the highest body weight, followed by Ross708 and HH1, LHR and RB and ACRB. Similar pattern was observed for feed intake. FGB were characterized by the lowest FCR ($P < 0.05$) while ACRB had the highest FCR ($P < 0.05$). Significant differences ($P < 0.05$) in body composition (bone mineral density and content, and percentage of lean mass and fat) were observed between SGB and FGB. Overall, no differences ($P > 0.05$) in α and β diversity in bacterial populations of ileum and cecum were observed between different broiler breeds. However, significant ($P < 0.05$) effect of breed or breed by age interaction were detected on bacterial composition at every taxonomic level in all 4 microbial populations (luminal and mucosal populations of ileum and cecum). These results indicate possibility of host genetic-specific microbiome interaction that could be involved in some of the performance differences seen between breeds.

Key Words: slow-growing chicks, fast-growing chicks, gastrointestinal tract, microbiome, 16S

104 Trends involved in bacterial transfer for day-of-hatch chick microbiome. J. Gruber*, E. Kim, M. Perry, and A. Thomas, *DuPont Animal Nutrition, Wilmington, DE, USA.*

Bacteria from breeders and the hatchery are predicted to be the primary sources of bacterial inoculum for day-of-hatch chicks. Breeders donate fecal bacteria as part of the normal laying process, while handling and transportation further adds bacteria onto the egg shell. As a model, *E. coli* has been used to study bacterial transfer from breeders to hatchery and chicks. From these studies, it was determined that *E. coli* could be successfully isolated on the egg shell and survive the hatching process. Chicks then maintained the initial *E. coli* strains up through the last day of monitoring at d 8. What is not yet understood are the larger dynamics of bacterial populations that are inoculated into chicks at day of hatch. To study bacterial transfer in production systems, a post-hoc study of bacterial populations was elucidated through 16s sequencing and monitored through linear breeder, hatchery surfaces, and day-of-hatch chicks. Bacterial populations were aligned to the areas sampled and then modeled for transfer from area to area. Bacteria deposited onto the egg shell from the breeder continued to be a primary source of bacteria that was maintained from collection through the hatching process. Day-of-chicks demonstrated significant bacterial populations related to density of bacteria on the egg shell. Much of day-of-chick gut resident bacteria was comprised of 4 separate bacterial species, while several minor bacteria were identified between 2 and 0.1% relative abundance to total reads. Together, although several bacterial species are co-localized on surfaces and are present to day-of-hatch chicks, density of bacteria appears to be key in bacterial



transfer. However, it cannot be ruled out how acclimation to gut environment of adaptation of transfer may also play a role. After day-of-hatch, repeated exposure to bacteria,

adaptation to the gut environment, and nutrition may further shape bacterial communities and development.

Key Words: breeders, microbiome, hatchery, transfer



Session 2: Oxidative State and Gut Health

106 Oxidative stress–gut health–animal performance.

J Ward*, *Camlin Fine Sciences, Urbandale, IA, USA.*

Oxidative stress is a term frequently used in articles concerning animal health and nutrition but is not necessarily well defined or understood. Oxidants play a central role in normal cellular function, providing an important feedback loop between metabolic activity and regulation of cellular functions. Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of oxidants and free radicals that damage all components of the cell including proteins, lipids, and DNA. The reduction of oxidative stress in food producing animals has been counteracted using antioxidant-rich foods, the formulation of proper vitamins and minerals levels in diets as well as enhancing productivity through antioxidant supplements. For aerobic organisms a mechanism to remove highly reactive oxygen species have been developed through the process of evolution. The consequences of active oxygen species and free radicals attack on molecules in biological membranes and tissues reduces the health and performance of animals and may increase their vulnerability to various diseases. These harmful health consequences resulting from oxidative stress and associated health issues have been measured using biomarkers in clinical and experimental studies. Considering the complexity of the number of interactions between antioxidants and body systems, it is conceivable that through a better understanding of antioxidant-animal interactions a deeper understanding of the effectiveness of antioxidant supplementation is achievable. Future research should not only focus on the establishment of a reference panel of biomarkers for oxidative stress but should also assess the issue of standardization of techniques and methodologies to study oxidative stress. A better understanding of the pathophysiology of oxidative stress will provide clarity to designing specific interventions.

107 Oxidative stress and animal health. T. Gaydos*, *Gaydos Technical Services LLC, Dallas, TX, USA.*

It is tempting to discuss metabolism, nutrition, antioxidants, and the immune system separately, because these systems are highly complex and deserve separate discussion. It is critical to remember that animals do not take classes, and every system is interconnected. All systems in an animal are subjected to oxidative stress, and many systems are the source of that stress. Oxygen is an essential part of cellular metabolism for all known life. Some organisms are not able to process or tolerate elemental oxygen,

because they do not have the enzymes necessary to protect themselves against oxidative damage and stress. All vertebrate animals require elemental oxygen for their survival, and have different mechanisms to prevent, limit, or repair the damage due to excess oxygen or oxidative compounds. The process of converting the energy stored in the chemical bonds of glucose, or other oxidizable compounds in the mitochondria, results in the production of hydrogen peroxide. Hydrogen peroxide is a strong oxidant that is toxic to the cell, and is neutralized by enzymes in the antioxidant system present in the cell and in the mitochondria itself. Phagocytic cells in the immune system produce reactive oxygen species to destroy the pathogens they phagocytize. The multicellular nature of the vertebrate allows the animal to use oxidation to destroy pathogens at the expense of some cells in the host. This creation and release of oxidizers creates a level of oxidative stress. For this reason, when an animal is battling an infection, the level of oxidative stress is extremely high. The ingestion of oxidized fats and oils is also a source of oxidative stress on the animal. The nature of the peroxidation of lipids creates a chain reaction of the resultant fatty acid radical, which can then react with another non-oxidized lipid molecule. This reaction is only terminated when the fatty acid radical oxidizes an antioxidant molecule such as vitamin E, or is neutralized by an enzyme. All of these sources of oxidative stress converge on the intestine. The impact of oxidative stress on the intestinal mucosa, antibodies, immune cells, and performance is well documented across species.

Key Words: oxidative stress, peroxide, antioxidant, immune

108 Profiling phytogetic inclusion level effects on the intestinal antioxidant capacity and the expression of protective genes against oxidation, stress, and inflammation in broilers.

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The effects of a phytogetic premix (PP) inclusion level on an array of genes relevant for host protection against oxidation (CAT, SOD1, GPX2, HMOX1, NQO1, Nrf2, and Keap1), stress (HSP70 and HSP90) and inflammation (NF- κ B1, TLR2 and TLR4) were evaluated along the broiler intestine in combination with determination of total antioxidant capacity (TAC). The proprietary PP "gut agility activator" used comprised of functional flavoring substances of ginger, lemon balm, oregano, and thyme. One-day-old Cobb broiler chickens (n = 500) were assigned into the following 4 treatments, depending on PP inclusion level in the basal diets (i.e., 0, 750, 1000 and 2000 mg/kg diet): CON, PP750, PP1000, and PP2000. Each treatment



had 5 replicates of 25 chickens with *ad libitum* access to feed and water. Data were analyzed by ANOVA and means compared using Tukey HSD test. Polynomial contrasts tested the linear and quadratic effect of PP inclusion levels. Overall, except for CAT, the inclusion of PP upregulated ($P \leq 0.05$) the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) / antioxidant response element (ARE) pathway genes (SOD1, GPX2, HMOX1, NQO1, Nrf2 and Keap1) evaluated. In particular, the majority of these genes were upregulated primarily in the duodenum and the ceca and secondarily in the jejunum. Moreover, genes were mostly upregulated in a quadratic manner with increasing PP inclusion level with the highest expression levels shown in treatments PP750 and PP1000 compared with CON. Similarly, intestinal TAC was higher in PP1000 in the duodenum ($P = 0.011$) and the ceca ($P = 0.050$) compared with CON. From the genes relevant for inflammation and stress assessed, NF- κ B1, TLR4, and HSP70 were downregulated with increasing PP level, the first one according to a quadratic pattern and the latter 2 linearly. As a conclusion, PP primed the expression of cytoprotective genes and downregulated stress and inflammation related ones, the effect being dependent on PP inclusion level and the intestinal site. Further investigation under stress-challenge conditions is warranted.

Key Words: poultry, Nrf2, antioxidant, phytogetic, gut

109 Intestinal homeostasis: The impact of the gut microbiota and its interaction with the host's epithelial cells. A. Byrd* and M. Kogut, *USDA, College Station, TX, USA.*

Neonatal animals are highly susceptible to enteric pathogens with the understanding that the gastrointestinal tract and the immune system are not mature. Homeostasis in the mature animal allows the animal to be resistant to the same pathogens but can become more susceptible under certain conditions. Understanding how our immune system maintains homeostasis is of interest in the gastrointestinal tract of the bird, because this organ harbors the largest microbial community in the animal. At the cellular level, the host's epithelial cells help maintain the balance with the microbiota. The mature epithelial cells help maintain the anaerobic environment which is ideal for the classes of *Clostridia* and *Bacteroidia*. As the epithelial cells become injured through chemical or mechanical means, the epithelial cells shift from an oxygen-consuming cell to an oxygen-producing cell through glycolysis which allows for the proliferation of the facultative anaerobic bacteria such as those found in the phylum *Proteobacteria* causing dysbiosis. This dysbiosis allows for other opportunistic pathogens such as *Salmonella*, *Clostridium* and *E. coli* to gain a strong-hold in the gastrointestinal tract and eventual invasion of the host. Intestinal inflammation is a driver of dysbiosis characterized by an expansion of

Enterobacteriaceae within the gut associated microbial community. Mechanisms driving this expansion include resistance of *Enterobacteriaceae* against antimicrobial host defenses induced during inflammation and the generation of respiratory electron acceptors generated as a by-product of the host inflammatory response, which favors growth of facultative anaerobic bacteria. Thus, the oxidative status of the lower intestinal of production animals plays a demonstrative role in the susceptibility to foodborne bacterial infections.

110 Dietary inclusion of various antioxidant, antimicrobial, or immunostimulatory compounds as prophylaxis against blackhead. T. L. Barros¹, L. C. Beer¹, J. D. Latorre¹, X. Sun¹, S. J. Rochell¹, G. Tellez-Isaias¹, A. L. Fuller², B. M. Hargis¹, and C. N. Vuong*¹, ¹University of Arkansas, Fayetteville, AR, USA, ²University of Georgia, Athens, GA, USA.

Blackhead (histomoniasis) is a re-emerging disease of major significance for commercial turkey production and some broiler breeders due to the lack preventative chemicals nor commercially available vaccine. *Histomonas meleagridis*, the protozoal pathogen which causes blackhead, is able to colonize the ceca, migrate to the liver, and cause up to 80% mortality in severely affected flocks. Maintaining healthy gut homeostasis while encouraging a responsive immune environment upon challenge is crucial to preventing disease. In an attempt to identify a possible prophylaxis to ameliorate blackhead disease in turkeys, various antioxidant, antimicrobial, or immunostimulating compounds were included in the diet and evaluated for prophylactic value. Screened compounds included resveratrol, capsaicin, curcumin, sodium chlorate, sodium nitrate, arginine, boric acid, and deoxycholic acid. Virulent blackhead disease was recreated with a wild-type field isolate of *Histomonas* and used as a challenge model to assess the efficacy of these compounds.

Key Words: antioxidant, antimicrobial, immunostimulatory, blackhead, turkey

111 Multi-omics case study: Combining microbiome, gene copy number, and metabolic pathway in the analysis. B. Shannon*¹, J. Johnson², and G. Weinstock², ¹Bio-Rankings, St Louis, MO, USA, ²Jackson Lab for Genomic Medicine, Farmington, CT, USA.

In this presentation, we are interested in answering the question of whether gut bacteria impact insulin sensitivity/resistance through the methionine degradation pathway. Using methods from ecology, we modified RLQ to combine microbial taxa read counts, gene copy numbers for the taxa, and subject covariates to answer this question. While our example is from the HMP2 pre-diabetes overfeeding study examining the transition from insulin



sensitive to resistance, the method is applicable to all animal health microbiome studies where there is interest in discovering possible metabolic mechanisms related to health and outcomes. In our example more copies of K01251 and K00558 Kegg genes were associated with insulin sensitivity, and fewer copies with resistance. This leads to a testable hypothesis: Could increasing taxa with more K01251 and K00558 decrease conversion to insulin

resistance? This presentation will show how to format the data and run analyses to generate testable hypotheses for any gut health problem. Replacing insulin S/R with health/performance traits of interest, RLQ can be used to develop microbial products to (1) decrease NE in chickens, (2) increase milk production in cattle, (3) fight viral diseases in pigs, or (4) reduce colic severity in horses.

Key Words: RLQ, metabolic pathway, analysis



Session 3: Feed Supplements and Gut Health

112 Optimizing feed additives in poultry using microbiome analyses. S. Ricke*, University of Arkansas, Fayetteville, AR, USA.

Development of feed additives for optimal poultry performance and gastrointestinal health has recently become more important for the poultry industry. It has become apparent that gut health and the microbial community composition and function are critical contributing factors to poultry performance. In addition, prevention of foodborne pathogen establishment and proliferation by selecting a population antagonistic to the pathogenic organisms is also important. There are a wide range of feed additives available that either eliminate foodborne pathogens already present in the gut or prevent their initial establishment. Among these feed additive that have been considered, prebiotics have received recent attention as candidates for commercial application. Prebiotics are defined as compounds which can be used by gut microorganisms beneficial to the host, but these same compounds cannot be used directly by the host. Prebiotic candidates that can consistently modulate the gastrointestinal tract microbial population to benefit the avian host and limit foodborne pathogen establishment have been the focus of recent research efforts. While traditional prebiotics are fairly well defined structurally, it is becoming more evident that other sources such as cereal grains may have components that elicit prebiotic-like properties when fed to birds. However, given their complex structure it is difficult to identify the compositional elements in these feed sources that are contributing to prebiotic activities. To assess impact of these more complex feed sources on the gut microbial ecology requires a systematic approach of initial screening with in vitro laboratory models followed by the appropriate bird trials to evaluate performance, gut health, and the ability to limit pathogen establishment. Eventual commercial application requires that the feed additive must be consistently effective as well as economical under different management practices.

113 Effects of supplemental butyrate on gene expression in the intestinal tissues of broiler chickens during *Eimeria maxima* infection. V. L. Hansen*¹, M. Proszkowiec-Weglarz¹, S. Ramos¹, S. Vaessen², and K. B. Miska¹, ¹United States Department of Agriculture, Agricultural Research Services, NEA, Animal Biosciences and Biotechnology Laboratory, Beltsville, MD, USA, ²Perstorp Waspik BV, Waspik, the Netherlands.

Coccidiosis is one of the most prevalent gastrointestinal diseases seen in the poultry industry leading to excessive economic losses. Over the last several years poultry producers have begun to look for substitutes to

chemotherapeutic agents to meet the demand for chicken raised without antibiotics. The addition of the short chain fatty acid butyrate has been previously reported to mitigate performance loss in chickens during coccidiosis. We hypothesized that addition of tributyrin in the form of glycerol esters of butyric acid can improve performance of *Eimeria maxima* infected broilers and change the expression of genes associated with gut performance and immune response. Ross 708 male broilers were fed starter diet supplemented with 0 or 0.25% triglycerides of butyric acid (ProPhorce SR 130, Perstorp) from day (d) 1. On d 21, half of the birds were infected via oral gavage with 10^3 *E. maxima* oocysts. Experimental groups consisted of birds which were either infected or not infected, consuming normal or supplemented diet (n = 6/group). Tissue samples from the jejunum, ileum, and ceca were collected 7 and 10 d post-infection (PI), and RNA was extracted followed by cDNA synthesis. Relative gene transcription levels for genes related to gut integrity and inflammation were measured by quantitative polymerase chain reaction (qPCR) and normalized to 3 reference genes. Statistical analyses for gene expression were performed using 3-way ANOVA with Holm-Sidak correction for multiple comparisons. At 7 d PI butyrate-supplemented infected chickens showed higher weight gain and lower FCR than those eating normal diet. However, these differences were not significant by 10 d PI. The majority of differences in gene transcription observed were between infected and non-infected samples, and the effect of infection was more prominent at 7 d PI. We conclude that short chain fatty acids may be a promising feed additive for broiler chickens infected with coccidia, but the downstream cellular mechanisms responsible for helping to protect the chicken gut are still unknown.

Key Words: chicken, immune response, nutrition, butyrate, *Eimeria*

114 Impact of oral prophylactics on gut microbiome and disease resistance in chickens. M. Mellata* and G. Redweik, Iowa State University, Ames, IA, USA.

Chickens are increasingly important food-producing animals due to the popularity of their food products. In the era of antibiotic-free products, it is crucial to implement practices that protect chickens against bacterial disease (i.e., *Escherichia coli*) and reduce food-contamination (i.e., *Salmonella*). To this end, probiotics and live vaccines are commonly used individually or together to improve animal productivity and food quality. However, how these prophylactics synergistically affect gut microbiota of chickens is vastly understudied. Our research objectives were to characterize how these prophylactics and their combination affect the gut microbiome and disease resistance. White Leghorn chicks were either not treated



(CON) or orally treated with probiotics (PRO), live *Salmonella* vaccine (VAX), or both (P+V). Chicks were fed probiotics daily. P+V and VAX chickens were orally inoculated with VAX at 4 d-old and boosted 2 weeks later. Ceca microbiota was analyzed using 16S sequencing and QIIME2. Resistance to bacteria was tested using either air sac [avian pathogenic *E. coli* (APEC)] or oral (*Salmonella*) challenges. Broad protection against multiple APEC serotypes was tested using in vitro whole blood bactericidal assays. In the ceca, microbiomes of VAX and P+V birds had highly similar β diversities and reduced levels of SCFA-producing bacteria. VAX birds had uniquely elevated levels of *Akkermansia*, whereas PRO birds had the greatest levels of *Enterobacteriaceae*, which were associated

with IgA levels. All treatments reduced the levels of gut pathogenic bacteria. P+V increased blood killing against multiple APEC strains in vitro and resistance to APEC challenge in vivo compared with CON. Additionally, only P+V birds were negative for fecal *Salmonella* at all-time points. In summary, combination of probiotics and live *Salmonella* vaccine improved resistance to bacteria, though probiotics did not prevent the massive changes in microbial composition induced by the live vaccine alone. This and future similar studies are needed to help better design oral treatments to improve animal productivity and food-quality.

Key Words: probiotics, live-vaccine, microbiota, disease-resistance, bacteria



Session 4: Mechanisms of Beneficial Microbes

115 A new probiotic technology that boosts the benefit of *Bacillus*-based probiotics. G. Everett*, J. Church, C. Zetena, and C. Greenwald, *NCH Life Sciences, Irving, TX, USA*.

Bacillus-based probiotics are an emerging trend in the probiotic market. These probiotics are formulated as spores to extend shelf life. The challenge of using spore-based probiotics is that in order for these probiotics to have direct effects, they must first germinate in the gut. NCH Life Sciences has developed a patent-pending technology, ACTPRO, which delivers billions of *Bacillus* spores that actually germinate in the small intestine. This is in contrast to *Bacillus*-based probiotics currently on the market that appear to either germinate late in the colon or pass through the gut without providing direct health benefits. Since most nutrient absorption occurs in the small intestine, it is vital that probiotics actively divide there to deliver full, direct benefits to the animal. Simulated gastric experiments show that ACTPRO bacteria germinate and grow in the duodenum. Controlled in vivo experiments have also shown an increase in efficacy over standard spore-based products. A study of broiler chickens was performed in collaboration with the National Pingtung University of Science and Technology in Taiwan. Chicks fed ACTPRO probiotics showed an improvement in FCR, reduction in fecal ammonia levels, decreased intestinal inflammation, and a significant reduction of pathogenic bacteria (*Escherichia coli* and *Salmonella*) in the gut.

Key Words: *Bacillus*, spore, probiotics, gut health, poultry

116 Assessment of a *Bacillus* spp. direct-fed microbial based on enzyme production to improve gut health integrity, immunological status, performance, and ability to counteract the toxic effects of aflatoxin B₁ in broiler chickens. G. Tellez-Isaias*¹, J. D. Latorre¹, D. Hernandez-Patlan², B. Solis-Crus², C. N. Voung¹, B. D. Graham¹, C. A. M. Selby¹, T. L. de Barros¹, and M. Arreguin-Nava³, ¹University of Arkansas, Fayetteville, AR, USA, ²Euxxis Bioscience, Fayetteville, AR, USA, ³National Autonomous University of Mexico, Mexico City, Mexico.

The increasing consumption of poultry meat globally, along with the utilization of grains for biofuel production, has led to the use of alternative and less digestible energy sources in poultry diets, which are related to an increase of digesta viscosity in monogastric animals. Some but not all *Bacillus* species have the capacity to produce exogenous enzymes, in addition to their well-recognized effect against several bacterial pathogens and enhancement of production parameters in poultry. In this talk, we present published research conducted on a *Bacillus* direct-fed microbial

(DFM), selected based on enzyme production profiles, to increase performance in high non-starch polysaccharide diets. In addition, the assessment of this DFM to improve gut health integrity, immunological status, and ability to reduce the severity of necrotic enteritis, *Salmonella* Enteritidis colonization and counteract the toxic effects of aflatoxin B₁ in broiler chickens are discussed.

Key Words: *Bacillus*-DFM, enzymes, necrotic enteritis, *Salmonella* Enteritidis, aflatoxin B₁

117 *Bacillus subtilis* 29784 significantly improved the growth performance of broilers, likely through beneficial effects on microbiota and host. D. Preveraud*¹, P. Choi⁶, J. Barton², J. Brackenridge², A. Nelson³, K. Mann³, P. Thiery¹, L. Rhayat⁴, E. Eckhardt⁴, K. Sidelmann Brinch⁵, F. Van Immerseel⁶, and E. Devillard⁴, ¹Adisseo France SAS, Antony, France, ²Adisseo USA Inc., Alpharetta, GA, USA, ³Novozymes North America Inc., Durham, NC, USA, ⁴Adisseo CERN, Commentry, France, ⁵Novozymes A/S, Lyngby, Denmark, ⁶Department of Pathology, Faculty of Veterinary Medicine, Ghent University, Ghent, Belgium.

A probiotic strain (*Bacillus subtilis* DSM 29784, BS29784) is capable of inducing beneficial effects on growth performance and could be therefore a reliable alternative to antibiotic growth promoters. The underlying mechanisms of probiotics, however, are often not fully understood. An in vivo investigation of microbiota profile aimed to observe a positive effect of BS29784 on butyrate producer bacterium such as *Ruminococcus* or *Lachnospirillum*. An in vitro approach, using Caco-2 cells line, showed a decrease of pro-inflammatory compounds (IL8, iNOS) following the supplementation of BS29784. This was mainly explained by an activation of the NFκB pathway. Finally, we also demonstrated the positive correlation of tight junction gene expression with transepithelial electrical resistance, a sensitive indicator of barrier tissue integrity. In this study, we used a 2-step approach to identify major metabolites produced by BS29784 known to have beneficial effects on broiler performance and health. The first step consisted in cultures of the BS29784 grown in Luria-Bertani and Tryptic Soy Broth culture media. After 4, 10, and 24 h, the supernatant of cultures was analyzed with UPLC/MS to identify the metabolites produced in vitro. The second step was an in vivo study, in which 1-d old broiler chicks were continuously administered BS29784 via the diet. At d 13, intestinal samples from different locations were collected and analyzed for a targeted metabolite analysis. A DNA extraction was performed on the intestinal samples to determine the relative abundance of *Bacillus* species in different intestinal locations (via qPCR). Nicotinic acid and hypoxanthine were the 2 main metabolites that were increased in the supernatant of BS29784 cultures.



An increase in their concentrations was also measured in ileum and jejunum samples of 13-d-old chickens to which the strain was administered. The wound healing assay confirmed the beneficial effect of these 2 metabolites on barrier function.

Key Words: *Bacillus subtilis*, chicken, microbiota, inflammation, metabolite

118 Are all probiotics the same when it comes to microbial profile of ceca? S. Ramirez*¹, D. Rodrigues², W. Briggs², C. Pender¹, R. Murugesan¹, and L. Bielke², ¹BIO-MIN America, Overland Park, KS, USA, ²The Ohio State University, Wooster, OH, USA.

Probiotics act primarily by competitive exclusion, taking up binding sites and utilizing nutrients that may otherwise be used by potentially pathogenic bacteria. In this way, probiotics are used in poultry to benefit intestinal health and growth performance; however, the extent of microbial modulation by different probiotics is not completely understood. The objective of this study was to determine the effect of probiotics on broiler ceca microbial profile. On day of hatch, 720 broiler chicks (15 birds/pen; 8 pens/treatment) were placed into 1 of 6 treatments: (1) control (CON), (2) yeast product (YST), (3) single-strain bacillus (SBAC), (4) multi-strain bacillus (MBAC)1, (5) MBAC2, and (6) live, multi-strain probiotic + prebiotic (LIVE) for a

42-d period. Performance was determined weekly and on d 28 and 42, 4 birds per pen were euthanized, ceca collected, and operational taxonomic units (OTUs) determined. On d 21 and 42, *Firmicutes* was the most abundant phylum regardless of treatment. On d 21, Lactobacillales was greater ($P < 0.05$) in LIVE birds compared with CON birds and other treatments were intermediate; however, all treatments were similar by d 42. On d 21, *Bifidobacteriales* was greater ($P < 0.05$) in SBAC birds compared with all other treatments, which were not different from each other; however, by d 42, SBAC birds had less ($P < 0.05$) abundance compared with MBAC1 birds and other treatments were intermediate. On d 21, there were no difference among treatments in *Lactobacillus* spp abundance; however, by d 42, MBAC1 birds had greater ($P < 0.05$) abundance compared with YST birds and other treatments were intermediate. Overall, all birds had relatively greater *Bifidobacteriales*, *Clostridiales*, and *Fecalibacterium prausnitzii* on d 42 compared with d 21. On d 21, BW was increased ($P < 0.05$) in LIVE, SBAC, and YST birds compared with MBAC2 and CON birds which were not different from each other and MBAC1 birds intermediate. However, by d 42, MBAC1 birds were heavier ($P < 0.05$) than MBAC2 birds and the other treatments were intermediate. Thus, not all probiotics modulated ceca microbiota to a similar extent nor resulted in improved BW. Grouping probiotics into different classes may help focus research to better predict their outcomes.



Session 5: Host–Microbe Interactions, Immunobiology

119 Delayed access to feed affects goblet cell distribution in chickens. K. Liu and E. A. Wong*, *Virginia Tech, Blacksburg, VA, USA.*

Newly hatched chicks are often subjected to delayed access to feed (DAF) and water up to 48 h posthatch, which impacts small intestinal development. Goblet cells arise from the stem cell pool present in the intestinal crypt and secrete mucin2 (Muc2), which contributes to the mucus layer. The objective of this study was to determine the effect of DAF on Muc2 mRNA abundance and the number and distribution of goblet cells. Cobb-500 broiler chicks hatching within a 12-h window were randomly distributed into 3 groups: control with no feed delay (ND), 24-h feed delay (D24), and 36-h feed delay (D36). Small intestinal samples were collected at 0, 24, 36, 72 and 120 h posthatch. Muc2 mRNA abundance was quantified by qPCR. Stem cells expressing olfactomedin 4 (Olfm4) and goblet cells expressing Muc2 mRNA were identified by in situ hybridization. Statistical analysis was performed using JMP Pro 14 and significant differences were determined by *t*-test and one-way ANOVA. Muc2 mRNA expressing cells were detected in both the crypt and along the villi. The Muc2 mRNA expressing cells in the crypt also express the stem cell marker Olfm4 mRNA. Intestinal crypt cells that express both Olfm4 and Muc2 mRNA have only been reported in chickens. The number and distribution of goblet cells in both the upper and lower half of the villi were determined and expressed as a ratio (VU/VL). The VU/VL for goblet cells was greater in D24 chicks compared with ND chicks at 24 h in the jejunum and ileum and greater in D36 chicks compared with ND and D24 chicks at 36 h in the jejunum. There was a corresponding decrease in the mRNA abundance of Muc2 mRNA. Muc2 mRNA abundance was lower in the duodenum of D24 and D36 chicks compared with ND chicks at 72 h and lower in the jejunum of D36 chicks compared with ND and D24 chicks at 36 h. Thus, DAF reduced the number of new goblet cells emerging from the crypt. The combined reduction in Muc2 mRNA expression and number of goblet cells may result in a decrease in the mucus layer and an increase in the risk of infection from pathogens.

Key Words: delayed access to feed, mucin 2, goblet cells, chicken

120 *Clostridium perfringens* toxins affecting the gut-brain axis as mechanism of colonization. L. Redondo¹, J. Diaz-Carrasco^{1,2}, N. Casanova¹, A. Cangelosi², P. Geoghegan², J. Goldstein^{3,4}, and M. Fernandez-Miyakawa^{*1,4}, ¹*Instituto de Patobiología Veterinaria, CICVyA-INTA, Buenos Aires, Argentina*, ²*Centro Nacional de Control de Calidad de Biológicos, ANLIS “Dr. Carlos G. Malbran,” Buenos Aires, Argentina*, ³*Departamento de Fisiología,*

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A comparatively few clostridia species are considered major pathogens, exerting their deleterious actions through several toxins which include the most toxic substances known, as epsilon toxin (ETX). Particularly, proliferation of type B or D *Clostridium perfringens* in intestines produces large amounts of ETX, which increases the permeability of the intestinal mucosal barrier, favoring their own absorption. Systemically, ETX produce prominent alteration of the central nervous system. In animals, ETX-enterotoxaemia is defined as an acute/hyperacute disease and research on *C. perfringens* and ETX pathogeny has been limited to understand acute neurological disorders. Information regarding chronic/non-lethal effects of ETX in apparently healthy animals remains limited and recent findings highlight the link between toxins secretion and *C. perfringens* fitness. In animals challenged with no lethal ETX, neurotoxicity was associated with changes in gut environment which might improve the conditions required for *C. perfringens* colonization and eventually proliferation, including alteration of intestinal permeability and gastrointestinal motility. Although some of these changes were transient, most severe changes were permanent, even in apparently healthy animals. Recent experiments show that systemic and non-lethal ETX also alters gut microbiota. A clear reduction in bacterial groups that compete with *C. perfringens*, like *Lactobacillus* spp., and an increase of other species (i.e., *Prevotella* spp.) is induced by sub-lethal systemic ETX. The observed changes suggest that ETX exposure can result in a significant perturbation of gut microbiota composition, increasing the biological fitness of *C. perfringens* in the intestinal environment. The accumulated evidence highlights the connections between brain-gut-microbiota and how intestinal pathogens can modulate gut-brain axis to improve gut colonization. ETX-enterotoxemia is probably accidental and potentially an undesired event occurred during life cycle of *C. perfringens*. These novel concepts might be considered when designing effective strategies to control *C. perfringens* associated diseases.

Key Words: *Clostridium*, colonization, gut-brain, toxin

121 The immunometabolic responses of heritage and modern broilers to immune challenge: Learning from the past to inform the future. R. Arsenault^{*1}, B. Aylward¹, C. Johnson¹, F. Perry¹, and R. Whelan², ¹*University of Delaware, Newark, DE, USA*, ²*Evonik Nutrition & Care, Hanau-Wolfgang, Germany.*

Here we characterize immune development over the course of grow-out by challenging both modern and



Athens-Canadian Random Bred (ACRB) heritage broilers with immune stimulants (CpG oligodeoxynucleotides or coccidiosis) over multiple trials and observe the impact of immune and metabolic signal transduction over time. A single dose of CpG resulted in a measurable reduction in growth of the modern birds only. Kinome analysis on the gut tissue over time showed that both strains displayed a marked increase in HIF-1, PI3K-AKT and Insulin signaling pathways. The CpG injected modern broilers had a noticeable dip in immune signaling response at d 15 post-hatch, while the ACRB had a more robust immune response throughout. Multiple doses of CpG during grow-out resulted in an acute response early on. At mid-grow out, the modern broilers still responded with an aggressive innate immune response while the ACRB had a more homeostatic response. In the final trial both bird types were vaccinated with Coccivac at d 1 post-hatch and challenged following vaccination at d 22 post-hatch in addition to a vaccine alone group and a control. The ACRB birds showed a strong response to vaccine throughout, while the modern broilers displayed a significantly reduced response. Unvaccinated challenged ACRB showed an aggressive immunometabolic reaction to the *Eimeria*, while the Ross had a lower response. Vaccine followed by challenge showed a distinct response between the 2 bird types, fully 78.8% of the differentially phosphorylated peptides were unique between these 2 groups. Responses unique to the challenge groups in both bird types pointed to a HIF-1 response, with differences in pathway activation between the 2 bird types. The results from this project allow us to better understand the changes to the modern broiler immune system due to selective pressures, this information will aid in formulating methods of modulating the immune response at key points in grow out to enhance the modern broiler's resistance to disease.

Key Words: kinome, immunometabolism, broiler, cocci, CpG

122 Segmented filamentous bacteria-based treatment improves intestinal health in layer chicks. G. Redweik* and M. Mellata, *Iowa State University, Ames, IA, USA.*

In mice, segmented filamentous bacteria (SFB) is a keystone taxon that intimately binds to the intestine and is crucial for gut maturation. SFB has been detected in chickens, but its colonization is not consistent between animals. Furthermore, its health benefits have not been directly investigated in poultry. To test the potential benefits of SFB for chicken gut health, we orally inoculated day-old chicks with chloroform-treated small intestinal scrapings (SISs) containing SFB (iSFB). SFB-negative SISs were orally inoculated to control birds (CON). At 3, 7, and 14 d post-inoculation (dpi), gut permeability was measured via FITC-dextran in serum. Additionally, SISs were collected to assess *Salmonella* resistance (5 strains) in vitro and levels

of total IgA. Lastly, distal ileum was fixed for SEM for SFB detection. At 3 dpi, iSFB birds exhibited significantly lower gut permeability versus CON. Using SISs collected 3 dpi, all *Salmonella* isolates tested exhibited greater growth in iSFB SISs versus CON. However, chicken SISs from 7 and 14 dpi significantly reduced load of each *Salmonella* isolate compared with CON. At 3 and 14, dpi, levels of total IgA did not differ between iSFB and CON birds. However, iSFB birds had significantly lower total IgA versus CON at 7 dpi. SFB were visually observable in the distal ileum via SEM in iSFB birds as early as 3 dpi. At d 7 and 14, all iSFB birds were colonized with SFB, whereas only 2 CON birds were colonized by SFB, only seen at 14 dpi. Altogether, these data suggest that oral inoculation with small intestinal spores significantly improved gut health, potentially due to SFB. Current work is underway to improve SFB treatment for poultry industry application.

Key Words: segmented filamentous bacteria (SFB), gut barrier, IgA, *Salmonella*, poultry

123 Early intestinal microbiota as a driver of poultry immunity. D. R. Rodrigues*, W. Briggs, K. Chasser, A. Duff, J. Bielke, K. Wilson, and L. Bielke, *Department of Animal Sciences, The Ohio State University, Columbus, OH, USA.*

The immune system-microbiota alliance has been highly associated with the establishment of the host immune competence. To gain deep insights into the cross-talk between intestinal pioneer colonizers and immune response, and further identify immune-biomarkers, we performed an *in ovo* application of 2 apathogenic *Enterobacteriaceae* isolates and lactic acid bacteria (LAB) to determine their influences on the intestinal proteome of broilers at day of hatch (DOH) and 10 d old. Embryos at 18 embryogenic days were inoculated with either saline (S), 10^2 cfu of *Citrobacter freundii* (CF), *Citrobacter* (C2) or LAB (L) into the amnion. At DOH (n = 20) and 10 d (n = 12), birds from each treatment were selected for a collection of intestinal samples for mass spectrometry. Based on the overexpressed proteins, it was conducted proteomic approaches as enrichment pathway and Gene Ontology (GO) function annotation analyses. In L treatment, there was an enrichment of GO terms associated with response to a stimulus, heterophil degranulation, and antimicrobial peptides pathways at DOH. While, by 10 d, the biological processes enriched were related to regulation of stress response, immune system process, and MHC class II antigen presentation pathway. C2 treatment enriched pathways associated with heterophil degranulation and TLR cascades at DOH, whereas, by 10 d, there was an enriched pathway allied to Rap1 signaling and response to stimulus. CF treatment did not affect the GO and pathways related to immune response. Afterward, we ranked proteins related to immunity based on a comparison



involving GO analyses. The key immune-related proteins included Leukocyte cell-derived chemotaxin-2, Avidin, High mobility group protein B1, Activated leukocyte cell adhesion molecule, Cathelicidin-2, and Lysozyme. Taken together, this study provides a novel understanding of how different early intestinal microbiota may drive the host immunity, in which manipulating the microbiota with the exposure of LAB may affect the development of immune functions. Besides, this study revealed a potential panel of proteins for evaluating mucosal immune response of broilers.

Key Words: *Enterobacteriaceae*, biomarkers, *in ovo* technique, probiotics, proteomics

124 High-throughput screening for natural host defense peptide-inducing compounds as alternatives to antibiotics. W. Lyu, Z. Deng, L. T. Sunkara, and G. Zhang*, *Department of Animal and Food Sciences, Oklahoma State University, Stillwater, OK, USA.*

A rise in antimicrobial resistance demands novel alternatives to antimicrobials for disease control and prevention. As an important component of innate immunity, host defense peptides (HDPs) are capable of killing a broad spectrum of pathogens and modulating a range of host immune responses. Enhancing the synthesis of endogenous HDPs has emerged as a novel host-directed antimicrobial therapeutic strategy. To facilitate the identification of natural products with a strong capacity to induce HDP synthesis, a stable chicken HTC macrophage cell line expressing a luciferase reporter gene driven by an avian β -defensin 9 (*AvBD9*) gene promoter was constructed through lentiviral transduction and puromycin selection. A high-throughput screening assay was subsequently developed using the stable reporter cell line to screen a library of 584 natural products. A total of 21 compounds with a minimum Z-score of 2.0 were identified. Secondary screening in chicken HTC macrophages and jejunal explants further validated most compounds with a potent HDP-inducing activity in a dose-dependent manner. A follow-up oral administration of a lead natural compound, wortmannin, confirmed its capacity to enhance the *AvBD9* gene expression in the duodenum of chickens. Besides *AvBD9*, most other chicken HDP genes were also induced by wortmannin. Additionally, butyrate was also found to synergize with wortmannin and several other newly identified compounds in *AvBD9* induction in HTC cells. Furthermore, wortmannin acted synergistically with butyrate in augmenting the antibacterial activity of chicken monocytes. Therefore, these natural HDP-inducing compounds may have the potential to be developed individually or in combinations as novel antibiotic

alternatives for disease control and prevention in poultry and possibly other livestock species.

Key Words: host defense peptides, antimicrobial peptides, HDP-inducing compounds, antibiotic alternatives, poultry

125 Effects of dietary protein source and litter condition on mitotically active cell and macrophage densities in the small intestine of broilers. A. J. Keel*, A. J. Calderon, O. J. Tejada, J. D. Starkey, and C. W. Starkey, *Auburn University, Auburn, AL, USA.*

Optimal function of the small intestine of broilers is necessary to facilitate efficient growth and performance as the small intestine serves as the site of nutrient absorption and a line of defense against ingested pathogens. As such, continuous renewal of enteric cells and the presence of macrophages are important to support optimal function. To test the effect of dietary protein source and litter condition on these parameters, a randomized complete block design experiment with a 3×2 factorial treatment arrangement was conducted. The 3 different dietary protein sources were soybean meal (SBM), a 50% poultry by-product meal and 50% feather meal blend (PFM), and porcine meat and bone meal (MBM). Birds were reared on either new (NL, fresh pine shavings) or used litter (UL, litter after 3 previous flocks). On d 0, Yield Plus x Ross 708, female broiler chicks (Aviagen, Huntsville, AL) were randomly allotted to 1 of 6 treatments and placed in an environmentally controlled, raised floor pen facility with 5 chicks per pen. On d 3, 8, 11, 15, and 21, 6 birds per treatment from 6 different blocks (total $n = 36$ per d) were injected intraperitoneally with 5'-bromo-2'-deoxyuridine 1 h before duodenal sample collection to label mitotically active cells. Samples were analyzed using cryohistology, immunofluorescence staining, and digital fluorescence microscopy to determine the density of mitotically active cells and macrophages. Neither dietary protein source nor litter condition influenced mitotically active cell or macrophage densities in the duodenum on d 11 or 21. However, broilers reared on UL had a greater mitotically active cell density than those reared on NL on d 3 ($P = 0.0126$) and d 15 ($P = 0.0292$). On d 8, broilers fed MBM had an increased macrophage density compared with broilers fed PFM and SBM ($P = 0.0401$). Mitotically active cell and macrophage density in the duodenum changed over time. Mitotically active cell density decreased from d 11 to d 15 ($P < 0.0001$), and this was accompanied by an increase in macrophage density ($P < 0.0001$).

Key Words: broiler chicken, macrophage, cell proliferation, immunofluorescence



126 Changes in the host transcriptome and microbial meta-transcriptome of the ileum of dairy calves subjected to artificial rumen contents dosing. W. Li*¹, A. Edwards², M. Cox², J. Skarlupka², S. Raabis², A. Steinberger², B. Murphy², A. Larsen², and G. Suen², ¹*US Dairy Forage Research Center, Madison, WI, USA*, ²*University of Wisconsin-Madison, Madison, WI, USA*.

Development of a properly functioning gastrointestinal tract (GIT) at an early age is critical for the wellbeing and success of dairy cattle. GIT development in calves is influenced by several factors, including colonization by microbes, which can accelerate immune response and promote overall health. However, the molecular changes associated with early microbial colonization on GIT development remain largely unknown, particularly for the small intestine. In this study, we performed artificial dosing of exogenous rumen fluid in the early life of the calf, starting at birth through the weaning transition at 8 wk. Six calves were included in this study, with 3 of them receiving artificial dosing and the remaining 3 receiving autoclaved rumen contents as a control. At 8 wk of age, tissue from the ileum were collected and subjected to host transcriptome and microbial meta-transcriptome analysis

using RNA-seq. A total of 333 genes showed significant differential expression (DE) (fold-change > 1.5; adjusted $P < 0.1$, mean read-count > 10) between the treated and control calves. Gene ontology analysis indicated that these DE genes were predominantly enriched in immunity response (P -value $\ll 0.0001$). The association analysis between the gene expression and the microbial species abundance identified 33 genes with significant correlation with the ileum microbial species (Pearson's r , P -value < 0.0001). Of these, 3 genes correlated with a large number of microbial species: *LYZ2* (73 species), *FABP5* (61) and *FUT1* (49 species). Specifically, *LYZ2* encodes an antibacterial lysozyme known to act against a wide range of bacteria. The profound increase in expression of *LYZ2* in treated calves suggested the activation of anti-bacterial activity and innate response from the host. This study's findings shed light on the impact of early exogenous introduction of microbes into the small intestine of calves. Further, this study provides a foundation for the development of probiotic treatments aimed at improving calf GIT development and host health.

Key Words: calf, ileum tissue transcriptome, microbial meta-transcriptome, artificial dosing



Section 6: Nutrition and Gut Health

127 *Tenebrio molitor* meal inclusion in broiler chickens diet: A multidisciplinary approach to gut health.

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Insect-derived feed represents a promising alternative to soybean meal in poultry nutrition thanks to the high quality and quantity of protein and the adequate amino acid profile. This study evaluated the effects of *Tenebrio molitor* (TM) larva meal inclusion in broiler diet. A total of 160 male broiler chicks were randomly allotted to 4 dietary treatments (control, TM 5%, 10% and 15%). At d 53, 10 broilers/diet were slaughtered. Cecal content was collected for direct DNA extraction and 16S rRNA amplicon based sequencing. Samples of duodenum, jejunum, and ileum were collected and processed for histomorphological investigations. Gut morphology was evaluated through morphometric indices: villus height (Vh), crypt depth (Cd) and villus height to crypt depth ratio (Vh/Cd). Periodic acid–Schiff, Alcian Blue and high iron diamine staining were performed on gut sections to characterize neutral, acidic sialylated and acidic sulfated mucins. Next-generation sequencing technology was used to investigate gene expression in jejunum. Data were analyzed by IBM SPSS Statistics V20.0.0 and R softwares (P -value < 0.05). Principal component analysis showed a separation of the cecal microbiota composition depending on diet ($P < 0.001$), with depletion of *Ruminococcus* being particularly evident in TM10 and TM15 groups. TM15 birds showed lower Vh ($P < 0.05$), higher Cd ($P < 0.05$), and lower Vh/Cd ($P = 0.001$) compared with C and TM5. Mucin staining intensity in crypt/villus depended on diet (crypts: $P < 0.01$), mucin type, gut segment and crypt/villus fragment ($P < 0.001$). In particular, TM5 and C showed higher mucin staining intensity than TM10 and TM15 birds. Greater acidic sialylated mucins staining intensity than neutral and acidic sulfated was also found. TM inclusion mainly modulates the expression of genes related to immune system, signal transduction, and metabolism. In conclusion, low levels of TM inclusion did not negatively affect intestinal morphology, microbiota, mucin composition, and gene expression, thus suggesting a potential use of insect-derived feed in poultry nutrition.

Key Words: *Tenebrio molitor*, gut health, chicken

128 *Hermetia illucens* meal inclusion in piglets: Effects on gut health. I. Biasato¹, E. Colombino², I. Ferrocino¹, S. Dabbou², V. Vincenti², A. Imarisio², A. Schiavone², L. S. Coccolin¹, L. Gasco¹, and M. T. Capucchio*²,

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Insects are considered a novel protein source for animal feed, because of their nutritive properties and rearing characteristics. The present study aimed to investigate the gut microbiota, mucin composition and histopathological findings in piglets fed *Hermetia illucens* (HI) larva meal. A total of 48 piglets were randomly allotted to 3 dietary treatments (control, HI 5% and 10%). Twelve animals per treatment were slaughtered at 61 d of age. Cecal content was collected for direct DNA extraction and 16S rRNA amplicon based sequencing. Samples of duodenum, jejunum, ileum, mesenteric lymph nodes, liver, spleen, lung, stomach, and kidney were collected and processed for histomorphological investigations. Gut morphology was evaluated through morphometric measurements of villus height, crypt depth and villus height to crypt depth ratio. Histopathological alterations were scored using a semiquantitative scoring system (0–3). Histochemical staining were performed on gut sections to characterize the 3 types of mucins (Periodic acid–Schiff: neutral mucins; Alcian Blue pH 2.5: acidic sialylated mucins; high iron diamine: acidic sulfated mucins) and percentage of mucin expression was evaluated with Image-Pro Plus software. Data were analyzed by IBM SPSS Statistics V20.0.0 and R softwares (P -value < 0.05). β -diversity calculation showed a separation of the cecal microbiota composition depending on diet ($P < 0.001$), with an higher prevalence of *Prevotella*, *Roseburia*, *Blautia*, *Ruminococcus*, (short chain fatty acids-producing bacteria) in HI piglets than C. Dietary HI meal inclusion did not influence gut morphology and histopathological alterations ($P > 0.05$). However, gut and stomach showed lymphoplasmacytic inflammation accompanied by reactive follicular hyperplasia and/or depletion in mesenteric lymph nodes. Lymphoplasmacytic inflammation and vacuolar degeneration were also identified in liver and kidney. Mucin expression was influenced by gut segment, type of mucin and interaction diet-mucin type ($P < 0.05$). In conclusion, dietary HI meal inclusion positively modulates gut microbiota of piglets with no negative effects on gut morphology and health status.

Key Words: piglet, insect, gut health



Section 7: Nutrition and Gut Health (continued)

129 Natural multifunctional solutions (Alquermix) in broilers. J. Pié*¹, D. Díez¹, C. Domenech¹, C. Gallardo², and M. E. Rosemberg², ¹Biovet, Constanti, Spain, ²Universidad Científica del Sur, Lima, Peru.

Alquermix line is composed of natural multifunctional products that contain a patented mycotoxin binder, feed preservatives, enzymes, probiotics, and active botanical molecules that optimize the organs' physiology. They are designed to replace additives commonly used in farms. An experiment was conducted to evaluate whether Alquermix products could replace several additives in broilers through the analysis of performance and resistance against coccidiosis. It was conducted with 248 one-day-old male broilers (Cobb 500) raised for 42 d and allotted to 2 treatments: (1) CN (control diet with organic acids, aluminosilicates, diclazuryl, bacitracin, a multivitaminic and an enzymatic with protease, amylase, β -mannase, xylanase, β -glucanase, cellulase, pectinase, phytase and probiotics); and (2) AX (basal diet without the mentioned additives + Alquermix products at 4 kg/ton during all the trial). There were 4 replicates per treatment. Body weight, feed conversion rate (FCR), mortality and uniformity were evaluated. Gut lesions were evaluated macroscopically and microscopically at the end of the trial to identify whether they were coccidiosis-related or not. Data were analyzed using PROC GLM procedures of SAS 9.2 and P value less than 0.05 was set as statistically significant. Weight in AX was significantly higher on d 7 and numerically higher on d 42 (24 more grams per bird). FCR was a 2.61% better in AX on d 42 ($P = 0.054$), this improvement was significant in the first growing stage (d 1–10). Weekly mortality was lower in AX ($P < 0.001$) compared with the control (2.99% vs. 4.57%, at the end of the trial). Uniformity was higher in AX group (83.7% vs. 78.0%, $P < 0.05$). Animals in CN showed more intestinal lesions (unspecific and related to coccidia). Histology findings were correlated with those in the macroscopic observation. It is possible to replace regular (including chemical) additives with natural ones. Replacement of feed additives with Alquermix multifunctional products can better productive parameters and improve birds' protection against coccidia without the need for AGPs, chemical or ionophore coccidiostats.

Key Words: natural additives, broilers, growth performance, coccidiosis, AGPs

130 Interaction effects of feeding *Bacillus subtilis* DSM 32315 and dietary protein on intestinal barrier function, microbial profiles, and growth performance in weaned piglets. W. Tang¹, J. C. González-Vega², J. Gao³, A. Menconi⁴, A. Sokale⁴, and K. Doranalli*², ¹Animal Nutrition Institute, Sichuan Agricultural University, and Key Laboratory of Animal Disease-Resistance

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The objective of the study was to determine to what extent *B. subtilis* DSM 32315 (*BS*) probiotic can ameliorate the negative effect of weaning stress on intestinal microbial profile and integrity, and growth performance, in piglets fed low or high crude protein (CP) diets. Seventy-two piglets (initial weight 7.61 ± 0.55 kg) were allotted to 4 diets in a randomized complete block design in a 2×2 factorial arrangement. Two protein levels included high CP (HCP; 0–14d, 20.5%; 15–42d, 19.5%) and low CP (LCP; 0–14d, 18%; 15–42d, 17%), and 2 added levels of *BS* included at 0 or 500 mg/kg diet. Results indicated that supplementation of *BS* increased ($P < 0.05$) *Bacillus* and *Bifidobacterium* in the ileum while there was a tendency ($P = 0.057$) for *Lactobacillus* counts regardless of CP level. However, in the colon, *Bacillus* and *Bifidobacterium* abundance was higher ($P < 0.05$) in LCP diets. In jejunum, supplementation of *BS* increased ($P < 0.05$) villus height (VH) in LCP diets, but this was not observed in HP diets (CP \times *BS* interaction; $P < 0.01$), and villus height: crypt depth ratio (VH:CD) was increased ($P < 0.01$) by *BS* supplementation. Supplementation of *BS* increased ($P < 0.05$) VH and VH:CD only in LCP diets (CP \times *BS* interaction; $P < 0.05$) in the ileum. In ileum, the relative mRNA expression of Occludin-1, and EGF and IGF-1R, increased ($P < 0.05$) when *BS* was added to LCP diets than to HCP diets (CP \times *BS* interaction; $P < 0.05$). Supplementation of *BS* increased ($P < 0.05$) the apparent total-tract digestibility (ATTD) of dry matter and gross energy regardless of CP level. Furthermore, feeding *BS* increased daily gain ($P = 0.02$) and reduced feed conversion ratio ($P < 0.01$). In conclusion, *Bacillus subtilis* DSM 32315 modified hindgut bacterial composition and maintained intestinal integrity of piglets, with more pronounced benefits in piglets fed low protein diets.

Key Words: *Bacillus subtilis* DSM32315, gut health, low protein diet, microbiota profile, piglets

131 Expression of genes encoding proteins associated with nutrient uptake at either the brush border or basolateral surface of the intestine in six strains of chickens with different growth potential. K. Miska*, L. Schreier, S. Kahl, B. Russell, and M. Proszkowiec-Weglarz, USDA/ARS, Beltsville, MD, USA.

This study was designed to compare expression of genes that encode proteins located at either brush border (BB) or basolateral (BL) of the gut epithelium among fast and



slow growing broilers. Six strains of chicks with different growth capacities were used: Ross 708, Hubbard H1 (HH1), Cobb500, Longnecker's Heritage (LHR), Red-Bro, and the Athens Canadian Randombred Control (ACRBC). Birds were sampled at embryonic d 19, day of hatch, and d 7, 14, 21, 28, and 35 post-hatch (PH) and a portion of the duodenum, jejunum, ileum, and ceca were snap frozen for RNA isolation. Ross 708, HH1, and Cobb500 birds had the greatest body weights (BW), reaching 2,172 g, 2,320 g, and 2,137 g respectively at d 35 PH. Red Bro and LHR birds attained BWs of 1,308 g and 1,436 g, while ACRBCs weighed only 421 g at d 35 PH. Quantitative RT-PCR was performed on 12 genes. Analysis of variance of 8 genes which encode proteins associated with the BB of the gut epithelium, including: Aminopeptidase N, 4 amino acid transporters, (ATB^{na+}, B^{na}AT, b^{na+}AT, EAAT3) a di- and tri- peptide transporter (PepT1), and 2 sugar transporters (GLUT5 and SGLT1). Also, analysis of 5 genes which encode proteins associated with the BL gut epithelium: 4 amino acid transporters (CAT1, CAT2, LAT1, and γ^+ LAT1), and a single sugar transporter (GLUT2) was carried out. The results indicate that while strain specific differences in gene expression were noted they did not correlate with growth rate. In most cases, genes encoding BB associated proteins increased in expression over time ($P < 0.05$) in the duodenum, jejunum, and ileum, while in the ceca the expression decreased. The genes encoding BL associated proteins decreased ($P < 0.05$) in expression over time in all gut segments, with the exception of GLUT2, which increased in expression in the small intestine. The temporal changes in gene expression were very consistent among bird strains. In conclusion temporal and strain differences in gene expression were noted, however, differences may not be associated with growth efficiency.

Key Words: broilers, nutrient transporter, growth

132 A unique hydrolyzed yeast product can prevent postweaning diarrhea in *Escherichia coli*-susceptible piglets. S. Hasan*, E. Valkonen, H. Kettunen, and J. Vuorenmaa, *Hankkija Oy, Hyvinkää, Finland.*

Postweaning diarrhea (PWD) frequently associated with enterotoxigenic *Escherichia coli* (*E. coli*) infection, is one of the major welfare and economic problems in pig production after weaning. The aim of this study was to examine whether hydrolyzed yeast (YD) based on whole brewery yeast (*Saccharomyces cerevisiae*) added to the creep feed of suckling and newly weaned piglets prevent PWD and improve performance. Piglets from 20 litters of F4 fimbriae receptor positive sows (therefore F4 receptor positive piglets) were randomly allocated to 2 treatment groups: YD group with 3 g/kg or 2 g/kg of YD added to the piglets creep feed from 2 week of age until 2 week post weaning (PW) and from wk 2 to 5 PW, respectively, and

control group (C) no added YD. At weaning (4 weeks of age) 2 piglets (n = 40) from each litter were individually housed and either experimentally *E. coli* challenged or placebo treated on d 1 and 3 PW. Further, performance was measured on 3 group-housed piglets (n = 60) from each litter. Fecal consistency scored (FCS) at 1 to 7, where 1 being hard, dry and 7 being foamy yellow. FCS was better in placebo than in *E. coli*-challenged piglets, and in YD piglets than in C. YD piglets had lower risk of PWD ($P = 0.001$) and the effect was evident in both placebo and challenged piglets ($P = 0.010$ and $P = 0.038$, respectively). In challenged piglets fecal shedding of hemolytic *E. coli* was significantly lower in YD than in C piglets ($P = 0.026$). In placebo piglets the latency time to first observation of PWD was longer in YD than in C piglets ($P = 0.048$). Feed intake from weaning to d 6 PW tended to be higher in YD piglets than in C both in challenged and placebo piglets. In group-housed piglets, less medical treatments against PWD were needed for YD than C pens during the first 3 weeks PW ($P = 0.078$). The average daily gain did not differ between the treatments. In conclusion, YD may prevent PWD after weaning at 4 week of age if added to the creep feed 2 weeks before weaning and post weaning.

Key Words: hydrolyzed yeast, postweaning diarrhea, piglet

133 Effects of zinc hydroxychloride on inflammation and intestinal integrity during feed restriction. E. A. Horst*¹, E. J. Mayorga¹, M. Al-Qaisi¹, S. Rodriguez-Jimenez¹, B. M. Goetz¹, M. A. Abeyta¹, R. S. Fry², S. K. Kvidera², and L. H. Baumgard¹, ¹*Iowa State University, Ames, IA, USA*, ²*Micronutrients USA LLC, Indianapolis, IN, USA.*

Objectives were to evaluate effects of dietary zinc hydroxychloride (HYD) on inflammation and intestinal integrity during feed restriction (FR) in lactating Holstein cows. Twenty-four cows (parity 3 ± 0.2) were randomly assigned to 1 of 4 treatments: (1) ad libitum-fed and control (ALCON; 75 ppm zinc from zinc sulfate; n = 6), (2) ad libitum-fed and HYD (ALHYD; 75 ppm zinc from HYD; n = 6), (3) 40% of ad libitum feed intake and control (FRCON; n = 6), or (4) 40% of ad libitum feed intake and HYD (FRHYD; n = 6). Before study initiation, cows were fed their respective diets ad libitum for 21d. The trial had 2 experimental periods (P) during which cows continued to receive their respective dietary treatments. Period 1 (5d) served as the baseline for P2 (5d), during which cows were fed ad libitum or restricted to 40% of P1 feed intake. In vivo intestinal integrity was evaluated on d4 of P1 and d2 and 5 of P2 using chromium (Cr)-EDTA. All cows were euthanized at the end of P2 to evaluate intestinal histology. Lipopolysaccharide-binding protein (LBP) concentrations increased (63%) in FR relative to AL-fed cows ($P = 0.02$),



and this difference was mostly attributed to increased LBP concentrations in FRHYD cows relative to all other treatments on d 1 and 3 of P2. Circulating serum amyloid A (SAA) and haptoglobin (Hp) from FR cows increased during P2, but both peaked higher (47 and 61%, respectively) in FRCON relative to FRHYD cows on d 5 ($P = 0.08$ and 0.05 , respectively). Overall during P2, plasma Cr area under the curve was increased (21%) in FR relative to AL-fed cows ($P = 0.01$), but this was mainly influenced by the difference in AL vs. FR treatments on d 2 of P2 ($P < 0.01$). Plasma Cr was not affected by HYD. Jejunum villus width increased (23%) and crypt depth decreased (22%) in FR relative to AL cows ($P = 0.02$). Overall, FR decreased jejunum mucosal surface area (24%; $P = 0.15$), along with ileum villus height and crypt depth (15 and 17%, respectively; $P \leq 0.14$), and HYD supplementation ameliorated the decrease in ileum villus height (25%; $P = 0.15$). In summary, HYD supplementation altered the acute phase response to FR and moderately influenced intestinal permeability.

Key Words: leaky gut

134 A high rumen degradable starch diet modulates jejunum microbiota and alters enterohepatic circulation of bile acids in dairy goats. J. Shen¹, J. Yao¹, and Y. Cao^{*1,2}, ¹Northwest A&F University, Yangling, Shaanxi, China, ²Harvard Medical School, Boston, MA, USA.

Fat is the major energy component in milk and accounts for many of the physical properties, manufacturing characteristics, and organoleptic qualities of milk and milk production. Milk fat can be affected by factors that influence the processes from dietary lipogenic precursors to milk fat, in the rumen, intestine, liver, and mammary tissues and so on. This study uncovered the effect of digestive processes and liver metabolism of lipogenic precursors on milk fat synthesis in dairy goats. Eighteen lactating goats were allocated equally into low RDS (LRDS = 20.52%), medium RDS (MRDS = 22.15%), and high RDS (HRDS = 24.88%) diet groups. After 5 weeks of feeding, the HRDS diet increased the relative abundance of *Firmicutes* and *Ruminococcus_2* in jejunum. *Firmicutes*, which are gram-positive bacteria, are the predominant bacteria able to deconjugate and dehydroxylate primary bile acids into secondary bile acids. *Ruminococcus* perform epimerization during the conversion from primary to secondary bile acids. The expression of bile acid receptor FXR in jejunum and ileum and TGR5 in jejunum were decreased by HRDS treatment, indicating the negative feedback regulation of bile acid synthesis was inhibited in liver. The increase expression of CYP7A1, the rate-limiting enzyme of the bile acid biosynthetic from cholesterol, indicate the increased bile acids synthesis in liver. We also found the

HRDS group increased the bile acids TCDCA and TDCA, and disordered the phosphatidylcholines in liver, as well as upregulated TNF α expression. We further measure the genes expression related to lipid metabolism, and found that HRDS group increased the expression of the transcription factor peroxisome proliferator-activated receptor α (PPAR α) and its downstream target gene CPT1. This study demonstrated that HRDS diet feeding modulates jejunum microbiota and alters enterohepatic circulation of bile acids, and promote lipid oxidation in dairy goats.

Key Words: bile acids, enterohepatic circulation, lipid metabolism, rumen degradable starch, dairy goats

135 Thymol modulates chemo-sensing receptors and inflammation markers in the gut of weaning pigs. A. Toschi^{*1}, B. Tugnoli², B. Rossi², A. Piva^{1,2}, and E. Grilli^{1,3}, ¹University of Bologna, DIMEVET, Ozzano Emilia, Bologna, Italy, ²Vetagro S.p.A., Reggio Emilia, Italy, ³Vetagro Inc., Chicago, IL, USA.

Thymol in vitro anti-oxidant and anti-inflammatory properties have been widely described, although details of the in vivo mechanism of action of thymol as a gut-health promoting agent are still lacking. More specifically, the involvement of thymol in gut chemo-sensing and intestinal function still has to be thoroughly elucidated. Aim of this study was to investigate the expression of olfactory receptor 1G1 (OR1G1) and of transient receptor potential vanilloid 1 (TRPV1), as well as inflammatory cytokines, in the gut of piglets fed with thymol in the 2 weeks postweaning. One hundred sixty pigs were fed 5 diets ($n = 8$) for 14 d: a pre-starter without (control, T1) or with thymol at 25.5, 51, 153, or 510 mg/kg of feed (T2, T3, T4, T5, respectively). Growth performance was recorded and, at d14, 8 pigs/group were euthanized to collect intestinal samples for mRNA analysis. Data were analyzed with ANOVA. Thymol did not affect growth performance although it tended to increase the expression of OR1G1, TRPV1 and tumor necrosis factor α (TNF α) in the duodenum ($P = 0.10$). In the ileum thymol significantly increased OR1G1 and TRPV1 in a dose-dependent manner ($P = 0.04$ and $P = 0.03$, respectively), whereas inflammatory cytokines expression was not affected. The upregulation of OR1G1 and TRPV1 by thymol all along the intestine implicates the possible role of these receptors as mediators of the gut-health effects associated with the use of thymol as a feed additive. Moreover, data suggest a possible association between the upregulation of these receptors and the modulation of the inflammatory state in the duodenum which was never described before.

Key Words: thymol, weaning pigs, chemo-sensing, inflammation



Posters

P100 Evaluation of feeding spray-dried plasma during heat stress on broiler growth, intestinal permeability, and bone mineralization. J. Ruff*¹, T. Barros¹, J. Cambell², R. Gonzalez-Esquerria², D. Graham¹, C. Selby¹, C. Young¹, S. Dridi¹, E. Greene¹, B. Hargis¹, and G. Tellez-Isaias¹, ¹University of Arkansas, Fayetteville, AR, USA, ²APC, Ankeny, IA, USA.

The purpose of this study was to evaluate feeding spray-dried plasma (SDP) during continuous heat stress as a model to induce leaky gut in broilers. On day of hatch, 480 chicks were allocated into 12 environmental chambers, 4 thermoneutral (TN-negative control); 4 heat stress (HS-positive control); and 4 heat stress treated with 2% SDP in the feed (HS-SDP) till d 28 followed by 1% SDP till d 42. At 21 d. the HS groups were exposed to 35°C from d 21–42; while thermoneutral ones were maintained at 24°C from d 21–42. Chickens were equipped with a ThermoChron temperature logger for continuous monitoring of core body temperature. Fluorescein isothiocyanate-dextran (FITC-d) was orally gavage to 2 chickens/replicate (n = 16) randomly selected on d 21, 28, 35 and 42. After one h of oral gavage, blood samples were collected to determine the passage of FITC-d. Tibias were removed to evaluate break strength on 21d and 42d. Feeding 2% SDP during the first 11 d of the trial, significantly increased ($P < 0.05$) body weight (BW) when compared with both control groups. Body temperature was significantly increased after 2 h of starting HS and remained that way until the end of the study. Chronic HS caused an increase in core body temperature which significantly decreased feed intake, BW, and feed efficiency (28, 35 and 42d) when compared with control TN chickens. However, feeding SDP gained 75 g (6%) and 135 g (8%) more weight at d 28 and 42, respectively when compared with HS-positive-control group. Similarly, serum FITC-d was significantly increased in HS chickens at all points of evaluation. Chronic HS also caused a significant reduction of bone strength at 42d when compared with the control chickens. Feeding SDP reduced intestinal permeability and bone strength when compared with HS-positive control group. The results from the present suggest that strategic use of SDP during period of stress such as heat stress could help to reduce its negative effects on broiler performance, improve bone strength, and improve gut integrity.

Key Words: chickens, enteric inflammation, heat stress, performance, serum FITC-d.

P101 Is the regulation of intestinal inflammation defective in high breast yield strain? G. Cardoso Dal Pont*¹, M. Kogut², B. Mallmann³, K. Feye³, C. M. Owens³, S. Ricke³, and C. N. Coon³, ¹Texas A&M, College

Station, TX, USA, ²USDA-ARS, College Station, TX, USA, ³University of Arkansas, Fayetteville, AR, USA.

The selection for rapid growth was already been associated with dysfunction of immune response in broilers. However, it is not known if animals selected for distinct body yield characteristics show differences in intestinal immunity. Therefore, an experiment with high breast yielding (HBY) and standard breast yielding (SBY) male broilers was conducted to compare their intestinal immunity and the interaction with wooden breast. Broilers were raised in floor pens, with feed and water *ad libitum*. All the birds received the same corn/soy based diet formulated to meet or exceed their requirements. On d 14 and 28 of age the animals were palpated to detection of wooden breast, then 10 animals from each strain were euthanized, 5 non-affected (N) and 5 with wooden breast (WB). Duodenum and ileum were collected to measure TNF, IL-8 and IL-10 expression. The results were submitted to ANOVA in a 2 × 2 factorial arrangement (2 strains × 2 breast categories), and means were compared by Tukey test at 5% probability. Birds affected by WB were not identified at 14 d of age, the myopathology was palpated from d 21 and up. At 14 d of age HBY broilers showed lower IL-10 expression on duodenum and ileum ($P < 0.01$) and higher IL-8 on ileum compared with SBY broilers ($P < 0.01$). At 28 d of age, differences in gene expression in the duodenum were observed; HBY presented higher IL-10 and IL-8 ($P < 0.001$) and an interaction between strain and wooden breast was observed to IL-10 ($P < 0.01$). Thus, the gene expression of pro-inflammatory cytokines suggest that birds selected for high breast yield present a higher inflammatory status in the intestine. Also, high breast yielding broilers have lower ability of downregulate the intestinal inflammation, especially the ones affected by wooden breast. Moreover, WB birds showed reduced expression of IL-10 ($P < 0.05$). The lower expression of regulatory cytokine suggest that WB birds have an impaired inflammatory regulation in the intestine, which may be an indicative of susceptibility to the development of wooden breast.

Key Words: wooden breast, genetic, gut health

P102 Investigating intestinal barrier integrity in heat-stressed modern broilers and their ancestor Jungle Fowl. T. Tabler*, E. Greene, S. Orłowski, J. Hiltz, N. B. Anthony, and S. Dridi, *University of Arkansas, Fayetteville, AR, USA.*

Heat stress (HS) is devastating to poultry production sustainability from its adverse effects on bird welfare, health, growth, and mortality. Although modern broilers have greater gut mass and higher energy use efficiency



than unselected birds, they are more vulnerable to HS that induces leaky gut syndrome. The aim of the present study was, therefore, to determine the effect of HS on gut barrier integrity in 3 modern broilers and in their ancestor Jungle Fowl. Four chicken populations: Giant Jungle Fowl, Athens Canadian Random Bred (1950s), 1995 Arkansas Random Bred, and Modern Random Bred (2015) were used. Day-old broiler chicks from each population were raised under thermoneutral conditions with feed and water intake measured daily. On d 28 the birds were subjected to 1 of 2 environment conditions: thermoneutral (24°C) or acute heat stress (2 h at 36°C). After the 2 h, samples from each section of the small intestine were harvested from 2 birds per line per treatment and flash frozen in liquid nitrogen. Gene expression was analyzed by 2-way ANOVA using real time quantitative PCR. At molecular levels in the duodenum, gene expression of HSP 70, 60, and 90, ZO-2, ZO-3, villin, PAT-J, cadherin, connexin-45, and calprotectin were shown to be significantly upregulated by heat stress. In the jejunum, gene expression of ZO-1, ZO-2, and ZO-3 was significantly upregulated by heat stress, while occludin was downregulated. This data provides evidence for a mechanistic understanding of the gut physiology and how it can be influenced by growth-rate and heat stress.

Key Words: leaky gut, tight junction, heat stress, GI tract, chicken

P103 Clostridia variation over time within a dairy cattle herd in southeastern Wisconsin. R. F. Teal*, A. LeMarche, V. G. Bretl, K. Van Zanten, J. S. Thompson, and T. G. Rehberger, *Arm & Hammer Specialty Products Division, Waukesha, WI, USA.*

Clostridium perfringens is an anaerobic, spore-forming, gram-positive rod-shaped bacterium which is known to cause enteric infections in a variety of hosts, including humans and livestock. In the bovine host *C. perfringens* has been linked to necro-hemorrhagic enteritis in calves, and hemorrhagic bowel syndrome in adult cows. Previous research within our lab has found that as total fecal clostridia loads increase within a dairy herd dry-matter intake and milk yields subsequently become highly variable. We have also seen evidence of widely varying total fecal clostridia loads within lactation groups on a single farm, as well as across farms within dairy-producing regions throughout the United States. The aim of this work is to begin to characterize this variation in 11 animals within a single farm over the course of 3 weeks. In addition to total clostridia enumeration, we also performed species-specific multiplex PCR and 16S sequencing analysis of clostridia isolates derived from these fecal samples. We attempted to correlate these data with the feed component clostridia community, as well as several atmospheric measurements. We observed a correlation between ambient air temperature

at time of fecal sample collection and total clostridia count data ($r = -0.79$, $P < 0.001$). We also observed several changes of community composition over the course of the 3 weeks between animals and overall within the farm, and that as the total clostridia load increases, so does the *C. perfringens* load ($r = 0.81$, $P < 0.00001$). Initial community composition data indicates that this herd is primarily challenged by *C. perfringens*, with *Paraclostridium bifermentans* the second largest proportion of clostridia present. Variation within the enumeration data and community data appeared to show no easily discernable pattern within such a small time-scale. Additional studies are currently being designed to more deeply explore the clostridia community within a single farm with plans to look at other organisms, atmospheric conditions, as well as additional KPIs such as milk quality and yield.

P104 Enumeration and identification of *Clostridium* along the gastrointestinal tract of dairy cows. M. N. Griffin*¹, J. S. Thompson¹, F. F. Cardoso², F. Cardoso², R. I. Mackie², A. H. Smith¹, and T. G. Rehberger¹, ¹*Arm and Hammer, Waukesha, WI, USA*, ²*University of Illinois, Urbana, IL, USA.*

Clostridium are anaerobic, spore forming bacteria that reside naturally in soils and the gastrointestinal tract (GIT). *C. perfringens* and other toxin producing species of *Clostridium* have been associated with enteric diseases in ruminants. Previous dairy feed and cow fecal surveys have indicated that the most abundant *Clostridium* species is *C. perfringens* with *Paraclostridium bifermentans* and *C. beijerinckii* being the next 2 most predominant species. The objective of this research was to understand where *Clostridium* colonizes along the GIT, the levels of colonization and the dominant species within each section of the GIT. Fourteen Holstein cows at the University of Illinois at Urbana-Champaign were challenged with a high, medium or low dose of *P. bifermentans* over 10 weeks, euthanized and their gastrointestinal tract harvested from the rumen to the rectum, in addition to a rumen fluid and fecal sample collected before euthanasia ($n = 210$). Each tissue sample was rinsed and only the epithelial mucosa used. Total clostridia counts ranged widely from <10 to $2.0E05$ cfu/g. The total clostridia counts were not different by challenge group, but the levels of total clostridia differed along the GIT when counts were combined from all groups. Seven sections had lower counts compared with the fecal counts ($P < 0.05$). From each sample, up to 10 isolated colonies were analyzed using a species-specific multiplex PCR and 16S analysis. The 2 most abundant species identified were *P. bifermentans* (50.7%) and *C. perfringens* (23.9%). Although not included in the challenge dose, *C. perfringens* was identified as the dominant population within both the duodenum and middle jejunum. This suggests that the *C. perfringens* is entering the system



naturally through the feed and inhabiting the GIT. *P. bifermentans* was identified as the dominant population in all other areas of the GIT. This research is important in understanding where these bacteria reside along the GIT and in the future, how they may be impacting the overall health and production of the animal.

Key Words: ruminants, *Clostridium*

P105 Feeding chestnut tannins stimulates pro-inflammatory immune response in broiler chicks. A. Lee*¹, G. Cardoso dal Pont¹, M. Battaglia², and M. Kogut³, ¹Texas A&M University, College Station, TX, USA, ²Silvateam/Indunor S.A., Buenos Aires, Argentina, ³USDA-ARS, College Station, TX, USA.

As the demand for alternatives of antibiotic growth promoters (AGPs) increases in food animal production, phytobiotic compounds gain popularity due to their ability to stimulate similar properties of AGPs. Chestnut tannins (CT; *Castanea sativa*) are one of many phytobiotic compounds utilized as feed additives, particularly in South America, for broiler chickens due to its bacteriostatic capabilities. While many studies have observed the microbiological effects of CT, there is a lack of studies determining the immunological function of CT in the host. Therefore, the aim of this study was to determine if chestnut tannins would affect host immunity compared with control birds based on the cytokine gene expression assay. One hundred fifty day-of-hatch Cobb 500 chicks were separated into 3 feed treatment groups: control (n = 50), 1% CT (n = 50), and 0.2% CT (n = 50). The different percentages of CT in feed were tested to see which concentration will provide the most significant modulation in the bird. Cecas were collected on d 2, 6, 8, and 10 post-hatch and immediately placed into liquid nitrogen for gene expression. Cytokine mRNA expression was assessed using TaqMan gene expression assays for pro-inflammatory and anti-inflammatory cytokines (specifically IL-1B, IL-6, IL-8, IL-10, IL-17 and IFN-gamma). Cecas from birds fed with 1% and 0.2% CT had increased levels of IL-6 expression ($P < 0.05$) during the entire experimental period, as compared with the control group. By d 6, the immune response of birds fed with tannins was regulated by IL-10 ($P < 0.05$), an anti-inflammatory regulatory cytokine. The remaining cytokines were not statistically significant when compared with the tannin-fed groups against the control. In conclusion, there seems to be an immunomodulatory role of CT in the broilers at early life stages, possibly indicating a temporary pro-inflammatory phase in the gut to enhance immunity via IL-10 regulated pathway.

Key Words: cytokines, phytobiotics, immunity

P106 Fermented cottonseed meal reduces fat deposition in white-feather broilers through cecum

bacteria-host metabolic cross-talk. J. Niu*¹, J. Zhang², L. Wei¹, X. Ma², W. Zhang¹, and C. Nie^{1,2}, ¹College of Animal Science & Technology, Shihezi University, Shihezi, Xinjiang, China, ²State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China.

Excessive fat deposition in broilers has been a serious problem in the commercial broiler industry. Gut microorganisms have the potential to influence fat deposition. However, the factors that affect fat deposition, cecum microbiota and metabolites response to fermented cottonseed meal (FCSM) diet in broilers are largely unknown. The aim of this study was to investigate the effects of FCSM on fat deposition, and cecum microbiota and metabolites as well as the interactions among them. A total of 180 1-d-old broilers were divided into 2 groups with 6 replicates of 15 birds in each. The 2 diets consisted of a control diet supplemented with 0% FCSM (CON) and an experimental diet with 6% FCSM (FCSM). Illumina MiSeq sequencing and liquid chromatography-mass spectrometry were used to investigate the profile changes of the cecum microbes and metabolites. Results showed that both abdominal fat and subcutaneous fat thickness significantly decreased ($P < 0.05$) in response to dietary FCSM supplementation at the age of 21d. The predominant microbe phyla in cecum were *Bacteroidetes* (53.55%), *Firmicutes* (33.75%), and *Proteobacteria* (8.61%). FCSM diet increased the relative abundance of *Bacteroides*, which belong to lean microbial, while decreased the relative abundance of obese microbial including *Fecalibacterium*, *Lachnospiraceae*, *Ruminococcaceae* and *Anaerofilum*. Cecum metabolomics analysis revealed that cecum lipids, organic acids, vitamins, peptides, and nucleic acids were significantly altered by adding FCSM in diet. Correlation analysis showed that abdominal fat and subcutaneous fat thickness were negatively associated with *Bacteroides*, while were positively associated with *Fecalibacterium*, *Lachnospiraceae*, and *Ruminococcaceae*. Moreover, abdominal fat and subcutaneous fat thickness were negatively associated with nicotinic acid, sebacic acid, (R)-pantothenic acid. These findings indicated that FCSM reduced the fat deposition by regulating cecum microbiota and metabolites, which are useful to improve the broilers production through utilization of FCSM.

Key Words: fermented cottonseed meal, fat deposition, cecum microbiota, cecum metabolites, broiler

P107 Effect of varying dietary crude protein concentration on performance and gut health in a subclinical necrotic enteritis challenge model. A. M. Villegas*¹, A. Menconi², A. O. Sokale², J. D. Liu¹, and T. J. Applegate¹, ¹Department of Poultry Science, University of Georgia, Athens, GA, USA, ²Evonik Corp., Kennesaw, GA, USA.



As a result of removing antibiotics, necrotic enteritis (NE) has re-emerged causing important economic losses. Several studies are being conducted to identify different nutritional interventions to help mitigate this challenge. Therefore, the objective of this study was to evaluate the interaction between dietary crude protein concentration and intestinal health in a subclinical NE (sNE) challenge model. Male broiler chicks (1,600 day-old Ross 308) were distributed in 4 different dietary treatments. Treatment groups were assigned to pens using a randomized complete block design and consisted of 2 protein concentrations (but similar formulated Lys, Met, Thr densities) fed to either NE challenged or unchallenged groups. Two protein diets included the high CP (HP) diets (0 to 14d, 24.5%; 15 to 28d; 21.5%; 29 to 39d; 19.5%) and the normal CP (NP) diets (0 to 14d, 22.5%; 15 to 28d; 19.5%; 29 to 39d; 18.5%). The sNE challenge model consisted of one dose of coccidial vaccine (Coccivac B52) at d of age and *Clostridium perfringens* (#CP6, *netB* positive) at d 14, 15, 16 and 17. Performance (BW, feed intake, and feed-to-gain) was recorded on d 0, 14, 28, and 39. Feed intake was conversely influenced by dietary CP on d 14, 28, and 39 ($P < 0.05$), high CP diets, reduced feed intake in all dietary phases. NE lesions and associated mortality was significantly higher in the challenged groups, but not affected by CP concentration. The concentration of *Clostridium perfringens* in ileal and cecal contents at 17 and 28 d of age was not influenced by crude protein concentrations nor challenge conditions in this experiment. Within the groups fed with the HP diets, the villus height: crypt depth ratio in the ileum was lower ($P < 0.05$) in the NE challenged group but was not different between the NP fed challenged and unchallenged birds. The present study shows that dietary CP concentrations did not influence the degree of NE under a mild challenge model. Further research is necessary to evaluate if same trends hold true under more severe challenge conditions.

Key Words: subclinical necrotic enteritis, *Clostridium perfringens*, poultry nutrition, crude protein concentration

P108 Effects of rumen-protected niacin on dry matter intake, milk yield, feed digestibility, and fecal microbiota in early-lactation dairy cows. N. Gaowa, G. Liu, S. Li, Z. Cao, and Y. Wang*, *College of Animal Science and Technology, China Agricultural University, Beijing, China.*

Many researchers have extensively studied rumen-protected niacin (NA) on dairy cows in early-lactation, but the effects of NA on changes in dry matter intake (DMI) and milk yield were conflicting. Besides, the function of NA on feed digestibility and the fecal bacterial community of early-lactation dairy cows is still unknown. Therefore, this study was conducted to investigate the effects of NA on DMI, milk yield, feed digestibility, and fecal bacterial community in early-lactation dairy cows. Twelve

multiparous Holstein dry cows were assigned into 2 dietary groups: (1) control diet (Con; not supplemented with NA, $n = 6$) and (2) supplemented diet (NA1; supplemented with 20 g NA/cow daily, $n = 6$). Experimental stage was from 49 d before expected calving to 21 d postpartum. Our results showed that NA supplement increased DMI and milk yield of cows during the first 3 weeks after calving ($P < 0.05$). Apparent total-tract digestibilities of DM, organic matter, crude protein, neutral detergent fiber, and acid detergent fiber were similar between Con group and NA group at 2 weeks after calving ($P > 0.05$). The 16S rRNA gene sequencing analysis showed that NA had no impact on α and β diversity, the relative abundance of the major phyla and most of the major genera in cow feces at 14 d after calving ($P > 0.05$). Linear discriminant analysis effect size analysis showed that 2 genera of family *Lachnospiraceae* increased and species *Clostridium butyricum* decreased in NA group compared with Con group. Overall, 20 g/d NA supplement to the diet could improve DMI and milk yield in 2 weeks after calving, while it had little influence on feed digestibility and fecal bacterial community.

Key Words: rumen-protected niacin, early-lactation dairy cow, DMI, milk yield, fecal bacteria community

P109 Effect of a protected blend of organic acid + essential oil on growth performance of broiler chickens undergoing several *Eimeria* challenge models. M. Pujol, R. Scott-Delaunay, and E. Santin*, *Jefo Nutrition Inc., Saint Hyacinthe, QC, Canada.*

The aim of this study was to develop a model of intestinal disorder in chickens and to evaluate the effect of a protected blend of organic acid + essential oils [P(OA+EO)] on growth performance of these animals. A total of 1,440 male broiler chickens (Ross 308) were reared in pen-floors, in 8 treatments (30 birds/pen) and 6 replicates for a 35 d trial. Treatments (T) were as follow: T1: unchallenged; T2: challenged-1 (50k) – 50k oocysts/bird; T3: challenged-2 (100k) – 100k oocysts/bird; T4: challenged-3 (200k) – 200k oocysts/bird; T5: T1 and [P(OA+EO) 300 g/t, Jefo]; T6: T2 and [P(OA+EO)-50k]; T7: T3 and [P(OA+EO)-100k]; T8: T4 and [P(OA+EO)-200k]. Oocysts were administered per bird by feed at 14 d in a proportion of 50% *Eimeria acervulina*, 30% *Eimeria maxima* and 20% *Eimeria tenella*. Feed intake, body weight gain (BWG) and feed conversion ratio (FCR) were evaluated. Each pen represented an experimental unit. Data were analyzed using one-way ANOVA, followed by Fisher LSD test. The *Eimeria* challenge impaired performance at 21, 28, and 35 d ($P < 0.05$). Seven d post-challenge, BWG was reduced by 6%, 12% and 16% and FCR worsened by 5%, 9%, and 9% on 50k, 100k and 200k groups, respectively. Fourteen d post-challenge, BWG was reduced by 6%, 10% and 12% and FCR worsened by 8%, 9%, and 9% on 50k, 100k, and 200k groups, respectively. At 21 d post-



challenge, BWG was reduced by 5%, 8%, and 8% and FCR worsened by 5%, 7%, and 6% on 50k, 100k, and 200k groups, respectively. Treatments supplemented with P(OA+EO) allowed a better FCR (3%) and a greater BWG (3%) than the respective non-supplemented treatments at 21 d. In conclusion, performance was affected depending on *Eimeria* oocyst amount administered and the protected blend of organic acids + essential oils improve growth performance of broiler chickens, undergoing different levels of intestinal challenge.

Key Words: broiler, *Eimeria*, challenge, performance

P110 Effect of yeast extract on early intestinal tract development of broilers. R. Raspoet^{*1}, M. T. Brufau², M. Castells-Valero², D. Moral-Anter², A. M. Perez-Vendrell³, E. Auclair¹, B. Vila³, J. Brufau³, R. Ferrer², and R. Martin-Venegas², ¹Phileo by Lesaffre, Marcq-en-Baroeul, France, ²Departament de Bioquímica i Fisiologia, Facultat de Farmàcia i Ciències de l'Alimentació, Institut de Recerca en Nutrició i Seguretat Alimentària (INSA-UB), Universitat de Barcelona, Barcelona, Spain, ³Institut de Recerca i Tecnologia Agroalimentàries (IRTA-Centre Mas de Bover), Constantí, Spain.

Over the past decades, genetic improvements have stimulated broiler production resulting in birds weighing \pm 3kg in 42 d. To reach this genetic potential, the absorption and digestion of nutrients and according morphological and functional development of the small intestine in the early life must be impeccable. Before hatch, uptake of nutrients by the chicken embryo is limited but rapid increase in villus length and formation of intestinal crypts, have been reported close to hatch and during the first 8 to 10 d of life. Considering the rapid development of the small intestine, it was the objective of this study to evaluate the effect of different concentrations of 2 yeast extracts on gut development in early life. Two thousand three hundred and four (2304) day-of-hatch male broiler chicks (Ross308) were divided into 7 treatment groups with either 6 or 7 replicates of 40 birds each. Broilers were fed *ad libitum* from hatching until 14 d of age, a control diet (non-supplemented control) or the control diet supplemented with either yeast extracts A or B at increasing concentrations (125, 250 and 500 g/ton of feed). At d 6, 2 randomly selected birds per replicate were sacrificed to investigate gut morphology in the duodenum, jejunum and ileum. Cryostatic slides were prepared and treated with periodic acid-Schiff to stain mucus glycoproteins. Villus length and number of goblet cells were measured in micrographs of known magnification. Statistical analysis was performed by Student's *t*-test comparing the mean values of the treatments with the non-supplemented control. In the duodenum, supplementation of both yeast extracts at 125 and 250 g/ton, resulted in an increased villus length. Additionally, supplementation of yeast extract A at 250 g/

ton was also able to increase villus length in the jejunum. No difference on either crypt depth or villus/crypt ratio could be found. Goblet cell counts were increased with supplementation of both yeast extracts at 250 and 500 g/ton in both duodenum and ileum. It can be concluded that the inclusion of yeast extracts in the diet have beneficial effects on the gut development by increasing the absorptive area and mucus layer.

Key Words: early gut development, gut morphology, yeast extract

P111 Comparative analysis of microbiota from commercial broiler farms of two integration companies using AGP or chestnut/quebracho polyphenol extracts. J. Diaz-Carrasco^{1,2}, L. Redondo^{*1}, N. Casanova¹, and M. Fernandez-Miyakawa^{1,2}, ¹Instituto de Patobiología Veterinaria, CICVyA – INTA, Buenos Aires, Argentina, ²CONICET, Buenos Aires, Argentina.

The importance of the microbiota in the gastrointestinal tract of animals is recognized to have an important role in host health and productivity. In this study, we sought to assess and characterize the microbiota in the feces of poultry chickens from several farms of 2 different integrators in the same geographical region of South America. These companies were changing AGP program to an AGP-free production with the use of a commercial mix of polyphenols of Quebracho/chestnut extracts (Silvateam Nutri P at 1 kg/ton from d 0 until end). Cecal contents were obtained at 21/40 d from chickens reared in ~30 commercial farms. Productive parameters and health status was monitored during the entire evaluation. Samplings were carried out in 5 productive cycles with a follow-up over a full year. DNA was extracted and the V3–V4 region of the 16S rRNA gene was amplified. HTS was performed in the Illumina MiSeq platform. Bioinformatics analysis was done with QIIME2. A significant variation in the number of observed species (OS) and phylogenetic diversity (PD) was found between both companies by Kruskal-Wallis statistical analysis of α diversity parameters ($P < 0.001$). Both metrics showed the highest diversity in summer and the lowest values in winter. Principal coordinate analysis based on unweighted UniFrac distances revealed that the samples corresponding to each season of the year tended to form groups, indicating a significant variation in microbiota profile of farms grouped in each integrator company (PERMANOVA, $P < 0.05$). The effect of “integration” was much more pronounced compared with that of additives or seasonality, which were consistent between cycles. In all farms, the use of Silvateam Nutri P showed an improvement of productive parameters with a concomitant stabilization of *Firmicutes/Bacteroidetes* ratio, number of species, and α diversity. This study highlights that microbiota is heavily conditioned by the integration company and should be



considered at the time of testing strategies of microbiota modulation in commercial poultry farms

P112 Effect of a blend of protected organic acids + essential oils on growth performance, nutrient digestibility, and intestinal health of broiler chickens undergoing an intestinal challenge. M. de Souza Vieira^{*1}, M. L. Moraes¹, J. C. Bodin¹, E. Santin¹, C. B. Adam², and C. Stefanello², ¹*Jefo Nutrition Inc., Saint Hyacinthe, QC, Canada*, ²*Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil*.

We investigated the dietary supplementation of protected organic acids + essential oils [P(OA+EO)] in challenged broilers. A total of 1,080 Cobb × Cobb 500 male chickens were reared in pen-floors in a climate experimental poultry house and allocated in a completely randomized design of 4 treatments and 10 replicates (27 birds/pen) for a 42 d trial. Treatments (T): unchallenged control (UC), challenged control (CC), antibiotic growth promoter (AGP, Enramycin at 10 ppm) and, P(OA+EO) at 300 g/t (Jefo). Except those on the UC group, all birds were challenged with *Eimeria* spp. at 1 d and *Clostridium perfringens* at 11, 12 and 13 d. Growth performance was evaluated weekly. At 17 d, intestinal permeability was evaluated by the FITC-d test. Intestinal alteration (I See Inside Scoring System methodology-ISI), mucin2, claudin1 and occludin jejunal mRNA expression and, ileal digestibility were evaluated at 21 d. From 1 to 42 d, birds on P(OA+EO) had respectively 6.5 and 4.7% better ($P < 0.001$) body weight gain (BWG) and feed conversion ratio compared than CC, while there was no significant difference to the UC group. For the same period, birds on P(OA+EO) had 2.9% even better BWG ($P < 0.001$) than birds on AGP. The birds on P(OA+EO), compared with CC ($P < 0.05$), had greater digestibility of dry matter (3.3%) and energy (110 kcal) and no difference compared with the AGP group. P(OA+EO) improved intestinal integrity by reducing intestinal permeability ($P < 0.001$) and increasing mucin2, claudin1 and occludin gene expressions ($P < 0.05$) compared with CC group. Birds on P(OA+EO) had reduced *Eimeria* lesions in the duodenum and lower inflammatory cell infiltration on epithelium and presence of oocysts in the ileum ($P < 0.05$), which resulted in the lowest total ISI score ($P < 0.05$) compared with the other treatments. In conclusion, the blend of protected organic acids + essential oils had a better or similar result to AGP in improving growth performance, nutrient digestibility, and intestinal health of broilers undergoing an intestinal challenge.

Key Words: broilers, essential oils, digestibility, gut health

P113 Effect of feeding glycerol esters of butyric and valeric acid on broiler performance. S. Vaessen¹, J. M.

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Feeding organic acids, particularly short-chain, have been demonstrated to improve intestinal health and efficiency, for instance by improving barrier function and nutrient absorption and moderating cytokine production and inflammation. An experiment was conducted to evaluate the effect of different organic acids [ProPhorce SR 130: glycerol esters of butyric acid (BA) and ProPhorce Valerins: glycerol esters of valeric acid (VA)] added to the feed on broiler performance. A total of 1,056 one-day-old Ross 308 male broilers were placed in 48 floor pens, with 22 broilers/pen. There were 3 experimental treatments (16 pens/treatment), T1: Control; T2: BA at 500 g/MT from 0 to 28d and 250 g/MT from 28 to 42d; and T3: BA at 500 g/MT from 0 to 14d, VA at 1,500 g/MT from 14 to 28d and BA at 250 g/MT from 28 to 42d. Feed intake and BW were recorded at 14, 28 and 42d and ADG and FCR was calculated. Data were analyzed as a randomized complete block design with treatment as main effect. From 0 to 14d, broilers receiving BA at 500 g/MT (T2 and T3) were heavier (T1 = 376.4^b, T2 = 396.6^a, T3 = 396.7^a g; $P = 0.0382$) and exhibited increased growth (T1 = 23.7^b, T2 = 25.1^a, T3 = 25.1^a g/d; $P = 0.0396$) than controls. From 14 to 28d, broilers receiving VA at 1,500 g/MT (T3) were heavier (T1 = 1327^b, T2 = 1362^b, T3 = 1414^a g; $P = 0.0013$) and exhibited increased growth (T1 = 67.9^b, T2 = 69.0^b, T3 = 72.8^a g/d; $P = 0.0003$) than T1 and T2. No significant differences ($P > 0.05$) between treatments were observed in performance at 28–42d. For the overall study (0–42d), the inclusion of VA during the grower period (T3) resulted in highest BW (T1 = 2,829^b, T2 = 2,884^{ab}, T3 = 2,951^a g; $P = 0.0078$) and growth (T1 = 66.3^b, T2 = 67.6^{ab}, T3 = 69.2^a g/d; $P = 0.0079$) and lowest FCR (T1 = 1.58^a, T2 = 1.54^{ab}, T3 = 1.53^b g/g; $P = 0.065$), with T2 (BA throughout) being intermediate. In conclusion, the supplementation of broiler diets with glycerol esters of butyric acid improved performance in young broilers. Moreover, the inclusion of glycerol esters of valeric acid during the grower phase improved BW, ADG, and FCR in broilers.

Key Words: glycerol esters of butyric acid, glycerol esters of valeric acid, performance, broiler

P114 Holofood: A holo'omic solution towards sustainable animal food production. J. Tarradas^{*1}, S. Marcos², D. Sandvang³, M. Limborg⁴, J. Zentek⁵, D. Jozefiak⁶, E. Johansen⁴, A. Estonba², N. Tous¹, E. Esteve-Garcia¹, A. Alberdi², and M. T. P. Gilbert⁴, ¹*Institute for Food and Agricultural Research and Technology (IRTA), Constanti, Spain*, ²*Department of Genetics, Physical Anthropology and Animal Physiology, University of the Basque Country (UPV/EHU), Leioa, Spain*, ³*Chr Hansen*



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With the planet's population approaching 9 billion, one of the key global challenges of this century is to secure that the growing food production is performed in a sustainable fashion and with a low-carbon signature. It is well known that gut microorganisms play a pivotal role in the homeostasis of animals, and a balanced gut microbiota is essential for an optimal food production. The gut microbiomes can be manipulated through the use of feed additives such as prebiotics and probiotics. However, the specific means of action of most additives on the microbiome and host organisms is not acknowledged. HOLOFOOD (<https://www.holofood.eu/>) is a European H2020 Innovation Action comprising 10 partners from 6 European countries that showcases a holistic approach to improve the efficiency of food production systems by deciphering the biochemical interactions between animals and their associated microorganisms. HoloFood will be running until 2022. Newly developed holo'omic framework will be implemented to understand the biochemical interactions between broiler chickens and their intestinal microorganisms through the analysis of whole-animal genomes, deep intestinal transcriptomes, microbial metagenomes, microbial metatranscriptomes and intestinal metabolomes, all of them in relation to key performance indices. A total of 1300 chickens will be biochemically, physiologically and phenotypically characterized through the analysis of over 15000 samples. The knowledge generated will be used to optimize the feed additive administration strategies of already implemented products, by tailoring them to the genetic background and developmental stage of the animals as well as production environment. The ultimate goal is to improve the quantity, quality, and safety of the produced food, as well as sustainability of food production and increased animal welfare. HoloFood will also serve to raise awareness about the importance of microbiomes in food production, and to establish bridges between companies and academia to foster science-based strategies.

Key Words: holistic framework, hologenomics, microbiome, probiotic, prebiotic

P115 Biomarkers to evaluate gut integrity in different models to induce intestinal inflammation in broiler chickens. G. Tellez-Isaias*, C. N. Voung, B. D. Graham, C. A. M. Selby, T. L. de Barros, and B. M. Hargis, *University of Arkansas, Fayetteville, AR, USA.*

Enteric inflammation models can help researchers' study methods to improve health and performance as well as evaluate various alternative compounds and dietary formulations targeted to improve performance in poultry. Our laboratory has developed several models to induce intestinal inflammation, including the use of high non-starch polysaccharide diets; dexamethasone; dextran sodium sulfate; feed restriction; and heat stress. In all those models, a significant increase in gut permeability can be easily measured by a non-terminal method such as serum concentration of fluorescein isothiocyanate- dextran (FITC-d; 3–5 kDa) 1 h after FITC-d oral administration or by quantification of bacterial translocation into the. We have incorporated other reliable serum biomarkers in our models such as antioxidant biomarkers (nitric oxide activity, superoxide dismutase activity, thiobarbituric acid reactive substances and total antioxidant capacity); enterocyte biomarkers (enterocellular signal-regulated kinase, citrulline, and MUC2); and immune biomarkers (interferon gamma and total/ specific secretory IgA). In this presentation, we discuss these biomarkers under different models to induce intestinal inflammation in broiler chickens.

Key Words: biomarkers, gut integrity, gut inflammation, poultry

P116 Analysis of rumen fermentation and microbial composition of cattle, dzo, and yak under grazing conditions. C. Zhao¹, Y. Li¹, F. Zhang¹, J. Yao¹, and Y. Cao^{*1,2}, ¹Northwest A&F University, Yangling, Shaanxi, China, ²Harvard Medical School, Boston, MA, USA.

The current study was investigated the rumen fermentation and microflora among cattle, dzo, and yak in Qinghai plateau. Each group contains 10 replicates. The results showed the total VFA concentration in cattle was significantly higher compared with dzo ($P < 0.05$). The ratios of acetic was significantly lower in yak and dzo in compared with cattle ($P < 0.05$), while the ratio of propionic and acetic-propionic ratio showed the opposite results. Yak had the strongest fiber degradation performance than the other groups, which was reflected in the activity of carboxymethylcellulose in rumen of yak was significantly higher than that of cattle ($P < 0.05$), and the activity of avicelase was significantly higher than that of cattle and dzo ($P < 0.01$). As for microflora, the rumen bacterial community diversity (ACE) of cattle and dzo were significantly higher than yak, and rumen fungi diversity (Chao1, ACE) and richness (Shannon, Simpson) in cattle were extremely significantly lower in yak and dzo ($P < 0.01$). By PCoA and ANOSIM analysis, the bacteria, fungi, protozoa and archaea community were quite distinct from each other among 3 groups, except for 2 special cases. The protozoa community in the cattle and dzo and the archaea structure between dzo and yak did not



cluster differently. It is also worth noting that for bacterial the genetic distance of cattle and dzo was closer and the fungi community in dzo was more similar with yak. It could be cautiously inferred that as a dominant species in the alpine plateau, yak has better adaptability than cattle and dzo, and the rumen microbial composition also might have undergone adaptive changes.

Key Words: ruminant, fermentation, microbiome, enzyme activities

P117 Changes in growth and motility of *Campylobacter jejuni* in response to serotonin. J. Lyte*¹, S. Shrestha¹, M. Lyte², and A. Donoghue¹, ¹USDA-ARS Poultry Production and Product Safety Unit, Fayetteville, AR, USA, ²Iowa State University, Ames, IA, USA.

Campylobacter is a leading cause of foodborne illness and strongly linked with the consumption of contaminated poultry products. Neuroendocrine crosstalk between host and microbe may play a critical role in enteric colonization/infection. We sought to identify how serotonin, a neurotransmitter synthesized in the intestinal tract, may affect *C. jejuni* growth and motility. Wild-type *C. jejuni* strains isolated from broiler chickens were inoculated into

a defined medium (CO₂-independent) with or without serotonin up to 48 h at 42°C in aerobic atmosphere. Bacterial growth was determined by culturing on Campylobacter-Line-Agar plates in microaerophilic atmosphere. Wild-type strain S-8 was selected to assess a dose-response relationship between bacterial inoculum size and serotonin concentration. To determine a dose-response effect of serotonin (0.0005, 0.005, 0.05 M) on *C. jejuni* motility, mid-log cultures of S-8 were stab-inoculated in the center of motility medium Petri plates that did or did not contain serotonin. Inoculated motility plates were incubated at 42°C for 48 h in a microaerophilic environment, the zone of motility was then measured. Examination of dose-response effect of serotonin on growth demonstrated that *C. jejuni* growth increased in response to the lowest but strongly inhibited at the highest concentration. Despite effects on growth, serotonin did not affect motility at any concentration. Data demonstrate that the neurotransmitter serotonin can influence *C. jejuni* growth, but not motility, in a dose-dependent manner. As serotonin production increases in the gut during times of stress, it is important to understand how changing concentrations of serotonin may alter growth and motility of *C. jejuni*.



NOTES



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