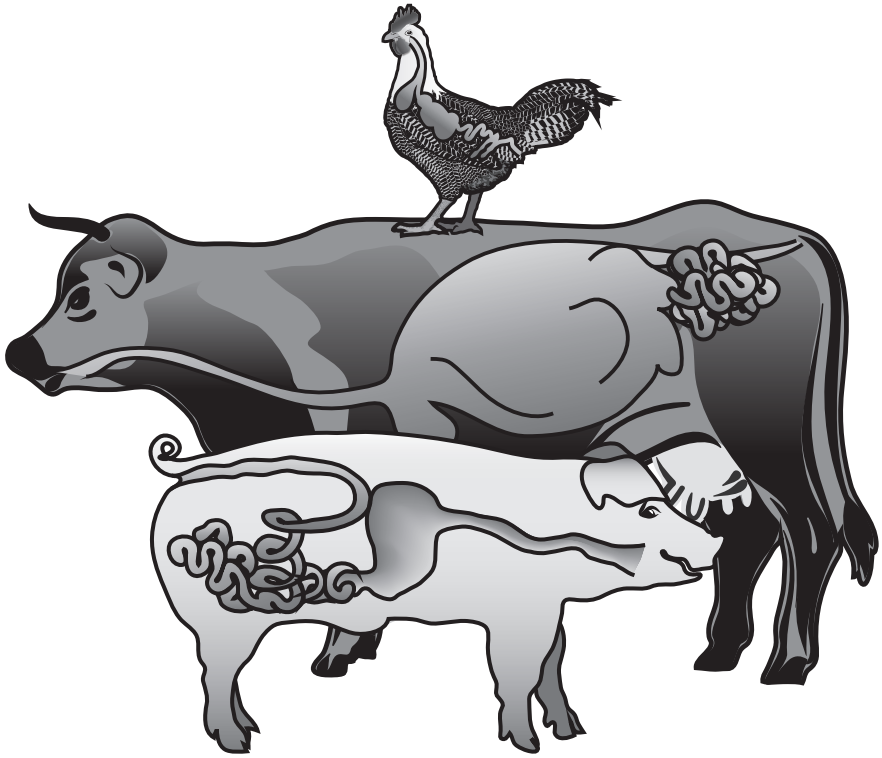
Symposium on Gut Health

in Production of Food Animals

November 5–7, 2018, St. Louis, Missouri



Program and Abstracts

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WELCOME

On behalf of the Organizing Committee for the 7th Symposium on Gut Health in Production of Food Animals, I welcome you back to St. Louis, Missouri! After a very successful 6th Symposium here in 2017, we decided a return trip was in order. I look forward to another scientifically and socially rewarding meeting in 2018.

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

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

Likewise, I encourage all of you to please take advantage of the informal nature of the symposium—it was planned this way to encourage interaction between scientists. I again ask that senior researchers make a special effort to engage with the graduate students who are attending and presenting. Remember 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

Program

Sunday, November 4

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

Monday, November 5

7:00 am – 8:00 am Breakfast
Sponsored by King Techina

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

SESSION 1

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

8:00 am – 9:00 am Microbial ecology of the gastrointestinal tract, mammary glands, and the reproductive tract of dairy cattle. (Abstract 100)
E. Khafipour[*1], J. Guo[1], Z. Zhang[2], K. Fehr[1], S. Sepehri[3], H. Derakhshani[1], and J. Plazier[1], [1]University of Manitoba, Winnipeg, MB, Canada, [2]University of Alberta, Edmonton, AB, Canada, [3]Children Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.*

9:00 am – 9:30 am Experimental colonization of poults with *Campylobacter jejuni* and selective media for culturing. (Abstract 101)
M. Sylte[*1], T. Looft[1], M. Inbody[1], E. Meyer[1,3], T. Johnson[1,2], Z. Wu[3], Q. Zhang[3], and E. Line[4], [1]USDA ARS National Animal Disease Center, Ames, IA, USA, [2]Purdue University, West Lafayette, IN, USA, [3]Iowa State University, Ames, IA, USA, [4]USDA ARS US National Poultry Research Center, Athens, GA, USA.*

9:30 am – 10:00 am New statistical method identifies cytokines that distinguish stool microbiomes. (Abstract 102)
B. Shannon[*1,2], [1]BioRankings, St. Louis, MO, USA, [2]Washington University School of Medicine, St Louis, MO, USA.*

10:00 am – 10:30 am Coffee Break: Grand Foyer
Sponsored by Silvateam

10:30 am – 10:45 am Effects of delayed feeding post-hatch on microbiome and expression of immune response genes in broiler chickens. (Abstract 103)
M. Proszkowiec-Weglarz, L. Schreier, K. Miska, S. Kahl, B. Russell, and T. Elsasser, USDA-ARS, NEA, ABBL, Beltsville, MD, USA.*

10:45 am – 11:00 am Effect of single dose of parenteral antimicrobials administration at birth on developmental dynamics of fecal microbiota and prevalence of selected antimicrobial resistance genes in piglets. (Abstract 104)
M. Zeineldin[*1,2], A. Megahed[1,2], B. Blair[1], B. Aldridge[1], and J. Lowe[1], [1]Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]Department of Animal Medicine, College of Veterinary Medicine, Benha University, Benha, Egypt.*

11:00 am – 11:15 am Characterization and understanding of early-life fecal resistome in dairy cattle. (Abstract 105)
J. Liu and D. Mills, University of California, Davis, Davis, CA, USA.*

11:15 am – 11:30 am Seasonality as a driver of cecal and litter microbiota variation in commercial broiler chickens. (Abstract 127)
J. M. Díaz Carrasco[*1,2], *E. A. Redondo*[1,2], *L. M. Redondo*[1,2], *and M. E. Fernández Miyakawa*[1,2], [1]*Instituto de Patobiología Veterinaria, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina,* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.*

11:30 am – 12:00 pm Evaluation of the antimicrobial activity of Activo liquid in porcine fecal cultures. (Abstract 106)
R. C. Anderson[*1], *S. R. Goodall*[2], *R. Cabrera*[3], *K. J. Genovese*[1], *H. He*[1], *M. E. Hume*[1], *R. C. Beier*[1], *R. B. Harvey*[1], *and D. J. Nisbet*[1], [1]*USDA/ARS, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX, USA,* [2]*Independent Consultant, Erie, CO, USA,* [3]*EW Nutrition USA, Des Moines, IA, USA.*

12:00 pm – 1:00 pm Lunch (Provided): Arch View Ballroom
Sponsored by Arm & Hammer

1:00 pm – 3:00 pm Poster Session: Grand Foyer

SESSION 2

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

3:00 pm – 4:00 pm Microbial endocrinology as an evolutionary-based language between host and microbiota influencing gut health. (Abstract 107)
M. Lyte, Iowa State University, Ames, IA, USA.*

4:00 pm – 4:30 pm Gene expression of molecules involved in nutrient processing and transport in fast- and slow-growing broilers. (Abstract 108)
K. B. Miska and R. H. Fetterer, USDA/ARS, Beltsville, MD, USA.*

4:30 pm – 4:45 pm Using a synbiotic to prime the broiler intestine for an enteric challenge. (Abstract 109)
S. Curry, C. Pender, and G. R. Murugesan, Biomin America Inc., Overland Park, KS, USA.*

6:00 pm – 8:00 pm Reception: Arch View Ballroom
Sponsored by Elanco

Tuesday, November 6

7:00 am – 8:00 am Breakfast: Arch View Ballroom
7:00 am – 5:00 pm Registration: Grand Foyer

SESSION 3

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

8:00 am – 9:00 am I See Inside: Precisely defining gut health. (Abstract 110)
E. Santin and A. Sanches, University Federal of Paraná, Curitiba, PR, Brazil.*

9:00 am – 9:15 am Characterizing the mycobiome in piglets during the weaning transition. (Abstract 111)
K. Summers, T. Ramsay, J. F. Frey, and A. Arfken, USDA, Beltsville, MD, USA.*

9:15 am – 9:30 am Cecal microbiome and short-chain fatty acids in Shaver White chickens fed *Bacillus subtilis* DSM29784 during grower, developer and laying phases. (Abstract 112)
M. Neijat[*1], J. Habtewold[1], R. B. Shirley[2], A. Welsher[2], J. Barton[2], P. Thiery[3], and E. Kiarie[1], [1]University of Guelph, Guelph, ON, Canada, [2]Adisseo USA Inc, Alpharetta, GA, USA, [3]Adisseo France, SAS, Antony, France.*

9:30 am – 10:30 am Coffee Break: Grand Foyer
Sponsored by Chr. Hansen

10:30 am – 11:00 am Transcriptome analysis of rumen epithelium and rumen microbial community in four-month-old calves with feed-induced acidosis. (Abstract 113)
W. Li[*1], S. Arnold[2], A. Edwards[3], and C. Riehle[4], [1]USDA Dairy Forage Research Center, Madison, WI, USA, [2]Department of Dairy Science, University of Wisconsin, Madison, WI, USA, [3]Department of Biology, University of Wisconsin-Madison, Madison, WI, USA, [4]Department of Genetics, University of Wisconsin-Madison, Madison, WI, USA, [5]Select Veal Feeds Inc., Harleysville, PA, USA.*

11:00 am – 11:30 am Virulence gene acquisition trends in avian pathogenic *Escherichia coli* under commercial conditions. (Abstract 114)
J. D. Gruber, D. Kroon, J. Walker, M. Perry, and E. Kim, DuPont Industrial Biosciences, DuPont Animal Nutrition, Wilmington, DE, USA.*

11:30 am – 12:00 pm Use of Alquernat Zycox as an effective manner to control induced coccidiosis in poultry. (Abstract 115)
E. H. Chowdhury[3], C. Domenech[4], M. T. Islam[1], J. Pié[4], and M. W. Rahman[*2], [1]Department of Medicine, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, [2]Department of Livestock Services, Dhaka, Bangladesh, [3]Department of Pathology, BAU, Mymensingh, Bangladesh, [4]Biovet S.A, Constantí, Tarragona, Spain.*

12:00 pm – 1:00 pm Lunch (Provided): Arch View Ballroom.

1:00 pm – 2:00 pm Poster Session: Grand Foyer

SESSION 4

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

2:00 pm – 3:00 pm Role of the gut microbiome on outcome following viral respiratory infections in nursery pigs. (Abstract 116)
M. Niederwerder, Kansas State University, Manhattan, KS, USA.*

3:00 pm – 3:15 pm Systemic and intestinal immunomodulatory effects of a *Bacillus* probiotic fed to turkeys reared in a commercial facility. (Abstract 117)
E. Davis, J. Christianson, E. Hutchinson, B. Wujek, T. Lavergne, T. Rehberger, and D. Karunakaran, Arm & Hammer Animal Production, Waukesha, WI, USA.*

3:15 pm – 3:30 pm Using probiotic performance assays and comparative genome analysis on *Lactobacillus johnsonii* strains to discover effective probiotics for use in commercial turkeys. (Abstract 118)
A. Johnson, B. Weber, and T. J. Johnson, University of Minnesota, Saint Paul, MN, USA.*

3:30 pm – 4:15 pm Coffee Break: Grand Foyer
Sponsored by Chr. Hansen

4:15 pm – 4:30 pm A post-biotic feed additive shows anti-inflammatory effects on immunometabolic signaling in broiler intestinal tissues. (Abstract 119)
B. Aylward[*1], C. Johnson[1], M. Kogut[2], S. Kazemi[3], and R. Arsenault[1], [1]Department of Animal and Food Sciences, University of Delaware, Newark, DE, USA, [2]USDA, Southern Plains Agricultural Research Center, College Station, TX, USA, [3]Pure Cultures, Denver, CO, USA.*

4:30 pm – 4:45 pm Quantification of the blood volume and pattern of organ permeability in the heat stressed pig. (Abstract 120)
W. Zhao[1], F. R. Dunshea[*1], Z. Zhang[1], J. B. Furness[1], M. T. Ringuet[1], K. DiGiacomo[1], B. J. Leury[1], E. Roura[3], G. Wijffels[2], D. Renaudeau[4], N. K. Gabler[5], and J. J. Cottrell[1], [1]The University of Melbourne, Parkville, VIC, Australia, [2]CSIRO, St. Lucia, QLD, Australia, [3]The University of Queensland, St. Lucia, QLD, Australia, [4]INRA, St-Gilles, France, [5]Iowa State University, Ames, IA, USA.*

4:45 pm – 5:00 pm Histological modulations of broiler gut under chronic heat stress with and without distillery yeast. (Abstract 121)
G. Abbas, H. Zaneb, S. Ashraf, S. Masood, I. Ahmad, M. M. Usman, H. F. Rehman, and H. Ur Rehman, UVAS, Lahore, Punjab, Pakistan.*

6:00 pm – 8: 30 pm Reception: Arch View Ballroom

Wednesday, November 7

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

7:00 am – 12:00 pm Registration: Grand Foyer

SESSION 5

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

9:00 am – 9:30 am Avian intestinal mucus modulates *Campylobacter jejuni* gene expression in a host-specific manner. (Abstract 122)
T. Looft, M. Sylte, and T. Casey, NADC-ARS-USDA, Ames, IA, USA.*

9:30 am – 10:00 am β-Mannanase supplementation reduced signs of intestinal inflammation in broilers. (Abstract 123)
M. A. Martinez-Cummer[*1], K. Poulsen[2], K. Baker[1], T. Kwiatkowski[3], M. H. Rostagno[1], and J. L. Snow[1], [1]Elanco Animal Health USA, Greenfield, IN, USA, [2]Elanco Animal Health Belgium, Antwerp, Belgium, [3]Elanco Animal Health Poland, Warsaw, Poland.*

10:00 am – 10:45 am Coffee Break: Grand Foyer

10:45 am – 11:00 am Maternal supply of methionine during late-pregnancy in Holstein dairy cows alters the fecal microbiome and metabolome in neonatal heifer calves during the preweaning period. (Abstract 124)
A. Elolimy[*1], M. Zeineldin[2], A. Alharthi[1], F. Batistel[1], C. Parys[3], and J. Loor[1,4], [1]Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL, USA, [2]Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA, [3]Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, [4]Division of Nutritional Sciences, Illinois Informatics Institute, University of Illinois, Urbana, IL, USA.*

11:00 am – 11:15 am Towards the replacement of antibiotics growth promoters in chicken: Meta-analysis approach. (Abstract 125)
A. Rouissi[*1], M. Boulianne[2], F. Guay[1], and M. P. Létourneau Montminy[1], [1]Animal Science Department, Laval University, Québec City, QC, Canada, [2]Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.*

11:15 am – 11:30 am Effect of PrimaLac on necrotic enteritis lesion scores and expression of tight junction proteins. (Abstract 126)
N. Emami[*1], A. Calik[1], M. White[1], M. Young[2], and R. Dalloul[1], [1]Virginia Tech, Blacksburg, VA, USA, [2]Star Labs/Forage Research Inc, Clarksdale, MO, USA.*

Poster Presentations

P100 Quinolone-resistant *Escherichia coli* in medically untreated dairy calves—How did they get there?
S. Finstad[*1], *H. Kaspersen*[2], *and A. Bjelland*[1], [1]*Faculty of Veterinary Medicine, Norwegian University of Life Science, Oslo, Norway,* [2]*Norwegian Veterinary Institute, Oslo, Norway.*

P101 Early colonizing microbiota of turkey poults.
J. Rehberger, R. Geier, E. Hutchison, S. Anderson, R. Wujek, E. Vang, and A. H. Smith, Church & Dwight, Waukesha, WI, USA.*

P102 Impact of probiotic supplementation on gastrointestinal functionality in young piglets: A meta-analysis and meta-regression.
A. A. Séon Simon[*1], *H. M. Golder*[2], *V. Verlhac-Trichet*[1], *F. Fru*[1], *I. J. Lean*[2], *and P. Celi*[3], [1]*DSM Nutritional Products France, Research Center for Animal Nutrition & Health, Saint Louis, France,* [2]*Scibus, Camden, Australia,* [3]*DSM Nutritional Products, Animal Nutrition and Health, Columbia, MD, USA.*

P103 Effects of probiotic interventions on chicken gastrointestinal functionality: A meta-analysis and meta-regression.
A. A. Séon Simon[*1], *H. M. Golder*[2], *M. B. De Ondarza*[3], *I. J. Lean*[2], *and P. Celi*[4], [1]*DSM Nutritional Products France, Research Center for Animal Nutrition & Health, Saint Louis, France,* [2]*Scibus, Camden, Australia,* [3]*Paradox Nutrition, West Chazy, NY, USA,* [4]*DSM Nutritional Products, Animal Nutrition & Health, Columbia, MD, USA.*

P104 Establishment of 3-dimensional organoids from chicken cecal crypts.
D. Zhao[*1], *M. Kogut*[2], *K. Genovese*[2], *L. A. Davidson*[3], *R. S. Chapkin*[3], *and Y. Farnell*[1], [1]*Department of Poultry Science, Texas A&M AgriLife Research, Texas A&M University, College Station, TX, USA,* [2]*Southern Plains Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, College Station, TX, USA,* [3]*Department of Nutrition and Food Science, Texas A&M AgriLife Research, Texas A&M University, College Station, TX, USA.*

P105 *Clostridium perfringens* enterotoxin induces chicken necrotic enteritis.
M. Abraha, M. Bansal, B. Al-Rubaye, A. Almansour, H. Wang, J. D. Latorre Cardenas, B. Hargis, and X. Sun, University of Arkansas, Fayetteville, AR, USA.*

P106 SPF-Anaerobe microbiota mediates *Campylobacter jejuni* clones and campylobacterosis in broiler chickens.
A. Almansour, B. Alrubaye, M. Bansal, M. Abraha, X. Sun, H. Wang, and B. Hargis, university of Arkansas, Fayetteville, AR, USA.*

P107 Identification and functional characterization of putative colonization factors of *Lactobacillus gallinarum* in poultry.
T. Duong, T. E. Askelson, T. J. Broderick, L. E. Froebel, L. K. Froebel, and M. L. Nash, Department of Poultry Science, Texas A&M University, College Station, TX, USA.*

P108 Review of immunomodulatory additives that are susceptible to being used as feed additives: Mode of action and identification of end-points for efficacy assessment.
J. Tarradas, N. Tous, E. Esteve, and J. Brufau, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Constantí, Spain.*

P109 Influence of probiotic metabolites on microbial diversity of the cecal microbiome in broiler chickens under heat stress conditions.
S. Yang[*1], *M. Roberts*[2], *J. McNaughton*[2], *M. Canady*[1], *K. Schuster*[1], *A. Blaszczak*[1], *and E. Wozniak*[1], [1]*Cytozyme Laboratories Inc., Salt Lake City, UT, USA,* [2]*AHPharma Inc., Hebron, MD, USA.*

P110 Evaluating the diverse immunological effects of chestnut tannins in broilers.
A. Lee[*1], *G. Cardoso dal Pont*[1], *M. Fernandez-Miyakawa*[3], *M. Battaglia*[4], *and M. Kogut*[2], [1]*Texas A&M University, College Station, TX, USA,* [2]*United States Department of Agriculture-ARS (SPARC), College Station, TX, USA,* [3]*Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina,* [4]*Silvateam/ Indunor S.A, Buenos Aires, Argentina.*

P111 Effect of dietary protein source and litter condition on immune response and mitotic cell activity in the duodenum of broiler chickens at 21 days of age.
A. J. Keel, A. J. Calderon, O. J. Tejeda, J. D. Starkey, and C. W. Starkey, Auburn University, Auburn, AL, USA.*

P112 Survey of *Clostridium* populations in dairy cattle feed samples across the United States.
T. L. March, J. S. Thompson, R. F. Teal, A. H. Smith, and T. G. Rehberger, Arm and Hammer Animal Nutrition, Waukesha, WI, USA.*

P113 The proliferation of *Clostridium* species in total mixed ration after 24-hour heat challenge.
V. G. Bretl, J. S. Thompson, T. L. March, A. H. Smith, and T. G. Rehberger, Arm & Hammer Animal Nutrition, Waukesha, WI, USA.*

P114 Nucleotide-mediated SPDEF modulates TFF3-mediated wound healing and intestinal barrier function during the weaning process.
H. I. Jung, S. I. Lee, and I. H. Kim, Dankook University, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

P115 Difructose dianhydride improves intestinal calcium absorption, wound healing, and barrier function.
H. I. Jung, S. I. Lee, and I. H. Kim, Dankook University, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

P116 Citrus flavonoid supplementation in weanling diets improves pig gut health.
D. Solà-Oriol[*1], *M. Paniagua*[3], *M. Saladrigas*[1], *F. J. Crespo*[2], *M. Serra*[2], *and J. F. Pérez*[1], [1]*Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [2]*Interquim S.A. (Ferrer HealthTech), Animal & Health Nutrition Division, Barcelona, Spain,* [3]*Quimidroga, Feed Department, Barcelona, Spain.*

Oral Abstracts
Session 1

100 Microbial ecology of the gastrointestinal tract, mammary glands, and the reproductive tract of dairy cattle. E. Khafipour*[1], J. Guo[1], Z. Zhang[2], K. Fehr[1], S. Sepehri[3], H. Derakhshani[1], and J. Plazier[1], [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*University of Alberta, Edmonton, AB, Canada,* [3]*Children Hospital Research Institute of Manitoba, Winnipeg, MB, Canada.*

The crucial role of rumen and hindgut microbiomes in intestinal and extra-intestinal diseases is emerging. Rumen and hindgut microbiomes have been shown to affect cow physiology, metabolism, and immune function and to confer direct and indirect (immune-mediated) resistance against enteric pathogens. Dysbiosis of the rumen and hindgut microbiomes affects the profile of microbially driven metabolites and compounds produced by the microbiota. These molecules influence the metabolic and immunological capacities of the host both within and outside of the digestive tract retroactively influencing the microbiomes of other body sites, such as the vaginal tract and the mammary glands. The shifts in the diversity and functionality of vaginal tract and mammary glands microbiomes in one hand can result in initiation or progression of infectious or inflammatory diseases in those systems; for example, mastitis, while on the hand can negatively affect the succession of gut microbiome in offspring hence increase their susceptibility to infectious diseases in early life. In this presentation, we will review the role of rumen and hindgut microbiomes in the context of their association with the vaginal and udder microbiomes and discuss available solutions that can improve gut microbiome and health in high-yielding dairy cows without compromising their productivity. We will also highlight the pressing need for development of synthetic microbial communities to improve gut, udder and vaginal health.

Key Words: rumen and hindgut microbiomes, mammary gland microbiome, vaginal tract microbiome

101 Experimental colonization of poults with *Campylobacter jejuni* and selective media for culturing. M. Sylte*[1], T. Looft[1], M. Inbody[1], E. Meyer[1,3], T. Johnson[1,2], Z. Wu[3], Q. Zhang[3], and E. Line[4], [1]*USDA ARS National Animal Disease Center, Ames, IA, USA,* [2]*Purdue University, West Lafayette, IN, USA,* [3]*Iowa State University, Ames, IA, USA,* [4]*USDA ARS US National Poultry Research Center, Athens, GA, USA.*

Consumption of contaminated poultry products is the main source of human campylobacteriosis, caused mainly by *Campylobacter jejuni*. Chickens, but not turkeys, have been experimentally colonized with different isolates of *C. jejuni*, and enumeration from intestinal samples can be challenging because routine *Campylobacter* selective media (Modified charcoal cefoperazone deoxycholate, Karmali or Campy cefex) support the growth of non-*Campylobacter* organisms. We sought to identify a) *C. jejuni* isolates that persistently colonize poults, and b) selective media to enumerate their abundance intestinal samples. For ease of isolation, mutants of *C. jejuni* strain NCTC 11168 were constructed resistant to chloramphenicol (CjCm) or kanamycin (CjK). Three-week-old poults were orally colonized with either CjCm or CjK or mock-colonized, and were euthanized up to 14 d post-colonization. Immunohistochemistry detected *Campylobacter* antigen in the cecum between the villi, and host-response was evaluated by qPCR on cecal tissue. Significant differences in IL-1β, IL-10, IL-13, IFNγ, IL-6, IL-17A and IL-22 mRNA expression were detected 2 d after colonization. CjCm and CjK were enumerated on Campy-Line agar with sulfamethoxazole (CLA-S) supplemented with chloramphenicol or kanamycin, respectively. Cecal colonization by CjCm and CjK significantly dropped after challenge, and neither was isolated from ileal samples. Next, poults were colonized with wild-type isolates NCTC 11168, 81-176 and NADC 20827, and different *Campylobacter* selective media (Campy cefex, CLA-S and CampyChrome) were evaluated for enumeration. Isolates NCTC 11168 and NADC 20287 persistently colonized the cecum for up to 21 d, and were enumerated using CLA-S and CampyChrome agar. Enumeration from ileal and colon samples diminished throughout the study, indicating that the cecum was the primary site of *C. jejuni* colonization in turkeys. Data from this study demonstrated that wild-type isolates NCTC 11168 and NADC 20827 persistently colonized the cecum, and CLA-S or CampyChrome agar were the best selective media to enumerate *Campylobacter* from poults. These findings will be useful to evaluate the host-response by wild-type *C. jejuni* colonization in turkeys and evaluate strategies to reduce its colonization to promote food safety.

Key Words: *Campylobacter*, turkey, selective media

102 New statistical method identifies cytokines that distinguish stool microbiomes. B. Shannon*[1,2], [1]*BioRankings, St Louis, MO, USA,* [2]*Washington University School of Medicine, St Louis, MO, USA.*

Researchers may want to know how microbiome composition (outcome or dependent variable) changes in relation to animal characteristics (inputs or independent variables) such as age, disease severity, or cytokine level. The value of this type of analysis is to understand how characteristics may directly affect microbial compositional and variability. For example, if we know the microbiome changes in a systematic way as patients age, those changes due to age can be accounted for and removed when studying disease processes and differences between healthy and sick animals. These results can indicate interventions for improving health. In this presentation, we introduce how this is done for microbiome taxa count data and introduce a new algorithm called Dirichlet-multinomial–recursive partitioning (DM-RP). To demonstrate the utility of DM-RP we test the hypothesis that changes in cytokines representing one or more physiological processes produce changes in the composition of the gut microbiome. These data consist of host-serum cytokines and gut microbiome samples collected from individuals during periods of self-reported viral upper respiratory infection. Twelve cytokines were selected that may represent one or more physiological process associated with antimicrobial activity (IL-17F, IL-17A, IL-21, IL-22, IL-23, IL-12p40), autoimmunity (eotaxin), allergy (IL-4, IL-13), and viral infection (IFNG), as

well as cytokines related to obesity (leptin) and regulation of inflammation (TGFB).

Key Words: data analytics, statistics, physiology

103 Effects of delayed feeding post-hatch on microbiome and expression of immune response genes in broiler chickens. M. Proszkowiec-Weglarz*, L. Schreier, K. Miska, S. Kahl, B. Russell, and T. Elsasser, *USDA-ARS, NEA, ABBL, Beltsville, MD, USA*.

Microbiome, intestinal barrier, and immune response are integral parts of a healthy and well-functioning gastrointestinal tract (GIT) influencing broiler's performance. In the current broiler system, chicks may not receive feed and water for 24–72 h due to variation in hatching time and hatchery treatments. Post-hatch (PH) feed delay affects body weight, FCR, mortality, PH growth, and GIT development. Little is known about the effects of delayed feeding PH on GIT microbiome and immune response in chickens. In this study, chicks received feed and water immediately after hatch or had 48 h delayed access to feed to mimic commercial hatchery settings (treatment, trt). Both groups were sampled (n = 6) at −48, 0, 4, 24, 48, 72, 96, 144, 192, 240, 288 and 336 h PH, and jejunum, ileum, ceca and digesta were collected for gene expression and microbiome analysis. Microbiota was determined by sequencing of the V3-V4 region of bacterial 16S rRNA and analyzed using Qiime2. The relative mRNA levels of immune response genes were measured by quantitative PCR and analyzed by 2-way ANOVA. Microbial a-diversity was affected ($P < 0.05$) by chicken age, and age*trt interaction in cecal content (CeC) and scrapings (CeS), in ileal content (IlC), and by trt in CeS. Beta-diversity analysis (Unweighted UniFrac) showed separation of bacterial communities in CeS, CeC, IlC due to age and trt. Significant differences in microbial community due to age and trt were also detected by PERMANOVA. Taxonomic composition was affected by age and age × trt in CeC, CeS, IlC, and IlS at every taxonomic level. Jejunal pIgR, IL-4, IL-6, IL-10, IL-18, TGFβ, INFγ and INFβ, and ileal IgA, IL-8, IL-10, IL-18, TLR2, INFβ mRNA expression was affected ($P < 0.05$) by age while jejunal IL-1β, IL-8 and TLR2, and ileal pIgR, IL-1β, IL-4, TGFβ, TLR4, INFγ mRNA levels were also affected by age*trt interaction ($P < 0.05$). Defensin mRNAs were affected ($P < 0.05$) by age, with defensin 8 in jejunum, and 8 and 9 in ileum also affected by age*trt. These results indicate that delayed access to feed affects microbiome development and the expression of genes related to immune response during first 2 weeks of development.

Key Words: delayed feeding, microbiome, immune response

104 Effect of single dose of parenteral antimicrobials administration at birth on developmental dynamics of fecal microbiota and prevalence of selected antimicrobial resistance genes in piglets. M. Zeineldin*[1,2], A. Megahed[1,2], B. Blair[1], B. Aldridge[1], and J. Lowe[1], [1]*Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA,* [2]*Department of Animal Medicine, College of Veterinary Medicine, Benha University, Benha, Egypt.*

The purpose of this study was to evaluate the effects of early life antimicrobials intervention on fecal microbiota development, and prevalence of selected antimicrobial resistance genes (ARG; *ermB*, *tetO*, *tetW*, *tetC*, *sulI*, *sulII*, and bla_{CTX-M}) in neonatal piglets. A total of 678 piglets were randomly allocated into one of 6 treatment groups soon after birth as follow; control, tulathromycin, ceftiofur hydrochloride, ceftiofur crystalline free acid, oxytetracycline, procaine penicillin G. Deep fecal swabs were collected from piglets at d 0 (before treatment), 5, 10, 15 and 20 after treatment. Sequencing analysis of the V3-V4 hypervariable region of 16S rRNA gene and the selected ARGs were performed using Illumina Miseq platform. Our results shows that, while antimicrobials treatment had no effect on individual weight gain, or mortality, it was associated with noticeable changes in the abundance of selected ARGs, and minor shift in the composition of the fecal microbiota during this developmental stage. Interestingly, the duration and extent of the observed changes were contingent on the class of antimicrobial administered. Relative to control, only TUL treated piglet exhibited significant decline in Chao1 richness index at d 20. Unifrac distance metrics revealed that only TUL treated piglets were significantly differentiated from CCFA and CHC treated piglets. Compared with CONT group, the early life PPG treated piglet exhibited a significant increase in the prevalence of *ermB* and *tetW* at d 20 of life. TUL intervention also resulted in significant increase in the abundance of *tetW* at d 10 and d 20, and *ermB* at d 20. Collectively, these results demonstrate that the shifts in fecal microbiota structure caused by perinatal antimicrobial intervention are modest and are limited to a particular group of microbial taxa. However, early life antimicrobial intervention could promote selection of ARGs in herds. While additional investigations are required to explore the consistency of these findings across larger populations, these results could open the door to new perspectives on the utility of early life antimicrobial administration to healthy neonates in swine management systems.

Key Words: microbiota, neonatal piglets, resistance genes

105 Characterization and understanding of early-life fecal resistome in dairy cattle. J. Liu* and D. Mills, *University of California, Davis, Davis, CA, USA.*

The increasing prevalence of antimicrobial resistance is a global concern to public health, and commensal bacteria serve as critical reservoirs of antimicrobial resistance genes (ARGs). Livestock play a significant role in selecting for antimicrobial resistance and maintaining such reservoirs. In dairy cattle, antibiotic-resistant bacteria display an age-dependent distribution, in which pre-weaned calves harbor the highest abundance of resistance. Despite this, knowledge of the ARGs possessed by commensal bacteria and the structure of the cattle resistome remains limited, and the relationship between the succession of resistome and the assembling bovine gut microbiome during early life is yet unclear. The intestinal microbiome assembled rapidly in newborn calves. A total of 329 ARGs conferring resistance to 17 classes of antibiotics were observed in dairy calves with Enterobacteriaceae predicted to harbor the most transferrable ARGs. The abundance of total ARGs declined markedly during nursing, however, some clinically relevant ARGs encoding resistance to macrolides-lincosamides-streptogramines and tetracyclines increased throughout this period. Network modeling indicated that ARGs in dairy calves co-occur with antibacterial biocide/metal resistance genes. Colostrum was predicted to be the source of over 90% of

ARGs observed in dairy calves at d 2. The early succession of resistome is a result of gut microbiome assembly which is likely driven by the diet transition in dairy calves. This is suggested by the fact that carbohydrates-associated enzymes (e.g., lactase), which are more prevalent during early days than later time, primarily originated in *Escherichia coli*, while the enzymes (e.g., amylase) which increased over time were mainly predicted from Bacteroidaceae. The fecal resistome in dairy calves changes dramatically during nursing exhibiting a progressive reduction of ARGs. Colostrum appeared to seed the vast majority of ARGs in dairy calves, and the assembly of the bovine resistome during early life appears to be driven by major dietary transitions.

Key Words: dairy calf, gut microbiota, resistome

106 Evaluation of the antimicrobial activity of *Activo* liquid in porcine fecal cultures. R. C. Anderson*[1], S. R. Goodall[2], R. Cabrera[3], K. J. Genovese[1], H. He[1], M. E. Hume[1], R. C. Beier[1], R. B. Harvey[1], and D. J. Nisbet[1], [1]*USDA/ARS, Southern Plains Agricultural Research Center, Food & Feed Safety Research Unit, College Station, TX, USA,* [2]*Independent Consultant, Erie, CO, USA,* [3]*EW Nutrition USA, Des Moines, IA, USA.*

Technologies are needed to maintain the health and wellbeing of livestock while minimizing dissemination of antimicrobial-resistant bacteria to the environment. Essential oils are potent antimicrobials against enteropathogens although efficacy differs between products. To test the effect of a commercial product, *Activo* liquid, containing an aqueous mixture of citric acid, oregano and cinnamon oils, freshly collected pig feces was mixed (0.5% wt/vol) with 1/2-strength Mueller Hinton broth (5 mL/tube) and inoculated with overnight-grown novobiocin (nov) and nalidixic acid (nal)-resistant challenge strains of *E. coli* or *Salmonella* to achieve 10^6 colony forming units/mL. Challenge strains were recovered from the fecal suspensions on MacConkey or Brilliant Green agar containing 25 µg of nov/mL and 20 µg of nal/mL. Half-strength broth was used to avoid a rapid pH decline during 24-h anaerobic incubation, at 39°C, of suspensions treated (in triplicate) with 0, 2, 8, 24, 75 or 225 µL of *Activo*/culture to achieve doses corresponding to 0, 5, 20, 60, 188 or 562 oz *Activo*/128 gal of water for weaned pigs and 0, 1, 4, 12, 38 or 112 oz *Activo*/128 gal of water for finishing pigs. Dose conversions were calculated based on daily water intake of 380 or 3840 mL/day and assumed gut volumes of 300 or 600 mL for weaned and finishing pigs, respectively. Results revealed a 3 to 5 $\log_{10}$ decrease in numbers of *E. coli* strains F18 and K88 in cultures treated with 24 to 75 µL *Activo* after 6 to 24 h, which equates to 12 to 38 oz *Activo*/128 gal drinking water for finishing pigs. The equivalent of 60 to 188 oz/128 gal was estimated to obtain the same bacterial decrease for weaned pigs due to lower expected water intake. A dose of 75 µL *Activo* achieved 5 $\log_{10}$ decreases in *Salmonella* Typhimurium and Choleraesuis, equivalent to 38 and 188 oz/128 gal for finishing and weaned pigs, respectively, whereas 24 µL (12 and 60 oz/128 gal for finishing and weaned pigs, respectively) was sufficient for similar control of wild-type *Campylobacter* enumerated on Campy Cefex agar. These results reveal that *Activo*'s recommended dose of 5 to 17 oz/128 gal may be sufficient for finished but not weaned pigs.

Key Words: antimicrobial, swine, pathogen

127 Seasonality as a driver of cecal and litter microbiota variation in commercial broiler chickens. J. M. Díaz Carrasco*[1,2], E. A. Redondo[1,2], L. M. Redondo[1,2], and M. E. Fernández Miyakawa[1,2], [1]*Instituto de Patobiología Veterinaria, Instituto Nacional de Tecnología Agropecuaria (INTA), Buenos Aires, Argentina,* [2]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina.*

Seasonal fluctuations in environmental parameters such as temperature and relative humidity are known to alter internal conditions of commercial poultry farms, and these changes have been shown to affect seasonal performance. Because the gut microbiome has been shown to modulate feed conversion efficiency, we hypothesized that these seasonal changes have an impact on the microbiome that inhabits litter and gut microbiota of chickens. The aim of this study was to assess this hypothesis using high-throughput sequencing (HTS) of 16S rRNA gene amplicons. Cecal contents and litter samples were obtained at 21 and 40 d from chickens reared in a commercial farm with 2 barns housing 20,000 animals each. One barn was treated with tannins in the diet, and the other was supplemented with antibiotics. Samplings were carried out in 6 consecutive 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 depending on the seasons of the year by Kruskal-Wallis statistical analysis of alpha diversity parameters ($P < 0.001$). Both metrics showed the highest diversity in summer and the lowest values in winter, with intermediate values in fall and spring, and this pattern was the same in both cecum and litter (Table 1). 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 of both cecal and litter microbiota by season (PERMANOVA, $P < 0.05$). The effect of seasonality was much more pronounced compared to that of dietary treatments or the age of the birds, which were not consistent between cycles. This study highlights that seasonal variation should be taken into account at the time of testing strategies of microbiota modulation in commercial poultry farms.

Table 1. Variation in the number of observed species (OS) and phylogenetic diversity (PD) by season

	Summer		Winter	
Item	OS	PD	OS	PD
Cecum	608	28.9	178	16.3
Litter	472	23.2	176	15.7

Key Words: high-throughput sequencing, intestinal microbiota, broiler chicken

Session 2

107 Microbial endocrinology as an evolutionary-based language between host and microbiota influencing gut health. M. Lyte*, *Iowa State University, Ames, IA, USA.*

The microbiota that inhabit the alimentary canal are a critical determinant of overall health, behavior and feed efficiency in farm production animals. The removal of antibiotics from feed has placed a renewed emphasis on understanding how digestive physiology and behavior may be dependent on the composition and activity of the trillions of bacteria that inhabit the gut. This talk will utilize an evolutionary-based approach to show that one element unites all facets that contribute to gut health; from nutrition to microbiota to behavior. That common element is a shared neurochemistry that has led to the development of the field of microbial endocrinology. The union of microbiology and neurobiology, termed microbial endocrinology, is the study of the ability of microorganisms to produce and respond to neurochemicals that originate either within the microorganisms themselves or within the host they inhabit. The perceived role of the microbiota as mainly concerned with digestion is changing to one that is highly interactive with components of the host not previously envisioned to be influenced by the microbiota. Neurochemicals, which are ubiquitous throughout nature, therefore serve as an evolutionary-based means of communication between host and microbiota. This means that nutrition, and the design of new feeds based on neurochemistry in addition to the more traditional concepts of energy, may represent new strategies that contribute to animal well-being. As will be discussed, the sensing and utilization of neurochemicals also enables bacteria to determine their physiology based upon their environment. This neurochemical sensing is particularly important during stress as it contributes to infectious disease pathogenesis. Further, the role of nutrition and its associated capacity to contribute to microbial endocrinology-mediated responses between host and microbiota due to the provision of neurochemical substrates and precursors will also be discussed. And critically, this neurochemical cross-talk includes the brain and behavior in what has become known as the microbiota-gut-brain axis. This talk will examine the new understanding of bacterial-host interaction mediated by neurochemicals in what has become known as the field of microbial endocrinology.

Key Words: neurochemicals, enteric nervous system, microbiota-gut-brain axis

108 Gene expression of molecules involved in nutrient processing and transport in fast- and slow-growing broilers. K. B. Miska* and R. H. Fetterer, *USDA/ARS, Beltsville, MD, USA.*

Within the last 60 years, genetics of broilers have changed to produce rapid growing birds that achieve market weight in 6 wk or less. To investigate the differences in factors that play a role in nutrient processing and uptake between modern fast-growing (Ross) and slow-growing broilers not selected for growth (ACRBC), we compared the expression of 13 genes that encode AA transporters (ASCT1, $ATB^{o,+}$, B^oAT, $b^{o,+}AT$, CAT1, CAT2, EAAT3, γ^+LAT1, and LAT1) and sugar transporters (GLUT2 and GLUT5), as well as aminopeptidase (APN) and the di- and tri- peptide transporter PepT1. The growth rate of Ross birds was approximately 4 times greater than that of ACRBCs, and the feed conversion ratio (FCR) was greater in ACRBCs at all time points. Gene expression in the duodenum, jejunum, and ileum was measured at 1, 3, 5, 10, and 14 d post hatch (PH). The expression of genes that encode proteins (especially ASCT1, $ATB^{o,+}$, and B^oAT located at the brush border of the gut epithelium was generally higher in ACRBCs especially at earlier time points. The expression of genes that encode proteins located at the basolateral surface of the gut epithelium was not as greatly affected. The expression of GLUT2 and GLUT5 was significantly decreased in ACRBCs at most time points and gut segments. We conclude that expression of brush border and sugar transporters in the small intestine can be correlated with growth. This data increases the identification of the factors that influence growth and will assist future studies of the function of these molecules.

Key Words: nutrient transport, ACRBC, broiler

109 Using a synbiotic to prime the broiler intestine for an enteric challenge. S. Curry*, C. Pender, and G. R. Murugesan, *Biomin America Inc., Overland Park, KS, USA.*

Probiotics have received increasing attention in recent years due to regulatory restrictions and consumer opinions regarding the use of sub-therapeutic antibiotics. Probiotics are live microbials used to improve host intestinal balance by modes of action including competitive exclusion, production of short chain fatty acids and bacteriocins, and enhancing barrier function. The use of probiotics has shown varying effects on performance, potentially due to differences in underlying health status of the birds. In a non-challenged broiler trial, 300 birds were allotted to a non-supplemented control group or a synbiotic (probiotic+prebiotic; PoultryStar me; 500 g/MT) supplemented group with 3 replicate pens per group (50 birds/pen). At d 35, birds fed the synbiotic had improved ($P < 0.05$) intestinal integrity as measured by transepithelial electrical resistance (TEER) and villus height while maintaining similar growth performance to that of control birds. A subsequent trial was performed to determine how a dual *Eimeria maxima* and *Clostridium perfringens* challenge would affect growth performance, intestinal morphology, and intestinal cytokine expression when birds are either not supplemented (NC), or supplemented with salinomycin (PC; 0.05 g/kg) or the same synbiotic (PS; PoultryStar me; 500 g/ton) for 35 d. At 14 d of age, all birds were orally inoculated with 1×10^4 *Eimeria maxima* oocysts followed by an inoculation of 1×10^3 cfu/bird of *Clostridium perfringens* at 17 d of age. At d 35, PC and PS birds had 8.2, 5.1% numerically greater ($P = 0.264$) body weight gain and 6.8, 5.1% lower ($P = 0.10$) FCR than NC birds, respectively. In addition, PC and PS birds had increased ($P = 0.085$) ileal villus height:crypt depth ratio by 26.8 and 21.2% than NC birds, respectively. Pro-inflammatory cytokine IL-1 mRNA was reduced ($P < 0.05$) 0.31, 0.23 fold and anti-inflammatory cytokine IL-10 mRNA was increased ($P < 0.05$) by 4.1, 5.6 fold in PC and PS birds, respectively, compared with NC birds. Overall, this synbiotic shows beneficial effects on intestinal integrity potentially allowing the birds to maintain growth performance during an enteric challenge.

Key Words: gut integrity, probiotic, broiler

Session 3

110 I See Inside: Precisely defining gut health. E. Santin* and A. Sanches, *University Federal of Paraná, Curitiba, PR, Brazil.*

The importance of the gastrointestinal tract (GIT) for nutrition has been the object of many studies. Recently, advances in immunology and microbiome knowledge have added a new function for the GIT as an immune organ in addition to the digestion and absorption. At this perspective, the precise definition of gut health does not only be related to the absence of disease, in instead, it should be related to the absence of all immune reaction that could affect the GIT functionality. For this reason, we developed the "I See Inside" (ISI) system that is a strategic plan to follow gut issues and correlated it with animal performance and profitability. Start with a description of animal production systems in terms of population, a statistically sampling plan, following by a translation of the tissues alteration, at histological and macroscopic levels in number, which allow it to be correlated with other numerical parameters as zootechnical performance and economic values. The numbers generated could help to interpret the influence of factors as feed ingredients quality, management, environmental, and others in the issues observed. Applying an equation model of morphohistological reading considering mild alteration associated with inflammation it was possible observed a correlation (−0.95) with lower animal performance in broilers. The ISI methodology has been constructed with the view that GIT issues affect the animal's zootechnical performance but are not always associated with the presence of moderate or severe lesions. When the ISI is systematically applied in a population, it allows us to predict the occurrence of GIT issues using an algorithm formula. The use of the ISI sampling methodology could help to improve the evaluation of microbiome, kinome, metabolome, etc. and would help to precisely define gut health in the future. During the presentation, some examples will be shown to explain how it works.

Key Words: histology, inflammation, zootechnical performance

111 Characterizing the mycobiome in piglets during the weaning transition. K. Summers*, T. Ramsay, J. F. Frey, and A. Arfken, *USDA, Beltsville, MD, USA.*

The importance of the microbiota in the gastrointestinal (GI) tract of animals is recognized as a critical player in host health. Recently, the significance of the mycobiome has been recognized, but culture-independent methods and studies are limited, especially in swine. In this study, we sought to assess and characterize the mycobiome in the feces of swine from birth through the critical weaning transition. Feces were sterilely collected from each piglet in 9 litters (n = 113) up to daily from birth through 2 wk post-weaning (d 35) and fungal species were grown with classical culturing techniques on Sabauraud Dextrose Agar (SDA) supplemented with 0.1mg/mL cefoperazone, to prevent bacterial growth, and *Candida* chromagar was used to assess the diversity of the fungi. At d 1, piglets carried a low-level or unculturable fungal population in their feces (0.825 ± 0.153) log cfu/g. The low level of fecal fungi persisted through d 21 (1.46 ± 0.245), but by d 35, significantly ($P < 0.0001$) elevated fungal burdens were detected in piglet feces (6.12 ± 0.144). By d 35, the piglet fungal burden is comparable to levels found in grower and finisher pigs (5–6 log cfu/g feces) regardless of sex, food type, or pen location (n = 37), suggesting stable colonization after weaning. Utilizing universal ITS primers, fungal diversity was seen to be highest at d 1 with a dynamic shift to predominantly *Saccharomycetaceae* by d 28 and persisting through d 35. Next, we tested environmental factors that could lead to piglet fungal colonization. No fungi were isolated from piglet food and water, sow milk, sow colostrum, or swabs of sow nipples. However, sow feces within the farrowing pen were colonized with a mean of 5.86 ± 0.27 log cfu fungi/g. Despite culturable fungi in the sows' feces, piglets did not maintain a stable fecal fungal population until after weaning. This study provides insights into the early colonization and subsequent establishment of fungi during the weaning transition in piglets. Future studies will investigate the effect of the mycobiome on piglet growth during the weaning transition.

Key Words: mycobiome, piglet, weaning

112 Cecal microbiome and short-chain fatty acids in Shaver White chickens fed *Bacillus subtilis* DSM29784 during grower, developer and laying phases. M. Neijat*[1], J. Habtewold[1], R. B. Shirley[2], A. Welsher[2], J. Barton[2], P. Thiery[3], and E. Kiarie[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Adisseo USA Inc., Alpharetta, GA, USA,* [3]*Adisseo France, SAS, Antony, France.*

Development of robust gut function is related to the composition of gut microbiome. In this study, the efficacy of Alterion-NE50, a single strain *Bacillus subtilis* (DSM29784, Adisseo, USA), hereafter SSB, was assessed based on the composition of cecal microbiota and short chain fatty acids (SCFA). Experimental treatments included a corn-soybean basal diet containing either no probiotic (control, CON), 1.1E+08 (Low, LSSB), 2.2E+08 (medium, MSSB) and 1.1E+09 (High, HSSB) cfu/kg of diet. There were 12 replicate cages per treatment, housing 15, 14, and 7 birds per cage in the grower (wk 5–10), developer (wk 11–16) and layer (wk 19–28) phases, respectively. Eight birds per treatment were euthanized and samples of cecal digesta were used to determine SCFA and microbiota composition. Genomic DNA was extracted and the V3-V4 hypervariable regions of the 16S rRNA gene were sequenced using the Illumina MiSeq platform. Regardless of phase and treatment, the predominant bacterial phyla were *Firmicutes* (~44%) and *Bacteroidetes* (~39%). Diversity decreased ($P < 0.05$) during the developer phase as SSB dose increased. However, a distinct clustering pattern ($P < 0.05$) of bacterial community noted during the developer phase may suggest the impact of SSB on cecal bacterial communities. At genus level, *Bacteroides* and *Fecalibacterium* were differentially enriched in the developer phase for SSB- compared with CON-fed birds. Although no differences in microbial diversity were detected in the grower and layer phases, iso-butyric acid was elevated in a dose response in growers (trend, $P = 0.089$) and layers ($P = 0.034$). In addition, increased propionate in grower ($P = 0.036$) and lactate in layer ($P = 0.014$) phases were noted due to *B. subtilis* supplementation. These increases coincided with a differential enrichment of *Butyricimonas* ($P = 0.037$) and *Butyricicoccus* ($P = 0.021$) for SSB- compared with CON-fed

birds, during grower and layer phases, respectively. Different species of *Clostridium* (XVIII, XIVa, IV, and XIVb) were also identified with stronger effect sizes for SSB- compared with CON-fed birds. The results suggest that supplementing chickens' diet with *B. subtilis* 29784 may selectively enrich beneficial bacterial communities, which in turn are critical in promoting growth and performance of chickens.

Key Words: *Bacillus subtilis*, chicken, microbiota

113 Transcriptome analysis of rumen epithelium and rumen microbial community in four-month-old calves with feed-induced acidosis. W. Li*[1], S. Arnold[2], A. Edwards[3], and C. Riehle[4], [1]*USDA Dairy Forage Research Center, Madison, WI, USA,* [2]*Department of Dairy Science, University of Wisconsin, Madison, WI, USA,* [3]*Department of Biology, University of Wisconsin-Madison, Madison, W, USA,* [4]*Department of Genetics, University of Wisconsin-Madison, Madison, WI, USA,* [5]*Select Veal Feeds Inc., Harleysville, PA, USA.*

Many of the common management practices used to raise dairy calves while on milk and during weaning can cause ruminal acidosis. Though ruminal pH has long been used to identify ruminal acidosis, few attempts have been undertaken to understand the effect of prolonged ruminal acidosis on the rumen microbial community or neonatal calf health. Thus, the molecular changes associated with prolonged ruminal acidosis in calves post-weaning are largely unknown. In this study, we induced ruminal acidosis by feeding a highly processed, starch-rich diet to calves starting from one week of age through 16 wk. Rumen epithelial tissues were collected at necropsy at 17 wk of age. Transcriptome analyses on the rumen epithelium and its associated microbial communities were carried out. Calves with induced ruminal acidosis had substantially lower rumen pH 8 h after feeding and showed significantly less weight gain over the course of the experiment. A total of 683 genes (fold-change, FC, $\geq$1.5, $P < 0.05$) showed significant differential expression between the 2 feed treatments. Biological pathways affected by the significantly differentially expressed genes include cell signaling and morphogenesis, indicating an impact of ruminal acidosis on rumen development. In calves with feed-induced acidosis, rRNA reads-based microbial classification indicated increased abundance of 30 genera, with *Olsenella*, *Desulfovibrio*, *Methanosarcina* and *Fusobacterium* showing the most dramatic changes. In the same group of calves, significantly decreased abundance of 19 genera ($P < 0.05$) were observed. Among these, *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* showed most significant decrease in abundance. Our study provides insight into host rumen transcriptome changes associated with prolonged acidosis in calves post-weaning. Shifts in microbial species abundance are promising for microbial species-based biomarker development. This knowledge could provide a foundation for better diagnosis and preventative management of rumen acidosis in dairy calves.

Key Words: transcriptomics, ruminal acidosis, rumen epithelium

114 Virulence gene acquisition trends in avian pathogenic *Escherichia coli* under commercial conditions. J. D. Gruber*, D. Kroon, J. Walker, M. Perry, and E. Kim, *DuPont Industrial Biosciences, DuPont Animal Nutrition, Wilmington, DE, USA.*

Lowered antibiotic usage, including no antibiotics ever (NAE), is a risk factor for emerging bacterial pathogens in semi-closed production systems. Of the pathogens currently involved in high-disease risk, avian pathogenic *E. coli* (APEC) continues to negatively affect production and initiate disease individually or in combination with other pathogens. Transition of *E. coli*, a commensal microorganism of the gastrointestinal tract, to APEC over time has been previously documented, but key adaptations in virulence-associated genes is not well understood. Importantly, the rate of APEC conversion and overall density may be a risk factor for production and/or indicative of sub-clinical disease. Select farms were surveyed for APEC isolates and studied for their clonal relationship and APEC genes. Transition of clonal relationships were compared together with APEC genes to document distribution among 10 virulence-associated genes that include iron acquisition, host survival, and toxicity. A general distribution of genes associated with host survival and iron acquisition were initially demonstrated to be involved in low-pathogenicity APEC transitioning to high-pathogenicity APEC. Both *traT* and *cvaC*, genes for host survival and toxin production respectively, were commonly associated with low-pathogenicity APEC. Genes *sitA* and *ompT*, for iron acquisition and antimicrobial peptide defense, respectively, were also commonly associated with low-pathogenicity APEC. High-pathogenicity APEC was then identified as having increased gene functions for iron acquisition and better host survival, specifically including *ibeA* and *irp2*. Gene adaptations provide *E. coli* key strategic growth advantages and plays a role in the maintenance of the microorganism in the host. Altogether, the transition of *E. coli* to virulent pathotypes is important to understand so that both prescriptive and defensive measures can be introduced.

115 Use of Alquernat Zycox as an effective manner to control induced coccidiosis in poultry. E. H. Chowdhury[3], C. Domenech[4], M. T. Islam[1], J. Pié[4], and M. W. Rahman*[2], [1]*Department of Medicine, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh,* [2]*Department of Livestock Services, Dhaka, Bangladesh,* [3]*Department of Pathology, BAU, Mymensingh, Bangladesh,* [4]*Biovet S.A., Constantí, Tarragona, Spain.*

An experiment was conducted to determine the effectivity of Alquernat Zycox, a gut immunobooster based on botanical active molecules, as a prevention for coccidiosis in broilers in comparison with a commonly used coccidiostat. Productive performance, mortality, feces appearance and cecal injuries were evaluated, as well as the oocyst counting per gram of cecal content. One thousand Cobb 500 broilers were divided into 3 treatment groups: (T1) infected control without coccidiostats in feed; (T2) Alquernat Zycox at 0.5 kg/mT; and (T3) a product composed of maduramicin and nicarbazin (0.8 and 8.0%, respectively) at 0.5 kg/MT. All animals were infected at d 16 of age with 2.5×10^4 oocysts of *Eimeria tenella* per broiler. Data were statistically analyzed using ANOVA SPSS v.20. Results showed that all treated groups (T2 and T3) improved FCR at the end of the trial by 8.61% and 9.80%, respectively. Alquernat Zycox (T2) obtained significantly lower mortality than T1 and T3, the mildest cecal injuries (both macroscopically and microscopically) and the greatest decrease of oocysts counting per gram of cecal content. In addition, mortality and lesion scores were lower and decreased earlier post-infection in the group with Alquernat Zycox (see Table 1). In conclusion, dietary supplementation of Alquernat

Zycox can prevent coccidiosis in broilers thanks to improving the condition of the gut immune system. Results suggest that this product can replace coccidiostats that are known to create resistances.

Table 1. Summary of results

	Mortality (%) on d 35	Oocysts (8 d post-infection)	Lesion score (10 d post-infection)
T1	14.24	14,900*	2.35*
T2	4.55	9,450	1.5
T3	8.25	12,000	1.1
Significance (*)		*$P < 0.01$	*$P < 0.01$

Key Words: coccidiosis, broiler, *Eimeria*

Session 4

116 Role of the gut microbiome on outcome following viral respiratory infections in nursery pigs. M. Niederwerder*, *Kansas State University, Manhattan, KS, USA.*

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) are 2 of the most widespread and significant pathogens affecting swine worldwide, costing the industry billions of dollars in losses over the last 30 years. Both viruses cause systemic infections, resulting in primary pathology of lymphoid and respiratory tissues, and are commonly detected in cases of polymicrobial respiratory disease. Polymicrobial respiratory infections are a significant challenge to swine production due to reduced weight gain, morbidities and mortalities, decreased animal welfare, and the need for increased antimicrobial administration. By utilizing the gut-lung axis, microbiome modulation is a potential alternative tool for reducing the effects of polymicrobial respiratory disease on growing swine. Our research has found that several gut microbiome characteristics, such as increased microbial diversity, shifts in microbial composition, and fecal microbiota transplantation, are associated with improved outcome following co-infection with PRRSV and PCV2 in nursery pigs. Improved outcome characteristics include reduced virus replication, increased antibody production, decreased lung pathology, as well as reduced morbidity and mortality. Future research is focused on understanding how microbiome modulation may be applied to improve the health and welfare of swine herds endemic for these diseases.

117 Systemic and intestinal immunomodulatory effects of a *Bacillus* probiotic fed to turkeys reared in a commercial facility. E. Davis*, J. Christianson, E. Hutchinson, B. Wujek, T. Lavergne, T. Rehberger, and D. Karunakaran, *Arm & Hammer Animal Production, Waukesha, WI, USA.*

Probiotics have been reported to modulate systemic and intestinal immune responses in poultry. This study evaluated the effects of a *Bacillus* probiotic feed additive on relative gene expression of immune factors in the peripheral blood, liver, and intestinal ileum of turkeys in commercial production. Approximately 3,800 male birds at 35 d of age were placed in 2 pens within a house and allocated to a control diet or a probiotic diet consisting of 2 *Bacillus subtilis* strains administered at 1.5×10^5 cfu/g of feed. Diets were fed throughout grow-out, and 10 birds/treatment were sampled to obtain tissue for analyses at 72, 107, and 144 d of age. Blood, liver, and ileal samples were frozen in RNALater, a blood serum sample was obtained for α-1-acid glycoprotein (AGP) analysis, and the intestinal tract was obtained to determine *E. coli* and *C. perfringens* counts. RNA was extracted and synthesized into cDNA for qPCR analysis to assess gene expression of immune factors. The probiotic reduced ($P < 0.05$) *E. coli* and *C. perfringens* counts at 72 d of age compared with controls, but there were no differences on the other sampling days (treatment × day, $P < 0.05$). Overall, serum AGP levels were lower ($P < 0.01$) in turkeys fed the probiotic compared with controls. The probiotic upregulated the expression of several genes associated with inflammation (IL-12, IL-17f, LITAF, and iNOS) in peripheral blood from turkeys at 72 d of age compared with controls, but no differences were observed on the other sampling days (treatment × day, $P < 0.05$). In the ileum, gene expression of IL-12 was downregulated ($P < 0.05$) in 72-d old birds compared with controls, but there were no differences on the other sampling days (treatment × day, $P < 0.05$). Gene expression of IL-17a and IL-6 in the liver did not differ between control and treated birds at 107 d of age, but the probiotic downregulated ($P < 0.05$) their expression compared with controls on d 144 (treatment × day, $P < 0.05$). Generally, inflammatory genes were upregulated in the peripheral blood and downregulated in organ tissues in the early grow-out period when turkeys were fed the *Bacillus* probiotic.

Key Words: turkey, direct-fed microbial, immune

118 Using probiotic performance assays and comparative genome analysis on *Lactobacillus johnsonii* strains to discover effective probiotics for use in commercial turkeys. A. Johnson*, B. Weber, and T. J. Johnson, *University of Minnesota, Saint Paul, MN, USA.*

Lactobacillus species are frequently used as probiotics in the poultry industry because their supplementation has been associated with positive gut health and increased growth performance. However, *Lactobacillus* spp. are vary in their ability to modulate poultry gut health and growth performance between species and even between strains. In this study, we compared strains of turkey-source, gut-derived *Lactobacillus johnsonii* (n = 116), representing different phylogenetic backgrounds, with the goal of identifying effective probiotic strains for use in commercial turkeys. We used multiple in vitro assays to compare the probiotic potential of each strain of *L. johnsonii*. These assays included acid tolerance, bile salt resistance, and production of bile salt hydrolase. We identified variability in strain performance ($P < 0.05$) in all assays, with patterns observed correlating to *L. johnsonii* phylogenetic clade. We performed comparative genome analysis on high- and low-performing strains within each assay to determine potential genes of interest associated with probiotic performance. We also compared chicken- and turkey-derived *L. johnsonii* strains to identify host specific adaptations in the genome. We were able to demonstrate that *L. johnsonii* strains vary phenotypically in probiotic performance assays, and have identified potential genetic loci associated with these differences. We have identified genes in these chicken versus turkey strains that can be used as markers of host specificity. Future work will utilize these tools to quickly screen isolate collections for strains with probiotic potential. This will also aid in the development of genetically modified probiotic strains aimed at enhancing bird performance.

Key Words: *Lactobacillus*, probiotic, genomics

119 A post-biotic feed additive shows anti-inflammatory effects on immunometabolic signaling in broiler intestinal tissues. B. Aylward*[1], C. Johnson[1], M. Kogut[2], S. Kazemi[3], and R. Arsenault[1], [1]*Department of Animal and Food Sciences, University of Delaware, Newark, DE, USA,* [2]*USDA, Southern Plains Agricultural Research Center, College Station, TX, USA,* [3]*Pure Cultures, Denver, CO, USA.*

Increasing demand for antibiotic-free chicken products, as well as consumer concerns over antibiotic resistant bacteria, has led to the withdrawal of antibiotics in commercial poultry production operations. This has resulted in loss of the growth-promoting effects of antibiotics and has allowed for the reemergence of diseases such as necrotic enteritis (NE). Feed additives such as post-biotics are being investigated as antibiotic alternatives. The term post-biotics refers to bacterial or fungal metabolites or cellular byproducts. These molecules have direct effect on immune cell functions in the gut. We investigated the impact of a post-biotic on immunometabolic signaling in the intestinal tissues of broiler chickens when the product was administered alone and within the context of an immune challenge. *Clostridium perfringens* and coccidiosis are known to induce NE so we included them in this study. There were 6 treatment groups: control birds, post-biotic alone (M), *C. perfringens* challenge (CP), post-biotic and *C. perfringens* challenge (M+CP), 10X CocciVac and the post-biotic (M+CV), and post-biotic, *C. perfringens*, and CocciVac (M+CP+CV). Duodenum and jejunum samples were taken from 5 birds per group on Day 21 post hatch. The tissues were analyzed using a custom chicken-specific kinome peptide array designed to measure changes in kinase activity within immunometabolic signaling pathways. The results showed that the treatments had a greater effect on signaling in the jejunum than in the duodenum, and the different treatments caused distinct patterns of change in signaling. Direct comparison of uniquely differentially phosphorylated peptides between the M group and CP group showed changes in the phosphorylation state of several key members of immunologically significant pathways. These shifts in phosphorylation suggest that the post-biotic treatment has countering effect on immune signaling as compared with the CP challenge, resulting in more anti-inflammatory responses. The anti-inflammatory signaling elicited by this additive may limit the damage to gut tissue caused by *C. perfringens* and/or coccidiosis observed in infected broilers.

Key Words: immunology, post-biotic, necrotic enteritis

120 Quantification of the blood volume and pattern of organ permeability in the heat stressed pig. W. Zhao[1], F. R. Dunshea*[1], Z. Zhang[1], J. B. Furness[1], M. T. Ringuet[1], K. DiGiacomo[1], B. J. Leury[1], E. Roura[3], G. Wijffels[2], D. Renaudeau[4], N. K. Gabler[5], and J. J. Cottrell[1], [1]*The University of Melbourne, Parkville, VIC, Australia,* [2]*CSIRO, St. Lucia, QLD, Australia,* [3]*The University of Queensland, St. Lucia, QLD, Australia,* [4]*INRA, St-Gilles, France,* [5]*Iowa State University, Ames, IA, USA.*

Under sustained heat load the pig enters an altered physiological, or stress state. This includes changes in acid-base balance due to elevated respiration rate (RR) and damage to organs such as the intestines, stemming from reductions in blood flow and increased vascular permeability. Therefore, the aims of this experiment were to (A) quantify changes in blood volume that may be linked to respiratory alkalosis buffering and (B) organ extravasation by using the marker Evans blue (EB). The experiment comprised 16 pigs, of which 13 were catheterized for EB bolus (15 mg.kg^{-1}). Pigs were fed at 2.5×ME and housed under thermoneutral (TN) conditions, or 1, 3 or 7 d of cyclic heat (HS, 8h 35°C and 16 h 27°C). Respiration rates (RR), rectal and skin temperatures (RT and ST) were measured 5 times during the heat period and the EB bolus performed on the final day of treatment before pigs were euthanized and tissues collected. Transepithelial resistance (TER) and permeability (FD4) were measured in the jejunum and ileum of all 16 pigs. Differences in groups were determined by ANOVA for the effect of time or by linear or quadratic regression in Genstat V16. Thermoregulatory parameters such as RR, RT, and ST increased with HS ($P < 0.001$). Blood volume, as measured by the EB volume of distribution (V_d) decreased linearly with duration of HS exposure (86, 85, 82 and 76 mL.kg^{-1}for TN; 1, 3, and 7 d HS, Lin $P = 0.038$). The ileum TER increased with HS (Lin $P = 0.022$), whereas the stomach, jejunum and colon were not affected. Furthermore, FD4 permeability in the ileum and colon was not influenced by HS. HS increased EB extravasation in the brain (up to 60%, d3 Quad $P = 0.056$), heart (62%, d3 Quad $P < 0.001$), liver (75%, d1 $P = 0.022$), kidney (54%, d1 quad $P = 0.011$), spleen (233%, d1 $P = 0.002$) and decreased in the ileum (−25%, d7 Lin 0.022). Organs where EB extravasation did not change with HS were the Semimembranosus muscle, jejunum, stomach, colon, and pancreas. In conclusion, these results highlight that HS negatively affects multiple organs in the pig, with increases in vascular permeability associated with tissue damage experienced in several vital organs. Furthermore, the total blood volume, which was expected to increase with water intake, decreased with HS, indicating compromised water balance.

Key Words: heat, barrier, stress

121 Histological modulations of broiler gut under chronic heat stress with and without distillery yeast. G. Abbas, H. Zaneb, S. Ashraf*, S. Masood, I. Ahmad, M. M. Usman, H. F. Rehman, and H. Ur Rehman, *UVAS, Lahore, Punjab, Pakistan.*

Production performance of broilers is a function of its gut which is negatively influenced by heat stress. Distillery yeast sludge is a waste product of fermentation of sugar, which has been reported to improve the growth performance of broiler. The present trial was designed to investigate the effect of distillery yeast; that is, Bio-Wache (B-W), on components of intestinal barrier in broilers raised under chronic heat stress (CHS). The study included 168 d-old broilers distributed in 6 groups (4 replicates/group) based on levels (0, 1, 1.5, 2, 2.5, and 3%) of B-W in basal diet. The group receiving 0% B-W and CHS served as control. The temperature and relative humidity (RH) on d 1 was 35 ± 1.5°C and 70 ± 5%, respectively. Temperature was reduced by 2.5°C/week until it reached 30 ± 1.5°C on d 14 with RH 70 ± 5%; thereafter, it was maintained until the end of experiment. Two birds from each replicate were randomly sampled. Results revealed an increase in the duodenal, jejunal and ileal villus surface area (VSA) of the supplemented groups when compared with the control with maximum duodenal VSA in the group receiving 1.5% B-W and jejunal and ileal in groups receiving 1.5% and 2.5% B-W. Histochemistry of the small intestinal samples also revealed an improvement in count of mixed and acidic mucins containing goblet cells (AGCs) in the supplemented groups when compared with control, with the highest number in duodenal AGCs in 2% B-W, jejunal in 1% B-W and ileal AGCs in 1.5% B-W group. Goblet cells containing mixed mucins were highest in the group receiving 1.5% B-W in all intestinal segments. Total goblet cell count in duodenum was highest in group receiving 2% B-W, while in jejunum and ileum it was highest in 1.5% B-W. The intraepithelial lymphocytes (IELs) count in duodenum, jejunum and ileum of the supplemented

birds were lowest in groups receiving 2% B-W, 2.5% B-W and 1.5% B-W in duodenum, jejunum, and ileum respectively. It is concluded that supplementation of B-W (particularly 1.5%) used in this experimental trial partially reduced the negative effects of chronic heat stress by improving the gut health and components of mucosal intestinal barrier

Key Words: distillery yeast, gut heat stress

Session 5

122 Avian intestinal mucus modulates *Campylobacter jejuni* gene expression in a host-specific manner. T. Looft*, M. Sylte, and T. Casey, *NADC-ARS-USDA, Ames, IA, USA.*

Campylobacter jejuni is a leading cause of bacterial foodborne illness in humans worldwide. However, *C. jejuni* naturally colonizes poultry without causing pathology where it resides deep within mucus of the cecal crypts. Mucus may modulate the pathogenicity of *C. jejuni* in a species-specific manner, where it is pathogenic in humans and asymptomatic in poultry. Little is known about how intestinal mucus from different host species affects *C. jejuni* gene expression. In this study we characterized the growth and transcriptome of *C. jejuni* NCTC11168 cultured in defined media supplemented with or without mucus isolated from avian (chicken or turkey) or mammalian (cow, pig, or sheep) sources. *C. jejuni* showed substantially improved growth over defined media, with mucus from all species, showing that intestinal mucus was an energy source for *C. jejuni*. We identified 241 differentially expressed genes from *C. jejuni* cultured in the presence of mucus after analyzing data across species, compared with defined media. Fucose transporter genes were increased regardless of the source of mucus. Seventy-three genes were differentially expressed when *C. jejuni* was cultured in avian vs. mammalian mucus. Genes associated with iron acquisition and resistance to oxidative stress were significantly increased in avian mucus. Many of the differentially expressed genes were flanked by differentially expressed antisense RNA asRNA, suggesting a role in gene regulation. This study highlights the interactions between *C. jejuni* and host mucus and the effect on gene expression, growth, and invasion of host cells, suggesting important responses to environmental cues that facilitate intestinal colonization.

123 β-Mannanase supplementation reduced signs of intestinal inflammation in broilers. M. A. Martinez-Cummer*[1], K. Poulsen[2], K. Baker[1], T. Kwiatkowski[3], M. H. Rostagno[1], and J. L. Snow[1], [1]*Elanco Animal Health USA, Greenfield, IN, USA,* [2]*Elanco Animal Health Belgium, Antwerp, Belgium,* [3]*Elanco Animal Health Poland, Warsaw, Poland.*

β1–4 Galactomannans are present in many feed ingredients. β-Mannan in soybean meal is relatively high and β-mannan concentration in wheat is more than in corn (Ferrel et al., 2014). Diets with high concentrations of β-mannans were demonstrated to have poor growth performance; however, performance was restored by dietary β-mannanase supplementation (Lee et al., 2005). The reduction in digesta viscosity was considered to contribute to the improved performance of birds fed diets with high concentration of β-mannan and supplemented with β-mannanase (Lee et al., 2003). However, it cannot fully explain the improved growth performance of birds fed less viscous diets such as diets based on corn and SBM (Jackson et al., 2004) or diets based on corn, wheat and SBM with supplemental xylanase (Van Eerden et al., 2014) were previously demonstrated to be potent activators of immune cells involved in innate inflammatory reactions (Tizard et al., 1989). This unnecessary innate inflammatory responses is wasteful because the process consumes energy and nutrients from the birds. Geneic et al. (2015) found reduced pro-inflammatory cytokine expression and less inflamed intestines were observed in broilers fed supplemental β-mannanase. A reduction in the number of activated pathways involved in immune signaling and an increase in the number of activated anabolic pathways was observed when β-mannanase was supplemented in diets fed to birds (Arsenault et al., 2018). Collectively, these studies suggest that the degradation of dietary reduced β-mannan-induced intestinal inflammation and may consequently improve intestinal health. The objective of this study was to evaluate the effect of β-mannanase supplementation on intestinal integrity of birds raised mostly under commercial conditions. Twenty experiences were selected from the Elanco Health Tracking System database. These commercial experiences were selected based on diets without and with supplemental β-mannanase being used at the same time. All diets were similar with the exception of the absence (control diet) or presence (treatment diet) of a β-mannanase; All diets contained an anti-coccidial medication but not antibiotic growth promotants, and completeness of data information in the database. Fifteen intestinal conditions associated with 23 lesion scores were used to evaluate the intestinal health of the birds and to calculate the I^2 scores.

124 Maternal supply of methionine during late-pregnancy in Holstein dairy cows alters the fecal microbiome and metabolome in neonatal heifer calves during the preweaning period. A. Elolimy*[1], M. Zeineldin[2], A. Alharthi[1], F. Batistel[1], C. Parys[3], and J. Loor[1,4], [1]*Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL, USA,* [2]*Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL, USA,* [3]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany,* [4]*Division of Nutritional Sciences, Illinois Informatics Institute, University of Illinois, Urbana, IL, USA.*

The objective of the current study was to investigate the effect of dietary methionine supply during late-pregnancy in dairy cows on the gut microbiome and metabolome, and their association with growth performance in neonatal calves from birth to weaning. Twenty-six Holstein heifer calves (n = 13/treatment) born to cows receiving a control diet (CON) or CON plus rumen-protected methionine (MET; Mepron, Evonik Industries AG, Germany) during the last 4 wk of pregnancy were used. Calves received 3.8 L of first-milking colostrum from the respective dam within 8 h after birth. Calves were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer, and had ad libitum access to a starter grain mix throughout the study. Fecal samples were collected at d 0 (i.e., at birth before colostrum feeding), 14, 28, and 42 (before weaning) for microbiome and metabolome analyses. Compared with CON, calves from MET-fed cows had greater body weight, hip height, and wither height, body length, and average daily gain calves. Maternal methionine supplementation induced greater *Ruminococcus*, *Lachnospiraceae*, and *S24–7* genera in the fecal microbiome at birth. In addition, MET calves had greater *Fusobacterium* community and lower *Fecalibacterium*, *Blautia*, *S24–7*, *Clostridiales*, *Coprococcus*, and *RF32* genera at d 14, 28, and 42. Fecal metabolome analysis revealed that maternal methionine supplementation upregulated phosphatidylcholine and phosphatidylethanolamines biosynthesis, and vitamin B2 and B6 metabolism at birth. In contrast, greater maternal MET

increased methionine metabolism, citric acid cycle, and vitamin B7 metabolism at d 14, 28, and 42. Overall, results indicate that maternal supply of methionine alters gut microbiome and metabolome in neonatal heifers. The mechanistic link between maternal methionine supply, performance, and gut metabolome/metabolome remains to be determined.

Key Words: methionine, microbiome, metabolome

125 Towards the replacement of antibiotics growth promoters in chicken: meta-analysis approach. A. Rouissi*[1], M. Boulianne[2], F. Guay[1], and M. P. Létourneau Montminy[1], [1]*Animal Science Department, Laval University, Québec City, QC, Canada,* [2]*Veterinary Medicine, University of Montreal, Saint-Hyacinthe, QC, Canada.*

The development and the transfer of antibiotic-resistance genes from animals to humans have led many countries to reduce their use, and even ban them as preventive use as growth factors in the diet (e.g., European Union, 2006). Consequently, research effort has been done to find alternatives. To quantify the effect of these alternatives and to identify factors of success to maintain growth performance while removing antibiotic growth factors, meta-analysis tool has been chosen in first approach. This is a relevant method for summarizing and quantifying the knowledge acquired through previously published research. The objectives of this work was to identify the main alternatives studied and when possible to quantify their effects in comparison to an antibiotic-free control diet. Publications were retained only if growth performance has been measured, the diet composition provided, as well as the dose. This results in 4 sub-databases, namely organic acids (OA; 32 trials), prebiotics (45 trials), probiotics (34 trials), and essential oils (64 trials). Level of energy and protein has been recalculated based on feedstuff composition tables and tested as X variables as well as the dose of alternatives. For OA, butyric acid was the most studied (78%), followed by blend with formic acid (24%) and blend without formic acid (8%). For prebiotics mannan-oligosaccharides was the most studied (76%), followed by fructo-oligosaccharides (22%). Only negative control without antibiotics and alternatives treatments were kept for this work and they has been compared within treatment with the random effect of the trial added in the models. Results for OA showed no effect on ADFI while ADG was increased linearly ($P < 0.01$) leading to a linear ($P < 0.001$) and quadratic ($P = 0.04$) decreased of FCR. Results for prebiotics, showed a linear ($P < 0.001$) and quadratic ($P < 0.001$) increase of both ADFI and ADG with increasing the dose. For FCR, there was a linear ($P = 0.02$) and quadratic ($P = 0.03$) decrease with increasing prebiotic dose, but the linear response of FCR depends of the level of ME (interaction ME × Dose, $P = 0.03$) showing that decrease of FCR with prebiotic addition is more marked when ME was limiting. Maximum effects for FCR are of 3% for OA and up to 9% for prebiotics.

Key Words: broiler, organic acid, prebiotic

126 Effect of PrimaLac on necrotic enteritis lesion scores and expression of tight junction proteins. N. Emami*[1], A. Calik[1], M. White[1], M. Young[2], and R. Dalloul[1], [1]*Virginia Tech, Blacksburg, VA, USA,* [2]*Star Labs/Forage Research Inc., Clarksdale, MO, USA.*

Necrotic enteritis (NE) continues to present major challenges to the poultry industry and the etiologic agent *Clostridium perfringens* is the fourth leading cause of bacterially induced food-borne illnesses in the US. The objective of this study was to evaluate the effects of the direct fed microbial (DFM) PrimaLac on intestinal lesion scores and RNA expression of the tight junction (TJ) proteins claudin-1, claudin-3, ZO-1, and ZO-2 during a naturally occurring NE. On day of hatch, 1,080 Cobb 500 male broiler chicks were randomly allocated to 3 experimental dietary groups (12 replicate floor pens, 30 birds/pen) including negative control (NC) fed a corn/soybean basal diet, positive control (PC) the fed basal diet with Virginiamycin, and additive group fed the basal diet supplemented with PrimaLac. One day post placement, all birds were challenged by a commercial live oocyst coccidia vaccine as a predisposing factor to naturally occurring NE. At the onset of NE on d 8, the small intestines of 2 birds/pen (24 per group) were examined for NE lesions, and jejunum samples from one of those birds were collected to measure gene expression of TJs. Statistical analysis was performed using the JMP 13 software and significance between treatments ($P < 0.05$) determined by LSD. The DFM (PrimaLac) and PC (Virginiamycin) groups had significantly reduced lesion scores in the duodenum and jejunum ($P = 0.006$ and $P = 0.04$, respectively) compared with the NC birds. Expression of claudin-3 was higher ($P = 0.03$) in PrimaLac-supplemented birds compared with NC. Furthermore, despite similar levels for claudin-1 and ZO-1, expression of ZO-2 tended ($P = 0.06$) to be higher in the PrimaLac group than in the NC birds. These findings showed that supplementation of PrimaLac during NE challenge helps reduce lesion scores in the duodenum and jejunum, suggesting a beneficial effect of this DFM. This may partially be due to increased expression of the TJ proteins claudin-3 and ZO-2 promoting gut integrity as *C. perfringens* toxins disintegrate TJ proteins, increasing the paracellular permeability of the gut.

Key Words: broiler, PrimaLac, necrotic enteritis

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Poster Presentations

P100 Quinolone-resistant *Escherichia coli* in medically untreated dairy calves—How did they get there? S. Finstad*[1], H. Kaspersen[2], and A. Bjelland[1], [1]*Faculty of Veterinary Medicine, Norwegian University of Life Science, Oslo, Norway,* [2]*Norwegian Veterinary Institute, Oslo, Norway.*

Quinolone is one of the most important antibiotics used in human medicine. Resistant bacteria of animal origin can be a source of antibiotic resistance affecting humans. In Norwegian dairy production, the use of fluoroquinolones is nearly negligible. Nevertheless, quinolone-resistant *Escherichia coli* (QREC) are frequently detected at low levels in bovine fecal samples with higher proportion in preruminants than older animals. The drivers of quinolone resistance are unknown. It is discussed whether QREC is transmitted between animals and their environment, or if intestinal factors drive QREC development in the bovine intestine. The aim of this work was to investigate the occurrence of QREC in Norwegian calves during their first 3 mo of life. Traditional microbiological methods and PCR as well as multilocus sequence typing analysis after whole-genome sequencing were used to detect potential transmission routes. Fecal swabs from calves and their mothers, in total, 12 individual from 2 different farms were analyzed for the within-sample prevalence of QREC over the duration of 3 mo with sampling every second week. QREC was identified in 29.8% (25/84) of the samples. Of the isolated QREC, 67.9% was found to have MIC values above clinical breakpoint (>0.5mg/L). Cohabiting calves were found to be simultaneously QREC positive for a short time before returning QREC negative, indicating an on/off-situation for QREC-shedding. Thus, QREC shedding did not correlate with age. Identical genotypes of QREC were identified from cow and calf on the same day of sampling, however changes in QREC genotype over time were observed. Also, the QREC genotypes were consequent different between farms. The results show a high prevalence of QREC above clinical breakpoint in clinical healthy animals fed with only milk, grass and feed concentrate without any use of antimicrobials. The coincide occurrence of QREC on calves within a farm, the periodically presence of resistance and the similar genotype between calves and cow indicate transmission of QREC at farm level and non-age dependent drivers for quinolone resistance.

P101 Early colonizing microbiota of turkey poults. J. Rehberger*, R. Geier, E. Hutchison, S. Anderson, R. Wujek, E. Vang, and A. H. Smith, *Church & Dwight, Waukesha, WI, USA.*

Commercial hatcheries sanitize eggs before placement in the hatchery to break the vertical transmission of pathogens. Unfortunately, this practice also disrupts the transference of commensal bacteria from hen to chick. To determine the early colonizing microbiota of turkey poults and to attempt to understand where these organisms are being acquired, samples were taken at 4 farms from the gastrointestinal tract (GIT) of breeder hens (3), day-old poults (10) and pretransfer in ovum birds (10) as well as the associated yolk sacs from poults and pretransfer eggs. Cell pellets were collected and stored at −20°C until DNA was extracted using the DNeasy PowerSoil Kit (Qiagen). PCR amplification was performed using V4 16S rRNA primers to investigate the total bacterial communities and Clostridia specific primers. Amplicons were pooled and Illumina sequencing was performed. Sequence analysis was performed using QIIME2 including quality filtering by DADA2, α- and β-diversity analysis, and closed reference taxonomic assignment by the full EZTaxon 16S database. Based on the V4 amplicons, breeders were the most diverse, with over 80 observed species, followed by DOH GITs (30 species), then DOH yolks and pretransfer GITs (20 species each) and finally pretransfer yolks (15 species). The most predominant organisms in the DOH GITs were *Clostridium* and *Enterococcus*. *Enterococcus* was not detected in the pretransfer yolks, pretransfer GITs, or DOH yolks but was detected in one of the breeders. *Enterobacteriaceae*, from the genera *Escherichia*, *Shigella*, or *Salmonella*, were detected across all sample types. Based on the Clostridia-specific primers *Paeniclostridium sordellii* was detected only in the GIT of the poult and pretransfer bird. *Clostridium paraputrificum* was detected only in the yolk and GIT of the poults. *Clostridium tertium* was abundant in the yolk and GIT of the poults and was detected in the breeder at low abundance (0.06%). The enterococcal and clostridial profiles of the poults suggests that early colonizing bacteria are being acquired from the hatchery environment.

Key Words: microbiota, turkey

P102 Impact of probiotic supplementation on gastrointestinal functionality in young piglets: A meta-analysis and meta-regression. A. A. Séon Simon*[1], H. M. Golder[2], V. Verlhac-Trichet[1], F. Fru[1], I. J. Lean[2], and P. Celi[3], [1]*DSM Nutritional Products France, Research Center for Animal Nutrition & Health, Saint Louis, France,* [2]*Scibus, Camden, Australia,* [3]*DSM Nutritional Products, Animal Nutrition and Health, Columbia, MD, USA.*

Evidence is increasing of positive effects of probiotic supplementation in young piglets on several aspects of gastrointestinal functionality. This study used meta-analytic methods to explore the effects of including probiotics (*Lactobacillus*, *Bacillus* spp., *Enterococcus and Saccharomyces*) on average daily gain (ADG), final body weight (FBW), average daily feed intake (ADFI), and gain to feed (G:F) and feed to gain (F:G) ratios. Other measures of interest included effects on fecal score, intestinal morphology, volatile fatty acid (VFA) production, and bacterial shedding. The effect of probiotics and diet composition on gastrointestinal functionality were also explored by using meta-regression methods. A comprehensive literature search from 1990 to 2017 was conducted to identify research studies involving treatments designed to evaluate the effects of probiotics on gastrointestinal functionality. Overall, probiotic supplementation resulted in an improved ADG, FBW and G:F, while no effect was noted for VFA. The effect of *Lactobacillus* on ADFI tended to increase with increased crude protein content of the diet. Efficiency of gain was improved with *Lactobacilli* supplementation, which also resulted in reduction in ammonia production. Increased dietary CP, lysine, and soybean meal resulted in higher FBW when *Bacillus* spp. were supplemented. There was also a tendency for G:F to improve with increased soybean meal in the diet. Finally, fecal score was markedly reduced in pigs fed *Bacillus* spp. While *Lactobacillus* and *Bacillus* spp. supplementation resulted in a reduction in *E. coli* fecal shedding, *E. faecium* supplementation resulted in an

increase in *Lactobacillus* in the feces. All interventions analyzed showed evidence of publication bias. Notwithstanding this bias, this study indicates that responses to probiotics were consistently positive in regards to improved ADG, FBW, F:G, and G:F ratios, but highly variable for all parameters. There were few variables studied that explained a significant amount of the variation in the studies suggesting that other factors such as strain differences and other aspects of study conduct influenced outcomes.

Key Words: probiotic, gastrointestinal functionality, piglet

P103 Effects of probiotic interventions on chicken gastrointestinal functionality: A meta-analysis and meta-regression. A. A. Séon Simon*[1], H. M. Golder[2], M. B. De Ondarza[3], I. J. Lean[2], and P. Celi[4], [1]*DSM Nutritional Products France, Research Center for Animal Nutrition & Health, Saint Louis, France, 2Scibus, Camden, Australia, 3Paradox Nutrition, West Chazy, NY, USA, 4DSM Nutritional Products, Animal Nutrition & Health, Columbia, MD, USA.*

Evidence is increasing of positive effects of probiotic supplementation in broiler chickens on several aspects of gastrointestinal functionality. This study used meta-analytic methods to explore the effects of including probiotics (*Lactobacillus*, *Bacillus*, *Enterococcus*, and *Saccharomyces*) on average daily gain (ADG), final body weight (FBW), average daily feed intake (ADFI), and feed to gain (F:G) ratios. Other measures of interest included effects on intestinal morphology, volatile fatty acid (VFA) production, and bacterial shedding. The effect of probiotics and diet composition on gastrointestinal functionality were also explored by using meta-regression methods. A comprehensive literature search from 2000 to 2018 was conducted to identify research studies involving treatments designed to evaluate the effects of probiotics on gastrointestinal functionality. Overall, probiotic supplementation resulted in an improved ADG, FBW and F:G. Cecal *E. coli*, *Salmonella* and *Clostridia* numbers were reduced with *Bacillus* supplementation. The limited observations on intestinal morphology show that villous height in the ileum is increased while crypt depth is reduced when *Bacillus* are included in the diet. While there was evidence that the variance of comparison was high, few of the variables studied explained the variation in the meta-regression analyses. This study indicates that responses to probiotics were consistently positive in regards to improved ADG, BWG and F:G. The interventions were effective at improving the performance of broilers as indicated by significant results for individual interventions and overall. However, all interventions had marked evidence of heterogeneity that was only rarely associated with sex, duration of trial or dietary factors investigated. This suggests that a considerable part of the variation resides with other factors, possibly including strain of organism selected, dose within organism and gastrointestinal microbiome. This heterogeneity and the evidence of unpublished experiments suggests that selection of organisms used and refinement of the methods of application will result in more consistent outcomes.

Key Words: probiotic, gastrointestinal functionality, chicken

P104 Establishment of 3-dimensional organoids from chicken cecal crypts. D. Zhao*[1], M. Kogut[2], K. Genovese[2], L. A. Davidson[3], R. S. Chapkin[3], and Y. Farnell[1], [1]*Department of Poultry Science, Texas A&M AgriLife Research, Texas A&M University, College Station, TX, USA,* [2]*Southern Plains Agricultural Research Center, Agricultural Research Service, US Department of Agriculture, College Station, TX, USA,* [3]*Department of Nutrition and Food Science, Texas A&M AgriLife Research, Texas A&M University, College Station, TX, USA.*

Intestinal organoids, known as mini-guts, are derived from intestinal stem cells, which are located at the bottom of the crypts. Recently, organoids derived from different organs have been used as a novel pathophysiological model to study the mechanism of host-pathogen interaction. However, there is a lack of long-term cultures of intestinal epithelial cells in chicken, and development of in vitro cell culture model is needed. Therefore, the objective of this study was to generate cecal crypt-derived organoids. Cecal samples were collected from layers and broilers with the age ranging from 2 d to 20 wk. Cecal crypts were isolated using a dissociation solution containing a chelating reagent, EDTA. Isolated crypts were embedded in basement membrane matrix (Matrigel), and cultured with conditioned medium collected from a supporting cell line that produced Wnt, R-spondin and Noggin factors. We found that isolated crypts have been successfully grown to form 3-dimensioanal epithelial structures which could be cultured up to 3 weeks. Characterization of crypt-derived organoids will be performed using immunofluorescence and reverse transcription-PCR. Long-term, organoid culture will help us understand host-pathogen interactions, eventually leading to discovery of intervention methods in poultry.

Key Words: chicken, crypt, organoids

P105 *Clostridium perfringens* enterotoxin induces chicken necrotic enteritis. M. Abraha*, M. Bansal, B. Al-Rubaye, A. Almansour, H. Wang, J. D. Latorre Cardenas, B. Hargis, and X. Sun, *University of Arkansas, Fayetteville, AR, USA.*

Necrotic enteritis (NE) caused by *Clostridium perfringens* and coccidiosis is one of the most important diseases with billions of dollars loss annually in the antimicrobial free era. However, the mechanism of NE pathogenesis remain elusive. We hypothesize that *C. perfringens* induces NE through producing enterotoxin (CPE). This was based on our previous findings that secondary bile acid deoxycholic acid (DCA) attenuated *Eimeria maxima* and *C. perfringens*-induced NE. NE was associated with sporulation of *C. perfringens* in ileum, while dietary DCA reduced the sporulation. To examine the role of *C. perfringens* sporulation on NE, we performed several in vitro and in vivo chicken experiments. *C. perfringens* were cultured in tryptic soy broth (TSB) overnight and then cultured in Duncan strong sporulation medium (DSSM) to induce sporulation. The supernatant was collected as CPE supernatant (Cpe-supe). RAW 264.7 cells were challenged with different doses of Cpe-supe to induce cell death. To further assess the Cpe-supe induction of NE in birds broilers were infected with 5,000 *E. maxima* oocysts/bird at 9 d of age and were then orally gavaged with the supernatant from d 14 to 16. The birds were killed at d 16 and ileal tissue was collected for histopathology assessment. We found that DSSM induced 10% *C. perfringens* sporulation at 6 h and 90% sporulation overnight. Interestingly, Cpe-supe at as low as 5 µL/mL overnight induced RAW cell death, showing as round and detached from culture plate. After staining the cells with propidium iodide (PI), the dead cell showed fluorescent red. Cpe-supe at 15 µL/mL induced 90% RAW cell death. Bird infected with *E. maxima* and gavaged with

Cpe-supe showed morbidity compared with *E. maxima* birds. Upon examination of the bird histopathology, small intestine of *E. maxima* birds with Cpe-supe displayed more obvious intestinal inflammation, showed as shortening of villi, hyperplasia of crypt, and infiltration of inflammatory cells into intestinal lamina propria than the birds infected with *E. maxima* alone. In conclusion, *C. perfringens* induced NE through production of CPE. These findings suggest that it is possible to prevent and treat NE by inhibiting NE sporulation.

Key Words: toxin, cell culture, chicken

P106 SPF-Anaerobe microbiota mediates *Campylobacter jejuni* clones and campylobacterosis in broiler chickens. A. Almansour*, B. Alrubaye, M. Bansal, M. Abraha, X. Sun, H. Wang, and B. Hargis, *university of Arkansas, Fayetteville, AR, USA.*

Campylobacter jejuni is the main cause of foodborne enteritis worldwide. Campylobacterioisis caused by consuming undercooked chicken meat is reduced with less contaminated chickens. However, few approaches are available to reducing *C. jejuni* chicken colonization because of limited knowledge on the interactions of microbiome and *C. jejuni* colonization. In this study, we aimed to investigate the role of microbiota on preventing *C. jejuni* chicken colonization. We cultured mouse specific-pathogen-free (SPF) mouse microbiota into anaerobe (SPF-Anaero) and aerobes (SPF-Aero) on Brain Hear Infusion plates. Birds raised on floor pens were colonized with 10^8 cfu/bird microbiota at d0 and infected with 10^9 cfu/bird C. jejuni AR101 at d10. Growth performance of body weight gain and feed intake were measured at d14, 21, and 28. Birds were killed at d 14, 21, and 28 to examine *C. jejuni* colonization in ceca. We found that the SPF-Anaero enhanced growth performance of body weight gain at d 14 (0.451 vs. 0.347 kg/bird, $P = 0.0008$) and at d 28 (1.745 vs. 1.54 kg/bird, $P = 0.0024$) compared with uninfected birds. Notably, birds colonized with SPF-Anaero and infected with *C. jejuni* grew faster compared with infected bird at d 14 (0.451 vs. 0.347kg/bird, $P = 0.0008$). Interestingly, birds colonized with SPF-Aero and infected with *C. jejuni* failed to improve body weight. At d14, *C. jejuni* AR101 was readily colonized intestinal tract at 2×10^3 cfu/g cecal digesta and reaching a plateau of 10^6 cfu/g cecal digesta at d 21. Importantly, SPF-Anaero reduced 92% of *C. jejuni* cecal colonization at d 28 (7×10^4 vs. 5×10^6 cfu/bird, $P = 0.0073$) compared with infected birds, while SPF-Aero did not change the bacterial colonization. In conclusion, SPF-Anaero reduces *C. jejuni* colonization in chickens and improves growth performance. These findings offer new antimicrobial alternative approaches to prevent *C. jejuni* chicken colonization.

Key Words: *Campylobacter jejuni*, microbiota, chicken

P107 Identification and functional characterization of putative colonization factors of *Lactobacillus gallinarum* in poultry. T. Duong*, T. E. Askelson, T. J. Broderick, L. E. Froebel, L. K. Froebel, and M. L. Nash, *Department of Poultry Science, Texas A&M University, College Station, TX, USA.*

Administration of probiotic *Lactobacillus* cultures in poultry has been demonstrated to improve animal health, growth performance, and microbial food safety. Although it is thought to be critical to their probiotic functionality, the mechanisms responsible for gastrointestinal colonization of *Lactobacillus* in poultry are not well characterized. We have previously demonstrated the chicken crop isolate *Lactobacillus gallinarum* ATCC 33199 to be readily transformed; effectively adhere to and inhibit adhesion of *Salmonella* to the LMH chicken epithelial cell line in vitro; and colonize the gastrointestinal tract of broiler chickens in vivo. The objective of this ongoing study is to characterize factors important to the colonization of this potentially important model probiotic microorganism in the gastrointestinal tract of poultry. Analysis of the draft genome sequence of *L. gallinarum* identified open reading frames (ORFs) putatively encoding cell surface adhesion proteins including collagen-binding, fibronectin-binding, mucus-binding, and surface layer domain containing proteins and environmental fitness factors including putative acid and bile tolerance proteins. Several ORFs were found to be truncated likely due to errors in sequencing and automated annotation. Targeted sequencing was performed to correct sequencing errors before manual annotation. Using the corrected sequences and updated annotation, PCR primers were designed for amplification and cloning of internal gene fragments for the construction of targeted gene insertion knockouts using the pORI28 chromosomal integration the system. Construction of these isogenic mutants will be used to evaluate the role of these factors in gastrointestinal colonization and competitive exclusion in poultry. A mechanistic understanding of *Lactobacillus* colonization and competitive exclusion in poultry is expected to improve to the selection and development of probiotic cultures for use in poultry production.

Key Words: *Lactobacillus,* probiotic, colonization

P108 Review of immunomodulatory additives that are susceptible to being used as feed additives: Mode of action and identification of end-points for efficacy assessment. J. Tarradas*, N. Tous, E. Esteve, and J. Brufau, *IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Constantí, Spain.*

This project tendered by EFSA (OC/EFSA/FEED/2014/01) and developed in IRTA sought to gather information on the potential of feed additives to improve the immune status of animals. A systematic review was carried out using Distiller SR software, with 15 chain searches run in WoS, Medline, and SciELo databases (1990–2014) obtaining 12,723 articles. Of these, 1144 were selected and reviewed, providing data for 185 substances/agents with potential to be used as feed additives (51 probiotics, 25 prebiotics, 92 plant extracts, 5 animal by-products, and 12 'other substances'). For each additive, we identified the main modes of action, interactions with dietary compounds, relevant end-points to demonstrate efficacy and methods for their measurement, safety, worldwide legislation, and patents. The most evaluated end-points in mammals and birds were microbiota, gut morphology, immunoglobulins, cytokines, and performance; and in fish: lysozyme and phagocytic activity, leucocytes, complement, immunoglobulins, respiratory burst, and performance. These end-points were classified into: (1) local immune response, (2) systemic immune response, and (3) health status. The beneficial effects of additives may be driven by down or upregulation of specific immune parameters. Some additives have already accepted as feed additives by the European Commission. Few interactions between diet ingredients and additives were noted. Some of the studies evaluated safety (although this review focused on immunomodulation). Many reports explore mixtures of additives for which specific modes

of action cannot be attributed to the individual compounds. The mode of action of plant extracts is poorly investigated. Few studies assess the dose response of additives on immune parameters. Minerals, amino acids, and vitamins can only be considered as immunomodulatory substances when the dosage is above the minimum requirements. Performance improvements, in parallel with the above endpoints, might be considered as indicatives of welfare. http://www.efsa.europa.eu/it/supporting/pub/en-905

Key Words: immunomodulation, feed additive, systematic review

P109 Influence of probiotic metabolites on microbial diversity of the cecal microbiome in broiler chickens under heat stress conditions. S. Yang*[1], M. Roberts[2], J. McNaughton[2], M. Canady[1], K. Schuster[1], A. Blaszczak[1], and E. Wozniak[1], [1]*Cytozyme Laboratories Inc., Salt Lake City, UT, USA,* [2]*AHPharma Inc., Hebron, MD, USA.*

The gastrointestinal tract's commensal microbiome plays an important role in the host's nutrition, health and immunity. The objective of our study was to investigate the effect of probiotic metabolites (PBM) supplementation, in an antibiotic-free diet, on the cecal microbiome of broiler chickens under heat stress conditions. A total of 832 one-day-old mixed-sex Ross 708 broilers were randomly allocated into 2 groups, with 8 replicated pens per group and 52 broilers in each pen, for a 49-d trial on used litter. Two groups of chickens were fed corn-soybean meal diets with and without probiotic metabolites at a concentration of 1 g/kg (PBM vs. CON, respectively). Heat stress was maintained at temperatures of 27–30°C (d 0 to 7), 38°C (d 8 to 14), and 41°C (d 15 to 49). At 49 d, the PBM group had significantly ($P < 0.05$) higher mean BW gain than the CON group (3.03 vs. 2.89 kg). Cecal samples of 5 randomly selected birds were pooled from each pen and used for genomic DNA isolation and PCR amplification of the 16S rRNA gene. Results of the pyrosequencing of the amplicons identified 12,174 and 10,299 sequence reads for the PBM and CON groups, respectively. Phylogenetic analysis indicated that probiotic metabolites supplementation increased the proportion of phylum *Firmicutes* (F) from 51.6 to 68.9% and decreased the proportion of phylum *Bacteroidetes* (B) from 44.8 to 27.6%, compared with the CON group. The PBM group had significantly ($P < 0.05$) higher F/B ratio than the CON group (2.68 vs. 1.37). Greater F/B ratios have been associated with bacterial profiles with higher capacity of energy harvesting and BW gain. The results of this study suggest that the growth-promoting effect of PBM supplementation in broilers may be facilitated via gut microbiome enhancement and modulation.

Key Words: probiotic metabolites, microbiome, *Firmicutes*:*Bacteroidetes* ratio

P110 Evaluating the diverse immunological effects of chestnut tannins in broilers. A. Lee*[1], G. Cardoso dal Pont[1], M. Fernandez-Miyakawa[3], M. Battaglia[4], and M. Kogut[2], [1]*Texas A&M University, College Station, TX, USA,* [2]*United States Department of Agriculture-ARS (SPARC), College Station, TX, USA,* [3]*Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina,* [4]*Silvateam/Indunor S.A., Buenos Aires, Argentina.*

For over 50 years, antibiotic growth promoters (AGPs) have been widely used in industry to improve growth performance and weight gain in poultry. However, over time, antibiotic resistance, consumer concerns, and persistent reoccurrence of pathogenic infection led producers to find alternatives to AGP. Tannins could be one alternative to AGPs since they have important biological activities. The phenolic compounds are capable of binding and precipitating proteins, which have led to anti-viral, anti-microbial, anti-parasitic and anti-inflammatory actions although the specific mechanism is still unknown. While previous studies have recognized tannins as anti-nutritional factors in monogastric animals, this was mostly based on relatively high concentrations. Recent studies have shown that moderate and lower tannin levels may bring positive effects to poultry growth performance. Therefore, our study attempts to evaluate the immunological and immunomodulatory effects of commercially available chestnut tannins (CT) in broiler chickens. In each replicate, d 1 chicks will be randomly distributed in 4 groups which will differ only on concentration of CT in feed offered. The treatments will consist of a negative control (NC) group with no AGPs, a positive control (PC) group with addition of antibiotic as a growth promoter level, a group with 1% CT, a group with 0.2% CT, a group with 0.08% CT, and a group with 0.03% CT. All chicks will receive corn and soybean-based diet formulated to meet the birds' nutritional requirements. Each replicate will last 10 d, with cecal tissues and contents taken at d 2, 4, 6, 8, and 10. Before each necropsy day, birds will be weighed for performance evaluation. The cecal tissue will be processed with a validated kinome peptide array analysis to determine what signal transduction pathways are being used for phosphorylation events. The cecal contents will be snap-frozen for future metagenomic studies. Looking into this aspect may provide valuable information regarding the identification and function of poultry immunometabolism.

Key Words: chestnut, tannins, immunometabolism

P111 Effect of dietary protein source and litter condition on immune response and mitotic cell activity in the duodenum of broiler chickens at 21 days of age. A. J. Keel*, A. J. Calderon, O. J. Tejeda, J. D. Starkey, and C. W. Starkey, *Auburn University, Auburn, AL, USA.*

Proper absorptive and protective functions of the gut are important for efficient utilization of feed, and these functions depend on the continuous self-renewal of intestinal epithelial cells and the presence and activity of immune cells necessary to defend against pathogens. A 3 × 2 factorial treatment arrangement in a randomized complete block design experiment was used to explore the combined impact of dietary protein source and litter condition on the density of mitotically active (proliferative) enteric cells and macrophages present in the duodenum of broiler chickens. The 3 different dietary protein sources were soybean meal (SBM), 50% poultry by-product meal and 50% feather meal (PFM), and porcine meat and bone meal (MBM). Birds consuming each protein source were reared on both new (NL, fresh pine shavings) and used litter (UL, litter after 3 previous

flocks). On d 0, Yield Plus × Ross 708, female broiler chicks (Aviagen, Huntsville, AL) were individually weighed, randomly allotted to 1 of 6 treatments, and placed in an environmentally controlled raised floor pen facility with 5 chicks per pen. On d 21, all birds and feed were weighed to calculate body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) on the birds sampled. On d 21, 6 birds per treatment from different pens (total n = 36) were injected intraperitoneally with 5′-bromo-2′-deoxyuridine (BrdU) 1 h before duodenal sample collection to label mitotically active cells. The duodenal samples were analyzed using cryohistology, immunofluorescence staining, and digital fluorescence microscopy to determine the density of mitotically active (BrdU+) cells and macrophages. Neither dietary protein source or litter condition significantly altered BWG ($P \geq 0.3427$) or FI ($P \geq 0.4994$). However, birds fed PFM had improved FCR compared with those fed MBM or SBM ($P = 0.0002$). Litter condition did not impact FCR ($P = 0.3659$). Neither protein source ($P = 0.5946$) or litter condition ($P = 0.9155$) altered duodenal macrophage or mitotically active cell densities.

Key Words: broiler chicken, local macrophage immune response, cell proliferation

P112 Survey of *Clostridium* populations in dairy cattle feed samples across the United States. T. L. March*, J. S. Thompson, R. F. Teal, A. H. Smith, and T. G. Rehberger, *Arm and Hammer Animal Nutrition.*

Clostridium are spore forming bacteria which commonly reside in soils and the gastrointestinal tract and have been associated with enteric disease such as hemorrhagic bowel syndrome (HBS) in ruminants. To understand the source of these pathogens in ruminants, research regarding the diversity and proliferation of clostridia in fermented forages and dairy feeds is a priority. The purpose of this research was to quantify total clostridia counts observed in total mixed rations (TMR), corn silage and haylage samples and determine the diversity of *Clostridium* species present in these samples to better maintain ruminant GI health. Samples collected for this research included TMR (n = 1,378), corn silage (n = 431), haylage (n = 326) and other feed stuffs (n = 877). Feed samples were collected from 326 farms across 27 states in the United States and enumerated on tryptose sulfite cycloserine (TSC) agar and representative isolates were harvested for further categorization. From these samples, 2,290 *Clostridium* spp. isolates were characterized of which 64.2% were *C. perfringens.* Of the *C. perfringens* isolates identified, 86.8% of the isolates were run on a multiplex PCR to determine the toxin type; toxin Type A was most prevalent at 88.4%. Other toxin types present included Type C at 0.7%, Type D at 0.9% and Type E at 0.9%. The remaining 10.7% were unidentified. Of the remaining *Clostridium*, the 2 predominant groups were *Paraclostridium bifermentans* group (12.8%), *Clostridium beijerinckii* group (10.1%) and *Clostridium butyricum* (5.2%). These species previously have been reported to produce 1,3-propanediol, acetone, butyrate and butanol which could be impacting rumen and GI function. When comparing the levels of total clostridia, TMR samples had higher counts compared with haylage and corn silage samples ($P < 0.0001$). This research is important in understanding *Clostridium* populations in feed to understand how these populations could be impacting ruminant health and production.

Key Words: ruminants, silage, *C. perfringens*

P113 The proliferation of *Clostridium* species in total mixed ration after 24-hour heat challenge. V. G. Bretl*, J. S. Thompson, T. L. March, A. H. Smith, and T. G. Rehberger, *Arm & Hammer Animal Nutrition, Waukesha, WI, USA.*

Clostridium are common inhabitants of soil and can lower the nutritional value or cause sub optimal fermentations by producing undesirable end products such as butyrate. Feed with a *Clostridium* challenge could also be a source for enteric diseases. To better understand the distribution of the clostridia populations in dairy feeds a survey was conducted of total mixed rations (TMR) (n = 1378) and fermented feeds (n = 757). The results indicated that clostridia levels were significantly higher in the TMR ($P < 0.0001$) compared with the fermented feeds. The average clostridia counts were 1900 cfu/g for TMR, 130 cfu/g for haylage, and 86 cfu/g for corn silage. We hypothesized from this survey that clostridia populations may proliferate in TMR. An experiment was conducted to better understand clostridia growth and populations in TMR. Clostridia populations were enumerated from 17 TMR samples on tryptose sulfite cycloserine (TSC) agar from 13 different farms before and after 24 h at 90% humidity and 37°C. The average counts for the 17 samples were 160 cfu/g at 0 h and increased to 289 cfu/g at 24 h. Results indicated that 5 TMR samples increased over 5 times the original level, while the remaining 12 TMR samples showed stable or reduced clostridia load. *Clostridium* species were identified by harvesting isolated colonies from both time points. The TMRs that increased in clostridia after 24 h had *C. perfringens* and *Paraclostridium bifermentans* at both time points. The TMRs with stable clostridia levels had *C. perfringens, P. bifermentans, C. beijerinckii,* and *C. butyricum* identified at both time points. The TMR with reduced levels of clostridia had all 4 *Clostridium* species identified at the first time point, while *P. bifermentans* and *C. beijerinckii* were identified at 24 h. Further testing will be done to determine shifts in the populations of other bacteria and in volatile fatty acid profiles.

P114 Nucleotide-mediated SPDEF modulates TFF3-mediated wound healing and intestinal barrier function during the weaning process. H. I. Jung, S. I. Lee*, and I. H. Kim, *Dankook University, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

Most alterations during weaning involve physiological changes in intestinal structure and function. Here, we evaluated the molecular mechanisms regulating the effects of nucleotides on weaning. Nucleotide treatment induced Trefoil factor 3 (TFF3) expression and IPEC-J2 cell growth and reduced wound width. Treatment with nucleosides and TFF3 in ipopolysaccharide-challenged IPEC-J2 cells increased intestinal transepithelial electrical resistance and decreased intestinal permeability. Additionally, nucleosides improved intestinal barrier function through induction of TFF3-mediated phosphatidylinositol 3-kinase/Akt, extracellular signal-regulated kinase 1/2, p38, and Janus kinase/ signal transducer and activator of transcription signaling pathways. Among selected differentially expressed genes, SAM pointed omain containing ETS transcription factor (SPDEF) expression was elevated by

nucleotides in a concentration-dependent manner. Moreover, SPDEF directly regulated TFF3 expression via binding to the promoter. In vivo, nucleotide supplementation improved growth performance, serum stress levels, and intestinal morphology. Our findings provide insights into the molecular mechanisms of intestinal development during weaning in pigs.

Key Words: nucleotide, weaning

P115 Difructose dianhydride improves intestinal calcium absorption, wound healing, and barrier function. H. I. Jung*, S. I. Lee, and I. H. Kim, *Dankook University, Cheonan-si, Chungcheongnam-do, Republic of Korea.*

The gastrointestinal tract (GIT) is critical for nutrient absorption and is an important barrier against harmful pathogens and antigens. Difructose anhydrides (DFA)-IVs are nondigestible disaccharides that enhance calcium and iron absorption by affecting the intestinal epithelial tissue. However, their effects on intestinal functions are not fully understood. In this study, we performed a feeding trial and found that dietary DFA-IVs improved growth performance, relative breast muscle and liver weight, and digestibility and blood calcium and iron concentrations in broilers. Additionally, dietary DFA-IVs increased expression of genes related to growth in the liver, muscle development, and absorption of calcium and iron in the intestine. In vitro experiments revealed that DFA-IV treatment improved intestinal wound-healing (migration, proliferation, and differentiation) after lipopolysaccharide (LPS) challenge in small intestinal epithelial cells. Furthermore, DFA-IV treatment enhanced the intestinal barrier function, which increased the transepithelial electrical resistance (TEER) and decreased the permeability of fluorescein isothiocyanate-dextran (FD-4) after LPS challenge in small intestinal epithelial cells. Collectively, these data indicate that DFA-IV could be used as a feed additive to enhance calcium and iron absorption by affecting the intestinal wound healing and permeability. This study may help improve our understanding of the molecular effects of DFA-IV on the intestine.

Key Words: difructose dianhydride, lipopolysaccharide, intestinal

P116 Citrus flavonoid supplementation in weanling diets improves pig gut health. D. Solà-Oriol*[1], M. Paniagua[3], M. Saladrigas[1], F. J. Crespo[2], M. Serra[2], and J. F. Pérez[1], [1]*Animal Nutrition and Welfare Service, Department of Animal and Food Science, Universitat Autònoma de Barcelona, Bellaterra, Spain,* [2]*Interquim S.A. (Ferrer HealthTech), Animal & Health Nutrition Division, Barcelona, Spain,* [3]*Quimidroga, Feed Department, Barcelona, Spain.*

Citrus flavonoid supplementation clearly improve piglet performance for the nursery period. It was hypothesized that flavonoid properties may play an important role on antimicrobial, antioxidant and anti-inflammatory function in the gut. A total of 252 male and female 21-d old piglets ([LW x LD] x Pt) were selected at weaning and randomly distributed according to BW (5.6kgSD = 0.86) into 18 pens (9 pens per block of BW and 14 pigs/pen) where 3 treatments were allotted. Treatments for PS (0–14d PW) and ST (15–35d PW) were, PS: T1) basal diet without antimicrobials (ZnO and AB), T2) T1 + ZnO (2.5kg/Tm) + Amoxicillin (300g/Tm) + Apramicin (100g/Tm), T3) T1 + Citrus flavonoids (300g/Tm) + Amoxicillin (300g/Tm); and ST: only T2 was adjusted as T1 + ZnO (1.5kg/Tm) + Amoxicillin (300g/Tm) + Neomicin (188g/Tm) + Tiamulin (100g/Tm). Intestinal tissue and ceacal content were collected on d7 (one pig/pen). An Open Array Real-Time PCR was performed to analyze the mRNA expression of 48 genes in jejunum and ileum samples. Cecal content was sequenced by the bacterial 16S rDNA gene using the Illumina MiSeq® System. Genes related with barrier function (*TFF3, MUC2, MUC13, OCL20*), nutrient transport (*SLC5A1.SGLT1, SLC13A1.NAS1, SLC15A1.PEPT1, SLC39A4.ZIP4*), digestive enzyme/hormones (*DAO*, *SI*, *HNMT*), antioxidant enzyme (GPX2) and digestive hormones (*GCG*) were overexpressed for T3 than T1 and T2 in jejunum ($P < 0.05$). However, at ileum level, barrier function (*TFF3*, *MUC2*), nutrient transport (*SLC5A1.SGLT1, SLC39A4.ZIP4*), antioxidant enzymes (*GPX* and *SOD*) and immune response (*IFNGR1* and *IL8*) genes were under-expressed for T3 and T2 than T1 ($P < 0.05$). Higher relative abundance of the families *Ruminococcaceae, Prevotellaceae*, *Veillonellaceae*, and *Lactobacillae* were observed for the animals fed the T3 than T1 and T2. Beta diversity showed that pigs not treated with antimicrobials and ZnO (T1) showed higher similarity than T3. The improved barrier function, nutrient transport and antioxidant capacity in both jejunum and ileum by flavonoid supplementation as well as beneficial gut microbial environment may explain the enhanced piglet performance by improving intestinal health, supporting a reduction of antimicrobial use.

Key Words: antimicrobial reduction, citrus flavonoids, pig gut health

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