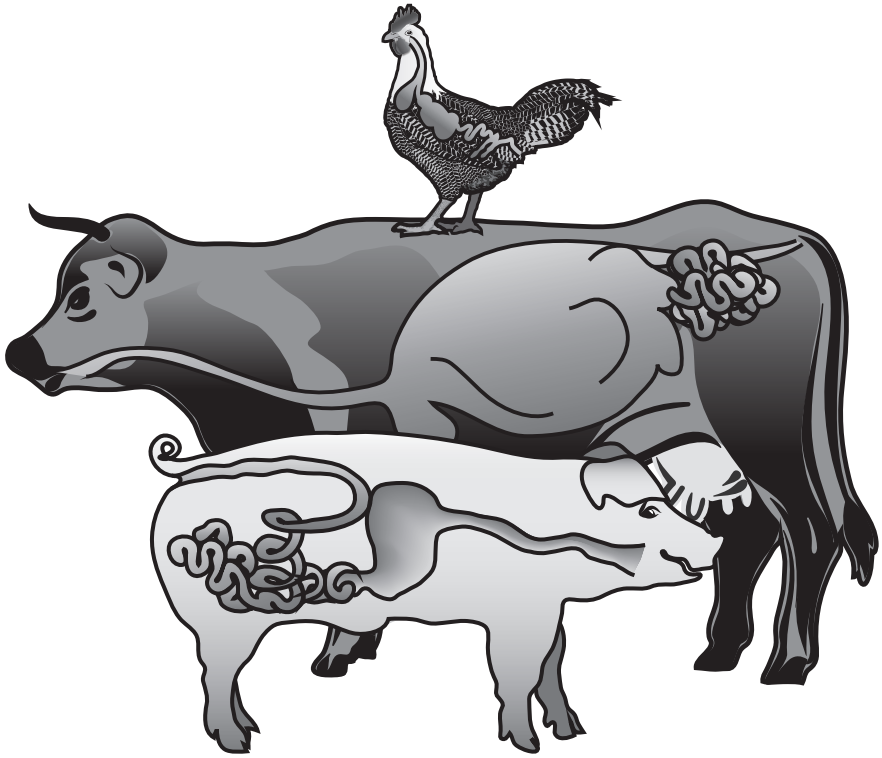
Symposium on Gut Health
in Production of Food Animals
November 11–13, 2013, Kansas City, Missouri



Program and Abstracts
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WELCOME

On behalf of the Organizing Committee for the 2nd Symposium on Gut Health in Production of Food Animals, I welcome you to Kansas City! I hope that the change in venue has made travel easier and less expensive.

Like the first Symposium organized around the topic of gut health in food animals, the aim this year 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 feedback that I received from last year's symposium was overwhelmingly positive. I believe that this is evident by the fact that we had twice as many abstracts submitted this year, covering a broader area of gut health in production animals.

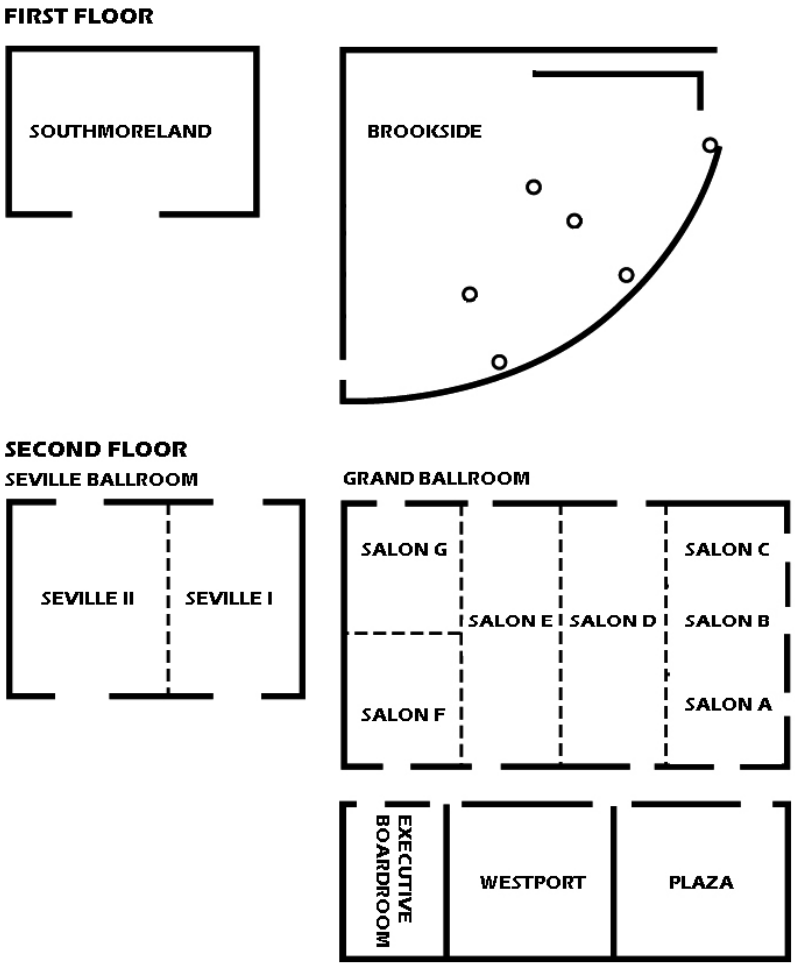
This year we have been fortunate enough to invite three distinguished plenary speakers who will cover current research topics in avian, porcine, and bovine gut health. Please take advantage of the presence of these scientists to engage in productive talks and develop collaborations between different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

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

Welcome again and enjoy the Symposium and your stay in Kansas City!

Mike Kogut
Chair, Organizing Committee

Marriott Kansas City Country Club Plaza



PROGRAM

Monday, November 11

8:00 am — Welcome.
Dr. Mike Kogut, USDA-ARS, Chair, Organizing Committee

SESSION I: IMPACT OF GUT MICROBIAL COMMUNITIES ON GUT HEALTH

Chair: Mike Kogut, USDA-ARS
Grand ABCD

8:15 to 9:15 am — **Invited Presentation:** The bovine gut microbiota: At the interface between nutrition, health, and development.
C. Yeoman, J. Swartz, and M. Lachman, Montana State University, Bozeman, MT, USA.*

9:15 to 9:45 am — Enterotoxigenic *Escherichia coli* induced changes in intestinal functionality in a pig model for early microbiota association.
S. J. Koopmans[1,2], J. Van der Meulen[2], A. Taekema[3], J. P. Lalles[3], and A. J. M. Jansman[2], [1]Wageningen University, Wageningen, the Netherlands, [2]Wageningen UR Livestock Research, Lelystad, the Netherlands, [3]INRA-ADNR, Saint-Gilles, France.*

9:45 to 10:00 am — Coffee break

10:00 to 10:30 am — An examination of the relationship of bacterial and fungal populations within the gastrointestinal tract of poultry.
J. A. Byrd, USDA-ARS, Food and Feed Safety Research Unit, College Station, TX, USA.*

10:30 to 11:15 am — Gastrointestinal microbiota and porcine immunity: Factors that influence *Salmonella* shedding in swine.
S. Bearson[1], S. Knetter[2], H. Allen[1], B. Bearson[3], B. Brunelle[1], T. Huang[2], T. Looft[1], A. Ramer-Tait[4], D. Nettleton[5], T. Stanton[1], M. Wannemuehler[6], and C. Tuggle[2], [1]USDA/ARS/National Animal Disease Center, Ames, IA, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, USA, [3]USDA/ARS/ National Laboratory for Agriculture and the Environment, Ames, IA, USA, [4]Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA, [5]Department of Statistics, Iowa State University, Ames, IA, USA, [6]Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA.*

11:15 to 11:45 am — Comparison of acute dose oral gavage and prolonged dose drinking water administration of dextran sodium sulfate in brolier chicks.
V. A. Kuttappan, E. A. Vicuna, O. B. Faulkner, J. D. Latorre, A. Menconi, A. D. Wolfenden, G. I. Tellez, B. M. Hargis, and L. R. Bielke, University of Arkansas, Fayetteville, Arkansas, USA.*

11:45 am to 12:45 pm — Lunch (boxed lunch provided)

12:45 to 2:30 pm — Poster Presentations: Grand Ballroom Foyer

SESSION II: INTESTINAL BARRIER FUNCTION/HOST-PATHOGEN INTERACTION/DIVERSITY OF MICROBIOME/MUCOSAL IMMUNOBIOLOGY

Chair: Mike Kogut, USDA-ARS
Grand ABCD

2:30 to 3:30 pm — **Invited Presentation:** Porcine intestinal health: Importance of epithelial barrier function, detoxification and cell protection systems.
J. P. Lalles, INRA-ADNC, F35590 Saint-Gilles, France.*

3:30 to 4:15 pm — Recombinant *Clostridium perfringens* α toxin as a potential vaccine against bovine enterotoxemia.
E. Goossens[*1], S. Verherstraeten[1], B. Valgaeren[1], B. Pardon[1], L. Timbermont[1], F. Haesebrouck[1], R. W. Titball[2], R. Ducatelle[1], P. Deprez[1], and F. Van Immerseel[1], [1]Ghent University, Merelbeke, Belgium, [2]University of Exeter, Exeter, United Kingdom.*

4:15 to 4:45 pm — Comparison of chitosan and modified chitosan adjuvants for induction of mucosal immunity with inactivated autogenous vaccines in poultry.
L. R. Bielke, B. M. Hargis, V. A. Kuttappan, E. A. Vicuña, N. R. Pumford, M. J. Morgan, J. D. Latorre, A. D. Wolfenden, A. Menconi, G. I. Tellez, and O. B. Faulkner, University of Arkansas, Fayetteville, AR, USA.*

4:45 to 5:00 pm — Coffee break

5:00 to 5:30 pm — Effects of nutrition and gut barrier function on the development of osteomyelitis complex in poultry.
J. Chen, K. J. Wedekind, J. J. Dibner, and J. D. Richards, Novus International Inc., St Charles, MO, USA.*

5:30 to 6:00 pm — Characterization of intestinal microbiota of the emu (*Dromaius novaehollandiae*).
J. E. Kim[1], H. M. Tun[2], F. C. Leung[2], D. C. Bennett[1], and K. M. Cheng[1], [1]Avian Research Centre, Faculty of Land and Food Systems, The University of British Columbia, Vancouver, British Columbia, Canada, [2]School of Biological Sciences, Faculty of Science, The University of Hong Kong, Hong Kong, SAR, China.*

6:00 to 6:30 pm — Evolution of the pig's gut microbiota depends on the feed presentation.
P. LeBel[*1,2], P. Fravalo[1,2], E. Yergeau[3], B. Laplante[4], and A. Letellier[1,2], 1NSERC Industrial Chair in Meat Safety, St-Hyacinthe, QC, Canada, [2]Groupe de Recherche et Enseignement en Salubrite des Aliments, St-Hyacinthe, QC, Canada, [3]National Research Council Canada, Energy, Mining and Environment, Montreal, QC, Canada, [4]F. Menard, L'Ange-Gardien, QC, Canada.*

6:30 to 8:30 pm — Reception
Main Street Grill

Tuesday, November 12

SESSION III: BENEFICIAL MICROBES AND GUT HEALTH

Chair: Mike Kogut, USDA-ARS
Grand ABCD

8:00 to 9:00 am — **Invited Presentation:** Communication between the microbiota and the gut mucosa to maintain optimal intestinal health: The case of butyrate.
F. Van Immerseel, Ghent University, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases.*

9:00 to 9:30 am — Modulation of intestinal integrity by direct-fed microbial on hepatic energy metabolism and performance of broiler chickens.
G. R. Murugesan[*1,2] and M. E. Persia[2], [1]Biomin America, Inc., San Antonio, TX, USA, [2]Iowa State University, Ames, IA, USA.*

9:30 to 10:00 am — Clostridial enteropathies: Microbes that mitigate the effects.
J. Schleifer, T. Lohrmann, and S. Johnson, Quality Technology International Inc., Elgin, IL, USA.*

10:00 to 10:15 am — Coffee break

10:15 to 10:45 am — Performance and rumen development of Holstein calves dosed with *Megasphaera elsdenii* NCIMB 41125.
M. C. Muya[1], K. A. Miller[3], C. C. Aperce[3], and L. J. Erasmus[2], [1]Agricultural Research Council-API, Irene , South Africa, [2]University of Pretoria, Pretoria, South Africa, [3]MS Biotec, Wamego, KS, USA.*

10:45 to 11:15 am — *Bacillus* producing NSP enzymes in situ increased the amounts of available sugars in feed.
B. K. K. Nielsen[1], J. Nielsen[2], T. Styrishave[1], M. D. Cantor[2], and P. M. F Derkx[2], [1]Chr.Hansen A/S, Animal Health, Hørsholm, Denmark, [2]Chr.Hansen A/S, Strains, Hørsholm, Denmark.*

11:15 to 11:45 am — In vitro enzyme production and viscosity determination by selective *Bacillus* spp. in different poultry diets.
J. D. Latorre[1], R. E. Wolfenden[2], A. Menconi[1], A. D. Wolfenden[1], L. R. Bielke[1], O. B. Faulkner[1], B. M. Hargis[1], and G. Tellez[1], [1]University of Arkansas, Fayetteville, Arkansas, United States, [2]Pacific Vet Group, Fayetteville, AR, USA.*

11:45 am to 12:15 pm — Evaluation of a novel probiotic formulation designed exclusively for neonatal broiler chicks.
M. F. Faulkner, J. D. Lum, J. L. Vicente, and R. E. Wolfenden, Pacific Vet Group, Fayetteville, AR, USA.*

12:15 to 1:15 pm — Lunch (boxed lunch provided)

1:15 to 3:00 pm — Poster Presentations: Grand Ballroom Foyer

SESSION IV: NUTRITION AND GUT HEALTH I

Chair: Mike Kogut, USDA-ARS
Grand ABCD

3:00 to 3:30 pm — Estimated metabolizability of diets fed to lactating dairy cows containing Immunis[3] or yeast culture.
C. A. Old[1], D. R. Daley[2], J. H. Killen[2], and K. M. Scallon[2], [1]Antelope Ranch, LeGrand, CA, USA, [2]Enz-A-Bac Advanced Products, Twin Falls, ID, USA.*

3:30 to 4:00 pm — Influence of diet's physical form (feed grinding/compaction) on colonisation and spreading of *Salmonella* Enteritidis in experimentally infected broiler chicks.
C. Ratert[1], S. J. Sander[1], J. Verspohl[2], and J. Kamphues[1], [1]Institut of Animal Nutrition, University of Veterinary Medicine Hannover - Foundation, Hannover, Germany, [2]Institute of Microbiology, University of Veterinary Medicine Hannover - Foundation, Hannover, Germany.*

4:00 to 4:30 pm — Effects of a *Lawsonia intracellularis* infection in young vaccinated and non-vaccinated pigs on the total-tract digestibility of nutrients and performance?
C. F. Visscher[1], J. Mischok[2], S. J. Sander[2], and J. Kamphues[2], [1]Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany, [2]Institute for Animal Nutrition, University of Veterinary Medicine Hannover, Hannover, Germany.*

4:30 to 5:00 pm — The effect of quaternary benzophenantridic alkaloids on *Salmonella* isolation, expression of CD3+ and goblet cells in the intestinal mucosa, blood immune cells and TER.
E. Santin[1], W. A. Gebreyes[2], B. A. King[3], and A. V. Vucskits[4], [1]Laboratory of Microbiology and Ornitopathology, Federal University of Paraná, Curitiba, Parana, Brazil, [2]College of Veterinary Medicine, Colombus, OH, USA, [3]Phytobiotics USA, Wentzville, MO, USA, [4]Phytobiotics Hungary, Budapest, Hungary.*

5:00 to 5:30 pm — Use of scanning electron microscopy to evaluate intestinal villi morphology in poultry fed butyric acid, mannan-oligosaccharide, and antibiotic feed additives.
R. D. Malheiros and P. R. Ferket, Prestage Departments of Poultry Science, NCSU, Raleigh, NC, USA.*

5:30 to 7:30 pm — Reception
Main Street Grill

Wednesday, November 13

SESSION V: NUTRITION AND GUT HEALTH II

Chair: Mike Kogut, USDA-ARS
Grand ABCD

8:15 to 8:45 am — Precision-delivery coated butyrate to control *Campylobacter* in poultry.
T. Goossens[1], M. Marien[1], J. Pierce[2], A. De Cesare[3], and G. Manfreda[3], [1]Nutriad, NV, Dendermonde, Belgium, [2]Nutriad Inc., Elgin, IL, USA, [3]Department of Agriculture and Food Sciences, University of Bologna, Bologna, Italy.*

8:45 to 9:15 am — Butyrate supplementation affects mRNA abundance of genes involved in glycolysis, lipogenesis and oxidative phosphorylation in the rumen epithelium of Holstein dairy cows.
A. H. Laarman[1], L. Dionissopoulos[1], O. AlZahal[1], M. A. Steele[1], J. C. Matthews[2], and B. W. McBride[1], [1]University of Guelph, Guelph, ON, Canada, [2]University of Kentucky, Lexington, KY, USA.*

9:15 to 9:45 am — Effect of a liquid whole-egg globulin protein supplement on broiler performance, intestinal histology, and bacitracin-resistant *Clostridium* growth.
M. Frank[1], M. Foy[1], T. Clark[1], P. Maharjan[1], J. Lutes[1], D. Zehendner[2], and S. Watkins[1], [1]University of Arkansas, Fayetteville, AR, USA, [2]R&D Life Sciences, Menomonie, WI, USA.*

9:45 to 10:15 am — Business: Next Meeting?
Grand ABCD

POSTER PRESENTATIONS
GRAND BALLROOM FOYER

P100 Comparative functional analysis of porcine-derived *Lactobacillus amylovorus* strains and the role of their S-layer proteins as adhesins.
U. Hynönen, R. Kant, S. Åvall-Jääskeläinen, and A. Palva, University of Helsinki, Faculty of Veterinary Medicine, Department of Veterinary Biosciences, Division of Microbiology and Epidemiology, Helsinki, Finland.*

P101 Effect of the human intestinal microbiota and *Bacteroides thetaiotaomicron* on *Escherichia coli* O157:H7 transcriptome: Multiple aspects of EHEC adaptation.
G. Le Bihan[1], P. Garneau[1], F. Beaudry[2], A. Bernalier-Donadille[3], C. Martin[3], G. Jubelin[3], and J. Harel[1], [1]Université de Montréal, Faculté de Médecine Vétérinaire, St-Hyacinthe, Québec, Canada, [2]Groupe de Recherche en Pharmacologie Animal du Québec (GREPAQ), Département de Biomédecine Vétérinaire, Faculté de Médecine Vétérinaire, Université de Montréal, St-Hyacinthe, Québec, Canada, [3]INRA, UR454 Unité de Microbiologie, St-Genes Champanelle, France.*

P102 Field evaluation of the live microbial product Calsporin on health, performance and carcass quality of finisher pigs.
T. Marubashi[1], S. Kritas[2], G. Filioussis[2], K. Papageorgiou[2], A. Govaris[3], D. Valoumas[4], and E. McCartney[5], [1]Calpis Co., Ltd, Japan, [2]Veterinary Faculty, Aristotle University of Thessaloniki, Greece, [3]Veterinary Faculty, University of Thessaly, Greece, [4]Creta Farm, Greece, [5]Pen & Tec Consulting, Spain.*

P102 Field evaluation of the live microbial product Calsporin on health, performance and carcass quality of finisher pigs.
T. Marubashi[1], S. Kritas[2], G. Filioussis[2], K. Papageorgiou[2], A. Govaris[3], D. Valoumas[4], and E. McCartney[5], [1]Calpis Co., Ltd, Japan, [2]Veterinary Faculty, Aristotle University of Thessaloniki, Greece, [3]Veterinary Faculty, University of Thessaly, Greece, [4]Creta Farm, Greece, [5]Pen & Tec Consulting, Spain.*

P103 Effect of *Saccharomyces cerevisiae* on ruminal microbial community during subacute ruminal acidosis.
O. AlZahal[1], L. Dionissopoulos[1], N. D. Walker[2], and B. W. McBride[1], [1]University of Guelph, Guelph, Ontario, Canada, [2]AB Vista, United Kingdom, Marlborough, United Kingdom.*

P104 Effect of dietary changes during broiler grow-out on gut microbiota composition, mucosal immunity, and intestinal barrier function.
M. H. Kogut[1], J. A. Byrd[1], K. J. Genovese[1], and B. Oakley[2], [1]USDA-ARS, Southern Plains Agricultural Research Center, College Station, TX, USA, [2]USDA-ARS, Richard B. Russell Agricultural Research Center, Athens, GA, USA.*

P105 Regulatory processes for prebiotics and microbial products used in animal food.
A. I. Orr, M. Alewynse, and S. Benz, US Food and Drug Administration Center for Veterinary Medicine, Rockville, MD, USA.*

P106 Evaluation of dextran sodium sulfate and xanthophyll pigment absortion for developing a gut inflammation model in broiler chickens.
E. A. Vicuna[1], X. Hernandez[2], A. Menconi[1], V. A. Kuttappan[1], J. D. Latorre[1], O. B. Faulkner[1], A. D. Wolfenden[1], G. I. Tellez[1], B. M. Hargis[1], and L. R. Bielke[1], [1]University of Arkansas, Fayetteville, AR, USA, [2]Universidad Nacional Autonoma de Mexico, Mexico D.F., Mexico.*

P107 AvrA^{-} *Salmonella* Typhimurium displays differential infection characteristics and altered patterns of host signaling in the gut and liver.
R. J. Arsenault[1], H. Wu[2], A. S. Neish[2], and M. H. Kogut[1], [1]United States Department of Agriculture, College Station, TX, USA, [2]Emory University School of Medicine, Atlanta, GA, USA.*

P108 The concerted action of *Clostridium perfringens* perfringolysin with α toxin to induce necrohemorrhagic enteritis in calves.
S. Verherstraeten, E. Goossens, B. Valgaeren, B. Pardon, L. Timbermont, S. Schauvliege, F. Haesebrouck, R. Ducatelle, P. Deprez, and F. Van Immerseel, Ghent University, Merelbeke, Oost-Vlaanderen, Belgium.*

P110 Heat stress: Intestinal barrier and immune disruption in pigs.
M. Bandrick[1], B. E. Bass[2], J. W. Frank[2], T. Looft[1], H. K. Allen[1], T. Casey[1], and T. B. Stanton[1], [1]USDA National Animal Disease Center, Ames, IA, USA, [2]Diamond V, Cedar Rapids, IA, USA.*

P111 High and low loads of cecal colonization by *Salmonella* Enteritidis in chickens triggers distinct immune kinome profiles.
C. L. Swaggerty, R. J. Arsenault, and M. H. Kogut, USDA/ARS, College Station, TX, USA.*

P114 Effect of dietary fructooligosaccharides supplementation on intestinal calcium and phosphorus transporter, ileal cytokine gene expression and apparent phosphorus digestibility of broiler chicks.
Y. Shang[1], A. Hunde[1], J. H. Kim[2], and W. K. Kim[1,3], [1]Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada, [2]Poultry Science Division, National Institute of Animal Science, Rural Development Administration, Chungnam, Republic of Korea, [3]Department of Poultry Science, University of Georgia, Athens, GA, USA.*

P116 Effect of a liquid whole-egg globulin protein supplement on broiler performance, intestinal histology, and bacitracin-resistant *Clostridium* growth.
M. Frank[1], M. Foy[1], T. Clark[1], P. Maharjan[1], J. Lutes[1], D. Zehendner[2], and S. Watkins[1], [1]University of Arkansas, Fayetteville, AR, USA, [2]R&D Life Sciences, Menomonie, WI, USA.*

P120 In vitro binding of lipopolysaccharide by processed calcium montmorillonite (Calibrin-Z).
E. D. De Boer, F. Chi, R. L. Cravens, S. Ching, and R. G. Goss, Amlan International, Chicago, IL, USA.*

NOTES

Session I: Impact of Gut Microbial Communities on Gut Health

100 The bovine gut microbiota: At the interface between nutrition, health, and development.

C. Yeoman*, J. Swartz, and M. Lachman,
Montana State University, Bozeman, MT, USA.

The gastrointestinal tract (GIT) microbiota of production animals are now firmly established as a key feature underscoring the animals health, development, and productivity. In particular, early gut colonization is of critical importance to the morphological and immunological development of the GIT, development of a functional fermentative environment, and providing neonatal resistance to pathogenic challenge. While perturbations of an animal's GIT microbiome at any age can have profound consequences, perturbations during early GIT development can be particularly severe and result in significant and long-lasting sequelae. As the GIT microbiome matures it exhibits significant diversity, a feature that appears to be an important indicator of ecosystem health. Recognition of the immense importance of the GIT microbiota to the host has led to the development of probiotic and prebiotic feedstuffs that strive to ensure animal health.

Key Words: microbiota, gut health, neonatal development

101 Enterotoxigenic *Escherichia coli*-induced changes in intestinal functionality in a pig model for early microbiota association.

S. J. Koopmans[1,2], J. Van der Meulen[2], A. Taekema[3], J. P. Lalles*[3], and A. J. M. Jansman[2],
[1]Wageningen University, Wageningen, the Netherlands, [2]Wageningen UR Livestock Research, Lelystad, the Netherlands, [3]INRA-ADNR, Saint-Gilles, France.

Neonatal microbiota diversity may affect intestinal functionality in later life. The aim of the present study was to investigate whether a neonatal complex microbiota is more effective than a simple microbiota in reducing the effects of an enterotoxigenic *Escherichia coli* (ETEC) challenge in later life. In addition, the effect of a diet enriched or not with medium chain triglycerides (MCT) was investigated as an approach to modify intestinal microbial colonization. Twenty four caesarean-derived piglets were administered orally at day 2 and 3 a simple microbiota (SM, n = 12) consisting of 10^6 to 10^7 cfu from each *Lactobacillus amylovorus*, *Clostridium glycolium* and *Parabacteroides* sp. Half of the piglets were administered additionally a fecal suspension (complex microbiota) from an unrelated sow at day 4 (CM, n = 12). From d 4 onward, half of the SM and CM piglets received a diet enriched with MCT (coconut oil), the other half received a diet without MCT (palm plus soyabean oil). The piglets were studied at 4 to 5 weeks of age using the "in situ" small intestinal perfusion technique. Two jejunal and two ileal segments per piglet were perfused with saline for 8 h with or without ETEC (5×10^9 cfu). Fluorescein sodium salt (NaF, 20 mg/kg BW) was injected intravenously 4 h after the start of perfusion as marker for intestinal permeability. Perfusion fluid leaving the intestinal segments was collected for 8 h and analyzed for NaF and intestinal alkaline phosphatase (IAP). Body weights were similar among treatment groups (8.3 ± 1.2 kg). ETEC perfusion caused a 5-fold reduction ($P < 0.001$) in net fluid absorption (μL/cm^2), a 50% increase ($P < 0.001$) in NaF permeability (ng/cm^2) and a 33% reduction ($P < 0.02$) in IAP concentration (μg/mg of protein). Neither neonatal microbiota association nor dietary MCT significantly affected gut net fluid absorption or permeability. Piglets expressing a complex neonatal microbiota, however, showed 2-fold higher IAP concentrations in the ileum ($P < 0.02$) but no change in the jejunum compared to piglets associated with a simple microbiota at young age. In conclusion, intestinal ETEC infection has profound effects on gut fluid homeostasis (induction of diarrhea), on gut barrier function (increase in permeability) and on IAP concentrations (decrease in detoxification capacity of bacterial LPS). The ability of neonatal microbiota association and dietary MCT to modulate severe physiological effects of ETEC in piglets of 4 to 5 wk of age is limited. Association with a complex microbiota in the neonatal phase, however, may enhance the capacity of 4- to 5-wk-old piglets to detoxify bacterial LPS in the ileum.

Key Words: gut functionality, neonatal microbiota diversity, enterotoxigenic *Escherichia coli*

102 An examination of the relationship of bacterial and fungal populations within the gastrointestinal tract of poultry.

J. A. Byrd*,
USDA-ARS, Food and Feed Safety Research Unit, College Station, TX, USA.

Fungus and yeast are consistent members of animal microflora that are poorly understood as related to the production of poultry. Fungus, like bacteria in the past, has been associated with the onset of disease. Little attention has been given to the beneficial effects of fungi (and particularly various yeasts) with regard to food safety and especially with the gastrointestinal tracts of food-producing animals. The goal of the present study was to record changes in fungi recovered from commercial poultry gastrointestinal tracts. Over 3000 broiler gastrointestinal samples were isolated and over 680 samples were further characterized using an automated repetitive sequence-based PCR (rep-PCR) methodology to track fungal genera changes during successive grow-outs. More than 24 different fungal and yeast genera were identified using rep-PCR including *Rhizopus*, *Aspergillus*, *Penicillium*, and *Fusarium*. The results from the present study will overall provide a normal fungi background genera under commercial conditions, relate these fungi to foodborne pathogens, and will be a stepping stone for investigating the effect of fungi on the gastrointestinal tract and overall health of poultry.

Key Words: poultry, fungi, pathogen

103 Gastrointestinal microbiota and porcine immunity: Factors that influence salmonella shedding in swine.

S. Bearson*[1], S. Knetter[2], H. Allen[1], B. Bearson[3], B. Brunelle[1], T. Huang[2], T. Looft[1], A. Ramer-Tait[4], D. Nettleton[5], T. Stanton[1], M. Wannemuehler[6], and C. Tuggle[2],
[1]USDA/ARS/National Animal Disease Center, Ames, IA, USA, [2]Department of Animal Science, Iowa State University, Ames, IA, USA, [3]USDA/ARS/National Laboratory for Agriculture and the Environment, Ames, IA, USA,

[4]Department of Food Science and Technology, University of Nebraska-Lincoln, Lincoln, NE, USA, [5]Department of Statistics, Iowa State University, Ames, IA, USA, [6]Department of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA.

Pigs are often asymptomatically colonized with the human foodborne pathogen *Salmonella* and can exhibit notable variation in severity and duration of *Salmonella* fecal shedding. Multiple factors affect the dynamics of *Salmonella* in swine, including features of the microorganism, responses from the pig, and contributions from the gastrointestinal microbiota. To characterize the immune capacity and intestinal microbiota in swine before and after *Salmonella* challenge, 54 cohabitating, crossbred pigs from multiple litters were intranasally inoculated with an equal dose of *Salmonella enterica* serovar Typhimurium (1×10^9 cfu); non-inoculated (NI) littermates were housed separately. Bacterial quantitation of *Salmonella* in swine feces over the 21-d study was performed, and the cumulative AULC was calculated to classify 8 low shedder (LS) and 8 persistent shedder (PS) pigs. Serum cytokine analysis and transcriptional profiling of blood RNA from the 2 shedding groups detected distinct immune responses within 2 d post-challenge: elevated serum IL-1β, TNF-α, and IFN-γ was observed in the PS pigs compared with LS pigs, while LS pigs had greater serum CXCL8 than PS pigs. PS pigs upregulated genes involved with STAT1, IFNB1 and IFNG networks, while upregulation of genes involved in immune response regulation were detected only in LS pigs. Furthermore, an ex vivo endotoxin stimulation assay of whole blood from the PS and LS pigs revealed an attenuated inflammatory response in the LS pigs. To examine the influence of the gastrointestinal microbiota on *Salmonella* shedding, the microbial communities were evaluated using a smaller subset of the extreme shedding groups (n = 5 pigs/group) as well as the NI control group. Total fecal DNA was isolated for 16S rRNA gene sequencing at 0, 2, 7 and 21 d post-challenge. Prior to inoculating the pigs with *Salmonella*, "will-be" LS pigs and "will-be" PS pigs had differences in their microbiota, including a higher abundance of the *Ruminococcaceae* family in the "will-be" LS pigs. Following *Salmonella* challenge, PS pigs had dramatic changes in their microbial communities compared with LS pigs; no differences were detected between LS and NI pigs. However, by 21 d post-challenge, the microbiota communities of LS and PS pigs were no longer different from one another, but were both different from NI pigs, suggesting that introducing *S.* Typhimurium into the porcine gastrointestinal tract altered maturation of the microbiota regardless of shedding status. The data indicate significant correlations between *Salmonella* shedding in pigs with both the porcine immune response as well as shifts in the gastrointestinal microbiota, thereby broadening our appreciation of the complex host-microbe-microbiota relationship for this important food safety and public health issue.

Key Words: gastrointestinal microbiota, porcine immune response, *Salmonella*

104 Comparison of acute dose oral gavage and prolonged dose drinking water administration of dextran sodium sulfate in broiler chicks.

V. A. Kuttappan*, E. A. Vicuna, O. B. Faulkner, J. D. Latorre, A. Menconi, A. D. Wolfenden, G. I. Tellez, B. M. Hargis, and L. R. Bielke,
University of Arkansas, Fayetteville, AR, USA.

Antibiotics, prebiotics, and probiotics are known to improve the growth performance of poultry. Even though the exact growth enhancing mechanisms are not yet well defined, many researchers suggest effects may be due to anti-inflammatory influences on the gastrointestinal tract. Therefore, development of an enteric inflammation model and permeability indicators for poultry could provide a means to explore various markers of inflammation and different methods to reduce such inflammation for improved growth performances. Dextran sodium sulfate (DSS) is a chemical which has been successfully used in murine models to induce both acute and chronic gut inflammation. The present study compared the effects of acute dose by oral gavage (ADOG) and prolonged dose drinking water (PDDW) administration of DSS in broiler chicks. At 3 d of age, birds were randomly assigned to either an ADOG group (control-no DSS, 0.45 g, 0.67 g, or 1 g of DSS/day/bird), or PDDW group (control, 1% and or 1.25% of DSS in drinking water). Body weight gain and mortality was recorded throughout the study. ADOG groups were administered the respective dose of DSS for 2 consecutive days (3d and 4d of age) and evaluated on 4d of age because birds given higher concentration of DSS began showing severe clinical signs and high mortality. PDDW group was provided the respective dose of DSS in drinking water for 3 consecutive days and processed on 6d of age. Both ADOG and PDDW groups were given an oral gavage of fluorescein isothiocyanate (FITC) dextran approximately 2.5 h before termination and blood samples were collected for plasma FITC measurement. Results showed an increase in mortality of birds as the dose of DSS increased in both ADOG and PDDW treatments. Furthermore, there was a decreasing trend in BWG as concentration of DSS increased in ADOG administration. In PDDW group, 1.25% group had lower ($P < 0.05$) BWG than control or 1% DSS. Plasma FITC levels showed an increasing trend with respect to increase in concentration of DSS. Both PDDW DSS groups had marginally higher plasma FITC when compared with their respective control. In conclusion, administration of ADOG at 0.45g DSS/day/bird could be an appropriate dose to induce inflammation because it resulted in reduced BWG and lower mortality compared with other groups. Plasma levels of FITC may not be a dependable marker, especially with lower doses of DSS and future experiments will investigate alternative markers of enteric inflammation and permeability.

Key Words: gut inflammation and permeability, dextran sodium sulfate, broiler chicks

Session II: Intestinal Barrier Function/Host-Pathogen Interaction/ Diversity of Microbiome/Mucosal Immunobiology

105 Porcine intestinal health: Importance of epithelial barrier function, detoxification, and cell protection systems.

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Monitoring of gut health is of utmost importance in animals and man. In pigs, gut barrier which is primarily made of enterocytes and colonocytes is sensitive to diet composition and level of feed intake. Also rearing conditions such as weaning, handling and transportation generate stress, all affecting gut barrier function through neuro-immune pathways. These include both central and enteric nervous systems, mast cells and various mediators acting upstream (e.g., CRF) and on (e.g., NGF) epithelial cell monolayer. As a result, trans- and/or paracellular permeability are altered at the levels of endocytotic processes and/or tight junction protein complexes, respectively. Furthermore, epithelial cells almost exclusively express inducible heat shock proteins (e.g., HSP27 and HSP70) that contribute to epithelial cell protection. Inducible HSPs control cellular protein quality and trafficking, integrity and functionality of cytoskeleton and tight junctions, and downregulate inflammation pathways. Inducible HSP expression level is mainly set by the microbiota and is modulated in turn by specific dietary components or nutrients (e.g., glutamine), but also toxic compounds and stress. The gut is in close contact with the microbiota and is exposed to both beneficial (e.g., lactobacilli, bifidobacteria) and potentially pathogenic (e.g., *Escherichia coli*) bacteria which send molecular signals to the host. Gut epithelial cells have developed detoxification systems to keep unwanted (e.g., pro-inflammatory) components outside the body and inflammation under tight control. One such system is intestinal alkaline phosphatase (IAP). This highly conserved membrane and intra-cellular enzyme detoxifies (by dephosphorylation) pro-inflammatory bacterial components (e.g., lipopolysaccharide, PS) and downregulates intestinal NFκB inflammatory pathway. If deleterious bacterial signals escape host control at the gut level, then they induce so-called chronic low-grade inflammation which in turn depresses IAP expression and activity, favors metabolic disorders and ultimately reduces feed utilization efficiency. Finally, an emerging concept in gut health is the early programming of selected functions that could affect gut (and body) homeostasis later in life. Recent data suggest an imprinting of IAP and maybe HSPs in pigs. Importantly, early malnutrition and stress may participate in gut programming, directly (e.g., dietary methyl donors) and indirectly through altering the nervous system and/or gut microbiota composition or functionality.

Key Words: gut barrier, heat shock protein, intestinal alkaline phosphatase

106 Recombinant *Clostridium perfringens* α toxin as a potential vaccine against bovine enterotoxemia.

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Clostridium perfringens type A has been shown to be the causative agent of a wide variety of enteric diseases in humans and animals, including bovine enterotoxemia. Enterotoxemia is a sudden death syndrome with necro-hemorrhagic lesions in the small intestine, which mainly affects suckling calves and veal calves [Muylaert et al., 2010 Vet. Rec. 167:64–65]. Predominantly veal calves of beef cattle breeds are affected, and losses due to enterotoxemia may be responsible for up to 20% of total mortality [Lebrun et al., 2010 Vet. Rec. 167:13–22, Pardon et al., 2012 BMC Vet. Res. 8:26]. Recently, α toxin has been proposed as an essential factor for induction of enterotoxemia in veal calves, which introduces the possibility to use it as a vaccine [Verherstraeten et al., 2013 Vet. Res. 44:45]. The use of only the main toxin instead of the whole arsenal of extracellular toxins and enzymes eliminates irrelevant or even immunosuppressive components and therefore may induce a stronger, protective immune response. In this study, we have compared a commercial multivalent vaccine with native α toxin, formalin-inactivated α toxin, and recombinant C-terminal domain of α toxin as vaccine candidates. The native and recombinant α toxin were capable of stimulating higher levels of immune responses compared with the formalin inactivated α toxin and the multivalent vaccine. In addition, the sera from calves immunized with the native and recombinant α toxin also showed higher inhibitory activity against the α toxin in a bioassay. These results suggest that the recombinant α toxin is a potential vaccine candidate against bovine enterotoxemia.

Key Words: bovine enterotoxemia, vaccination, recombinant α toxin

107 Comparison of chitosan and modified chitosan adjuvants for induction of mucosal immunity with inactivated autogenous vaccines in poultry.

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Mucosal immunity is a crucial component of the humoral immune response for prevention of infection, and has been traditionally induced only through live-attenuated or recombinant vaccination. However, for autogenous vaccines, attenuation or recombination is not often a practical strategy, largely forcing producers to either inject vaccines at day of hatch and rely on systemic IgG production to prevent disease, or accept sub-optimal protection through killed orally administered vaccination. Chitosan and modified chitosan adjuvants, with inactivated bacterins, were evaluated for stimulation of mucosal immunity through combinations of parenteral and oral administration. In Exp. 1, chitosan and chitosan plus alum were administered with *Clostridium septicum* bacterin by subcutaneous injection (SQ) on day of hatch to poults. At 14 d, 0.5% chitosan injected birds had significantly ($P \leq 0.05$) higher specific IgG titers than the alum combination and controls, which is consistent with field evaluations of a protective effect. Chitosan and modified chitosan (MCA) with killed *Bordetella avium* were compared in poults by SQ on day of hatch, followed by drinking water (DW) boost on d 14 in Exp, 2. MCA vaccinated poults had significantly higher IgG titers with MCA vaccination

than both controls and chitosan vaccinated birds. Additionally, poults vaccinated with MCA-*B. avium* by SQ/DW combination responded with similar specific IgA titers as poults vaccinated with a SQ/SQ combination (Exp. 3). Chitosan and MCA were further evaluated in broilers with killed *Salmonella* by SQ on day of hatch, followed by oral gavage on d 12 in Exp, 4. While chitosan significantly increased specific IgG and IgA response, MCA vaccinated chicks exhibited further increased titers. A subsequent study (Exp, 5) showed such increased titers correlated with decreased recovery after *S.* Enteritidis challenge in broilers. Likewise, in Exp, 6, DW primary and boost vaccination with MCA resulted in decreased, but not significantly different, specific IgA titers when compared with SQ/DW combination. Furthermore, vaccination followed by autogenous challenge with *S.* Heidelberg in poults was moderately improved with MCA combinations when compared with identical chitosan vaccinations in Exp, 6. Taken together, these studies show that certain modifications to chitosan can improve killed bacterin oral vaccination in poultry. The improved IgA responses following oral vaccination suggest that actual protection of flocks from infection may prove to at least be equal to parenteral administration for pathogens with a mucosal portal of entry.

Key Words: mucosal immunity, adjuvants, vaccine

108 Effects of nutrition and gut barrier function on the development of osteomyelitis complex in poultry.

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Structural integrity issues, including lameness, are potential consequences of poor gut health that are often overlooked. Lameness can manifest itself in several ways, including bacterial chondronecrosis with osteomyelitis (BCO), turkey osteomyelitis complex (TOC), and other structural defects such as synovitis, tibial dyschondroplasia (TD), broken bones and tendons. BCO and TOC are among the most common forms of lameness and are associated with opportunistic bacterial infections in the proximal long bones, often occurring in microfractures in the bone. Some of the bacterial species identified from BCO and TOC lesions originate in the gut, indicating that the diseases are associated with gut barrier failure. One strategy to reduce incidence of BCO is to improve gut health via probiotics, presumably by enhancing barrier function. Depending on the nature of the barrier failure, additional nutritional interventions for BCO and TOC could include trace minerals. A model to reliably induce BCO and lameness in broilers has been developed (Wideman et al., 2012). In this model, broilers are raised at relatively low densities in large wire flooring pens with water and feed at opposite ends, allowing normal levels of activity. This wire flooring model allows researchers to investigate the etiology, pathology, prevention and intervention strategies of BCO and lameness in broilers. We used this model to test the efficacy of probiotics and chelated trace minerals in reducing BCO symptoms including femoral and tibial head lesions. We found that Sporulin (Pacific Vet Group-USA Inc.) a direct-fed *Bacillus* spore-based probiotic, reduced femoral and tibial head lesions in one study, and Mintrex (Novus International Inc.) chelated minerals decreased femoral head lesions in another study. In a separate field trial, feeding turkeys with the same chelated minerals reduced the incidence of synovitis and TD by 51% and 44%, respectively, in birds exhibiting TOC. Synovitis was correlated with lameness. TD has been reported to be associated with TOC. The combination of Sporulin and Mintrex is a potential intervention strategy to reduce BCO and TOC in the poultry industry.

Key Words: bacterial chondronecrosis with osteomyelitis (BCO), probiotics, chelated minerals

109 Characterization of intestinal microbiota of the emu (*Dromaius novaehollandiae).*

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Emus (*Dromaius novaehollandiae*), large flightless ratites native to Australia, are farmed for their fat and meat. They are omnivorous, feeding on a wide variety of plants and insects, preferring high quality items in which nutrients are concentrated. They have a simple gastrointestinal (GI) tract and a short digesta retention time, but little is known about GI microbial diversity. In this study, we evaluated the phylogenetic diversity of the intestinal microbiome of 4 adult emus (2 males, 2 females) fed a barley-alfalfa-canola based diet, using 454 pyrotag sequencing after amplification for v3-v5 region of bacterial 16s rRNA gene. After quality trimming, a total of 165,585 sequence reads were obtained from the small intestine (duodenum, jejunum, ileum), and 69,194 sequence reads were obtained from the ceca. A total of 700 operational taxonomic units (OTU) belonging to 19 bacterial phyla were identified in the small intestine, while 822 OTU belonging to 9 bacterial phyla were identified in the ceca. The most predominant bacterial phyla were Firmicutes (14–99% of total classified diversity) and Proteobacteria (0.5–76%) in the small intestine, and Bacteroidetes (48–61%) and Proteobacteria (5–44%) in the ceca. OTUs common to the small intestine of all 4 emus ranged from 2 to 14 depending on the section. Two of these OTUs, belonging to *Escherichia* and Sinobacteraceae, were common to all 3 sections of the small intestine. In contrast, 100 OTUs were common to all 4 ceca samples. The 4 most abundant were identified as members of the *Bacteroidetes*, *Escherichia*, and *Fusobacterium* genera. This study is the first to characterize the microbiota of different compartments of the emu gastrointestinal tract. The results indicate the emu's ceca have a higher microbial richness and diversity than that of the small intestine.

Key Words: emu, intestinal microbiota, pyrosequencing

110 Evolution of the pig's gut microbiota depends on the feed presentation.

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If many studies have proven that a modification in the pig feed composition can alter its gut microbiota, the effect of feed

presentation is still largely to be determined. A previous study (LeBel et al., 2013) demonstrated that after 21 d of specific diet (varying only by their presentation) fecal bacteria (as many as 65 different genera) differ in proportion between the different diets. But to demonstrate the potential of the feed presentation to increase (or decrease) the presence of a given bacterial genus, a specific analyze of the evolution of the gut microbiota components must be made. To do so, 96 pigs were assigned a specific diet (24/diet) of the same composition varying only by their particle size (500 or 1250 µm) and/or texture (pellet or mash feed). Feces samples were taken at d 0 and 21. Non-specific PCR amplifications of the 16S gene from a DNA extraction of the feces were analyzed by an Ion-Torrent Semiconductor Sequencer (Illumina) and sequences were attributed using Ribosomal Database Project Pipeline. The proportion of the microbiota that represent a given bacterial genus obtained at d 0 was subtracted to the same data obtained at d 21 to measure the evolution of a given genus from one date to the other. For more than 30 different genera the evolution of the given genus was significantly different from one diet to the other between the 2 dates. While many genus containing potential pathogens seem to be advantaged ($P < 0.05$) by a small particle size (500 µm) and/or a pellet texture (for example: *Corynebacterium*, *Salmonella*, or *Treponema*) some, such as *Clostridium* cluster I, seem to be advantaged by a larger particle size (1250 µm) and a mash texture. It must be noted that many bacteria of the *Clostridium* genus are short-chain fatty acid producers which is reported to provide benefits to the animal by inhibiting some potential intestinal pathogens. Also, some of the reputed good gut health promoting bacteria genus (e.g., *Bifidobacterium* or *Lactobacillus*) have shown a significantly greater evolution ($P < 0.05$) in the pigs feed large particle size and/or mash feed. This study demonstrated that feed presentation alone is able to modify piglet's fecal microbiota, analyses indicated that mash or large particle size feed would promote a healthier microbiota.

Key Words: mash, pig, microbiota

Session III: Beneficial Microbes and Gut Health

200 Communication between the microbiota and the gut mucosa to maintain optimal intestinal health: The case of butyrate.

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In recent years, there has been a change in the types of diseases affecting animals. Due to restrictions on the use of antibiotics in the European Union, and a general attitude of limiting antibiotic usage worldwide, diseases caused by gastrointestinal pathogens such as *Clostridium perfringens* emerged and aspecific multifactorial gut disorders became dominant. An example of the latter is the so-called dysbiosis, a condition in which the altered composition of the gut microbiota, often induced by nutritional changes, leads to changes in the gut wall, including morphological changes and inflammatory reactions, ultimately interfering with digestive processes, leading to poor performance. In some specific inflammatory gastrointestinal disorders characterized by a dysbiosis, a lack of butyrate producing bacteria from the Firmicute Clostridial clusters IV and XIVa are a hallmark. These bacterial species have, due to the production of butyrate and possibly other metabolites, anti-inflammatory effects and are able to restore the intestinal integrity losses caused by toxic insults on the gut mucosa. While this has been well-studied in mice models and in humans, data start being generated on the isolation of these bacteria in animals, and on the interaction between these bacterial species with other bacteria and the host. While currently butyrate is added to animal feed to improve gut health, novel strategies are being exploited to ensure stable high concentrations of butyrate in the animal gut. As an example, administration of butyrate producing species is an option, as well as steering the gut microbiota toward a butyrate producing one using nutritional strategies. These methods have already been demonstrated to decrease gastrointestinal pathogen (e.g., *Salmonella*) colonization, to decrease lesion induction by pathogens (e.g., necrotic enteritis), and to improve animal performance by beneficially affecting gut morphology and functionality.

Key Words: butyrate, inflammation, intestinal integrity

201 Modulation of intestinal integrity by direct-fed microbial on hepatic energy metabolism and performance of broiler chickens.

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The removal of antibiotic growth promoters from poultry diets in Europe due to regulatory restrictions and recent moves toward reduction of these compounds in North American poultry diets have put tremendous pressure on the poultry industry to look for viable alternatives. In this context, direct-fed microbial (DFM) organisms have been explored to improve gut health and there by performance. Two experiments were conducted in broiler chickens by feeding different formulations of DFM to examine their effects on intestinal epithelial barrier function, hepatic energy metabolism and performance. Two treatment groups of Ross-308 broiler chicks were fed a corn-soybean meal based control (CON) and CON + DFM diets. Chicks were allocated on a complete randomized design on d 1 to experimental units (EU) with 8 EU of 8 chicks in Experiment 1 and 16 EU of 8 chicks in Experiment 2. All the sampling was done on d 21. MIXED procedure from SAS was used to differentiate the means with significance observed at $P \leq 0.05$. Ileal mucin (MUC2) mRNA expression and colon trans-epithelial electrical resistance (TER) were increased ($P \leq 0.01$), while colon endotoxin permeability ($P = 0.04$) was reduced by DFM in Experiment 1. No significant difference was observed in the ileal TER. In a follow-up experiment, cecal molar proportions of propionate and butyrate as well as total short-chain fatty acids were increased ($P \leq 0.01$) with DFM addition. Serum endotoxin concentration was reduced in chicks fed DFM under fasting as well as fed state ($P \leq 0.05$). No significant differences were observed in hepatic glyceraldehyde 3-phosphate dehydrogenase and fatty acid synthase activity. However, hepatic glucose-6-phosphate dehydrogenase (G6PDH) activity as well as glycogen concentration were increased ($P \leq 0.01$) by DFM supplementation. Addition of DFM increased ($P \leq 0.01$) body weight gain and feed efficiency during the 1–21 d period. Supplementation of DFM increased the intestinal integrity while elevated hepatic G6PDH and glycogen activity for DFM fed chicks indicate increased glucose-sparing which may be associated with the effect of butyrate.

Key Words: MUC2, epithelial resistance, glucose-6-phosphate dehydrogenase

202 Clostridial enteropathies: Microbes that mitigate the effects.

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Clostridial enteropathies continue to plague commercial livestock production in the United States. Recent reports indicate an increase in diseases such as clostridial enterotoxemia and *Clostridium difficile* infections in swine, and necrotic enteritis in broilers. The results of these diseases are decreased production performance, heightened cost of production and increased mortality. Corresponding to the rise of clostridial diseases is reduced antibiotic usage. This production dynamic is being driven by market demands and regulatory initiatives. Thus, alternatives to commonly used therapeutic agents are being investigated. Recent studies have reported that the use of direct fed microbials (DFMs) can ameliorate the effects of intestinal clostridial diseases. The objectives of this presentation are to delineate the current incidence of clostridial enteropathies in livestock production, particularly in monogastric animals. An examination of published literature describing the efficacy and mode of actions that DFMs express toward the amelioration of clostridial enteropathies will be discussed. Finally, a survey of current research regarding the mitigating effect DFMs have on clostridial enteropathies will be addressed.

Key Words: direct-fed microbial, clostridium, enteropathy

203 Performance and rumen development of Holstein calves dosed with *Megasphaera elsdenii* NCIMB 41125.

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Twenty-six Holstein calves (BW = 34.5 ± 1.65 kg) were randomly assigned to treatments at birth to evaluate the influence of an oral dose of *Megasphaera elsdenii* NCIMB 41125 (*M. elsdenii*) on pre- and post-weaning intake, performance, and ruminal development. Treatments were a control group, which did not receive *M. elsdenii* (Cont) and a *M. elsdenii* group, which received a 50-mL oral dose of *M. elsdenii* NCIMB 41125 (10^8 cfu/mL) at 14 d of age (Me14). Calves were given colostrum for the first 3 d of life followed by free choice access to whole milk during feedings at 0800 and 1400 h. Milk was offered in a 5 L bucket, which was refilled as needed until voluntary intake ceased. From d 52 until weaning (d 56) milk intake was limited to 4 L/d, offered once daily at 0800 h. A calf starter was offered ad libitum starting at 4 d of age until the end of the study. Fresh water was available throughout the study. Intake of milk and starter feed were measured daily and body weights were taken weekly. Blood samples were collected on d 7, 21, 28, 42, and 56 via jugular venipuncture to determine β-hydroxybutyrate (BHBA) concentrations. Intake and performance were measured for an additional 14 d following weaning. During the pre-weaning period (d 1–55) milk intake was greater for Cont calves compared with Me14 calves ($P = 0.01$). The inverse was true for starter feed DMI as Me14 calves consumed more starter feed ($P < 0.01$) than Cont calves. Overall DMI was not different between treatments ($P = 0.4$) and neither was estimated metabolizable energy intake ($P = 0.14$). Average daily gain during the pre-weaning period was not different between treatments ($P = 0.3$), but Me14 calves were 5.8 kg heavier ($P = 0.01$) at weaning compared with Cont calves. Gain efficiency was greater ($P < 0.01$) for Me14 calves compared with Cont calves. On d 7 BHBA concentrations tended to be greater for Cont calves compared with Me14 calves, however the average BHBA concentration between d 21 to 56 were greater for Me14 calves ($P = 0.02$). Post-weaning (d 56–70) DMI and estimated metabolizable energy intake were greater ($P \leq 0.03$) for Me14 calves compared with Cont calves. During the post-weaning period Me14 calves gained 0.37 kg more per day ($P < 0.01$) than Cont calves and weighed 11.6 kg more ($P < 0.001$) than the Cont calves at the end of the study. There was a trend ($P = 0.07$) for Me14 calves to have better gain efficiencies than the Cont calves. Administering *M. elsdenii* to Holstein calves at 14 d of age improved pre- and post-weaning performance and starter feed intake. Improvements in starter feed intake suggest greater ruminal VFA concentrations to stimulate rumen development. In addition higher blood β-hydroxybutyrate concentrations may indicate greater metabolic activity of the rumen epithelium for calves given *M. elsdenii*.

Key Words: *Megasphaera elsdenii*, rumen development, weaning

204 *Bacillus* producing NSP enzymes in situ increased the amounts of available sugars in feed.

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Increasing raw material prices worldwide are a challenge for animal production that is facing poor economy and today feed prices accounts for about 70% of pig and poultry production costs. An improved feed utilization catalyzed by exogenous enzymes or probiotics has been used to counteract poor production economy, but a more efficient solution is required. Feed utilization might be improved by degrading nonstarch polysaccharides (NSP) that cannot be digested by monogastrics and affects the overall digestion resulting in 15 to 25% of the feed not being digested by pigs and poultry. By supplementing probiotic microorganisms that can synthesize NSP enzymes not only the health of the animal, but also the nutritional value of feed ingredients is improved. An increased microbial fiber digestibility increases the availability of other nutrients, vitamins and minerals that are fixated by the fiber matrix and it also increases the energy supply from the fiber itself resulting in reduced nutrient loss to the environment. NSP enzyme activity can be analyzed for each single enzyme (i.e., endo-cellulases) or by measuring the degradation products from fiber digestion: reducing sugars. Reducing sugars include glucose, glyceraldehyde and galactose as well as disaccharides, such as lactose and maltose, can be measured by the dintrosalicylic acid (DNS) method. A new feed-based assay was developed to examine the potential of *Bacillus* strains in a complex environment. Commercial *Bacillus* strains have been tested in this newly developed assay based on pig feed and the amount of reducing sugar after incubation in feed measured. All *Bacillus* products supplied more nutrients to the animal by delivering more reducing sugars. BioPlus and GalliPro delivered about 3 times more reducing sugars than the control, whereas another commercial product delivered less than 2 times more.

Key Words: *Bacillus*, probiotic, enzymes

205 In vitro enzyme production and viscosity determination by selective *Bacillus* species in different poultry diets.

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Increased global demand for poultry meat, combined with increased utilization of grains for biodiesel production, has led to the use of alternative energy sources in poultry diets. Wheat, barley and rye as well as biofuel byproducts (DDGS) have become common ingredients in many regions. However, these raw materials provide a higher level of less digestible carbohydrates, known as non-starch polysaccharides (NSP), which are related to increased digesta viscosity. An alternative to optimize digestibility of NSP is the inclusion of bacterial enzymes, such as xylanase. The purpose of the present study was to evaluate and select *Bacillus* species from environmental and poultry sources as

candidate direct-fed microbials (DFM) based upon their enzyme production capacity. Thirty *Bacillus* isolates were screened in vitro for relative enzyme activity of cellulase, protease, lipase, xylanase and phytase using selective media. The selection criteria were based on colony size and area of clearance around each colony at specific times of incubation. Nine out of the 30 strains showed specific and considerably larger enzyme production zones, confirming that not all *Bacillus* synthesize the same type and amount of enzymes. *Bacillus* candidates were identified as *B. subtilis* (7/9), *B. megaterium* (1/9), and *B. licheniformis* (1/9). One of these 9 strains is currently present in the commercial direct-fed microbial, DFM/Sporulin, and was experimentally included at a concentration of 10^9 spores/gram in 5 different poultry diets: corn-soybean, wheat-soybean, rye-soybean, barley-soybean, and oat-soybean. An in vitro digestion assay was performed for each diet and the supernatants were further tested for viscosity and compared with the same diet without DFM inclusion. In contrast to the control, and DFM treated corn-soybean diets, a significant reduction in viscosity ($P < 0.05$) in DFM treated diets was observed in the wheat, rye, barley and oat based-diets, which contained high concentrations of NSPs, suggesting the synthesis of xylanase and β-glucanase by the *Bacillus*-DFM. The results of this study suggest that the selection and consumption of a *Bacillus*-DFM, producing a variable set of enzymes in either conventional or especially high NSP diets, may contribute to enhanced performance through improving digestibility, reducing intestinal viscosity, and promoting healthy intestinal integrity in commercial poultry. Further studies to quantify the concentration of international enzyme units as well as in vivo performance trials are in progress.

Key Words: *Bacillus*-DFM, enzymes, viscosity

206 Evaluation of a novel probiotic formulation designed exclusively for neonatal broiler chicks.

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At the time of hatch, the intestines of broiler chicks are not fully developed and the resident microflora of the GI tract have yet to become fully established. The first organisms to colonize the GI tract have a distinct advantage in becoming the pioneer colonizers in the chick and have been shown to be important for proper gut and immune development. In a commercial situation, the chicks are hatched in a relatively sterile environment, processed, shipped, and then placed on a farm. This process has separated the chick from the hen and has retarded normal microflora development in the chick. This has left the chick more open to pathogens from the hatchery and farm, and may lead to non-uninform development of microflora within a flock. There is evidence that early application of probiotics give beneficial microbes time to colonize the GI tract of poultry. This has been shown to inhibit the colonization of harmful bacteria as well as induce more rapid intestinal growth. In these studies we used a novel, hatchery-applied probiotic, FloraStart (FS), which comprises *Lactobacillus plantarum* (TY036) and *Enterococcus faecium* (MFF109). Treated chicks were given 1×10^6 of MFF109 and TY036. The birds were weighed on day-of-hatch and at 7 d to determine 7-d weight gain. In the first experiment, the average 7-d weight gain from the FS group was 108.0 ± 3.4 g compared with the 7-d weight gain of the controls, 78.3 ± 4.7 g ($P \leq 0.01$). In the second experiment, the FS group had 7-d weight gain of 104.4 ± 1.3 g compared with control, 94.6 ± 1.2 g ($P \leq 0.01$). The results of these experiments also show a marked increase in the rate of intestinal villus development. At 3 d, villus length of FS treated birds was also significantly increased from 255.8 ± 3.7 µm in control groups compared with 309.2 ± 1.5 µm in FS-treated birds. This data indicates that FloraStart is beneficial to the growth and GI development of neonatal broiler chicks.

Key Words: probiotic, poultry, gut

Session IV: Nutrition and Gut Health I

207 Estimated metabolizability of diets fed to lactating dairy cows containing Immunis[3] or yeast culture.

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Lactating dairy cows were allocated into one of 2 groups at approximately 25 d post-calving to evaluate effects of products containing either hydrolyzed yeast, live yeast, lactic acid bacteria (LAB) and digestive enzymes (Immunis[3], I3) or yeast culture (YC) on feed intake and milk energy content. Dry matter intakes for either group were determined daily using a commercial utility (Feed Watch), individual milk production and composition were determined every 14 d. Intake energy (IE, Mcal/cow·d^{-1}) was calculated from diet composition and milk energy content (LE, Mcal/cow·d^{-1}) from milk production (kg/d) and composition (fat, protein and lactose). Intake energy was partitioned into that required for maintenance functions and LE; estimates of metabolizability (Q), maintenance (ME_m) and efficiency of ME use for lactation (k_l) were determined using a non-linear model. It was assumed that neither k_l nor ME_m differed between groups and, if differences existed, they were due to differences in Q for cattle fed either I3 (Q_{I3}) or YC (Q_{YC}). Variability in parameter estimates Q, ME_m and k_l were evaluated using Markov Chain Monte Carlo (MCMC) simulation. Ten runs (2,500 simulations) were performed; the first 1000 of each were excluded from analysis. Intake energy for I3 was 110 Mcal/d and for YC, 113 Mcal/d ($P = 0.087$), LE for I3 was 32.9 Mcal/d and for YC, 32.6 Mcal/d ($P = 0.350$). Gross efficiency of energy utilization (LE/IE) was greater ($P < 0.001$) for I3 (0.300) than for YC (0.288). Non-linear estimates of Q_{I3} and Q_{YC} were 0.730 and 0.690, respectively; k_l was estimated to be 0.562 and ME_m, 22.0 Mcal/d, which is approximately $0.160W^{0.75}$. Markov Chain Monte Carlo estimates of ME_m and k_l were 30.0 Mcal/d and 0.762; the former is approximately $0.220W^{0.75}$ Mcal/d. While MCMC estimates of ME_m and k_l are greater than those determined using conventional linear procedures, ME_m is consistent with non-linear estimates of maintenance in growing beef cattle and k_l is less than the theoretical maximum (0.79). Metabolizability estimates, from MCMC simulations, were 0.667 (Q_{I3}) and 0.632 (Q_{YC}) and were different ($P < 0.001$). Estimated IE were 110 Mcal/d (I3) and 115 Mcal/d (YC), observed IE were 110 Mcal/d (I3) and 113 Mcal/d (YC). The model accounted for 99.8 percent of the variability in IE for I3 cows and 102 percent of the variability in IE for YC cows. Adjusting Q_{YC} to account for 100 percent of the variability for YC gives a value of 0.642, still different ($P < 0.01$) from Q_{I3}. Because IE × Q = ME, increases in Q are indicative of reduced fecal, urinary and gaseous energy losses consistent with the role of hydrolyzed yeast, yeast and LAB in enhancing utilization.

Key Words: metabolizability, lactic acid bacteria, yeast culture

208 Influence of diet's physical form (feed grinding/compaction) on colonization and spread of *Salmonella* Enteritidis in experimentally infected broiler chicks.

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Zoonotic diseases due to food of animal origin, in particular infections with *Salmonella* spp. are of major public interest. As a result the pressure on livestock production to ensure food safety increases ("from farm to fork"). The hypothesis of this study was that a coarse feed structure, determined by grinding and further treatment, may decrease the spread of infection and therefore should lower *Salmonella* prevalence in poultry flocks. A total of 312 male broilers (Ross 708; 7 d old) in 3 consecutive trials were fed 1 of 4 botanically and chemically identical diets, which only differed in grinding and further compaction. Only two 14-d-old broilers per group were inoculated directly into the crop with *Salmonella* Enteritidis (10^8 cfu; SE 147; Methner et al., 1995 J. Vet. Med. B 42:459–469) and placed back into the group ("seeder birds"). The spread of infection was measured by cloacal swabs (on d 2, 4, 6, 13 postinfection) and by cecal content and liver tissue samples taken at 21.5 and at 35.5 d of age in average. Each sample was analyzed for *Salmonella* Enteritidis after qualitative enrichment. To follow up the spread of the infection in each group, only the contact birds were monitored and therefore their data are exclusively shown in Table 1. According to this study only the diet including 22% whole wheat significantly reduced the spread of infection and the frequency of colonization and translocation of *Salmonella.* species. The causative mechanisms are, in contrast to pigs, still in debate and need further investigations for elucidation. This project was supported by the Federal Ministry of Food, Agriculture and Consumer Protection of Germany based on a decision of the Parliament of the Federal Republic of Germany

Table 1. Characterization of the experimental diets (13 MJ of ME; 234 g of XP/kg of DM) and results of *Salmonella* spp. testing of cloacal swabs, cecal content and liver tissue

	Pellet			
Item	Fine	Coarse	Whole wheat	Extrudate
Grinding form/intensity[1]	HM (fine)	RM[2] (coarse)	HM (fine) + 22% whole wheat[2]	RM (coarse)
Compaction	pellet	pellet	pellet	extrudate
Particle size distribution[3] (% of DM)				
>1 mm	12.4	38.8	30.7	19.4
<0.2 mm	42.9	32.4	37.3	58.9
Birds with proof of *Salmonella* (%)	47.2[b]	52.2[ab]	30.0[c]	65.6[a]
Cecal content (%)	23.6[a]	37.7[a]	10.0[b]	36.1[a]
Liver tissue (%)	26.4[a]	31.9[a]	11.4[b]	34.7[a]

[ab]Denotes statistical differences between the groups ($P < 0.05$).
[1]HM = hammer mill; RM = roller mill.
[2]Added before pelleting.
[3]By wet sieve analysis (extrudate: modified wet sieve analysis).

Key Words: feed structure, whole wheat, *Salmonella*

209 Effects of a *Lawsonia intracellularis* infection in young vaccinated and nonvaccinated pigs on the total-tract digestibility of nutrients and performance.

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Introduction: In this study, it should be investigated to what extent performance issues due to typical signs of proliferative enteropathy in piglets are also caused by changes in nutrient digestibility. Material and methods: A total of 27 potentially natural *L. intracellularis* infected pigs were divided into 3 groups (3 trials with 9 animals; bw: 19.0 ± 1.50 kg): not vaccinated, without clinical findings = VAC^{-}*CF*$^{-}$; clinical signs (soft feces) = VAC^{-}*CF*$^{+}$; vaccinated and without clinical manifestations (Enterisol Ileitis; vaccinated in the farrowing unit) = VAC^{+}*CF*$^{-}$. A standard diet was fed (ad libitum) to individually housed pigs for 10 d (CP: 176 g, CF: 23.5 g, CL: 33.6 g, ME: 13.8 MJ/kg diet). The feed contained a marker (0.05% Cr_2O_3). Five days were used for adaptation to the new environment. In the next 5 d the feces were collected to determine app. digestibility of organic matter (OM), crude protein (CP), and starch. In the collection phase, the number of *L. intracellaris* bacteria in the feces was determined by qPCR. Statistical analyses were done by one-way ANOVA (procedure GLM; $P < 0.05$). Results and discussion: In all groups *L. intracellularis* was detected in the feces. The highest number of genome equivalents (GE) was found in the group VAC^{-}*CF*$^{+}$ (VAC^{-}*CF*$^{+}$: lg GE = 7.70 ± 1.83; VAC^{+}*CF*$^{-}$: lg GE = 6.00 ± 2.89; VAC^{-}*CF*$^{-}$: lg GE = 5.83 ± 2.35). The animals in the non-vaccinated, clinically affected group (VAC^{-}*CF*$^{+}$) had the lowest dry matter contents in the feces (Table 1). In the clinically symptomatic group the digestibility of OM tended to be lower (p = 0.0537), the digestibility of CP was significantly reduced. The pigs of group VAC^{-}*CF*$^{+}$ had the lowest daily feed intake, daily weight gain and the worst feed conversion ratio. Conclusion: Even clinically mild cases of *L. intracellularis* infection lead to, at least partly, reduced digestibility of nutrients. The experimental study was short and performed under optimal management and housing conditions. Thus, in practice, the effects of *Lawsonia* infection may therefore be more distinctive.

Table 1. Feces quality (DM content), total-tract digestibility rates (OM, CP, starch), daily feed intake, daily BW gain and feed conversion ratio in young pigs shedding *L. intracellularis*

Item	VAC^{-}*CF*$^{-}$	VAC^{-}*CF*$^{+}$	VAC^{+}*CF*$^{-}$
DM content of feces (g/kg)[1]	245 ± 16.8^{a}	211 ± 19.8^{b}	236 ± 18.4^{a}
Total-tract digestibility rate (%)			
OM	86.9 ± 1.81	84.8 ± 2.19	86.4 ± 1.46
CP	83.9 ± 2.03^{a}	80.7 ± 2.57^{b}	83.0 ± 1.72^{a}
Starch	99.0 ± 0.15	98.8 ± 0.26	98.9 ± 0.27
Daily feed intake (g)[2]	1,207 ± 119	1,165 ± 148	1,320 ± 142
Daily BW gain (g)[2]	852 ± 100^{ab}	800 ± 139^{b}	920 ± 60.0^{a}
Feed conversion ratio (feed:gain)[2]	1.42 ± 0.08	1.47 ± 0.15	1.43 ± 0.12

[1]Determined during the digestibility trial.
[2]Because of technical problems, only trial 2 and 3 were considered.

Key Words: *Lawsonia*, qPCR, digestibility

210 The effect of quaternary benzophenantridic alkaloids on *Salmonella* isolation, expression of CD3+ and goblet cells in the intestinal mucosa, blood immune cells and transepithelial electrical resistance.

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A strong and well performing immune system is needed to be able to "maintain" the eubiotic state of the intestine. This defense mechanism has to be well synchronized and executed on an optimal level to maintain gut health. In this process inflammation plays, and "inflammation management" may play a key role. Plants that contain quaternary benzo[c]phenantridine alkaloids (QBA) has been used as anti-inflammatory medication for a long time in traditional medicine. QBA containing feed additives has been used in animal nutrition for more than 10 years with beneficial effects on the economy of animal production. It can be hypothesized that the anti-inflammatory property of QBA contributes to the advantageous results. To prove the described hypothesis 2 experiments were carried out to investigate the effect of QBA on selected parameters of broilers and piglets. Experiment 1 evaluated of the effects of the use of QBA (sanguinarine) in the drinking water on the performance, *Salmonella enteritidis* count, intestinal morphology, and expression of immune cells in the blood and intestinal mucosa of broilers challenged with *S. enteritidis*. The treatment of *S. enteritidis* challenged broilers with QBA via drinking water reduced *S. enteritidis* isolation in the cecum and the crop 7 d post inoculation compared with the control group. Broilers treated with sanguinarine alkaloids via drinking water presented significantly lower expression of goblet and CD3^{+} cells in the duodenum and jejunum, and higher expression of cells positive for the markers CD4, CD8α, CD8α^{bright}, CD8α^{dim}, CD8β, TCR Vβ1, and CD28 in the blood as compared with the non-treated birds. Experiment 2 was conducted to evaluate

the influence of QBA (sanguinarine) on the intestinal mucosa integrity and inflammation processes. Ten 5-wk-old piglets were challenged via oral drench with *Salmonella typhimurium* and allocated to 1 of the 2 treatment groups: (1) QBA preparation (1,5 mg/kg) and (2) chlortetracycline (60 mg/kg). At the end of the trial (d 40) pigs were euthanized, intestinal mucosa was collected and transepithelial electrical resistance (TER) was determined in vitro using the Ussing chamber model. Ileal samples from pigs fed the diet supplemented with 1.5 mg of QBA preparation/kg of feed had a higher mean TER (62 ± 9 W/cm^2), which indicated enhanced health of the mucosal barrier, compared with the mean TER for pigs fed the diet supplemented with chlortetracycline (41 ± 2 W/cm^2) or uninfected control pigs (50 ± 2 W/cm^2). The QBA preparation fed animals showed a significant higher TER than the positive control (CTC). This indicates significantly ($P < 0.05$) less lesions and inflammation processes in the intestinal mucosa in the QBA preparation group than either in the control or CTC group at d 40.

Key Words: quaternary benzo[c]phenantridine alkaloids (QBA), *Salmonella*, transepithelial electrical resistance (TER)

211 Use of scanning electron microscopy to evaluate intestinal villi morphology in poultry fed butyric acid, mannan-oligosaccharide, and antibiotic feed additives.

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There is growing interest in the use of alternatives to antibiotics (alternative growth promoter, AGP) feed additives to promote gut health for commercial poultry. Butyric acid and mannan-oligosacharides from yeast are popular AGP alternatives that may enhance enteric health of poultry by positive effects on the morphological development and maintenance of intestinal villi. Two trials were done to evaluate the effect of these feed additives on the morphological characteristics of villi using scanning electron microscopy (SEM) in comparison to histomorphometric evaluation using conventional light microscopy. The first trial evaluated a dry coated sodium butyrate product (30% activity) that has more favorable handling characteristics for feed manufacturers than the concentrated liquid butyric acid (BA). Commercial broilers were randomly assigned to 32 floor pens, within 4 dietary supplementation levels of BA (0, 0.015, 0.03, and 0.06%) were subjected to 8 replicate pens per treatment from 1 to 14 d. Subsequently, all birds were fed common grower and finisher diets in pelleted form. At 3, 8, and 14 d, 4 birds/treatment were sampled for intestinal histomorphometric and SEM evaluation. The positive starter feed treatment effects was observed throughout the experiment with 0.015% BA, resulting in a 3% and 2% improvement by 42 d ($P < 0.02$). By light microscopy, an increase in villi height and villi surface area was observed among broilers fed BA. However, by SEM, we were able to clearly see the BA-fed birds had less distressed villi morphology (wrinkled villi with microvilli clumping) and less mucus than the control-fed broilers. The second trial with turkeys evaluated the use of a mannose-rich oligosaccharide derived from cell wall of a specific strain of *Saccharomyces cerevisiae* in comparison to virginiamycin, a common AGP. Although few significant treatment effects were observed on growth performance and villi histomorphometric evaluation, significant differences in SEM images of ileal villi were observed. SEM images of villi from poults fed the mannan-oligosaccharides were observed to exhibit abundant of mucus secretion, whereas those fed the AGP exhibited considerable colonization of segmented filamentous bacteria (SFB). Mucosal SFB colonization has been associated with enhanced the luminal IgA production and reduced mucosal inflammation. We conclude that SEM of intestinal villi is a useful method to evaluate the effect of feed additives that affect enteric health, even when no differences in growth performance and histomorphometric analysis can be observed.

Key Words: intestinal villi, butyric acid, mannan-oligosaccharide

Session V: Nutrition and Gut Health II

300 Precision-delivery coated butyrate to control *Campylobacter* in poultry.

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Campylobacter is the leading cause of foodborne illness in the US and in Europe. Epidemiological studies indicate that poultry and poultry products are significant sources of human infection. In this context, *Campylobacter* colonization in the intestinal tract of broilers is an important parameter to consider, as intestinal *Campylobacter* count is correlated with broiler carcass contamination. Since it has been estimated that a reduction of intestinal *Campylobacter* counts of 2 Log10 would result in a 30-fold decrease of human campylobacteriosis cases, and given that certain butyrate-based feed additives have already been described to decrease colonization of certain zoonotic pathogens in broilers, we decided to explore the effect of a target release coated butyrate, able to deliver butyrate in the distal parts of the gastrointestinal tract (Ultramix C), on *Campylobacter* counts in broiler ceca. To this end, we set up 2 different experiments. In a first study, natural *Campylobacter* infection was mimicked. Three broilers groups were raised in separate isolators; one group received 3 kg coated butyrate product per ton finished feed, a second group 5 kg per ton, while the third group served as a negative control. Microbiological analysis of cloacal swabs demonstrated that the birds of all 3 groups were *Campylobacter*-negative at d 10, while they were positive for *Campylobacter jejuni* at d 18 (between 5.12 and 5.77 $\log_{10}$ cfu/g cecal content), without artificial infection. While cecal *Campylobacter* count remained high in the negative control group, counts for the groups receiving butyrate were found to be below the detection limit of 3 $\log_{10}$ at d 29 (for the 5 kg/T group) and at d 39 (for both butyrate groups). In a second trial, the effect of supplementing coated butyrate during different feeding phases (starter-grower-finisher) was evaluated after broilers were orally infected with a *Campylobacter jejuni* on d 18. From the results it can be concluded that in this experimental setup, 3 kg/T of butyrate product was able to reduce cecal *Campylobacter* by minimum 2 $\log_{10}$ units by d 39, when included in the feed from the moment of infection onwards. Apart from its well-documented trial on zootechnical performance, the tested butyrate product can therefore prove to be a valuable approach to reduce the number of human campylobacteriosis cases, by significantly limiting *Campylobacter* colonization in broilers.

Key Words: butyrate, *Campylobacter*, poultry

301 Butyrate supplementation affects mRNA abundance of genes involved in glycolysis, lipogenesis and oxidative phosphorylation in the rumen epithelium of Holstein dairy cows.

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Energy availability in epithelial cells is a crucial link for maintaining barrier integrity; energy depletion is linked to impaired barrier function in several epithelia. This study aimed to elucidate the effects of exogenous butyrate on mRNA abundance of genes indirectly involved in rumen epithelial barrier integrity. Sixteen mid-lactation Holstein cows fed a total mixed ration received a concentrate mix to induce subacute ruminal acidosis (SARA). For 7 d, while being fed the concentrate mix, cows were assigned either a control treatment or a butyrate treatment, in which cows were fed butyrate at 2.5% daily dry matter intake in the form of a calcium salt. On d 6 and 7, rumen pH was measured continuously and on d 7, rumen biopsies took place. Rumen pH fell below 5.6 for more than 3 h per day in both treatments, confirming the occurrence of SARA. Microarray and pathway analysis, confirmed by real time PCR, showed that exogenous butyrate significantly increased the mRNA abundance of hexokinase 2 (fold change: 2.07), pyruvate kinase (1.19), Cytochrome *b*-complex 3 (1.18) and ATP synthase F0 subunit (1.66), which encode important glycolytic enzymes. Meanwhile, butyrate decreased mRNA abundance of pyruvate dehydrogenase kinase 2 (−2.38), ATP citrate lyase (−2.00) and mitochondrial CoA transporter (−2.27), which encode lipogenesis enzymes. These data suggest exogenous butyrate induces a shift toward energy mobilization in the rumen epithelium, which may aid barrier function in the rumen epithelium during SARA.

Key Words: rumen epithelium, glycolysis, lipogenesis

303 Effect of a liquid whole-egg globulin protein supplement on broiler performance, intestinal histology, and bacitracin-resistant *Clostridium* growth.

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Protemace is a liquid whole egg globulin protein supplement, applied to the drinking water, that provides a source of immunoglobulins. This investigational effort was designed to measure the effect of Protemace on various aspects of broiler performance. Parameters measured included live performance, duodenal and ileal histology, and mid-gut bacitracin-resistant *Clostridium*. Day-old males from the Cobb 500 female line were allocated to 48 pens (30 chicks/pen; 12 pens/treatment) and grown to 42 d. There were 4 water treatments. Control had 2 subgroups, one used only municipal water and the other included diluted stabilized hydrogen peroxide provided at the same time as treatment 4. Treatment 2 supplemented Protemace from d 1–7, followed by 24 h treatment of diluted stabilized hydrogen peroxide. Treatment 3 supplemented Protemace from d 1–7, d 21–28, followed by a 24 h treatment of diluted stabilized hydrogen peroxide dilution on d 8 and 29. Treatment 4 supplemented Protemace from d 1–42, followed by a 24-h treatment of diluted stabilized hydrogen peroxide on d 8, 15, 22, 29, 36, and 40. Live weights, feed conversions and livability were evaluated at 14, 35, and 42 d. Gastrointestinal histology and Clostridium were evaluated at d 42. No significance existed in live performance ($P < 0.05$). Duodenal villi height in treatments 2 and 3 were both significantly longer ($P < 0.05$) than the control, while crypt depth was significantly deeper ($P < 0.05$) in treatments 2, 3, and 4 versus

controls. Treatments 3 and 4 had crypt depth significantly deeper ($P < 0.05$) than treatment 2. Ileum villi height of treatments 2, 3, and 4 were significantly longer ($P < 0.05$) than control, and crypt depths of treatments 2, 3, and 4 were all significantly deeper ($P < 0.05$) than control, with treatments 3 and 4 being significantly greater ($P < 0.05$) than treatment 2. Of the 12 birds per treatment sampled, the control group had a 77% incidence of bacitracin-resistant Clostridium species in the mid-gut while treatments 2 and 3 each had 33% positive incidence and treatment 4 had 0% positive. These results suggest supplementing with Protemace could improve gut health and may also have a role in alleviating Clostridia challenges in broilers.

Key Words: globulin, *Clostridium*, broiler

POSTER PRESENTATIONS

P100 Comparative functional analysis of porcine-derived *Lactobacillus amylovorus* strains and the role of their S-layer proteins as adhesins.

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Surface (S) layers are regular paracrystalline protein arrays commonly found on lactobacilli and other bacterial species. The identical subunits of S-layers are non-covalently linked to each other and to the underlying cell surface and form the S-layer lattice by an intrinsic self-assembly process. The biological functions of *Lactobacillus* S-layer proteins are not well understood. The purpose of this study was to gain knowledge about the adhesive and pathogen inhibitory properties of several *L. amylovorus* pig intestinal isolates and, more specifically, to characterize the putative role of the S-layer proteins as adhesins in these lactobacilli. *L. amylovorus* type strain DSM 20531, as well as the strain DSM 16698, a previously well-characterized probiotic, were included in the study. None of the *L. amylovorus* strains studied bound to either porcine gastric or intestinal mucus in vitro. In contrast, the strains showed differences in their adhesiveness to porcine small intestinal cells (IPEC-1): 3 strains were efficient in binding, while the rest were less adherent. Differences between the *L. amylovorus* strains were also observed in their abilities to inhibit the binding of an enterotoxigenic, K88-expressing *E. coli* strain to IPEC-1 cells. If the *Lactobacillus* strains were added beforehand or simultaneously with the pathogen at a pathogen-*Lactobacillus* ratio 1:10, half of the strains studied were able to inhibit pathogen binding, with some variation in the inhibition efficiency between the strains observed. If added afterward, none of the strains exhibited significant pathogen inhibition ability. To study the role of *L. amylovorus* S-layer proteins in adhesion to IPEC-1 cells we have created a protein presentation system based on the use of isolated cell wall fragments. This system, allowing the assembly of S-layer proteins in their native symmetric orientation, was developed because of the poor water-solubility properties of *Lactobacillus* S-layer proteins and the unfeasibility to create S-layer negative mutants of *L. amylovorus*. The results obtained by this method suggest that the S-layer proteins of several *L. amylovorus* strains have a role in the binding of these strains to IPEC-1 cells. We have also initiated in vitro studies to identify the receptor of *L. amylovorus* DSM 16698 S-layer protein on IPEC-1 cells. The method used is a pull-down system based on S-layer protein -coated cell wall fragments incubated on confluent layers of IPEC-1 cells, combined with the chemical cross-linking of the adhesin to the receptor by a UV-activatable biotin label transfer reagent.

Key Words: S-layer, *Lactobacillus*, adhesion

P101 Effect of the human intestinal microbiota and *Bacteroides thetaiotaomicron* on *Escherichia coli* O157:H7 transcriptome: Multiple aspects of EHEC adaptation.

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Enterohemorrhagic *Escherichia coli* (EHEC) are human pathogens frequently responsible for important outbreaks in developed countries. EHEC O157:H7 produce the Shiga toxins and colonize the epithelium of the colon using a type III secretion system (T3SS) which is involved in the formation of attaching and effacing lesions. When EHEC reach the large intestine, they face the digestive environment and compete with the gut microbiota to colonize the epithelial cells. We hypothesize that EHEC adapt their gene expression in response to the intestinal metabolome modulated by human microbiota (HM) and *Bacteroides thetaiotaomicron*, a predominant bacterial species of the gut, Using microarrays and qRT-PCR we monitored the adaptations of EHEC to the HM and *B. thetaiotaomicron* by comparing global gene expression of the EHEC O157:H7 strain EDL933 cultivated in the intestinal content of germ-free rats with that of the strain cultivated in the intestinal contents of rats associated with the HM and mono-associated with *B. thetaiotaomicron*. The metabolic profile of EHEC changed in response to the HM switching from glycolytic to gluconeogenic pattern. Pathways involved in the degradation of sialic acid, ethanolamine, amino acids and microbiota-derived compounds were upregulated in response to the HM while genes required for the utilization of sugars and glycerol were downregulated. Moreover, the expression of genes encoding for the T3SS and its secreted effectors was decreased in the intestinal content of rats associated with the HM and more importantly with *B. thetaiotaomicron*. The identification of the nutritional niche of EHEC in the gut as well as compounds regulating virulence genes would allow novel approaches to prevent and fight EHEC infections in human.

Key Words: enterohemorrhagic *E. coli*, Human gut microbiota, *Bacteroides thetaiotaomicron*

P102 Field evaluation of the live microbial product Calsporin on health, performance and carcass quality of finisher pigs.

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Interest in live microbial products has intensified due to problems and concerns relating to antimicrobial resistance. Numerous publications document beneficial effects of live microorganisms on health and growth performance in nursery pigs. However, few published studies show their positive effects on live performance in growing-finishing pigs. This study assessed the efficacy of Calsporin (Calpis Co. Ltd., Japan), a product containing viable spores of *Bacillus subtilis* C-3102, in fattening pigs (88 to 186 d of age). The study was performed on a commercial 1,800-sow farrow-to-finish pig farm. Pigs were routinely vaccinated against enzootic pneumonia, PRRS (modified live vaccine) and porcine

circovirus type 2. All fattening units on this farm contain separate rooms, each with 14 pens (25 pigs/pen). The pigs were grouped by size, allocated to mixed-sex pens, and thereafter weighed by pen. The study was run in 2 time blocks. In the first block, young pigs derived from 4 sequential (weekly) weaning batches were used. Pigs of batches 1 and 3 were housed in one room and fed basal diets, whereas pigs of batches 2 and 4 were housed in a separate room and fed test diets (basal feeds containing *Bacillus subtilis* C-3012 at 1.5×10^5 cfu/g). This separation prevented cross-contamination between the groups. The same procedure was used for the second block, but the room allocations were reversed to compensate for any room effect (crossover design). In total, 89 control and 89 test pens (replicates) were used, with 1,923 and 1,869 pigs, respectively. At study start, mean pen age, nº pigs/pen or pen bodyweight did not differ significantly between groups. Health, growth and carcass parameters were recorded for the entire experimental period. The viable spore supplementation improved growth significantly by 17 g/pig/day ($P < 0.05$). Microbiological examination of fecal samples indicated that the treatment tended to reduce *Escherichia coli* and *Clostridium* spp. excretion, at both 50 d on trial, and at study end. With regard to carcass quality at slaughter, the treatment significantly improved backfat thickness, and there was a tendency for a better overall meat score ($P = 0.058$) in the test group. Significantly more carcasses from the treated pigs were classified in the highest E category (63%) than those from the control (49.5%) ($P = 0.007$). Supplementation with *Bacillus subtilis* C-3012 improved growth in fattening pigs under commercial farm conditions and carcass quality.

Key Words: *Bacillus subtilis* C-3102, fattening pig, field evaluation

P103 Effect of *Saccharomyces cerevisiae* on ruminal microbial community during subacute ruminal acidosis.

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Active dry yeast has been used as a ruminant feed supplement to improve productivity and health. Our previous research (AlZahal et al., 2013, Page 401 in Proc. 64th Annu. Mtg. EAAP, Nantes, France) demonstrated that supplementing dairy cows with active *Saccharomyces cerevisiae* (SC) prevented subacute ruminal acidosis (SARA) and the associated reduction in feed intake and production. The objective of this study was to investigate changes in key microbes within the rumen of lactating dairy cows supplemented with SC and challenged with SARA. Sixteen multiparous, rumen-cannulated lactating Holstein cows were randomly assigned to 1 of 2 dietary treatments that included SC (4 g/d, AB Vista, UK) or placebo. During the first 49 d, all cows received a high-forage diet (77:23, F:C; NFC = 35). Cows were switched on d 50–51 to a high-grain diet (50:50, F:C; NFC = 48) and remained on the high-grain diet until the end of the experiment (d 73). Quantitative real-time PCR was used to assess relative abundance of microbial rRNA gene (Table 1). Total bacteria were used for normalization using the DCt method. Cows supplemented with SC had a 9-fold increase in SC, a 2-fold increase in *F. succinogenes*, a 6-fold increase in *A. lipolytica*, and an 8-fold increase in anaerobic fungi compared with control cows (Table 1), which suggested an increase in cellulolytic microbes within the rumen. Cows supplemented with SC had 2.2-fold reduction in *P. albensis*, which is a gram-negative bacterium predominant during SARA. *Prevotella* spp. are suggested to be an important source of lipopolysaccharide responsible for inflammation within the rumen. Cows supplemented with SC had a 2.3-fold increase in *S. bovis* and a 12-fold reduction in *M. elsdenii*. The reduction in *M. elsdenii* may reflect lower concentration of lactic acid within the rumen for SC cows. In conclusion, SC supplementation to dairy cows was proven in a previous study to alleviate SARA; the current study showed that SC supplementation can also improve rumen function as indicated by increased numbers of cellulolytic microorganisms within the rumen. *Saccharomyces cerevisiae* can alleviate the effect of SARA in dairy cattle.

Table 1. List of microbes tested, fold change, and *P*-values

Microbe	Log_2 fold change	SE	*P*-value
Saccharomyces cerevisiae	3.13	0.65	**
Prevotella albensis	−1.16	0.33	*
Fibrobacter succinogenes	0.99	0.35	*
Anaerovibrio lipolytica	2.56	1.03	*
Streptococcus bovis	1.19	0.49	*
Megasphaera elsdenii	−3.59	1.74	†
Anaerobic fungi	2.98	1.50	†
Ruminococcus albus	0.42	0.22	
Succinimonas amylolytica	−0.61	0.32	
Prevotella brevis	−0.35	0.22	
Total protozoa	1.17	0.81	
Succinivibrio dextrinisolvens	0.31	0.29	
Butyrivibrio fibrisolvens	0.18	0.18	
Selenomonas ruminantium	0.37	0.37	
Ruminococcus flavefaciens	0.29	0.31	
Lactobacillus spp.	−0.06	0.17	
Prevotella bryantii	−0.15	0.49	
Escherichia coli	0.05	0.68	

†$P < 0.10$, *$P < 0.05$, **$P < 0.01$.

Key Words: dairy cow, SARA, yeast

P104 Effect of dietary changes during broiler grow-out on gut microbiota composition, mucosal immunity, and intestinal barrier function.

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The intestinal microbiota and the host interact in a variety of dynamic and symbiotic relationships including the development and stimulation of immune development and function. The composition of the gut microbiota strongly correlates with diet where within hours after dietary changes over 50% of the

microbiota community changes possibly due to profound effects on host innate immunity. Broilers undergo 3 dietary changes during grow-out, but the effect of these dietary changes on the microbiota composition and intestinal immune responses is unknown. Thus, the objectives of these experiments were to define changes in gut microbiota and measure innate immune gene expression in the intestine caused by dietary changes in broiler grow-out. We combined 454 pyrosequencing of broad-range 16S rRNA gene amplicons of feces and intestinal contents to profile the poultry-associated microbiome at 72 h after changes of broiler diets (starter diet = 10 d; grower diet = 18 d; and finisher = 31 d post-hatch) and RT-qPCR of intestinal tissues to profile changes in expression of local immune gene expression. Significant differences were observed in the microbiome by sampling location and time. At 10 d post-hatch, bacterial genera over-represented in the fecal samples included *Gallibacterium* and *Lactobacillus* significantly ($P < 0.006$; metastats comparison), while *Bacteroides* was significantly more abundant in the cecum. By 31 d post-hatch, *Clostridium* and *Caloramator* (also a Clostridiales) had increased significantly in the cecum and *Lactobacillus* remained over-represented in fecal samples. Interestingly, in the ceca, the relative abundance of sequences classified as *Clostridium* increased by ca. 10-fold each sampling period from 0.1% at 10 d, to 1% at 18 d, and 18% at 31 d. Dramatic temporal and spatial differences in the poultry G-I microbiome were observed according to a variety of ecological metrics and increases in network complexity. Likewise, the cytokine gene expression changed radically over time. At 10 d post-hatch, the response was skewed toward pro-inflammatory with Il-6, IL-1β, and IL-18 upregulated. Following change in diet from starter to grower and grower to finisher, the cytokine profile changed to predominately anti-inflammatory/regulatory where IL-10 and TGF-β4 were significantly upregulated while the pro-inflammatory cytokines were significantly downregulated following the dietary changes. These results provide some interesting data on the cecal changes in both microbiota and immune responses. The results suggest that the microbiota induce a tolerant-type of condition in the ceca that results in a more homeostatic local environment.

P105 Regulatory processes for prebiotics and microbial products used in animal food.

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There is increasing interest in the marketing of prebiotics and microbial products for use in animal food. Animal food includes both livestock feed and companion animal food. Food falls under the regulatory authority of the US Food and Drug Administration (FDA). Prebiotics have been defined as nondigestible food ingredients that beneficially affect the host by stimulating the growth and/or activity of bacteria in the colon. Direct-fed microbial or probiotic products intended for use in animal food are products that are purported to contain live (viable) microorganisms. The promotion and marketing of these types of products have increased greatly the past few years and the types of microorganisms and their intended uses have become of greater concern to the FDA. Prebiotic and probiotic products may be regulated as foods or drugs under the Federal Food Drug and Cosmetic Act (FFDCA), depending on the intended use. FDA's Center for Veterinary Medicine (CVM) regulates both food and drugs intended for animals. Substances intended to be food or that are added to a food must be safe and achieve their intended purpose. Two regulatory pathways are available for new substances added to animal foods. The food additive petition process is described in regulation 571 in Title 21 of the *Code of Federal Regulations* (21CFR571). The safety of the substance at the intended use rate must be addressed for both the animal and the environment. For food producing species, the safety of human food obtained from the animals must also be addressed. When FDA approves a food additive petition (FAP), a regulation in 21CFR573 is established addressing the proposed use of the substance in animal food. The second pathway is for qualified experts to determine that a particular use of a substance in animal food is exempt from the premarket requirements of the FFDCA because this use is generally recognized as safe (GRAS). A GRAS determination generally demands the same quantity and quality of data/information needed for a FAP with the added requirement that this information be in the public domain. Sponsors can notify CVM about a GRAS determination through the animal food GRAS notification program. CVM maintains an internet list of animal food GRAS notices and CVM's conclusions about each notice. More information about these processes is available at http://www.fda.gov/safefeed. Another pathway for substances that raise no safety concerns is provided by the Association of American Feed Control Officials, which may establish an ingredient definition in its *Official Publication*.

Key Words: prebiotic, direct-fed microbial, probiotic

P106 Evaluation of dextran sodium sulfate and xanthophyll pigment absorption for developing a gut inflammation model in broiler chickens.

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Intestinal integrity is a major factor for control of disease and optimal production standards in poultry. Multiple agents contribute to inflammatory processes within the gut, clearly affecting performance. An enteric inflammatory model in chickens would be a useful tool for poultry research to reproduce the intestinal inflammation sequence of events caused by different types of insults such us viral, bacterial, toxic, physical and chemical insults. A method for consistently replicating this situation in the intestine would permit researchers to evaluate substances that may modify innate immune responses and associated inflammation, caused by these insults through development prophylactic and therapeutic treatments. Additionally, in some regions of the world, skin pigment is an important quality factor in poultry, and is considered a sign of health for poultry. Thus, in these studies trials were conducted to test dextran sulfate sodium (DSS), a common chemical used in murine models, for induction of intestinal inflammation, and to measure the effects on absorption of xanthophyll pigment.. Though an initial study in which DSS was administered in the drinking water to chicks, from d 3 to d 12 of age, at a rate of 0.75%, was able to significantly reduce pigment absorption from 2.1 µg/mL in plasma for control to 0.7 µg/mL for DSS treated birds, subsequent experiments investigating different doses of DSS, duration of treatment, and age of birds were largely unsuccessful at affecting pigment absorption levels. These

studies showed that the murine model does not directly transfer to chickens and further studies to evaluate DSS and markers of intestinal absorption are needed to develop a reproducible model and marker for measurement.

Key Words: dextran sulfate sodium, broiler, gut inflammation

P107 AvrA⁻ *Salmonella* Typhimurium displays differential infection characteristics and altered patterns of host signaling in the gut and liver.

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Salmonella Typhimurium is a serious infectious disease throughout the world, a major reservoir for *Salmonella* is chicken. Chicken infected with *Salmonella* do not develop disease, this may be the result of important host interactions with key virulence proteins. To study this we infected chickens with mutant *Salmonella* Typhimurium which lacked the virulence protein AvrA. AvrA is a bacterial effector protein which has been shown to have several diverse functions in *Salmonella* infection. We infected chickens with either wild-type *Salmonella* Typhimurium, mutant AvrA$^-$ *Salmonella*, or mutant ad-back AvrA$^+$ *Salmonella*. We then compared immune cellular signaling events induced by the various bacteria in cecum, jejunum and liver tissue. Cultures from infected birds over the 14 d course of infection showed that the AvrA$^-$ mutant *Salmonella* was more persistent and invasive than wild type *Salmonella*. Chickens infected with the AvrA$^-$ showed reduced weight gain. Kinome analysis indicated that there were significant differences in the host kinase activities in animals infected with bacteria lacking AvrA compared with those containing AvrA. However, pathway analysis showed that the signaling pathways affected by *Salmonella*, either containing or lacking AvrA, were substantially similar. These results indicated that while significant changes in cellular signaling was being caused by the presence or absence of AvrA, these changes were localized to select pathways. For example, the ErbB signaling pathway in jejunum is altered by infection with both mutant and wild type *Salmonella*. Thirteen phosphorylation sites displayed differential phosphorylation following infection with AvrA$^-$ *Salmonella* but were not affected by wild type *Salmonella*. Five sites were differentially phosphorylated in the ErbB pathway by wild type *Salmonella* that were not affected by *Salmonella* lacking AvrA. That constitutes 18 host phosphorylation events distinctly influenced by *Salmonella* differing only in a single virulence protein and within a single pathway. A similar result is observed in the MAPK signaling pathway. Only a relatively small number of pathways are affected by *Salmonella* containing AvrA that are not influenced by *Salmonella* lacking the protein. Examples in the cecum include the mTOR signaling pathway, natural killer cell-mediated cytotoxicity, and the Wnt signaling pathway. Our results indicate significant changes within the pathways related to *Salmonella* infection when comparing wild type to AvrA$^-$ mutants. These changes in key physiological and immune pathways appear to alter the infection characteristics of *Salmonella* within chicken.

Key Words: *Salmonella*, kinome, AvrA

P108 The concerted action of *Clostridium perfringens* perfringolysin with a toxin to induce necrohemorrhagic enteritis in calves.

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Bovine necrohemorrhagic enteritis or enterotoxemia is a major cause of mortality in veal calves, causing important economic losses. *Clostridium perfringens* is considered as the causative agent, but there has been controversy on the toxins responsible for this disease. Recently, it has been demonstrated that a variety of *C. perfringens* type A strains can induce necrohemorrhagic lesions in a calf intestinal loop assay. This puts forward a potential role for α toxin and perfringolysin, since both are produced by nearly all *C. perfringens* strains. The importance of perfringolysin in the pathogenesis of bovine necrohemorrhagic enteritis has not been studied before. Therefore, the objective of the current study was to evaluate the role of perfringolysin in the development of necrohemorrhagic lesions in calves and its possible synergism with α toxin. After experimental inoculation in calf intestinal loops, a perfringolysin-deficient mutant, an α toxin-deficient mutant and a perfringolysin α toxin double mutant were less able to induce necrotic lesions in comparison with the wild-type strain. Only complementation with both toxins could restore the activity to that of the wild-type. In addition, a perfringolysin-deficient mutant and an α toxin-deficient mutant were less cytotoxic on bovine endothelial cells as compared with the wild-type, but the cytotoxic effect of a perfringolysin α toxin double mutant was even lower. The cytotoxicity of a double-complemented mutant was comparable to that of the wild-type. These results underscore the importance of endothelial cell damage and potentially explain why capillary hemorrhages are an initial step in the development of necrohemorrhagic lesions as observed in the calf intestinal loop assay. Taken together, these results show that perfringolysin acts synergistically with α toxin in the development of necrohemorrhagic lesions in the bovine intestine and both toxins may act by targeting the endothelial cells.

Key Words: necrohemorrhagic enteritis, calves, *Clostridium perfringens*

P110 Heat stress: Intestinal barrier and immune disruption in pigs.

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Economic losses to the livestock industry due to heat stress (HS) are estimated to be greater than $2.0 billion annually. HS morbidity is linked to disruption of normal intestinal tract (IT) absorptive and barrier functions, is often manifested as decreased performance; however, extreme HS can have long-term negative effects or result in mortality. HS models often consist of constant exposure to high heat, unlike the cyclical heat pattern pigs are exposed to during production. In addition, knowledge regarding the effects of HS on specific and non-specific immune function in pigs is lacking. The goal of this study was to compare 2 HS models, constant heat (CH) versus variable heat (VH), in terms of health and performance statistics, IT histology, and specific

and non-specific immune responses. Pigs were divided into 3 treatment groups (n = 12/group) at weaning (21 d old): CH, VH, and control (C). The HS period started 4 weeks later and lasted for 7 d. CH pigs were subjected to 33°C during the entire HS period. VH pigs were subjected to a repeated cycle of 40°C for 5 h then 25°C for 19 h. C pigs were maintained at 25°C. Pigs in both HS groups were stressed by our criteria. Both CH and VH animals had lower ADG and ADFI and higher respiratory rates and skin and rectal temperatures during HS compared with controls. Further, HS pigs experienced disrupted specific and non-specific immune responses. Natural killer activity, a measure of non-specific immune function, was lower in VH animals compared with control. Only HS pigs had lymphoproliferative responses to a sonicate of 12 different bacterial species isolated from the IT mucosa of healthy pigs. These lymphoproliferative responses serve as an indicator of IT barrier disruption and a measure of specific immune function. IT barrier disruption is supported histologically by IT architectural changes including desquamation and lower villus height and crypt depth in the jejunum of HS pigs on d 1 and 3 of HS. Both CH and VH temperature stresses are acceptable models of HS in this age pig; the VH design is more representative of what the commercial pig experiences. Future studies will apply the VH design to optimize HS mitigation at the level of the IT to strategically reduce the burden of HS and to quicken recovery following a HS event.

Key Words: heat stress, immune, pig

P111 High and low loads of cecal colonization by *Salmonella* Enteritidis in chickens triggers distinct immune kinome profiles.

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Salmonella enterica serovar Enteritidis are facultative intracellular bacteria that cause disease in numerous species. *Salmonella*-related infections originating from poultry and/or poultry products are a major cause of human foodborne illness, and *S.* Enteritidis is the leading cause worldwide. Despite the importance of *Salmonella* to human health and chickens being a reservoir, little is known of the response to infection within the chicken gastrointestinal tract. Using chicken-specific kinome immune peptide arrays we compared a detailed kinomic analysis of the chicken gut immune response in birds with high and low *Salmonella* loads. Four-d-old chicks were challenged with *S.* Enteritidis (10^5 cfu) and cecal content and a section of jejunum collected on d 4, 7, 10, 14, 17, 24 and 37 post-infection (pi, [n = 5]). *Salmonella* colonization was enumerated and birds with the highest and lowest loads were selected for kinomic analyses. A small number of peptides were differentially phosphorylated between birds with high and low *Salmonella* loads including IGF2R, Pyk2, VIM, and BLNK. Identification of specific proteins associated with increased resistance against *S.* enteritidis provides breeders additional biomarkers to identify birds naturally more resistant to this important foodborne pathogen potentially reducing the need for antibiotics and creating a safer food supply for the consumer.

Key Words: chicken, kinome, *Salmonella*

P114 Effect of dietary fructooligosaccharides supplementation on intestinal calcium and phosphorus transporter, ileal cytokine gene expression and apparent phosphorus digestibility of broiler chicks.

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Fructooligosaccharides (FOS) are non-digestible carbohydrates that have prebiotic properties. They are a good source for intestinal microflora fermentation and thus have the potential ability to improve mineral bioavailability and enhance immune function in the gastrointestinal tract of the animals. A study was conducted to investigate the effects of FOS supplementation on calcium (Ca) binding protein (CaBP), sodium dependent phosphate transporter 2 (SLC20A2), ileal interleukin (IL)-2, IL-8, IL-18 and interferon (IFN)-? gene expressions as well as total-tract apparent phosphorus digestibility (APD) of broiler chicks. A total of 90, 1-d-old, male Ross × Ross 308 chicks were randomly assigned to 3 dietary treatments with 6 replicate pens and 5 birds per pen. The 3 treatments include (1) PC: wheat-corn-soybean meal basal diet with adequate Ca and available phosphorus (aP) (1% Ca and 0.45% aP), (2) NC: basal diet with reduced Ca and aP (0.8% Ca and 0.25% aP), and (3) NC+0.5%FOS: NC diet supplemented with 0.5% FOS. A 0.3% of titanium dioxide (TiO_2) was incorporated in the diet as an indigestible marker. The diets were fed for 21 d, and the birds had *ad libitum* access to feed and water. On d 21, segments of ileum were collected for determination of gene expressions using quantitative real-time polymerase chain reaction (qRT-PCR), and digesta samples were collected for measuring P concentrations by an inductively coupled plasma optical emission spectrometer (ICP). The mRNA expression of intestinal mineral transporters, CaBP and SLC20A2, was not significantly different among the dietary treatments. However, total tract APD was significantly increased ($P = 0.002$) in NC and NC+0.5%FOS treatments when compared with the PC treatment. Expression of IL-2 and IFN-? gene in the ileum of the broiler chicks were significantly upregulated ($P < 0.0001$, $P = 0.003$, respectively) in chicks fed 0.5% FOS diet. In the same time, the expression of IL-8 and IL-18 did not differ among the treatments. In conclusion, these results indicated that the low Ca and aP diet and the supplementation of FOS did not affect Ca and P transporter gene expression in the intestine of broiler chicks. The low Ca and aP diet improved total-tract P utilization. The dietary supplementation of FOS in broiler ration demonstrated significant influence on ileal cytokine gene expression. It would be necessary to further investigate molecular mechanisms of FOS on the immune responses of broiler chickens.

Key Words: fructooligosaccharides, cytokine gene, intestinal mineral transporter

P116 Effect of a liquid whole-egg globulin protein supplement on broiler performance, intestinal histology, and bacitracin-resistant *Clostridium* growth.

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Protemace is a liquid whole-egg globulin protein supplement, applied to the drinking water, that provides a source of immunoglobulins. This investigational effort was designed to measure the effect of Protemace on various aspects of broiler performance. Parameters measured included live performance, duodenal and ileal histology, and mid-gut bacitracin-resistant *Clostridium*. Day-old males from the Cobb 500 female line were allocated to 48 pens (30 chicks/pen; 12 pens/treatment) and grown to 42 d. There were 4 water treatments. Control had 2 subgroups, one used only municipal water and the other included diluted stabilized hydrogen peroxide provided at the same time as treatment 4. Treatment 2 supplemented Protemace from d 1–7, followed by 24 h treatment of diluted stabilized hydrogen peroxide. Treatment 3 supplemented Protemace from d 1–7, d 21–28, followed by a 24 h treatment of diluted stabilized hydrogen peroxide dilution on d 8 and 29. Treatment 4 supplemented Protemace from day1–42, followed by a 24 h treatment of diluted stabilized hydrogen peroxide on d 8, 15, 22, 29, 36, and 40. Live weights, feed conversions and livability were evaluated at 14, 35, and 42 d. Gastrointestinal histology and *Clostridium* were evaluated at d 42. No significance existed in live performance ($P < 0.05$). Duodenal villi height in treatments 2 and 3 were both significantly longer ($P < 0.05$) than the control, while crypt depth was significantly deeper ($P < 0.05$) in treatments 2, 3, and 4 versus controls. Treatments 3 and 4 had crypt depth significantly deeper ($P < 0.05$) than treatment 2. Ileum villi height of treatments 2, 3, and 4 were significantly longer ($P < 0.05$) than control, and crypt depths of treatments 2, 3, and 4 were all significantly deeper ($P < 0.05$) than control, with treatments 3 and 4 being significantly greater ($P < 0.05$) than treatment 2. Of the 12 birds per treatment sampled, the control group had a 77% incidence of bacitracin-resistant *Clostridium* species in the mid-gut while treatments 2 and 3 each had 33% positive incidence and treatment 4 had 0% positive. These results suggest supplementing with Protemace could improve gut health and may also have a role in alleviating clostridia challenges in broilers.

Key Words: globulin, broiler, *Clostridium*

P120 In vitro binding of lipopolysaccharide by processed calcium montmorillonite (Calibrin-Z).

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Endotoxic lipopolysaccharides (LPS) are components of the outer membrane of the cell walls of gram-negative bacteria, such as *Escherichia coli*. They can range in size from 10,000 to 1,000,000 Da. Endotoxins are toxic to animals; the degree of toxicity varies with the species of animal and the amount of the endotoxin, since their toxicity is associated with the animal's immune system's responses to their presence in the animal's bloodstream. If the intestine is compromised by diseases or mycotoxins, endotoxins may be able to enter the animal's bloodstream. However, if the endotoxin could be bound and retained in the intestine it would be unable to enter the bloodstream and cause toxicity. Processed calcium montmorillonite, Calibrin-Z, has been shown to effectively bind a broad range of mycotoxins and bacteria toxins, such as α toxin and NetB from *Clostridium perfringens*, preventing them from entering the body and causing damage; thus it was hypothesized that it might also bind LPS. Therefore, an in vitro test was run using the *Limulus* amebocyte lysate assay method in which LPS solutions with concentrations of 5, 10, 50, or 500 ppm were treated with Calibrin-Z. After removal of the Calibrin-Z with a 0.2 µm filter, solutions were tested for the presence of LPS. Results are shown in the following table, and show that the processed calcium montmorillonite bound lipopolysaccharide.

Table 1. Binding of lipopolysaccharide by processed calcium montmorillonite in vitro

Initial LPS, ppm	Calibrin-Z:LPS	Endotoxin units/mL		% Removed
		Untreated	Treated	
500	20	2,544,000	1,620,000	36
50	200	258,500	96,700	63
10	1,000	48,900	24,000	51
5	2,000	34,000	5,730	83
1	10,000	5,240	260	95
0	—	0.841	0.005	—
0	—	0.814	None detected	—

Key Words: lipopolysaccharide, endotoxin, calcium montmorillonite

NOTES

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