



Oral Abstracts

Session 1: Nutrition and Gut Health

100 Functional properties of dietary complex carbohydrates and minerals as modulators of gut microbiota and digestive physiology in pigs.

B. Metzler-Zebeli*,

University of Veterinary Medicine Vienna, Vienna, Austria.

The commensal gut microbiota play an important role for the host, being involved in nutrient digestion, metabolism and gut health. In view of the demand to reduce antibiotic use in pig production, enhancing porcine gut health after weaning by dietary intervention is of utmost importance. Similar to humans, complex carbohydrates may act as functional ingredients in pigs but they are often regarded as detrimental for growth. Increasing gastrointestinal butyrate fermentation and lactobacilli are assumed beneficial in young pigs; hence, results from our and other research would categorize β -glucans as functional component, whereas resistant starch-rich cereals may favor beneficial bifidobacteria. Research on resistant starch (RS) further showed that the introduction of new RS types need to be critically evaluated to ensure promotion of intestinal saccharolytic bacteria and fermentation profiles toward higher propionate and butyrate proportions. Dietary minerals, such as calcium-phosphorus (CaP), may have a stabilizing effect on the gut microbiota too, either by meeting special microbial nutrient needs or influencing luminal conditions in the gut. Using microbiome analysis, our research indicated a distinct and specific promotion of beneficial lactobacilli at the stomach mucosa of weaned pigs fed high CaP levels, whereas low dietary CaP may limit butyrate fermentation in the porcine hindgut. Changes in the fermentation profiles in the upper digestive tract indicated a modulatory effect of CaP on microbial metabolic activity or increased host absorption which was supported by changes in expression of nutrient transporters and cytokines in the small intestine. We could show an effect of the dietary CaP level on jejunal expression of tight-junction proteins and toll-like receptor 2 which may affect barrier function in young pigs. Data of our research emphasize the need to carefully evaluate the effects of complex carbohydrates and dietary minerals on the gastrointestinal bacterial homeostasis and the consequences for the host to formulate “gut healthy” diets.

Key words: gut health, pig, microbiota

101 Effect of zinc oxide sources and dosages on intestinal coliform counts and gut integrity of weaned piglets.

J. Michiels^{*1}, N. Van Noten¹, J. Degroote¹, W. Wang¹, and A. Romeo²,

¹Ghent University, Gent, Belgium, ²ANIMINE, Sillingy, France.

Zinc oxide (ZnO) can be supplied at pharmacological dosage (2400 mg/kg of Zn) in diets of weaned piglets to improve performance through adjusting gut health. In this study, the effects of a potentiated ZnO source (HiZox®) at low dose were compared with the regular ZnO. The conventional ZnO was evaluated at 110 and 2400 mg/kg of Zn, vs. 110 and 220 mg/

kg of Zn for the potentiated ZnO. High iron level was used to induce gastro-intestinal disturbances: diets including regular ZnO contained 100 or 500 mg/kg of Fe from FeSO₄, vs. 500 mg/kg of Fe for diets with the potentiated ZnO. Each of the 6 treatments was replicated in 4 pens (2 piglets/pen, 20d of age at start), during 15 d. Animal performance, coliform and *E. coli* counts by plating in intestinal contents and gut barrier and chloride secretion upon secretagogues in distal jejunum were assessed. Groups fed with conventional ZnO at 2400 mg/kg of Zn and potentiated ZnO at 220 mg/kg showed higher growth than the other groups, irrespective of Fe content ($P < 0.05$). Piglets fed the conventional ZnO at 2400 mg/kg of Zn had significantly lower numbers of coliform bacteria and *E. coli* in distal small intestine than the groups fed with 110 mg/kg of Zn from regular ZnO ($P < 0.05$). Also, supplementation with potentiated ZnO reduced coliform counts compared with 110 mg/kg of Zn from regular ZnO ($P < 0.05$), and numerically reduced *E. coli*. Transepithelial electrical resistance (TEER) of jejunal mucosa was significantly ($P < 0.05$) higher for groups fed with potentiated ZnO, compared with groups fed with 110 mg/kg of standard ZnO, suggesting a better intestinal epithelial integrity. In conclusion, the potentiated ZnO at low dosage showed positive effects on the reduction of the coliform counts and improved gut epithelial barrier integrity, albeit similar to the effects of pharmacological dosage of standard ZnO.

Key words: zinc, piglets, weaning

102 Effect of exogenous nucleotide supplementation on gut health and cytokine profile of newly weaned piglets fed a high-soybean meal diet.

C. Camacho^{*1}, A. Garcia², S. Solorio², and T. G. Kiros²,

¹Instituto Nacional de Ciencias Medicas y Nutrición Salvador Zubiran (INCMNSZ), Mexico City, Mexico, ²Phileo-Lesaffre Animal Care, Marcq-en-Baroeul, Lille, France.

Weaning is often characterized by reduction in feed intake (anorexia) resulting in intestinal damage leading to the “Leaky gut” syndrome if not managed well. Soybean meal is a source of quality protein which may help in repairing the intestinal damage associated with post weaning anorexia. However, its inclusion in nursery diets is often restricted due to the presence of dietary antigens that induce severe inflammation and tissue damage in young pigs. Nucleotides are often used to enhance growth and repair of tissues with rapid turnover of cells like the intestine. The objective of this study is therefore, to evaluate the effect of dietary nucleotides in ameliorating the disturbance in immune response and intestinal damage associated with feeding high soybean meal diet and weaning stress in nursery pigs. A total of 45 piglets weaned at 21 d of age with an average weight of 5.5 ± 0.9 kg were randomly assigned to one of 3 diets containing high soybean meal (25%): without, with 500ppm, or 1000ppm yeast extract rich in nucleotides. Morphometric measurement was conducted on small intestinal samples, as well as pro and anti-inflammatory cytokines measured in serum using Luminex assay. Yeast extract rich in nucleotide supplementation both at 500ppm and 1000ppm significantly increased intestinal villi length in the



duodenum ($P < 0.05$) and Jejunum ($P < 0.001$), while crypt depth was significantly decreased ($P < 0.001$) only in the duodenum of piglets supplemented with 1000ppm. Supplementation with 500ppm showed significantly higher levels of anti-inflammatory cytokines ($P < 0.05$), mainly IL-10, but also IL-1ra and IL-4. On the other hand, there was no significant effect of nucleotides ($P > 0.05$) on pro-inflammatory cytokines except for IL-1b which seems to increase in the 500ppm group. In conclusion, we have shown here that supplementation of piglets with 500ppm of yeast extracted nucleotides can suppress the inflammatory response associated with high soybean meal diet and ameliorate the intestinal damage associated with post-weaning nutritional stress.

Key words: gut health, nucleotide, pigs

103 Type B trichothecene mycotoxins in US feed and corns samples from 2014 to 2016.

E. G. Hendel^{*1}, T. Jenkins², S. M. Mendoza¹, and G. R. Murugesan¹,

¹BIOMIN America, Inc., San Antonio, TX, ²BIOMIN Holding GmbH, Getzersdorf, Austria.

Mycotoxins are harmful fungal metabolites commonly found in commercial crops. Type B trichothecenes, mycotoxins produced by *Fusarium* fungi, are detrimental to gut health, decreasing mucus production, compromising intestinal epithelial tight junctions, increasing intestinal inflammation, and suppressing immune function. The Type B trichothecenes (B-Trich) include deoxynivalenol (DON), nivalenol (NIV), and fusarenon-X. Additionally, fungi produce acetylated forms of DON (3-ADON, and 15-ADON). Mycotoxins can also be masked by plants with added glucose or sulfate molecules, rendering them undetectable to conventional analysis. Masked B-Trich (DON-3-glucoside; DON 3-G, and NIV-glucoside; NIV-G), are broken down to DON and NIV, respectively, within the stomach and are thus equivalent in toxicity to unmodified toxins. Emerging mycotoxins are newly identified fungal metabolites. Tenuazonic acid (TA), produced by *Alternaria* and *Phoma* fungi, inhibits protein synthesis and is equivalent to DON in toxicity. TA is known to cause gizzard erosion in poultry. These masked and emerging mycotoxins present a challenge for mycotoxin risk management; these metabolites are not tested by US diagnostic laboratories, and literature examining their presence, toxicity, kinetics, and multiple mycotoxin interactions is sparse. To investigate the prevalence of masked B-Trich and TA in the US, we further analyzed results from 96 samples submitted from 2014-2016 for multi-mycotoxin testing at the University of IFA-Tulln, Austria (Spectrum 380®). These samples consisted of corn, corn silage, and complete feed for multiple livestock industries (cattle, swine, and poultry). Of the 96 samples, 63% had detectable DON, 5% 3-ADON, 12% 15-ADON and 49% had detectable DON-3-G. The median positive level of DON-3-G was 37 ppb with a maximum of 326 ppb. NIV was less commonly detected than DON (20% of samples) and only 2% of samples tested positive for NIV-G. The emerging mycotoxin TA was present in most samples (70%) with some high values (median 54 ppb, max 4942 ppb). Collectively these results indicate an increased risk to animal gut health beyond what is suggested by current detection methods in the US.

Key words: mycotoxin, leaky gut, TER

104 Nutritional rehabilitation responses in two genetic lines of chickens.

M. F. A. Baxter^{*1}, J. D. Latorre¹, N. Anthony¹, S. Dridi¹, D. A. Koltes¹, S. C. Ricke¹, S. Park¹, E. S. Greene¹, R. Merino², S. Bickler³, B. M. Hargis¹, and G. Tellez¹,

¹University of Arkansas, Fayetteville, AR, ²Universidad Nacional Autónoma de México, Ciudad De México, México,

³University of California, San Diego, CA.

Previously, we have reported that poultry fed a rye-based diet had significantly higher gut permeability, viscosity and altered gut morphology and bone mineralization when compared with poultry fed a corn-based diet. Two independent experiments were conducted to determine if broiler chickens from a 1995 commercial line (95 line; Exp 1) or birds from a jungle fowl line (JF; Exp 2) could rehabilitate the intestinal epithelial barrier after consumption of a rye-based diet and determine if physiological damage could be repaired. In both experiments, chickens from hatch to 10 d-of age were fed either corn-based (C) or rye-based (R) diets. At d 10, diets were switched to one of 4 treatments; corn to corn (C-C); corn to rye (C-R); rye to rye (R-R); or rye to corn (R-C). At 10 and 20d, chickens received an oral gavage of fluorescein isothiocyanate dextran (FITC-d). One hour post gavage, birds were humanely euthanized and blood samples were collected from the femoral vein to determine the passage of FITC-d. The liver was collected from each bird to evaluate bacterial translocation (BT). Samples from duodenum and ileum were collected for morphometric analysis and tibias were collected for bone diameter and strength. At 10 d of age, the 95 line fed a corn-based diet had significantly ($P < 0.05$) higher body weight (BW), and tibia diameter and significantly lower serum FITC-D than chicks fed a rye-based diet. At 20 d of age, C-C chicks had significantly higher BW and tibia breaking strength compared with other treatments. C-C chicks had significantly lower BT and duodenal villus height to crypt depth (VH: CD) ratio than the R-R chicks. Interestingly, R-C chicks, showed a compensatory growth and bone mineralization when compared with R-R chicks. Similar results were observed in jungle fowl. These results confirm that, regardless of the genetic line, high rye diets negatively impact the gut permeability and stunt growth; however switching diets allows chicks to rehabilitate their gut resulting in compensatory growth and improved bone quality. The effects of these diets on microbiome composition as well as gene expression of tight junction proteins are currently being evaluated in both genetic lines.

Key words: nutritional rehabilitation, enteric inflammation, NSP

105 The use of gut health additive as an alternative to antibiotic growth promoters in broiler production.

K. Hogan¹, S. Webster¹, and P. J. Roubos-van den Hil^{*2},

¹Trouw Nutrition USA, Highland, IL, ²Trouw Nutrition R&D, Boxmeer, The Netherlands.

With the introduction of new veterinary feed directive rules limiting the use of antibiotics in feed, a need has arisen in finding new alternatives that can replace the growth performance effects of antimicrobial growth promoters (AGP). This study explored the use of Elarom ESP (Trouw Nutrition, Highland, IL), a commercially available blend of short chain fatty acids (SCFA),



medium chain fatty acids (MCFA), slow release MCFA and a phytogetic compound. The study was performed at Southern Poultry Research, Inc. in Athens, Georgia. A total of 3,600 male chicks (Ross 708) were randomly and equally assigned to 4 treatments (50 chicks per pen). The treatment included: 1) negative control, 2) Elarom ESP standard (1.5 – 1.0 – 0.5 kg/t starter - grower - finisher, 3) Elarom ESP Elevated (2.0 – 1.5 – 1.0 kg/t starter – grower - finisher) and 4) positive control bacitracin methylene disalicylate (BMD; 50 g/ton). The chicks were placed on litter that was not removed from the facility from the previous flock and received a corn soy diet that is typically fed in the US. Half of the broilers were grown to a final weight of approx. 2.5 kg (49 days) and the other half to 3.6 kg (63 days). Bird weights by pen were recorded at days 0, 15 and 30 and 49 for the 2.5 kg group and 0, 15, 30, 40 and 63 for the 3.6 kg group. The results from the elevated Elarom ESP group indicated that there was no

observed performance advantage to an increased inclusion, even in the larger sized broilers. It was observed that the FCR of the birds in the Elarom ESP group was significantly lower than that of the negative control group. The FCR observed from the BMD group was similar to that of the Elarom ESP group (standard) at the end of the growing periods for both sizes of broilers. The body weight gain of the Elarom ESP group was significantly higher than the negative control in the finisher period of the heavy birds ($P = 0.002$). The results in body weight gain of the Elarom ESP group are comparable with the AGP group. The results of the Elarom ESP group compared to the group fed a diet containing BMD indicate that Elarom ESP could replace AGPs in broilers of both a 2.5 kg and 3.6 kg final weight.

Key words: antibiotic growth promotor, gut health additive, broilers



Session 2: Nutrition and Gut Health

106 Nutrition 2.0—Diet components at the gut/microbe interface.

B. J. Bradford* and S. E. Gragg,
Kansas State University, Manhattan, KS.

The first century of nutritional science focused largely on defining animal requirements for various nutrients, based on their roles as anabolic substrates, metabolic cofactors, and fuels. However, evolving methods in molecular biology and physiology have now revealed far more diverse and far-reaching impacts of dietary components, a development we refer to as Nutrition 2.0. Nutrients and even non-nutritive feed components can impact animal biology by influencing microbial communities, activating cellular receptors, or by directly altering enzyme activity. These primary effects can result in short-term impacts on animal physiology as well as long-term effects via epigenetic modifications. At the mucosal interface, these developments suggest that several routes may offer opportunities for nutrient-mediated modulation of gut/microbe interactions. Proof-of-principle studies demonstrate that nutrients not only influence commensal microbial communities, but can amplify or attenuate the immunology response to the presence of those commensals. Nutrients released by microbial metabolism help to program the mucosal immune system, and the gut is known to “feed” commensals during nutrient restriction. Opportunities likely also exist to influence the pathogenicity of opportunistic pathogens either through direct effects or via alterations to mucosal immunity or the surrounding microbial community. Further understanding the complex role that competitive exclusion and microbial metabolites play in decreasing pathogenicity and improving mucosal immunity is important for improving gut health. In summary, the vast number of mechanisms included in Nutrition 2.0 point to sustained hope that dietary alterations remain a fruitful area of investigation in the quest for improved gut health and function.

Key words: nutraceutical, host/microbe interactions, mucosal immunity

107 Managing necrotic enteritis in antibiotic-free production.

C. M. Pender* and G. R. Murugesan,
Biomim America Inc., San Antonio, TX.

As consumer and regulatory opinions have evolved, poultry producers are increasing the number of flocks raised without antibiotics. Standard antibiotic-free programs prohibit the use of several antimicrobial agents such as antibiotics and ionophores. Eliminating these tools brings legitimate concerns for producers regarding performance, flock uniformity, and disease incidence—particularly necrotic enteritis (NE). Several factors can have a considerable impact on the proliferation of *Clostridium perfringens* and the successful shift to antibiotic-free production. Unique phytogenic formulations have been effective in overcoming the challenges posed by predisposing and causative factors of NE. The first study evaluated the effects of a phytogenic feed additive (PFA; Digestarom®) on broiler performance during an *Eimeria* challenge. A total of 504, day-old broiler chicks were randomly assigned to one of 3 treatment groups, each consisting of 12 replicate pens (14 chicks/pen). Treatment groups

consisted of a non-challenged control, an *Eimeria* challenged control, and a challenged group supplemented with PFA (125 g/ton). Supplementation of PFA was able to improve body weight gain and feed conversion to levels similar to the non-challenged control. The second study investigated the effects of PFA on broiler performance during a NE challenge. A total of 570 broiler chicks were randomly divided among 3 treatment groups consisting of 8 replicate pens (24 chicks/pen). The treatment groups included a non-challenged control, and *C. perfringens* challenged control, and a challenged group supplemented with PFA (125 g/ton). Supplementation of PFA was able to improve body weight gain, feed conversion, and mortality and alleviate the negative consequences associated with NE. Ultimately, the main challenges producers face as they transition to antibiotic-free hinge upon intestinal health. Necrotic enteritis is a multifaceted disease with many overlapping predisposing factors, thus numerous adjustments are necessary to succeed. Phytogenic feed additives may provide an opportunity to reduce incidence of NE as part of an overall gut health program.

Key words: antibiotic-free, necrotic enteritis, phytogenics

108 EAAT3 regulates proliferation of porcine intestinal epithelial cells through the mammalian target of rapamycin pathway.

M. Zhu*, J. L. Ye, C. Q. Gao, X. G. Li, W. G. Sui, H. C. Yan, and X. Q. Wang,
College of Animal Science, South China Agricultural University/ National Engineering Research Center for Breeding Swine Industry, Guangzhou, Guangdong, P. R. China.

Excitatory amino acid transporter 3 (EAAT3) is primary glutamate (Glu) transporter, and Glu plays a critical role as fuel source for intestinal epithelial cells. While, there is limited information about the action of EAAT3 in the intestine. Therefore, 2 experiments were conducted to investigate the effects of EAAT3 on cell proliferation and mammalian target of rapamycin (mTOR) pathway in porcine jejunal epithelial cells (IPEC-J2). In the first experiment, IPEC-J2 cells were cultured in Glu-deficient medium supplemented with 50 $\mu\text{mol/L}$ Glu (Control), and 50 $\mu\text{mol/L}$ Glu plus 100 $\mu\text{mol/L}$ L-trans pyrrolidine-2,4-dicarboxylic acid (PDC, the EAAT3 inhibitor), respectively. In the second experiment, to further verify whether the expression level of EAAT3 is associated with the IPEC-J2 cell proliferation, transfection EAAT3-pcDNA3.1+ or siRNA were carried out to overexpress or knockdown the EAAT3 expression. Proliferation of IPEC-2 cells was determined by MTT and cell counting assays. The expression levels of mTOR pathway related proteins were analyzed by Western blot. The results showed that the proliferation of IPEC-J2 cells increased ($P < 0.05$) with EAAT3 overexpression group, and reduced ($P < 0.05$) by EAAT3 knockdown or PDC supplementation. The protein expression levels of phosphorylation of mTOR (Ser2448), ribosomal protein S6 kinase-1 (S6K1, Thr389) and ribosomal protein S6 (S6, Ser235) were increased by EAAT3 overexpression and decreased by EAAT3 knockdown or PDC supplementation ($P < 0.05$). In summary, EAAT3 can promote cell proliferation of IPEC-J2 by activating the mTOR signaling pathway. These findings may lay the foundation for improving the efficiency of



amino acids utilization, and provide reference target for treatment of glutamate metabolic diseases. [This study was supported by the National Basic Research Program of China (2013CB127302) and National Natural Science Foundation of China (31330075); Correspondence to: X. Q. Wang, e-mail: xqwang@scau.edu.cn].

Key words: EAAT3, intestinal epithelial cells, mTOR

109 Biomarkers of gut health in farm animals.

P. Celli^{*1,4}, A. Cowieson², A.-M. Klünter², F. Fru-Nji², and V. Verlhac³,

¹DSM Nutritional Products, Animal Nutrition and Health, Columbia, MD, ²DSM Nutritional Products, Animal Nutrition and Health, Wurmisweg 576, 4303 Kaiseraugst, Switzerland, ³DSM Nutritional Products, Animal Nutrition and Health, Village-Neuf, F-68128, France, ⁴Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia.

The study of gut health is a relatively young field of research in animal nutrition and health. However, while gut health is an increasingly important topic in animal nutrition, a clear scientific definition is still lacking although it has been used repeatedly in animal health. A clear definition of gut health and how it can be measured is required to monitor animal health and to evaluate the effects of any nutritional intervention on animal performance. As animal performance can be impaired with any overt clinical signs of disease, we propose to combine the principal components of gut health, namely diet, effective structure and function of the gastrointestinal barrier and normal and stable microbiota, with effective digestion and absorption of feed and effective immune status. All these components play a critical role in digestive physiology, animal health and welfare and therefore a more comprehensive definition of gut health would be “a steady state where the microbiome and the intestinal tract exist in symbiotic equilibrium and where the welfare and performance of the animal is not constrained by intestinal dysfunction.” Clarity of understanding of gut health will require the characterization of the interactions between all of these components. The development of biomarkers of gut health is imperative to gain clarity of understanding of the patho-physiological events that influence the intestinal barrier, its functionality and the ecology of the gastrointestinal microbiota. While there is considerable knowledge in biomarkers that are indicative of the gastrointestinal ability to digest, absorb, transport and secrete major macro and micro-nutrients, a large gap in the literature exists in relation to biomarkers of gastrointestinal permeability, gastrointestinal barrier function, or biomarkers that are indicative of the functional presence of beneficial microbiota or their metabolites. Therefore, future research should focus on the establishment of a reference panel of biomarkers of gut health to be used in farm

animals and address the issue of standardization of techniques and methodologies to study gut health.

Key words: gut health, biomarkers, inflammation

110 Transient and persistent grain-rich feeding affects differently the ruminal absorption and endotoxin concentration, but not the permeability of the ruminal wall in cattle.

Qumar Muhammad¹, Ratchaneewan Khiaosa-Ard¹, Fenja Klevenhusen², Joerg Aschenbach¹, and Qendrim Zebeli^{*1},
¹University of Veterinary Medicine Vienna, Vienna, Austria, ²Freie University of Berlin, Berlin, Germany.

Enhancement of the absorption of short-chain fatty acids (SCFA) and lactate across reticuloruminal wall while maintaining its epithelial integrity is instrumental in increasing energy supply and preventing health disorders in cattle. The present study investigated whether the reticuloruminal absorption of SCFA and lactate, permeability, and endotoxin concentration in the gastrointestinal tract, as well as the systemic acute phase response was altered by different strategies of high-grain feeding. Eight rumen-cannulated, nonlactating Holstein cows were fed a forage-only diet (baseline) and then gradually adapted to a 60% grain level in a crossover 2 × 2 experimental design. The grain-rich diet was fed for 4 wk either continuously (CON) or interruptedly (INT) with a 7-d break in-between in the INT model. Ruminal absorption of SCFA and permeability to lactulose across the reticuloruminal wall were determined in vivo with the washed reticulorumen technique. Compared with baseline, ruminal and fecal endotoxin concentrations dramatically increased ($P < 0.001$) by grain-rich feeding, though cows in CON group tended ($P < 0.10$) to have greater endotoxin than INT cows. In contrast, the grain feeding did not affect the absorption of lactulose across rumen wall and the activation of a systemic acute phase response. Data showed that the absorption rates of individual and total SCFAs were higher in CON vs. INT cows ($P < 0.05$). Lactate was not absorbed during forage-only feeding and 1-wk grain feeding, but after 4-wk of grain feeding lactate was absorbed from reticulorumen wall independent of the feeding model. In conclusion, SCFAs absorption across the reticulorumen was enhanced by the 4-wk continuous grain feeding, which seems to be more advantageous in terms of rumen acidosis prevention compared with the interrupted grain feeding model. The study also provided evidence of lactate absorption across the reticulorumen of nonlactating cattle after both continuous and interrupted 4-wk grain feeding. The feeding of 60% grain either transiently or continuously did not compromise rumen permeability or trigger an acute phase response in cows.

Key words: high-grain diet, rumen permeability, ruminal absorption



Session 3: Beneficial Microbes and Gut Health

111 Cultivating the enteric ecosystem to resist enteric pathogens and maintain gut health in poultry.

P. R. Ferket*,

Prestage Department of Poultry Science, North Carolina State University, Raleigh, NC.

Gut health has a great influence on the growth performance and welfare of poultry, as it affects feed digestion, nutrient absorption, protein and energy utilization, immunity and disease resistance, metabolism, and physiology. To cultivate gut health, we must understand the fundamentals ecology and enteric development: 1) development and condition of the physical ecosystem; 2) nutrient-substrate balance; and 3) microflora symbiosis. Developing a robust ecological environment in the gut is paramount to establishing lasting gut health and nutrient utilization efficiency, beginning by enteric conditioning during the perinatal period. Supplementing the perinatal chick's first meal (the amnion) by in ovo feeding greatly advances gut development, skeletal health, immune function, meat yield, and growth performance efficiency. Access to feed soon after hatch also has a critically positive effect on subsequent health and growth performance. Optimizing nutrient balance within the appropriate parts of the gut to favor a symbiotic enteric ecosystem is a critical. Poultry depend upon dietary texture and particle size to normalize gizzard function and gut motility, which helps establish distinct microbial ecosystems within the gut that favor the proliferation of symbiotic microflora. Strategic use of supplemental enzymes improves foregut digestion, which starves pathogens of nutrients and leaves fermentable substrates for symbiotic bacteria in the hindgut. Finally, strategic use of feed additives help maintain the symbiotic microflora stability throughout the productive life of broilers. Dietary supplementation of herbs, spices, essential oils, and organic acids have been shown to reduce the microbial load of pathogens, while probiotics and functional carbohydrates favor of microflora that symbiotically support gut health. The strategic combination of factors affecting early development, feed manufacturing, and feed additives formulation is necessary to cultivate gut health in poultry.

112 *Bacillus subtilis* improves performance of broilers fed medicated or non-medicated feed.

L. Rhayat¹, V. Jacquier¹, P. A. Geraert¹, E. Devillard¹, and A. Ghane*²,

¹*Adisseo France SAS, Antony, France,* ²*Adisseo USA Inc., Alpharetta, GA.*

Bacillus-based direct-fed microbials are of particular interest to improve gut health and performance, due to their ability to modify gut microbiota, and to remain viable after pelleting. The objective of the present experiment was to investigate the effect of *Bacillus subtilis* DSM 29784 on the performance of broilers compared with or in combination with bacitracin methylene disalicylate (BMD). A total of 2400 d-old male broiler chickens, Cobb 500, were randomly allocated according to a factorial design with 4 treatments (12 replicates of 50 birds) and reared until 35 d in floor pens. The experimental treatments were: T1, negative control (basal diet, corn-based); T2, T1 + BMD at 55 ppm; T3, T1 + *Bacillus* strain DSM 29784 at 5.10^8 cfu/kg of feed;

T4, T1 + *Bacillus* strain DSM 29784 + BMD (5.10^8 cfu/kg of feed and 55 ppm, respectively). Feed intake (FI) and body weight gain (BWG) were measured at 21 and 35 d and feed conversion ratio (FCR) calculated. At 21 d, the 3 treatments (groups T2, T3 and T4) significantly improved BWG and FCR ($P < 0.05$). There was also a numerical improvement when T4 was compared with T2 and T3. At d35, all treatments increased BWG, significantly ($P < 0.05$) for T3 and T4 with an improvement of 7.7% and 6.8%, respectively. T2 and T3 improved significantly ($P < 0.001$) the FCR by 3.3% and 3.7%, respectively. For T4, there was also a significant ($P < 0.001$) FCR improvement of 4.1%, with a numerical ($P > 0.05$) increase compared with T2 and T3. These results showed that *Bacillus subtilis* strain DSM 29784 improves broiler performance, and the level of improvement is similar to that obtained with BMD. There were no antagonistic interaction between the 2 products and a trend for performance increase was obtained with the combination. In conclusion, *Bacillus subtilis* strain DSM 29784 can be added to non-medicated as well as medicated diets to improve broiler chicken performance.

Key words: direct-fed microbial, *Bacillus subtilis*, broiler

113 What can we learn from the small intestinal mucosal glutathione redox status during the weaning transition of piglets?

J. Degroote*¹, H. Vergauwen², W. Wang¹, N. Van Noten¹, C. Van Ginneken², S. De Smet¹, and J. Michiels¹,

¹*Ghent University, Ghent, Belgium,* ²*University of Antwerp, Antwerp, Belgium.*

Weaning of young mammals is a critical process which interferes with the functionality of the digestive tract. In pigs, weaning is associated with small intestinal villus atrophy, disruption of the tight junction barrier function and an increased inflammatory status of the gut. It has also been shown that the enterocyte oxidative status affects the severity of these alterations. With regard to the oxidative status, the tripeptide glutathione (γ -glutamyl-cysteinylglycine) is the most abundant (1–10 mM) and potent intracellular reducing agent. Because of these properties, glutathione is the main determinant of the cellular redox status, which is involved in the modulation of intestinal epithelial cell proliferation and cell-cell adhesion. Next to the important antioxidant and redox functions, glutathione is also involved in the phase II conjugation of xenobiotic metabolites. Taken together, it can be stated that glutathione and its redox status are of special interest during the weaning transition. This presentation aims to describe the alterations of the glutathione redox status after weaning that were observed when different weaning systems were compared. The results indicate that the small intestinal mucosal glutathione redox status can be maintained at the homeostatic level during the post-weaning period. However, maternal separation and feeding a milk replacer at a very young age (3d post-natal) severely affected the redox status of different tissues, including the small intestinal mucosa. When these artificially reared piglets were subsequently weaned at 3 weeks of age, a similar evolution of the small intestinal mucosal glutathione redox status was observed as seen after maternal separation. Furthermore, in all cases the changes in the mucosal glutathione redox status were accompanied with a



transient decreased barrier function of the small intestine. This was measured by assessing the FITC-dextran (FD4; 4 kDa) paracellular flux in Ussing Chambers. These observations are in line with earlier in vitro research where it is shown that a disturbed redox status can affect the enterocyte functionality.

Key words: glutathione, weaning, piglets

114 Ecology of broiler cecal bacteria following *Clostridium perfringens* infection and effects of supplemental bacitracin or Avi-Lution®.

L. A. Krueger*¹, D. A. Spangler¹, A. M. Temple¹, C. A. Johnson¹, D. R. Vandermyde¹, M. D. Sims², and G. A. Ayangbile¹,

¹Agri-King, Inc., Fulton, IL, ²Virginia Diversified Research Corp., Harrisonburg, VA.

Avi-Lution® is a defined, patented, synbiotic formula containing *Saccharomyces cerevisiae*, *Bacillus* spp., and *Enterococcus faecium*. Broiler chickens (n = 1250) were experimentally treated as unchallenged controls (uCon), infected controls (iCon) with *Clostridium perfringens*, or infected and treated with bacitracin (BMD) or Avi-Lution® at 1.0 (AvL1) or 2.0 (AvL2) g/kg in feed for 42 d. AvL1, AvL2, and BMD-treated pens showed improved growth and feed efficiency compared with iCon pens. Despite improved performance, AvL1 pens documented the greatest severity of infection-associated intestinal lesions. Concentration of acetic acid and total volatile fatty acids (VFA) were significantly decreased ($P < 0.05$) in AvL1 jejunal contents, which potentially stimulated ecological changes in the cecum. Cecal propionic and valeric acids were significantly correlated ($R^2 > 0.57$) for all treatment groups except AvL1 ($R^2 = 0.13$), and AvL1-treated birds documented decreased concentrations of isobutyric, butyric, and valeric acids despite no difference in total VFA. A unique and distinct correlation ($R^2 = 0.77$) between propionic and isovaleric acids was also observed in AvL2 samples. Relative abundances of cecal bacterial operational taxonomic units (OTU), classified on the basis of 97% 16S V4 gene similarity, significantly predicted the concentration of cecal propionic acid in each treatment group via stepwise linear regression analyses ($R^2 > 0.75$), but disparity in prediction efficiencies among treatments for a combined model suggests that the OTU associated with propionic acid metabolism were central to ecological changes induced by antibiotic or synbiotic treatments.

Key words: *Clostridium perfringens*, broiler, probiotic

115 Influence of maternal microbial communities on the intestinal mucosal microbiome of the neonatal pig.

N. Maradiaga*¹, M. Zieneldin^{1,2}, J. Lowe¹, and B. Aldridge¹,
¹University of Illinois at Urbana-Champaign, Urbana, IL,
²Benha University, Benha, Egypt.

Colostrum is vital to the newborn pig and cross-fostering is employed to equalize the number of piglet between litters ensuring colostrum intake for their survival and growth. However, little is known about its impact on the intestinal microbiome of the neonatal pig. Twenty-four piglets were enrolled in the study to determine the influence of maternal microbial communities on the mucosal microbiome of the young pig. Piglets were randomly assigned to 1 of 3 treatments, according to colostrum source and postcolostral milk feeding for 21 d, as follow: treatment 1 (n = 8), received colostrum and post-colostral milk feeding from their

own dam; treatment 2 (n = 8), received colostrum from foster dam and returned to their own dam for post-colostral milk feeding; and treatment 3 (n = 8), received colostrum and post-colostral milk feeding from foster dam. DNA was extracted from nasal, fecal, and gastrointestinal (GI) tract of the piglets and from colostrum, vaginal, and fecal samples of the sows. Discriminant analysis revealed that bacterial communities varied with biogeographical location in the GI tract, with colon being the most diverse section. *Firmicutes* and *Bacteroidetes* were the dominant phyla in the GI tract of the young pig. Bacterial communities in both maternal colostrum and vaginal samples were significantly associated with those present in the GI tract, feces, and nasal passage of piglets. Treatment did not affect bacterial communities present in the piglet GI tract, however, the bacterial communities present in piglet fecal and nasal samples changed over time. Although cross-fostering did not impact microbial communities in the piglet, this study suggests an impact of colostrum and maternal influence on the development of the microbiome of the piglet.

Key words: cross-fostering, colostrum, piglet

116 Metatranscriptomic profiling of rumen microbiomes in beef cattle with different feed efficiency.

F. Li* and L. L. Guan,

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada.

Exploring compositional and functional profiles of the rumen microbiome can improve our understanding of rumen fermentation mechanisms and thus to further optimize this process. In this study, rumen digesta samples were collected from 20 beef steers with different feed efficiency (efficient, n = 10; inefficient, n = 10). Metatranscriptomic data sets were generated by sequencing total RNA using Illumina HiSeq platform. Bacterial and archaeal compositions were estimated based on 16S rRNAs, and microbial metabolic functions including carbohydrate-active enzymes (CAZymes) were predicted based on mRNAs from metatranscriptomic data sets. “Core active phylotypes” were identified including 8 bacterial families (*Succinivibrionaceae*, *Prevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, *Veillonellaceae*, *Spirochaetaceae*, *Dethiosulfovibrionaceae*, and *Mogibacteriaceae*), and 4 archaeal clades (*Methanomassiliicoccales*, *Methanobrevibacter ruminantium*, *Methanobrevibacter gottschalki*, and *Methanosphaera*), which represented $76.0 \pm 2.6\%$ (mean \pm SEM) and $89.4 \pm 3.3\%$ of total bacterial and archaeal abundance, respectively. “Core active pathways” were affiliated with 79 KEGG pathways, representing $59.7 \pm 3.3\%$ of mapped mRNAs with carbohydrate metabolism ($12.1 \pm 0.6\%$) and translation ($7.9 \pm 0.4\%$) being the most abundant functions. Through comparative analysis, the relative abundances of *Lachnospiraceae*, *Lactobacillaceae*, *Veillonellaceae*, and *Methanomassiliicoccale* tended to be different ($P < 0.10$) between high and low efficiency animals. Meanwhile, 22 metabolic pathways (such as “pentose phosphate pathway,” “vitamin B6 metabolism,” etc.) were enriched (FDR < 0.15), and 14 CAZymes were only present in inefficient animals, suggesting that the rumen microbiomes of inefficient cattle may have higher and more diverse activities than those of efficient cattle. This study is the first to link rumen metatranscriptomes with feed efficiency in beef cattle, indicating that different activities of rumen microbiomes may be related to the variations in host feed efficiency.

Key words: rumen microbiome, metatranscriptome, cattle



Session 4: Development and Diversity of the Microbiome

117 16S Characterization of chicken cecal microbiome during long-term heat stress.

J. Hsieh*¹, N. Barrett², T. Looft³, M. Persia², C. Schmidt⁴, and S. Lamont¹,

¹Iowa State University, Ames, IA, ²Virginia Polytechnic and State University, Blacksburg, VA, ³National Animal Disease Center, Ames, IA, ⁴University of Delaware, Newark, DE.

The gut microbiome (especially the bacterial population) has garnered attention in the recent years for its contribution toward host response to stress. For production chickens, both broilers and layers, heat stress has a significant negative impact on production traits and overall well-being of the birds. However, the gut microbiome of chickens under heat stress is currently poorly understood. In this study, we used 16S rRNA sequencing to characterize the chicken cecal microbiome during a 4-weeks heat stress experiment. A group of 80 production egg-layers were randomly split into 2 groups: heated and not heated. Eight birds from each group were euthanized to harvest samples at 5 time points during heat treatment: day 1, and weekly in weeks 1-4. Bacterial DNA were isolated from cecal digesta, and the V1-V3 region of the 16S rRNA gene was amplified. The barcoded amplicons were pooled and sequenced on the Illumina MiSeq. The sequences were analyzed with MG-RAST. We identified subtle changes in the microbial community. Divergence of the 2 treatment groups became apparent by 2 weeks of heat stress based on alpha-diversity analysis. However, the alpha-diversity level were similar between the two groups by 4 weeks. The Firmicutes/Bacteroidetes ratio decreased initially (week 1), showed recovery (week 2), but then declined again (weeks 3 and 4). 16S rRNA characterization gave unprecedented insights into the dynamic nature of heat-induced changes in the gut microbiome that may enable the rationale design of interventions to reduce the negative impacts of heat on layer performance. Support for this work is from an USDA-NIFA-AFRI grant and Hatch project #5358.

Key words: chicken cecal microbiome, heat stress, 16S rRNA sequencing

118 Bacitracin modulates metabolomic profiles in turkey cecal microbiomes.

T. Looft*¹, T. Johnson¹, L. Chandra¹, and M. Sylte¹,

¹USDA-ARS-NADC, Ames, IA.

Many antimicrobial compounds currently being used in US for disease prevention or treatment and feed efficiency in food producing animals will be withdrawn from the market in 2017, highlighting the need to define their mode of action and aid the search for alternatives. Here we describe the effects of bacitracin methylene disalicylate (BMD), a commonly used antibiotic feed additive, on turkey microbial communities and metabolomes over 14 weeks. Two-hundred-40 poults were divided into 3 treatment groups [no antibiotic control, sub therapeutic BMD (50 g/ton), and therapeutic BMD (200 g/ton)]. After euthanasia, cecal contents were collected to characterize microbial population shifts using high-throughput 16S rRNA gene sequence analysis and evaluate global metabolomic profiling. Both concentrations of BMD had immediate and lasting impacts on the microbiota structure, reducing species richness in the BMD-treated animals. Metabolomic analysis identified 712 named biochemical,

including overlap between the metabolic profiles of the therapeutic and subtherapeutic treatments, with 75 metabolites differentially present from the control animals ($q < 0.1$). These included markers for increased protein and dietary triglyceride catabolism, as well as microbial metabolism of complex plant carbohydrates such as hemicellulose and pectin. While many effects were sustained, some metabolic changes in the therapeutic group were transient. For example, birds fed both concentrations of BMD had early reductions in metabolic pathways associated with growth (i.e., protein and aromatic amino acids catabolism products), while only the therapeutic group had reduced metabolites associated with glycolysis. These temporal metabolic effects may be due to an early antibiotic disturbance followed by partial recovery of bacterial function even in the presence of continued antibiotics. Connecting the microbiome structure and metabolomic response during antibiotic disturbance may improve microbiota modulation strategies. Metabolic shifts within the bacterial community resulting from antibiotic consumption may be related to “beneficial” microbiome functions that can be targeted to improve animal health and production.

Key words: antibiotics, microbiome, metabolomics

119 Characterizing intestinal epithelial and immunological gene expression in healthy calves.

K. Wade*, E. Bichi, J. Lowe, and B. Aldridge,
Integrated Food Animal Medicine Systems, College of Veterinary Medicine, University of Illinois, Urbana, IL.

Intestinal disease is a significant cause of calf morbidity and economic loss to dairy and beef farmers. An understanding of the interplay between the intestinal immune system, epithelium, and microbiome, together called the “gut health triad,” will be essential in designing novel and effective intervention strategies. In this study, we characterized mucosal immunological and epithelial gene expression for various biogeographical intestinal sites in healthy calves during the first 3 weeks of life. We utilized 12 calves from second parity dams on a single commercial dairy. The calves were removed from their mothers upon delivery and immediately fed clean, high quality colostrum. Calves were fed milk replacer and were health-monitored daily. A subset of calves ($n = 3$) was sacrificed on d 1, 3, 7, and 21, and mucosal tissue samples were taken from the duodenum, proximal jejunum, middle jejunum, distal jejunum, ileum, and colon, along with associated lymph tissues and Peyer’s patches. Immune and epithelial health marker gene expression levels were analyzed using real-time quantitative PCR to determine the relative mRNA expression of interleukin 4 and 10, toll-like receptors 2, 4, and 10, tumor necrosis factor- α , interferon- γ , claudin 1 and 2, F11 receptor, cathelicidin 4, mucin 2, kruppel-like factor 4, and keratin 8 genes. The results showed that the mucosal immunological profile of this group of healthy calves was subdued. There was a range in epithelial health marker gene expression across biogeographical locations. Garnering a better understanding of the gut health triad could lead to the development of new management solutions for GI disease, reducing the need for antibiotics and improving overall health and production.

Key words: PCR, immunological gene expression, epithelial gene expression

**120 Effect of *in ovo* gram-negative bacterial inoculation on microbial profiles of chicks.**K. M. Wilson*¹, W. R. Briggs¹, A. F. Duff¹, K. D. Teague², L. E. Graham², and L. R. Bielke¹,¹The Ohio State University-OARDC, Wooster, OH, ²University of Arkansas, Fayetteville, AR.

Improved GIT development of chicks may be influenced by early exposure to bacteria during hatching. To promote early colonization of beneficial bacteria, *in ovo* administration of bacteria has been suggested as a potential efficient mechanism of administration of desirable strains, but the impact of gram-negative bacteria on gut health is not well known, limiting research on *in ovo* probiotics. The objective of these preliminary studies was to determine the impact of select apathogenic gram-negative isolates on microbial profiles of chicks. In all experiments, D18 embryos were inoculated into the amnion with either saline (CON), 2×10^2 cfu (LOW), 2×10^3 cfu (MED) or 2×10^4 cfu (HIGH) of an apathogenic gram-negative strain. On day of hatch (DOH), intestinal samples were collected for microbial recovery on McConkey's (MC) and tryptic soy agar (TSA). After *Citrobacter* inoculation, foregut (duodenum-Meckel's diverticulum) and hindgut (Meckel's diverticulum-cloaca) TSA and MC recovery were higher ($P < 0.0001$) in LOW and MED compared with CON and HIGH. After a repeated trial with similar results, separate D18 embryos were inoculated with CON LOW or MED doses of *Citrobacter* and GIT collected at DOH and 72 h post-hatch for microbial recovery on MC, TSA, and MRS. At 72 h post hatch, foregut and hindgut TSA and MC recovery were significantly higher in LOW and MED ($P < 0.01$), and hindgut MRS recovery was significantly higher ($P < 0.0001$) in LOW and MED compared with CON. Next, similar to *Citrobacter* experiments, D18 embryos were injected with CON LOW or MED of *Klebsiella oxytoca* and bacterial recovery determined on DOH and 72h. TSA and MC microbial recovery showed no difference among treatments in CON LOW and MED at DOH, and this trend continued to 72h. Taken together, these studies show that *Citrobacter* administered *in ovo* may have a stronger influence on microbial profile of the GIT than *Klebsiella oxytoca*. This suggests that different gram-negative bacteria may have different effects on diversity and establishment of pioneer colonizers in the GIT and further studies on the specific impact of these differences are warranted.

Key words: *in ovo*, pioneer colonizers, microbial diversity

121 Bmi1 or Lgr5 promote proliferation of porcine intestinal epithelial cells by activating Wnt/ β -catenin signaling pathway.

X. G. Li*, R. Q. Chen, C. Q. Gao, M. X. Chen, H. C. Yan, and X. Q. Wang,

College of Animal Science, South China Agricultural University/ National Engineering Research Center for Breeding Swine Industry, Guangzhou, Guangdong, P. R. China.

Polycomb ring finger oncogene (Bmi1) and Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) are widely used as markers of quiescent intestinal epithelial stem cells and actively dividing intestinal epithelial stem cells, respectively. The objective of this study was to clone pig Bmi1 and Lgr5 gene and to investigate their effects on proliferation of pig intestinal epithelial cells. Proliferation of pig intestinal epithelial cells was determined by MTT and cell counting assays. The expression

levels of Wnt/ β -catenin pathway related proteins were analyzed by Western blot. The results showed that pig Bmi1 cDNA is 3,193 bp in length and the 981 bp coding sequence encodes 326 amino acids. Lgr5 cDNA is 2832 bp in length and the 2724 bp coding sequence encodes 907 amino acids. Bmi1 or Lgr5 overexpression increased ($P < 0.05$) proliferation of porcine intestinal epithelial cells, decreased ($P < 0.05$) expression levels of Axin2 and GSK-3 β proteins, and increased ($P < 0.05$) levels of β -catenin, c-Myc and cyclin D1 proteins at 72 h after seeding, respectively. Furthermore, 10 μ M XAV939 (a small molecule inhibitor of Wnt/ β -catenin signaling which stimulates β -catenin degradation by stabilizing Axin2) supplementation decreased the proliferation of porcine intestinal epithelial cells, increased level of Axin2 protein, and decreased ($P < 0.05$) level of β -catenin protein, respectively. Interestingly, the expression levels of Bmi1 or Lgr5 proteins were also decreased significantly with the XAV939 supplementation. Taken together, our findings demonstrated that pig Bmi1 or Lgr5 can promote the proliferation of pig intestinal epithelium cells by activating Wnt/ β -catenin signaling pathway. [This study was supported by the National Natural Science Foundation of China (31330075) and National Basic Research Program of China (2013CB127302); Correspondence to: X. Q. Wang, e-mail: xqwang@scau.edu.cn].

Key words: Bmi1, Lgr5, intestinal epithelial cells

122 Expression of toll-like receptors and inflammatory cytokines in gut-associated lymphoid tissues in pigs subjected to cross-fostering.N. Maradiaga*¹, A. Pineda², M. Zeineldin^{1,3}, J. Lowe¹, and B. Aldridge¹,¹Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL ²Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, ³Benha University, Egypt.

Gastrointestinal (GI) disease is the leading cause of preweaning mortality and thus of great economic loss for the swine industry. Newborn pigs must receive colostrum during the first 3 d of life to receive a proper maternal immune protection. This study sought to establish a baseline for mucosal immune gene expression in young pigs reared in cross-fostering model given high quality colostrum from birth dam or foster dam upon birth. Thirteen piglets were euthanized at d 21 (a common weaning time in the pig industry) to characterize the mucosal inflammatory gene expression profiles at different biogeographical locations. Tissues from intestinal mucosa in jejunum, colon, ileum, peyer's patches and associated lymph nodes were utilized. Piglets were randomly assigned to 1 of 3 treatments, according to colostrum source and postcolostral milk feeding for 21 d, as follow: treatment 1 (n = 8), received colostrum and post-colostrum milk feeding from their own dam; treatment 2 (n = 8), received colostrum from foster dam and returned to their own dam for post-colostrum milk feeding; and treatment 3 (n = 8), received colostrum and post-colostrum milk feeding from foster dam. Quantitative real time PCR analysis was performed to quantify the expression of toll-like receptors (TLR) 2, 4, and 10, tumor necrosis factor- α (TNF α), interleukin (IL) 4, 10 and interferon gamma (IFN γ). Results show that all mRNAs were detected in all tissues. Transcript levels of the toll-like receptors and cytokines were higher ($P < 0.01$) in ileum lymph nodes and peyer's patches tissues. Transcripts of TLR2 and 4 were equally



expressed in colon. Furthermore, TRL10 and inflammatory cytokines were also expressed in jejunum peyer's patches. Overall, our data suggests that expression of toll-like receptors and cytokines were more consistent in the peyer's patches and lymph nodes tissues. Thus, revealing novel information about the distribution and expression patterns of these in the GI tract of the piglet.

Key words: piglet, gene expression, cytokines

123 Synergistic induction of chicken host defense peptide gene expression by sugars and butyrate.

G. Zhang*, L.-A. Fong, and L. T. Sunkara,
Department of Animal Science, Oklahoma State University, Stillwater, OK.

Routing use of in-feed antibiotics increases the risk of developing antibiotic-resistant pathogens. Alternatives to antibiotics are urgently needed to reduce resistance and ensure animal health and productivity. Modulating the synthesis of endogenous host defense peptides (HDPs) is being explored as a novel antibiotic alternative approach. In this study, we revealed that several

chicken HDP genes such as avian β -defensin 9 (AvBD9), AvBD14 and cathelicidin B1 (cath-B1) are upregulated by mono- and disaccharide sugars in both dose- and time-dependent manners in chicken HD11 macrophage cells. Among a panel of sugars tested, most induced AvBD9 and AvBD14 gene expression, whereas only lactose, galactose and trehalose stimulated cath-B1 expression. We further revealed a strong synergy in inducing AvBD9 and cath-B1 expression between butyrate and sugars in both chicken HD11 cells and jejunal explants. Although lactose alone had no impact on histone acetylation, it was synergistic with butyrate in enhancing histone acetylation. Several signaling pathways including NF- κ B, MAPK, and cAMP were also found to be involved in AvBD9 induction in chicken HD11 macrophage cells mediated by butyrate and lactose. In summary, mono- and disaccharides are capable of synergizing with butyrate in inducing chicken HDP gene expression. This study revealed the potential of using a combination of a sugar and butyrate as an antibiotic-alternative approach to disease control and prevention in poultry and possibly in other animal species as well.

Key words: host defense peptides, antimicrobial peptides, mucosal immunity



Session 5: Impact of Gut Mucosal Communities

124 Microbiome analysis reveals temperate dietary protein restriction improves the composition and metabolism of gut microbiota in adult pig model.

X. Ma*,

China Agricultural University, Beijing, China.

Low protein diets in animal husbandry could relieve global environmental nitrogen excretion, but its effects on gut microbiota and metabolism are unclear. Herein, the effects of dietary protein restriction on microbiota of finishing pigs (16%, 13% and 10% crude protein (CP) in diets) were investigated using Illumina Miseq sequencing, and microbial metabolites were analyzed. We observed that ileal bacterial abundance and diversity which reflected from Chao and Shannon index significantly increased when the dietary CP level reduced from 16% to 13%, but decreased in 10% CP group. Ileal abundance of Firmicutes obviously decreased whereas Proteobacteria increased accompanied with CP level reduction. Notably, *Lactobacillus* had a much larger proportion in 13% CP group. Meanwhile, colonic bacterial diversity decreased with CP level reduction, along with increased abundance of Firmicutes and decreased Bacteroidetes. Additionally, intestinal concentrations of SCFAs and biogenic amines decreased with reduction of dietary CP level. The ileal morphology was impaired in 10% CP group, whereas expression of tight junction proteins in 13% CP group were higher than other 2 groups. In conclusion, temperate dietary protein restriction (13% CP) could alter the bacterial community and metabolites, promote colonization of beneficial bacteria in both ileum and colon, and improve gut barrier function.

Key words: dietary protein level, finishing pigs, gut microbiota

125 Microbiota development in young animals will affect health and performance in later life.

P. Roubos-van den Hil*, M. Davids, and C. Smits,

Trouw Nutrition R&D, Boxmeer, The Netherlands.

The development of the microbiota in the gastrointestinal tract of neonates is characterized by rapid and large changes in abundance, composition and diversity. This development not only has short-term effects on health and growth of the animals but also will affect disease resistance and performance throughout life. From the first day of life, the gastro-intestinal tract is growing and undergoing major developments, changes in the microbiota coincide with maturation of intestinal cells and development of the immune system. Actually, microbial colonization of the intestinal tract starts already during birth of the animal. Sow study results show that vertical transmission from birth canal to neonatal piglet is determining to a large extent the initial microbiota. Subsequently, exposure to excreta and colostrum further play an important role in microbiota setting. Recent findings indicate that oral antibiotic treatment of sows has a significant effect on microbiota composition and intestinal development of their offspring. In broiler chickens, vertical transmission of the microbiota from the mother to newly hatched chicken is interrupted by hygiene practices in the hatchery. Birds will pick up randomly the microbiota that is present in the environment and this may lead to a highly variable and unfavourable initial flora. The absence of feed in the first hours of life may be an additional

risk factor for impaired microbiota development and immunity. Feed interventions during this critical perinatal period may have a large impact with long term consequences. Organic acids, pre- and probiotics are widely applied nowadays in neonatal and young animal feeds. Study results show various impacts of those compounds on microbial diversity and proliferation of specific groups of bacteria, which could be related to specific health benefits and growth performance. Since microbiota is established mostly in the neonatal period, this period is critical for gaining a stable and healthy microbiota that can alleviate the effects of stressors later in life. During the maternal and neonatal period feed plays an important role in modulation of a healthy intestinal system with lifelong effects on health and performance.

Key words: microbiota development, lifelong effects, feed

126 The link between the microbiome and immunological health in the intestines of healthy calves.

K. Wade*¹, E. Bichi¹, S. Ishaq², C. Yeoman², J. Lowe¹, and B. Aldridge¹,

¹Integrated Food Animal Medicine Systems, College of Veterinary Medicine, University of Illinois, Urbana, IL, ²Montana State University, Department of Land Resources and Environmental Sciences, Bozeman, MT.

Intestinal health plays an important role in the dairy and beef industries, and the presence of disease can cause significant economic loss to farmers. Essential to developing effective treatments is understanding the interaction between the “gut health triad,” which is made up of the intestinal immune system, epithelium, and microbiome. To that end, we compared the mucosal immunological gene expression of various biogeographical intestinal sites in healthy calves to the microbiome of the same population. Twelve calves from second parity cows on a single commercial dairy were removed from their dams immediately after birth and fed clean, high quality colostrum. The calves were clinically normal, and showed excellent feed intake and growth rates over the course of the experiment. A subset of calves (n = 3) was sacrificed on d 1, 3, 7, and 21, and mucosal tissue samples were taken from the duodenum, proximal jejunum, middle jejunum, distal jejunum, ileum, and colon. Immune gene expression (IGE) levels were analyzed using real-time quantitative PCR, and 16S rRNA sequencing was employed to determine the taxa of bacteria present. In the ileum, there were certain taxa of bacteria, including *Ruminococcaceae*, *Veillonella*, *Prevotella*, and *Lactobacillus*, that were negatively correlated with IGE, implying an immune regulatory role for these microbes. Contrastingly, there were bacteria that had a strong positive correlation with IGE in the colon. These bacteria included *Bacteroides* and *Gallibacterium*. In future studies, we will compare the intestinal microbial communities and immunological gene expression in immunologically compromised or sick calves to better understand the role that mucosal microbial communities play in gut health. Acquiring an understanding of the connection between the immune system and microbial populations could lead to the development of novel management solutions that improve GI health and reduce the volume of antimicrobials employed in these production systems.

Key words: microbiome, immunological health



127 *Bacillus subtilis* 29784 contributes to control the effect of *Clostridium perfringens* on broiler performance.

L. Rhayat¹, G. Mathis², C. Hofacre³, V. Jacquier¹, P. A. Geraert¹, E. Devillard¹, and A. Ghane^{*4},

¹Adisseo France SAS, Antony, France, ²Southern Poultry Research, Athens, GA, ³University of GA Veterinary Medicine, Athens, GA, ⁴Adisseo USA Inc., Alpharetta, GA.

Clostridium perfringens contribute to necrotic enteritis (NE) syndrome which causes significant economic losses in the broiler industry. *Bacillus subtilis* 29784 has been previously shown to inhibit several strains of *C. perfringens* in different types of in vitro tests. The study aimed to examine the effect of supplementing diet with *B. subtilis* 29784 on growth parameters. A battery trial from 0 to 28d of age used Cobb male broiler chicks challenged with 0 or 5,000 oocysts of *Eimeria maxima* on d14 and with 0 or 1×10^8 cfu of *C. perfringens* per bird once daily on d19, 20, and 21. There were 8 cages of 8 birds each per treatment fed with an unmedicated US standard broiler starter diet. Six treatments were: (1) Non-infected chicks, unmedicated diets; (2) infected chicks, unmedicated diets; (3) infected chicks, bacitracin methylene disalicylate (BMD, 55 ppm); (4) infected chicks, *B. subtilis* 29784 (5.10^7 cfu/kg), (5) infected chicks, *B. subtilis* 29784 (1.10^8 cfu/kg); and (6) infected chicks, *B. subtilis* 29784 (5.10^8 cfu/kg). Body weight gain (BWG) and feed intake were measured on d28 to calculate the feed conversion ratio (FCR). The challenge strongly decreased the performance of the animals up to -24.5% on BWG and +32.8% on FCR. *B. subtilis* 29784 restored the BWG of challenged animals by +19.8% to 29.8% and their FCR by -15.5% to -17%. The level of improvement obtained with *B. subtilis* 29784 was the same for all 3 doses tested.

Key words: direct-fed microbial, *Bacillus subtilis*, *Clostridium perfringens*

128 Effects of coccidial vaccination, supplemental dietary protease and vitamin C on intestinal barrier and immune parameters.

S. Hutsko^{*1}, M. Lilburn¹, A. Cowieson², and M. Wick¹,

¹The Ohio State University, Columbus, OH, ²DSM Nutritional Products, Belfast, UK.

In an era of increased transparency and an overall reduction in the use of medications in poultry diets and rearing programs, anti-coccidial vaccines have increasingly re-emerged as a one component of anti-coccidial rotation cycles. These vaccines are largely composed of low levels of live or attenuated oocysts and may cause low level inflammatory responses within the intestinal tract leading to the potential for secondary bacterial infections. In the current study, day old broiler chicks (n = 960) were obtained from a commercial hatchery. One half the chicks were administered an anti-coccidial vaccine (VAC; n = 480) and the other half were not vaccinated (CON; n = 480). One half of the VAC and CON chicks were fed either a control diet (CON) or the same diet with supplemental vitamin C and a protease (SUPP). The chicks were assigned to one of 2 rooms, each containing 24 replicate pens and the experimental design was a 2 × 2 complete randomized block design and. Oocyst concentrations in each pen was determined weekly and 2 birds per pen were euthanized on d 28 to collect ileal mucosal samples.

The transcription levels of 3 genes, *MUC2*, *TFF2* and *TLR4* were analyzed via RT-PCR and the PROC MIXED procedure of SAS 9.4 was used to analyze the data. The determined differences in oocyst concentrations in the VAC and CON chicks increased with age but there were no vaccination treatment effects on *MUC2*, *TFF2*, or *TLR4* transcription levels. The SUPP diets consistently increased transcription of all 3 genes although the treatment differences were not highly significant (*MUC2*, $P = 0.157$; *TFF2*, $P = 0.177$; *TLR4*, $P = 0.06$). There were no diet by vaccination interactions. The results of this experiment suggest that a low level of coccidiosis induced by vaccination was not sufficient to elicit transcription differences in 3 intestinal proteins associated with cellular homeostasis. The small transcriptional differences associated with the SUPP diet, particularly the vitamin C, could be in association with other beneficial intestinal lumen effects as this diet has been shown to augment iNOS production as a result of increased macrophage activity in vaccinated chicks.

Key words: coccidiosis, mucosa, transcription

130 A non-invasive technique to evaluate transcriptional changes in the GI tract of neonatal dairy calves undergoing a mild diarrhea.

F. Rosa^{*1}, S. Busato², F. C. Avaroma², M. Bionaz¹, and J. S. Osorio³,

¹Oregon State University, Corvallis, OR, ²Escuela Agrícola Panamericana El Zamorano, El Zamorano, Francisco Morazan, Honduras, ³South Dakota State University, Brookings, SD.

Diarrhea is the most common disease during the neonatal stage of dairy calves. Exfoliated gastrointestinal epithelial (EGE) cells isolated from fecal samples of newborn humans have been used to study GI transcriptomics associated with nutrition. Similarly, EGE from neonatal dairy calves can be used to evaluate nutritional effects as well as health conditions such as diarrhea. The aim of this study was to use the RNA isolated from EGE cells to evaluate the transcriptional adaptations to mild diarrhea by assessing the expression of genes related to the inflammatory response and cell membrane transporters. Eight newborn Jersey male calves were used from birth to 5 wk of age and housed in individual pens at the Oregon State University Dairy Center. After birth, calves received 1.9 L of colostrum from their respective dams. Calves had ad-libitum access to water and starter grain (22% CP) and were fed twice daily a total of 5.6 L whole milk. Starter intake, BW, fecal score, withers height (WH), and rectal temperature (RT) were recorded during the experiment. RNA isolated from EGE fresh fecal samples (~200 mg) collected weekly was used for RT-qPCR analysis. Data were analyzed using the PROC MIXED procedure of SAS. Statistical significance and tendencies were declared at $P < 0.05$ and $P \leq 0.15$, respectively. Starter intake, BW, and WH increased ($P < 0.01$) over time. Fecal score was greatest (2.6 ± 0.3 ; $P < 0.01$) during 2 wk. The mRNA expression of inflammation-related genes *TLR4* and *TNFA* increased ($P < 0.01$) over time and a trend was observed for *NFKB1* ($P = 0.07$), while a quadratic effect over time ($P < 0.01$) was observed for *IL8* and *IL1B*. In contrast, *IFNG* and *TLR2* expression decreased ($P < 0.01$) over time. A time effect ($P = 0.01$) was observed for *SLC5A1* expression, a sodium/glucose transporter, because of a decreased during 3 wk of age. The fecal RNA method utilized in this study was able to detect transcriptional changes during a mild diarrhea



event in neonatal dairy calves, which underscore the usefulness of such method for future research including nutritional effects and health conditions.

Key words: dairy calves, gut health, gene expression



Poster Abstracts

P100 Diving deeper into functionality of a probiotic product for livestock.

A. M. Temple*, D. A. Spangler, L. A. Krueger, G. A. Ayangbile, and C. A. Johnson,
Agri-King, Inc., Fulton, IL.

Avi-Lution® is a patented, direct-fed probiotic containing viable *Enterococcus faecium* (EF), *Saccharomyces cerevisiae* (Y) and *Bacilli*. Several animal studies have been performed to prove efficacy of this product. In one study, 20 non-Swiss albino CF-1 mice were challenged with *Salmonella typhimurium* ATCC #14028 (ST) and treated as control, with EF, Y, or EF and Y in combination. Mice treated with EF and Y in combination weighed more 7 d post-challenge, had lower fecal ST, and more rapid ST clearing effect ($P < 0.05$). In another study, 1,250 broilers were challenged with *Clostridium perfringens* and supplemented with Avi-Lution® at 1.0g/kg feed. Avi-Lution® improved body weight and mortality-adjusted feed conversion rate ($P < 0.05$). To investigate the action of EF within the gut, jejunal contents were analyzed for lactic acid. Challenged broilers treated with Avi-Lution® were statistically similar to the uninfected control ($P = 0.19$), while infected control had significantly lower lactic acid than uninfected control ($P = 0.03$). An in vitro study was done to identify the function of Y in the gut. Pig jejunal epithelial cells (IPEC-J2) were used to evaluate how Y interacts with ST and the impact on interleukin 8 (IL-8). IPEC-J2 cells were treated with Y for 20 min before the addition of ST, then incubated 2 h. IPEC-J2 cells were washed with antibiotic supplemented media, and incubated 6 h. TEERs measurements were taken at 0, 2, and 8 h, and IL-8 sampling at 8 h. Y did not improve tight cell junctions, but Y reduced ST internalization into IPEC-J2 cells ($P < 0.001$). We hypothesized Y may impede nutrient availability to IPEC-J2 cells due to reduction in tight junctions, so heat-killed Y (YK) was added to ST treated IPEC-J2 cells resulting in higher TEERs ($P < 0.001$) and reduced ST internalization ($P < 0.001$). Y and YK numerically reduced IL-8 secretion due to ST. We hypothesize EF acts to produce lactic acid in the jejunum of the broiler hindering growth of pathogenic bacteria. Y and YK reduced ST in IPEC-J2 cells, and YK also restored tight junctions. Avi-Lution® acts as a multi-functional probiotic to prevent the harmful effects of bacterial challenges.

Key words: probiotic, IPEC-J2

P101 Analysis of *Lactobacillus* species in the ceca of breeder hens.

B. Adhikari* and Y. M. Kwon,
Department of Poultry Science, University of Arkansas,
Fayetteville, AR.

The genus *Lactobacillus* has been widely studied due to their beneficial effects on health and performance of chicken. For isolation of lactobacilli, de Man, Rogosa and Sharpe (MRS) agar medium has been used most commonly, yet there is little information regarding their selectivity to different *Lactobacillus* species. In this study, we hypothesized that (1) *Lactobacillus* population recovered from MRS agar may not represent the *Lactobacillus* population in gastrointestinal tract of chickens accurately, and (2) the *Lactobacillus* colonies recovered from

higher serial dilutions represent dominant *Lactobacillus* species in the samples, which may be an important feature for isolation of probiotic candidates. For this purpose, cecal samples were collected from 10 32-weeks old breeder hens, and 10^2 fold (LOW), 10^4 fold (MEDIUM), and 10^6 fold (HIGH) dilutions of the contents were plated on MRS agar and incubated under a microaerophilic condition. Genomic DNA was extracted from the pools of the colonies recovered from MRS plates as well as directly from the cecal contents. After 16S rRNA gene (V1-V3) was amplified, the amplicons were pooled, sequenced by MiSeq, and analyzed by QIIME. Taxonomic analysis among MRS-selected groups revealed *Firmicutes* as dominant phylum (95.92%) followed by *Proteobacteria* (3.68%). *L. salivarius* was found significantly higher ($P < 0.0001$) in all 3 MRS-selected groups as compared with other *Lactobacillus* species. Alpha diversity (chao1) was significantly ($P < 0.05$) higher in total bacterial group, but there was no difference among different dilutions of MRS-selected groups. Normalized *Lactobacillus* sequence reads revealed relative abundance of *L. salivarius* as 89.85% and 31.82%, followed by *L. crispatus* as 3.44% and 25.70% for MRS-selected groups and total bacteria, respectively. This result indicates selective bias of MRS for *L. salivarius* in comparison to other *Lactobacillus* species. Observation of *L. aviarius*, *L. delbrueckii*, *L. amylovorus*, and *L. ceti* only on total bacterial group indicates that they are either strictly anaerobic or unculturable on MRS agar medium.

Key words: *Lactobacillus*, breeder hen, MRS agar

P102 Yeast cell fractions inhibit EPEC adhesion onto T84 intestinal epithelial cells.

L. Dunière^{*1,2}, C. Verdier^{1,2}, F. Chaucheyras-Durand^{1,2}, and M. Castex¹,
¹Lallemand Animal Nutrition, F-31702 Blagnac, France,
²INRA, UR454 Microbiologie, F-63122 Saint-Genès Champanelle, France.

Limiting pathogens introduction in the food chain is of great concern for the industry and upstream efforts should be deployed to maintain and improve health of production animals. Adhesion properties of intestinal pathogenic microorganisms are a major target to increase pathogens clearance and limit their spread at the farm level. Yeasts are commonly used as direct fed microbials to improve livestock nutrition and health through modifications of the gut microbial ecology. Yeasts or part of their cell wall might induce a positive effect on limiting pathogen adhesion in the gut. Inhibition capacity of a processed yeast cell fractions (YCF) obtained from a proprietary strain (Lallemand SAS) on pathogen adhesion was investigated in vitro with the intestinal epithelial cell line T84. First, we focused on the interaction between immature T84 cells and one Enteropathogenic *E. coli* (EPEC) strain isolated from a diarrheic calf gut, in presence of YCF, second, we performed experiments with differentiated T84 cells. Dosages of YCF ranging from 0.5 mg/mL to 10 mg/mL were tested in 2 types of experiments i) co-infection, i.e. addition at the same time of EPEC and YCF incubated on T84 cells for 4h; ii) post-infection, i.e. a first step of T84 cells infection by EPEC followed by YCF addition for a total duration of 4h incubation. Results were expressed in percent of adhered EPEC with negative



control showing 100% adhesion. On immature T84 cells, a dose-response effect was observed from 3 mg/mL of YCF with a strong and significant decrease in EPEC adhesion ($13.9 \pm 9.4\%$ of adhered EPEC only). Inhibition capacity of YCF also increased in a dose dependent manner post-infection: pathogen adhesion was of: $50.9 \pm 5.5\%$ at 5 mg/mL and $8.4 \pm 7.3\%$ at 10 mg/mL. On differentiated T84 cells, stronger inhibition was measured as EPEC adhesion decrease was already significant with the lowest dose of product tested (1 mg/mL) both in co-infection and post-infection experiments ($P < 0.05$). Efficient limitation of EPEC adhesion on intestinal cells and dislodgment of the pathogen was achieved with YCF either on immature or differentiated cells. This product requires *in vivo* studies to confirm its potential to limit pathogen adhesion.

Key words: pathogen adhesion, yeast cell fraction, intestinal epithelial cells

P103 Neonatal Jersey calves supplemented with BIOTIX, a blend of probiotic bacteria, improved the pathophysiological response to an oral *Salmonella enterica* challenge.

Y. Liang*, R. E. Hudson, and M. A. Ballou,
Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX.

Objectives were to determine if supplementing calves with a blend of specific strains of *Lactobacillus casei* and *Enterococcus faecium* influenced the pathophysiological response to a *Salmonella enterica* challenge. Twenty 4 1d old Jersey calves were blocked according to TSP and BW, and randomly assigned to 1 of 3 treatments: (1) Control (CON), (2) Control + *Salmonella enterica* (CON+Salm), and (3) BIOTIX + *Salmonella enterica* (BTX+Salm). The BTX+Salm were supplemented with 2×10^{10} and 2×10^9 cfu of BIOTIX Dairy per d for the first 3 d and the remainder of the experiment, respectively. Calves were fed 485 g of milk solids and had ad libitum access to a common starter and water. On d 7, the CON+Salm and BTX+Salm calves were challenged with 5.3×10^6 cfu of a *Salmonella enterica*. Peripheral blood samples collected on d 0, 7, 10, 14, and 21. Calves were euthanized at d 21 and duodenum and ileum analyzed for histopathology and mesenteric lymph nodes for microbiology. There were no treatment or treatment x time ($P \geq 0.364$) effects on ADG; however, there was a tendency ($P = 0.065$) for the CON+Salm and BTX+Salm to have reduced ADG from d 8 to 14 when compared with CON. Calves challenged with *Salmonella enterica* had elevated rectal temperatures ($P = 0.035$) on d 9, 10, and 11. There was a treatment x time interaction ($P = 0.028$) on serum *Enterococcus faecium* concentrations, whereas before the challenge, Control calves had greater haptoglobin than the BTX calves, and following the challenge CON+Salm had increased haptoglobin where the BTX+Salm did not. There were treatment differences in the villi: crypt ratio in the duodenum (1.34, 0.74, and 1.29 ± 0.206 ; $P = 0.063$) and ileum (1.66, 1.50, and 2.43 ± 0.095 ; $P \leq 0.001$) for CON, CON+Salm, and BTX+Salm, respectively. Mesenteric lymph nodes were positive for *Salmonella* in 0, 3, and 1 for CON, CON+Salm, and BTX+Salm, respectively. These data indicate that supplementing calves with BIOTIX Dairy probiotic did not influence performance, but limited the acute phase

response and intestinal damage following a *Salmonella enterica* challenge.

Key words: calf, gastrointestinal health, probiotic

P104 A comparison of fungal populations in broilers from high and low producing farms.

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

Molds or Fungi have been associated with the onset of disease. Little attention has been given to the beneficial effects of fungi with regard to food safety and especially with the gastrointestinal tracts of food-producing animals. In this study, we surveyed the changes that occur in both fungal and microbial populations in health commercial broilers. Four complexes were selected from the southern United States over a 12 mo period. For microbiome analysis, crop, duodenum, jejunum and cecal, samples were collected from each bird ($n = 5/\text{house}$) and 10 farms per complex on d 30–36. These samples were used for microbiome analysis ($P \leq 0.05$). Fungal populations were isolated by conventional culturing as well as pyrosequencing. In the cecal samples, dramatic changes were observed in the fungal populations of, and *Eupeniidiella* and *Acremonium* genes in the ceca of birds sampled on the high versus low producing farms. Understanding the fungal and bacterial changes that occur in gastrointestinal tracts of commercial poultry may help us develop a gut health model that will help us target area for potential production and poultry health improvements as well as may be utilized in the development of intervention strategies to control food borne pathogens.

Key words: fungi, broilers

P105 Impact of parenteral antimicrobial administration on the structure and diversity of porcine fecal microbiota.

M. Zeineldin*^{1,2}, B. Aldridge¹, N. Maradiaga¹, and J. Lowe¹,
¹*Integrated Food Animal Management Systems, Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois at Urbana-Champaign, Urbana, IL,* ²*Department of Animal Medicine, College of Veterinary Medicine, Benha University, Egypt.*

Antibiotics administration in swine husbandry systems has been used for prevention and treatment of several forms of infections as well as a growth promoter, but concerns regarding the emergence of antibiotic-resistant and adverse effect on the microbial colonization have led to novel approaches in development of alternative strategies. Therefore, the purpose of this study was to characterize the impact of antibiotics administration on the composition and diversity of the resident fecal microbiotas in pigs. Five antimicrobial treatment groups each consisting of 4, 8 week old piglets were treated with one of the antimicrobials (Tulathromycin, Ceftiofur Crystalline free acid, Ceftiofur hydrochloride, Oxytetracycline, and Procaine Penicillin G) at label dose and route. Individual fecal swab was collected before antibiotics administration (d 0) and again on d 1, 3, 7, and 14. Genomic DNA was extracted, and the V1-V3 region of 16S rRNA gene was amplified and sequenced using Illumina- based sequencing. Our result demonstrated



that the core fecal microbiome was dominated by *Firmicutes* and *Bacteroidetes*. Discriminant analysis showed pronounced microbial shift in the fecal microbiota after different antibiotics administration. *Prevotella*, *Ruminococcaceae*, *Streptococcus*, *Christensenellaceae*, *Clostridium*, *Lachnospiraceae* and *Oscillospira* were the main bacterial taxa associated with the microbial shift after Tulathromycin, Ceftiofur Crystalline and Ceftiofur hydrochloride administration. Only minor alterations were noted after the administration of Oxytetracycline and Procaine Penicillin G. Based on our results, exposure to various antibiotics administration has distinct effects on the composition of the porcine fecal microbiotas with no significant effect on bacterial diversity as measured using the Shannon, Chao1, Observed species and PD whole tree indices. Understanding these effects is a critical step in designing comprehensive health management programs that optimize local immunity to minimize the disease and the need for antibiotics.

Key words: Piglet, antimicrobials, fecal microbiota

P106 Impact of environmental management on the host microbial ecosystem in growing pigs.

K. Evans*¹, M. Zieneldin^{1,2}, J. Lowe¹, and B. Aldridge¹,
¹Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Champaign, IL, ²Department of Animal Medicine, College of Veterinary Medicine, Benha University, Benha Egypt.

Due to substantial world population increases and food production variability through external factors such as climate change, economic change, and demographic change, food supply security is at risk. Constraining infectious disease within animal food production is a key approach in working toward the sustainability of the world animal-source food production chain. Antimicrobial use in livestock-based food production has allowed the industry to produce efficiently, and constrain such infectious diseases worldwide. Unfortunately, antimicrobial use is not a long-term solution as it causes resistance and reduces the ecology of the microbiome. Considering a post-antibiotic era means a reduction in production efficiencies and a risk to the security and sustainability of food production. Industries will have to manage livestock in ways that do not coincide with the available environmental resources. Understanding the ecology of the livestock-based food production system is essential in optimizing animal health and productivity without the use of antibiotics. *This project objective is to establish an alternative model livestock system that allows for the identification of various drivers within swine microbiome associated with health, production, and the development of antimicrobial resistance.* The use of a model production ecosystem approach allowed for the managing of 2 different environments of 5 batches of 35 growing pigs with one barrow sacrificed at the time of placement. This ecosystem was measured through changes in host microbiome, mucosal immune responses, and the influence of these on animal growth and performance. Results have indicated a high probability of variance among the environment and biological geographical site within various microbiota of the genus and phylum. In the future, the approach will be to investigate and explain the long term impacts of commercial growing pig management practices on growth and performance including antibiotic use over multiple generations. These significances will be measured by changes in and interactions between the host microbiome, host immune

system, and prevalence of known pathogens and antimicrobial resistance genes.

P107 A comparative analysis of the ileal and cecal microbiome and performance of turkeys fed diets containing either antibiotic growth promotors (AGP) or medium chain fatty acids (MCFA).

T. J. Johnson¹, T. P. Karnezos*², C. L. Novak³, M. Masadeh³, B. W. Koppen³, and R. A. Dvorak²,
¹University of Minnesota, Saint Paul, MN, ²PMI Nutritional Additives, Arden Hills, MN, ³Purina Animal Nutrition, Shoreview, MN.

A 133d, 82 bird/pen, 4 treatment, 8 replication turkey study was conducted to compare the impact of feeding turkeys diets supplemented with MCFA's (Vitacy[®] P) or a typical AGP on the ileal and cecal bacterial communities, and bird performance and feed conversion. All birds were vaccinated with Coccivac[®]-T. Treatments were 1) AGP; BMD[®] 50 g/ton in starter through finisher 1 + STAFAC[®] 20 g/ton in finisher 2 and 3 diets; 2) Negative control (NC); no additives; 3) MCFA; 4.0 lb./ton in starter + 3 lb./ton in grower + 2 lb./ton in the finisher. Ileal and cecal samples were harvested at 56 and 133 d of age. Pen and feed weights were collected at 56, 83 and 133d. Alpha diversity of the microbiome was significantly different due to bird age and similar across the treatments. Ileal vs. cecal β diversity together with time was different, whereas the treatments were similar. Ileal taxa were dominated by Firmicutes (Bacilli) at d56 and by Firmicutes (Bacilli and Clostridia) and Bacteroidetes on d133, whereas the ceca were dominated by Firmicutes (Bacilli and Clostridia) on d56 and Firmicutes (Clostridia) and Bacteroidetes (Bacteroidia) on d133. At 56d, body weights were not different ($P > 0.05$) among the treatments and FCR of the AGP and MCFA treatments were better ($P < 0.08$) than the NC. At 83d, there were no treatments differences for body weight or FCR. At 133d, body weights were heavier ($P < 0.098$) for the AGP (39.08 lb.) and MCFA treatments (39.04 lb.) than the NC (37.88 lb.). Mortality adjusted feed conversion at 133d was significantly ($P \leq 0.05$) better for MCFA (2.415) than the NC (2.551) and similar to the AGP (2.455) treatment. These data demonstrate that the bacterial communities of turkeys fed a diet supplemented with MCFA or AGP's were significantly affected by the age of the birds and location in the gastro-intestinal tract more than the effects of the treatments, although some treatment-specific changes in diversity and taxa were observed. MCFA supplementation resulted in a mortality adjusted feed conversion and weight gain similar to that of the AGP treatment and better than the NC.

Key words: microbiome, medium chain fatty acids, turkey

P108 A survey of early colonizing bacteria in US broiler and turkey flocks.

E. Hutchison*, J. Rehberger, A. Smith, S. Anderson, E. Vang, R. Wujek, and T. Rehberger,
Agro BioSciences, Inc., Wauwatosa, WI.

Early colonizing bacteria in young turkeys or broilers set the stage for immune function, bacterial homeostasis and nutritional processing for the duration of the bird's life. In commercial production systems, newly-hatched birds are generally passively inoculated by a varying array of organisms in their immediate



hatching environment. This can lead to high variability among the enteric microbiota in birds, even within the same flock. To better understand the early poultry microbiome, this present survey examined turkey poults and broiler chicks at day-of-hatch (DOH) across multiple companies. Gastrointestinal tracts from 263 poults representing 5 turkey companies and 505 chicks from 8 broiler companies, including 10 broilers from a smallholder farm, were sampled and their predominant populations were analyzed using 2 methods; 16S rRNA sequencing of culturable bacteria grown anaerobically on MRS agar, and terminal restriction fragment polymorphism (TRFLP) using primers designed to be selective for lactic acid bacteria. Microbial profiles of each flock were constructed using 16S rRNA sequencing data and compared by principal component analysis (PCA). *Enterococcus faecalis* and *E. gallinarum* were predominant in both broilers and turkey flocks, however, multiple turkey flocks from a single company yielded many *E. coli* isolates. PCA analysis of TRFLP profiles of individual birds showed that *Pediococcus* is more associated with broilers while *Clostridium*, *Streptococcus*, and *Lactobacillus casei* are more associated with turkeys and confirmed that Enterococci were present in the majority of DOH birds. The smallholder birds were distinct from conventional turkey and broiler flocks in both 16S rRNA and TRFLP analyses. Simpson diversity indices of turkey poults and broilers chicks showed that turkeys had a more diverse microbial profile. This data begins to elucidate the early core microbial populations of commercial turkeys and broilers in the US and may help in defining normal and abnormal microbiomes as a predictor for flock health and productivity.

Key words: poultry, microbiome

P109 Microbial community succession of the piglet gastrointestinal tract during the lactation period.

J. Sawall¹, J. Rehberger¹, X. Smith¹, K. Friesen², D. Murray³, and E. Davis^{*1},

¹Agro BioSciences, Inc., Wauwatosa, WI, ²NutriQuest, Mason City, IA, ³New Fashion Pork, Jackson, MN.

The early establishment of a diverse microbial consortium in the gastrointestinal tract of young pigs is a dynamic process that is influenced by management and environmental conditions during early microbial exposure. This study defines the major bacterial contributors of the microbiome and temporal population shifts from early life to weaning in piglets reared in commercial swine production facilities. Gastrointestinal sections were sampled from a total of 72 commercially raised piglets representing one, 10, and 20 (weaning) days of age, with 24 pigs representing each sampling age. Bacterial enumeration of *E. coli* and lactic acid bacteria (LAB) were performed on esophagus, jejunum, cecum and colon sections, and traditionally cultured isolates were genotyped via RAPD PCR analysis and identified by 16S rRNA gene sequencing. Bacterial DNA was extracted from each gastrointestinal section for microbial community analysis and total bacterial and LAB populations were characterized by TRFLP analysis of respective 16S rRNA gene targets. Cultured isolate diversity and 16S identification showed Streptococci populations constituted roughly 20% of the relative abundance across ages, and *L. reuteri* was the predominant *Lactobacillus* species, constituting roughly 30% of the relative abundance. Total bacterial community analysis revealed greater relative abundance of *Lactobacillus* populations in the esophagus and jejunum

sections compared with the cecum and colon, whereas Bacteroides and Enterobacteriaceae comprised larger proportions of the small and large intestinal communities. Decreasing proportions of Enterobacteriaceae were observed with age, complementary to relative increases in *L. crispatus* and *L. delbrueckii* populations, demonstrating the establishment of the commensal *Lactobacillus* populations in the gastrointestinal tract. These data contribute to a better understanding of the progression of enteric microbial establishment during the young pig's early development and for the identification of key microbial contributors associated with health and performance of commercially reared pigs.

Key words: swine, neonatal, microbiota

P110 In vitro and in vivo antimicrobial activity of cinnamaldehyde and chemical derivatives against the pig gut microbiota.

J. Michiels*, J. Degroote, A. Ovyne, and S. De Smet, Ghent University, Ghent, Belgium.

Cinnamaldehyde, an α,β -unsaturated aldehyde with a non-substituted phenyl ring, has well known antimicrobial properties. However, it shows high reactivity to amino acid residues. As an alternative, 20 chemical derivatives were chosen; having different functional moieties, i.e., alcohol, ether or carboxylic acid, having different degrees of α,β -saturation or substituents on the α carbon atom, and bearing substituents on the phenyl ring. The antimicrobial activity was tested in an in vitro incubation model simulating the digestion in the small intestine at 0, 100 and 400 mg/L and at pH 5 and 7. 4-nitrocinnamaldehyde had the highest antimicrobial activity, with reductions for all tested bacterial groups. However, this compound is carcinogenic and was not further issued. Cinnamaldehyde had the second highest activity, particularly against coliform bacteria and *E. coli*, followed by 4-methoxycinnamaldehyde, 2-methoxycinnamaldehyde and hydro-cinnamaldehyde. All other derivatives showed lower potency, but they were consistently more bactericidal against coliform bacteria and *E. coli* as compared with G+ bacteria. At pH 7, aldehydes were stronger than their corresponding carboxylic acids, but not at pH 5 suggesting a different mode of action. In the in vivo experiment, diets of newly weaned piglets were supplemented for 13 d with cinnamaldehyde (100 and 400 mg/kg), 2-methoxycinnamaldehyde (491 mg/kg) and 4-methoxycinnamaldehyde (491 mg/kg). No significant improvements of animal performance were observed. Only 4-methoxycinnamaldehyde reduced significantly total anaerobic bacterial counts in the proximal small intestine. In contrast to the clear antibacterial effect in the in vitro experiments, the in vivo trial did not reveal a similar outcome.

Key words: cinnamaldehyde, antimicrobial, piglets

P111 Design of a first-generation bacteria-specific kinome peptide array for the study of gut microbe signaling.

G. J. Pagano^{*1,2} and R. J. Arsenault^{1,2},

¹Department of Animal and Food Sciences, University of Delaware, Newark, DE, ²Center for Bioinformatics & Computational Biology, University of Delaware, Newark, DE.

The ubiquity of signaling pathways in cell regulation makes them an important area of study in determining how hosts and



pathogens alter their phenotypes in response to different infections and treatments. While genomics can provide useful information about changes in signaling pathways, a proteomics approach can give insight into the period immediately before phenotypic changes occur. Changes in protein activity are controlled by post-translational modifications, including phosphorylation by enzymes known as kinases. One method for quantifying kinase activity employs peptide arrays, which contain a series of peptides that mimic kinase target sites. By comparing relative levels of phosphorylation between 2 samples, significant differences in phosphorylation can be identified, as well as what types of signaling pathways may be involved. Species-specific peptide arrays have been previously used to study eukaryotic targets, but limited work has been done for prokaryotic organisms. We describe here methods for developing and testing a first-generation prokaryotic peptide array for use with *Salmonella typhimurium* and *Escherichia coli*. Serine, threonine and tyrosine target sites from *E. coli* were mined from online databases and standardized to a length of 15 residues. Using reciprocal BLAST tests, the 15-mers were compared against the *S. typhimurium* proteome to find matches based on sequence conservation, protein name and target residue position. These matches were combined with predicted *S. typhimurium* serine and threonine sites, also verified with BLAST, to produce a list of 163 peptides for the array. A subset of this list was then printed on an array, and *S. typhimurium* grown under different metabolic conditions were used to test for differential phosphorylation. We plan to expand upon our list of peptide sites for different prokaryotes, and possibly develop sets of target sites that can be used to distinguish bacteria in a mixed population. In the long term, peptide arrays could be used to reveal aggregate signaling patterns found in the gut microbiome, or study microbial imbalances to better understand the microbiome's role in gut health.

Key words: microbiome, kinome, signaling

P112 Evaluation of different disinfectants and antiseptics to surface sterilize turkey eggs and hatch germ-free turkey poults.

M. Sylte*, L. Chandra, and T. Looft,

USDA ARS National Animal Disease Center, Ames, IA.

Bird eggs are protected from bacterial translocation by an outer glycoprotein cuticle layer, a mineralized porous shell and an internal shell membrane, and are in contact with intestinal microbiota before oviposition. Generating germ-free poults for research requires sterilization of the egg's surface without damaging the developing embryo. In a preliminary study, untreated turkey eggs were hatched in a germ-free environment. *Firmicutes* were detected in the intestinal microbiota of these poults, suggesting that poults may acquire spore-formers by exposure to shell contents while hatching. However, disinfectants and antiseptics may affect embryo viability due to toxicity. The goals of this study were to evaluate: 1) the ability of different disinfectants and antiseptics to sterilize the egg shell surface without harming the developing embryo, and 2) the hatching of germ-free poults. Different classes of disinfectants and antiseptics (halogens, biguanidines and oxidants) were selected to kill spores and vegetative bacteria likely present on the egg's cuticle and shell. Contact time is vital for microbial killing. Eggs were fully immersed in disinfectant or antiseptic solutions for up to 15 min and shells were aseptically harvested for aerobic and

anaerobic culturing of bacteria. Embryotoxicity was evaluated by incubating the treated eggs for up to 27 d of embryonation. Embryos were grossly evaluated for developmental changes. Sequential immersion of turkey eggs in acidified sodium hypochlorite, chlorine dioxide and povidone iodine was selected to evaluate for hatching germ free poults because it produced minimal embryotoxicity and sterilized the egg's surface. Other compounds tested produced embryotoxicity or failed to fully sterilize the egg's surface. Surface sterilized eggs were incubated in a germ-free isolator until hatching. Immediately after hatching, poults were euthanized and sterility of intestinal contents was determined using bacterial culture and qPCR of 16S rRNA gene. Generation of germ-free poults is important to evaluate the host-*Campylobacter* spp. interaction and the development of the turkey's mucosal immune system.

Key words: germ-free, turkey, egg

P113 Rumen metagenome of rumen liquid and solid fractions in response to inclusion of corn, sorghum, and treated sorghum distillers' grains in finishing diets.

E. A. Latham*^{1,2}, W. W. Gentry², J. S. Jennings², and W. E. Pinchak³,

¹Texas A&M, College Station, TX, ²Texas A&M AgriLife Research, Amarillo, TX, ³Texas A&M AgriLife Research, Vernon, TX.

Calcium hydroxide treatment can increase the digestibility of distiller's grains via solubilization of hemicellulose and pH buffering. In vitro microbial populations respond dramatically to calcium hydroxide treatment; however we do not know in vivo responses in the diversity, structure, or function of the rumen community. To determine rumen microbial populations structural and functional adaptations to calcium hydroxide treatment of sorghum distillers grain in finishing diets, 6 ruminally cannulated crossbred Angus steers (444 ± 4.0 kg of BW) were used in a replicated 3 × 3 Latin square design with 21 d periods consisting of a 17 d adaptation period. Rumen sample were collected on d 18–21 and separated into liquid and solid fractions and frozen immediately. Dietary treatments included steam-flaked corn finishing diet (57.1% ± 0.6) with either 30.0% ± 0.2 corn wet distillers' grains (CDG), sorghum (SDG), or calcium hydroxide treated sorghum WDGS (2.67% Ca(OH)₂ DM basis). Reads were analyzed using the metagenome rapid annotation using subsystem technology (MG-RAST) server. Statistical analyses were performed using PROC MIXED on SAS 9.4 (SAS Institute Inc., Cary, NC), R software, and QIIME. Significant differences in ruminal fermentation characteristics such as pH, ammonium and VFA concentration, and digestibility were correlated with microbial changes in terms of relative abundance and functional gene expression that previously have in vitro accounted for these measured changes. For example, *Eubacterium* (21.9% difference) and *Clostridium* (11%), both known butyrate production and acetate consumers, were elevated in the rumen of steers fed corn ($P < 0.05$), which was found to have elevated butyrate and depressed acetate compared with sorghum fed steers. This was also reflected in a 35% increase in the butyryl-CoA dehydrogenase gene ($P = 0.001$). Overall, the microbial community structure of treated sorghum most closely matched that of untreated sorghum, however it appears that treatment with calcium hydroxide has a



buffering effect as the community structure trended toward the corn community.

Key words: metagenome, cattle, sorghum

P114 *Clostridium perfringens* infection of the chicken induces immunometabolic alterations in the duodenum that includes the glycolytic and insulin signaling and NLRP3 inflammasome-mediated inflammatory cell death.

M.I Kogut*¹ and R. Arsenault²,

¹USDA-ARS, College Station, TX, ²University of Delaware, Newark, DE.

Clostridium perfringens (CP) infection of the gut is a central requirement for the establishment of necrotic enteritis (NE), despite CP very often being a member of the commensal microbiota in broiler chickens. Little is known about the immune mechanisms directed against CP during necrotic enteritis infections. An integral component of immune regulation is through the metabolic pathways necessary to support energetically demanding protective or pathogenic responses. Understanding these links in immunometabolism is critical to understanding basic immune responses to CP. Using a species-specific kinome immunometabolism peptide array, we investigated changes in signaling pathways in the duodenum of broilers given a live-attenuated vaccine against IBD followed by a CP challenge. In these experiments, all birds received a commercial infectious bursa disease vaccine on d 10 of age followed by an orally administered CP challenge on d 15, 16, and 17. The studies were terminated at d 21 when birds were sacrificed and a 40 mg sample of duodenal tissue was collected from each bird, flash frozen, homogenized, and applied to the peptide array protocol. We observed metabolic changes that affected glucose metabolism through the glycolytic and the insulin signaling pathways. Within 4 d of challenge infection, we observed changes in the duodenal phosphorylation state of the enzymes up and down the glycolytic pathway. In addition, changes to a large subset of the protein intermediates of the insulin pathway were altered by infection. Immunologically, infection induces pyroptosis by increased phosphorylation of several peptides in both the TLR1/NFAT and NLRP3 (Caspase 1, CARD9, PRTPIP1) signaling pathways. This is the first report of significant regulatory metabolic and inflammatory signaling pathways induced by CP infection and provide new insights in the mechanisms essential for the establishment of NE in chickens.

Key words: necrotic enteritis, kinome, immunometabolism

P115 Survey of *Clostridium perfringens* populations in dairy cattle from Wisconsin and Texas.

J. S. Thompson*, J. J. Mouradian, J. Schissel, M. N. Griffin, A. H. Smith, and T. G. Rehberger, Agro BioSciences Inc., Wauwatosa, WI.

Clostridium is a genus of gram-positive, spore-forming bacteria that are common residents of the gastrointestinal tract. Several *Clostridium* species have been linked to enteric disease in ruminants including hemorrhagic bowel syndrome (HBS), a disease often correlated with elevated levels of *Clostridium perfringens* Type A. The purpose of this research was to characterize the distribution and diversity of *C. perfringens* in dairy cattle as a prerequisite for developing effective tools

to control these organisms. Cow and calf fecal samples were collected from Wisconsin (n = 24) and Texas (n = 7) dairies. Clostridia were enumerated and isolated from Perfringens Agar Base media. Isolates were tested for *C. perfringens* toxin genes by multiplex PCR. Multiplex PCR identified 95.3% of the isolates from Wisconsin and 97.1% of the isolates from Texas as Type A. Estimated *C. perfringens* levels were calculated using the percentage of positive isolates multiplied by total Clostridia levels for each sample. Average *C. perfringens* levels were lower in cows than calves in Wisconsin. Genetic fingerprints were generated with RAPD-PCR technology for all *C. perfringens* isolates. Isolates were compared by fingerprints and clustered based on 75% similarity. Diversity indices indicated slightly lower diversity in calves compared with cows, Shannon-Weiner indices 4.22 and 4.53 respectively. Texas displayed lower diversity, Shannon-Weiner index 3.88, which was expected given the lower number of sample sites. The survey results indicate a difference in average levels of *C. perfringens* between calves and cows in Wisconsin. However there did not appear to be specific fingerprint clusters of *C. perfringens* isolates specific to cows, calves, or state. This survey can offer comparative levels of *C. perfringens* identified in dairies in Wisconsin and Texas and can serve as a benchmark to help identify high levels of *C. perfringens* associated with enteric diseases such as HBS.

Table 1.

	Wisconsin cow	Wisconsin calf	Texas cow
Samples	133	95	470
Clostridia (cfu/g)	5.8 x 10 ⁴	1.2 x 10 ⁶	8.7 x 10 ⁴
<i>C. perfringens</i> (cfu/g)	3.5 x 10 ⁴	4.3 x 10 ⁵	8.2 x 10 ⁴

Key words: ruminant, *C. perfringens*

P117 A target for intervention: Necrotic enteritis in broilers triggers changes in the PI3K-Akt signaling network that are distinct between duodenum, jejunum, and ileum.

C. N. Johnson*¹, J. A. Byrd², M. H. Kogut², and R. J. Arsenault¹,

¹Department of Animal and Food Sciences, University of Delaware, Newark, DE, ²United States Department of Agriculture, Agricultural Research Service, SPARC, College Station, TX.

Necrotic enteritis (NE) is regarded as one of the most common and costly diseases in the modern broiler industry having an economic impact exceeding \$2 billion dollars annually. *Clostridium perfringens* is the etiologic agent of NE, although, predisposing factors are required for disease development. In this study, an experimental model of NE was induced using a commercial infectious bursal disease virus (IBDV) vaccine and *C. perfringens* infection. Chicken-specific immunometabolic kinome peptide arrays were used to measure differential phosphorylation between infected and control (uninfected) birds in duodenal, jejunal, and ileal tissues. Data analysis revealed statistically significant differential phosphorylation between infected and control groups in several signal transduction pathways including those interacting with the PI3K-Akt signaling hub (insulin signaling, AMPK/mTOR, and apoptosis). Within this signal transduction network kinase targets were differentially phosphorylated



between the infected gut tissues. Differences were observed in CREB (immunity, cell survival), RPS6KB1 (cell survival/growth), AKT3 (insulin signaling, immunometabolism), CDC37 (cell stress response), EIF4EBP1 (protein translation regulation), IRS1 (insulin signaling, immunometabolism), MAPK1 (cell growth), mTOR (immunometabolism), PIK3CB (cell growth, immunity), PIK3CG (immunity), and PRKAA1 (energy sensor, immunometabolism). NE is a multifactorial disease that includes nutritional and infectious causes and effects and results in distinct pathology in the different gut segments. The differences in the PI3K-Akt signaling network between the gut segments, which impact cell growth, development, metabolism and immune responses, may be important in understanding the mechanism of NE and designing targeted intervention strategies.

Key words: kinome, clostridium, immunometabolism

P118 Phage endolysins as alternative antimicrobials for treating *Clostridium perfringens*, a causative agent of necrotic enteritis.

D. T. Rowley¹, B. Oakley², J. Foster-Frey¹, D. M. Donovan^{*1}, S. M. Swift¹, and S. Rahimipour³,

¹Animal Bioscience and Biotechnology Laboratory, USDA-ARS, Beltsville, MD, ²College of Veterinary Medicine, Western University of Health Sciences, Pomona, CA, ³Bar-Ilan University, Ramat-Gan, Israel.

Clostridium perfringens is a major necrotic enteritis causing bacterial pathogen in poultry, and a source of food poisoning and gas gangrene in people. *C. perfringens* can also cause mild to severe enteritis in pigs. In the EU, the occurrence of *C. perfringens* associated necrotic enteritis in poultry has increased as antibiotic use has decreased. As the US moves away from use of antibiotics in animal feed, we can expect an increase in necrotic enteritis with subsequent losses from morbidity and, in subclinical cases, losses from decreased chicken weights. Alternatives to antibiotics in animal feed will be needed in the near future. The genomes of 43 unique *C. perfringens* isolates from chicken were sequenced, examined for peptidoglycan hydrolase enzymes by homology to known enzymes. There were more than 120 putative peptidoglycan hydrolases (primarily phage endolysins) identified that clustered into 15 groups according to homology [less than 50% amino acid identity between groups and more than 90% amino acid identity within group]. Of 15 representative lysins (one from each group) 4 lysins were shown to have high lytic activity against all 43 of the initial isolates in plate lysis assays but not other gram-positive or gram-negative species tested. Activity was also demonstrated in both zymogram, and turbidity reduction assays. The domain architecture and relative activity of the 4 lysins has been determined. Sonication production of nanoparticles composed of phage endolysins as alternative antimicrobials will be described.

Key words: necrotic enteritis, poultry, alternative antimicrobials

P119 Effect of medium and short chain fatty acids on performance and gut health in piglets.

C. Sol^{*}, N. Hillis, M. Puyalto, and J. J. Mallo, NOREL SA, C/Jesus Aprendiz, 19, Madrid, Spain.

The objective of this study was to evaluate the effect of sodium salts of coconut fatty acids distillates (DICOSAN) and sodium butyrate protected with sodium salts of palm fatty acids (Gustor

N'RGY) on performance and gut microbiology in weaned piglets. A total of 72 piglets, with 21d of age (7.1 ± 0.15 kg) were distributed into 18 pens and 3 experimental groups: control diet (CTR) or the same diet supplemented with DICOSAN (DIC; 0.3%); minimum content of lauric acid 32%); or Gustor N'RGY (N'RGY; 0.1% minimum content of sodium butyrate 70%), all offered *ad libitum*. The trial lasted 14 d and intake and live weight were recorded at the end. On d 14 one piglet per pen was euthanized and ileum and cecum content were sampled to evaluate microbial counts. Data were analyzed by one-way ANOVA using GLM procedure of SAS. No significant differences were observed in final BW, although piglets in N'RGY and DIC groups were heavier than CTR group (8.96, 9.64 and 9.95 kg, for CTR, N'RGY and DIC, $P = 0.14$). ADFI was numerically higher in the DIC fed animals at 35d (407, 403 and 456 g/d, for CTR, N'RGY and DIC, $P = 0.30$). DIC treatment also tended to improve FCR when compared with the other treatments (2.32, 1.55 and 1.35, for CTR, N'RGY and DIC, $P = 0.09$). Regarding microbial counts, no significant differences were seen in *lactobacillus* at ileum or in cecum level, however, N'RGY and DIC groups numerically increased that count. The use of N'RGY numerically reduced and DIC significantly reduced *coliforms* in ileum (7.33, 5.76 and 4.79 Log cfu/g, for CTR, N'RGY and DIC, $P = 0.03$) and in cecum (7.30, 5.30 and 4.75 Log cfu/g for CTR, N'RGY and DIC, $P = 0.03$) compared with CTR group. For *E. Coli* and enterobacteria in ileum and in cecum all treatments followed the same pattern, being a significant reduction of counts with DIC treatment compared with CTR. Results suggest a higher antibacterial effect of medium-chain fatty acid (DICOSAN) in the intestinal lumen and it was reflected in a better performance of the animals. In addition, even the low dosage of protected sodium butyrate (Gustor N'RGY), showed moderate effects on performance and on intestinal microflora.

Key words: protected sodium butyrate, medium-chain fatty acid salts, piglets

P120 Influence of a direct-fed microbial on growth performance, digestibility, methane production, and gut health across multiple livestock species.

J. Barnes^{*}, M. Showell, and R. Carpenter, BiOWiSH Technologies, Inc., Cincinnati, OH.

Direct-fed microbials (DFM's) provide a viable alternative to antibiotics for growth enhancement, improved digestibility and gut health across species. The effects of a DFM containing bacteria from the genera *Bacillus* and *Lactobacillus* are reported for poultry, cattle, and swine. Feed conversions for broiler chicks on both high protein and low protein pelleted diets improved 3.5% and 6.6%, versus the respective controls when the DFM is added. This benefit derives from improved feed ingredient utilization. In a simulated rumen environment, a dosage equivalent to 25 g DFM per metric ton of feed improves *in vitro* digestibility of a poor quality feed like hay. This traces to more complete digestion of crude protein, higher nitrogen production per kilogram of feed and production of higher levels of key volatile fatty acids, like propionate, while shifting the ruminal microbiome. Analysis of the ruminal contents using TRFLP showed a microbiome shift at the genus level over time. Steers fitted with rumen cannulas were fed a growing beef cattle diet consisting of hay, corn, soybean meal, dried distillers' grains (DDGs) and molasses. Results from this study showed a 42% reduction in methane production for



the group fed the DFM versus the control, with corresponding improvements in feed digestibility, and fecal reduction. Swine studies showed a 15.1% and 13.2% improvement in Average Daily Gains (ADG) for both female and male groups versus controls when fed 200 g/Ton of DFM. Feed Conversion Ratios (FCR) for the same groups also showed improvements of 2.03% and 8.67% versus their control counterparts. These studies show that diets supplemented with a DFM can enhance growth, improve digestibility and potentially improve gut health by shifts in the microbiome. Additionally, studies on *in vitro* intestinal cell lines show an up-regulation of certain cytokines associated with the inflammatory pathway signaling a potential DFM pro-inflammatory response that may prime the immune system for rapid response to irritation and infection.

Key words: DFM, growth performance, digestibility

P121 Increases in volatile fatty acid production and stimulation of key microbes by Original XPC™ and NutriTek® in an *in vitro* rumen microbial model.

T. Kwan*, A. Brainard, C. Reedy, T. Werner, J. Butler, M. Scott, and I. Yoon,
Diamond V, Cedar Rapids, IA.

The objective of this study was to measure the effect of 2 *Saccharomyces cerevisiae* fermentation products—Diamond V Original XPC (XPC) and NutriTek (NutriTek)—on VFA production and microbial populations *in vitro*. Three experiments were conducted with 10 replicates per treatment. A total mixed ration formulated for lactating dairy cows served as substrate. To separate the effect of nutrients in the unfermented grain portion from the effect of the fermented portion of each product, a grain only treatment served as a blank (GB). A negative control (Control) with no treatment was also included. Tubes were inoculated with buffered rumen fluid and incubated for 12 h. As expected, GB had greater acetate, propionate, butyrate, and total VFA concentrations than Control ($P < 0.0001$). XPC had greater ($P < 0.0001$) acetate (21.3 vs. 19.9 mM), propionate (9.0 vs. 8.3 mM), butyrate (3.9 vs. 3.6 mM), and total VFA (35.5 vs. 32.8 mM) than GB. NutriTek had greater ($P < 0.0001$) acetate (22.8 mM), propionate (9.9 mM), butyrate (4.3 mM), and total VFA (38.4 mM) than both GB and XPC. Relative abundance of cellulolytic and lactate-utilizing bacteria was determined using real-time qPCR. XPC resulted in greater ($P \leq 0.0013$) *Ruminococcus albus* (0.018 vs. 0.011%), *R. flavefaciens* (0.016 vs. 0.012%), *Fibrobacter succinogenes* (0.27 vs. 0.23%), *Selenomonas ruminantium* (0.035 vs. 0.024%) and *Anaerovibrio lipolytica* (0.00071 vs. 0.00039%) compared with GB. NutriTek resulted in greater ($P = 0.0013$) *F. succinogenes* (0.27%) than GB, and greater ($P < 0.0001$) *R. albus* (0.026%), *R. flavefaciens* (0.027%), *S. ruminantium* (0.062%), and *A. lipolytica* (0.0012%) than both GB and XPC. However, XPC and NutriTek resulted in less ($P = 0.0008$) *Butyrivibrio fibrisolvens* (0.36 and 0.38%) when compared with GB (0.47%). No treatment effect was observed for *Megasphaera elsdenii* ($P = 0.7$). In conclusion, XPC and NutriTek enhanced VFA production and stimulated certain cellulolytic and lactate-utilizing bacteria *in vitro*. NutriTek further enhanced ruminal VFA production and some functional rumen bacteria compared with XPC.

Key words: XPC, NutriTek, *in vitro* rumen microbial model

P122 Effects of oral administration of various essential oils on intestinal characteristics and intestinal microbiota in broilers.

Won Yun*¹, Ji Hwan Lee¹, Chang Hee Lee¹, Seo Young Oh¹, Hyeun Bum Kim², and Jin Ho Cho¹,
¹Chungbuk National University, Cheongju, Chungcheongbuk, South Korea, ²Dankook University, Cheonan, Chungcheongnam, South Korea.

A study was carried out to examine the effect of oral administration of various essential oil on intestinal characteristics and intestinal microflora in broiler chicks experimentally infected with *Salmonella enterica* Lipopolysaccharide (LPS). A total of 40 21day-old mixed sex ROSS 308 broilers separated into 4 equal groups were used in a 2-week experimental period. These groups, half of group infected with *Salmonella enterica* LPS and other injected saline, with essential oils was administered 200 μ L by oral. It was administered orally to contained minimum 98% pure essential oils for broilers at 18:00 h. Treatment groups were as follows: CON) basal diets, T1) basal diets with oral administration of 200 μ L carvacrol, T2) basal diets with oral administration of 200 μ L thymol, T3) basal diets with oral administration of 200 μ L oregano. Fecal score, villi height, and goblet cell counts were improved in broilers fed a diet with essential oils compared with broilers fed a diets without essential oil supplementation ($P < 0.05$), moreover, it showed that better appearance of villi morphological structure in the groups fed with essential oils. Administration with dietary carvacrol, thymol and oregano resulted in *Lactobacillus* and *E.coli* counts not differing from the administration without essential oils, but, *Salmonella* counts of oral administration with essential oil has lower than non-administration with essential oils ($P < 0.05$). In conclusion, broilers administered orally various essential oils improved villi height, goblet cell counts, villi morphological structure and fecal score by inducing the growth inhibition of *Salmonella* in gut.

Key words: essential oil, intestinal characteristics, broiler

P123 Effect of supplementation of rumen protected live yeast on site and extent of digestion in the digestive tract of beef heifers fed high-grain diet.

P. X. Jiao^{1,2}, F. Z. Liu², S. Ding¹, N. Walker³, and W. Z. Yang*¹,
¹Agriculture and Agri-Food Canada, Lethbridge Research and Development Centre, Lethbridge, AB, Canada, ²North West Agriculture and Forestry University, Yangling, Shaanxi, China, ³AB Vista, Marlborough, Wiltshire, UK.

Several mechanisms whereby probiotics (yeast or bacteria) may improve gut health, intestinal microbial balance and production efficiency have been proposed, but few of these have been directly examined in experiments with cattle. With ruminants, the challenge is to deliver probiotics with high activity post-ruminally due to the highly proteolytic environment of the rumen. The objective of this study was to determine the effect of adding live yeast (LY) on site and extent of feed digestion in the digestive tract of finishing cattle. The LY was encapsulated using barley protein hordein and glutelin extracted from barley grain. The stability of encapsulated yeast in the rumen and its release in the intestine were valid *in vitro*. Five rumen cannulated beef heifers



(body weight, 650 kg) were used in a 5×5 Latin square. Heifers were fed ad libitum diet containing 10% barley silage and 90% barley concentrate (DM basis). Five treatments were: 1) control (CON; no additives); 2) antibiotics (ANT; 28 mg/kg monensin + 11 mg tylosin/kg diet DM); 3) 4 g/head/d LY as-is, LY; 4) 4 g encapsulated LY (EY); and 5) 4 g of each LY and EY (MY). Yb and 15N were labeled, respectively, digesta and rumen microbes. Omasal sampling technique was used to measure the flows out of the rumen. Intake of DM (kg/d) ranged from 11.3 to 12.1 and was not affected by treatment. Flows (kg/d) of OM to omasum tended ($P < 0.08$) to be lower for heifers fed MY (4.5) than for CON (5.2) and ANT (5.3). Ruminant OM digestibility was greater ($P < 0.02$) with LY (71%) or MY (72%) than with CON (67%) and ANT (66%). However, intestinal digestibility (% of intake) of OM was not affected by treatments. As a result, the total digestibility of OM tended ($P < 0.07$) to be higher with LY (80) and MY (81) than with CON (78) and ANT (77%). Microbial N production (averaged 140 g/d) was not affected, whereas microbial efficiency was lower ($P < 0.02$) for MY versus CON (15 vs. 22 g/kg rumen fermented OM). These results indicate that supplementation of high-grain diet with LY slightly improved rumen OM digestion, thus in the total digestive tract, which suggest that improvement of postruminal digestion by adding protected LY is not apparent.

Key words: beef heifer, digestibility, live yeast

P124 Effect of yeast extract rich in nucleotides on gut health and performance of broiler chickens.

A. Garcia*¹, S. Solorio¹, M. Forat², V. Navaro², R. Raspoet¹, and T. G. Kiro¹,
¹Phileo-Lesaffre Animal Care, Marcq-en-Baroeul, Lille, France, ²Instituto Internacional de Investigacion Animal (IIIA), Mexico.

The GIT of the chicken grows more rapidly than the rest of the body in the first few days post hatching undergoing a rapid morphological, molecular and biochemical changes. Human and animal studies have shown that dietary nucleotides play a major role in the growth and maturation of tissues with higher turnover of cells such as the intestine and the immune system. Nucleotides are not essential nutrients as they can be synthesized de novo by the animal, but exogenous supply in the form of feed additives may improve gut health and hence performance of animals. Therefore, the objective of this study is to investigate the effect of 2 different concentration levels of yeast extract rich in nucleotides (500 g/ton vs 750 g/ton) supplemented under 2 different feeding programs (d 0 to d 21 vs d 0 to d 35 of age) on the performance and gut health of broiler chickens. A total of 95 male Ross 308 birds were randomly allocated into 5 different treatment groups with 19 birds per treatment. Zootechnical parameters were measured at different time points during the study and finally 5 birds per group were sacrificed at d 21 and d 35 of age to collect tissue samples for morphometric studies in the duodenum, jejunum and ileum. All birds which received yeast extract supplement were heavier than the control birds at the end of the experiment with higher ADG; however, the effect was significant ($P < 0.001$) both for final weight and ADG only in birds supplemented with 500 g/ton yeast extract in both feeding programs. Birds which receive 500g/ton yeast extract rich in nucleotides also show a significantly better ($P < 0.0001$) feed conversion ratio. Supplementation for only 21 d significantly increased ($P < 0.001$) villi height and width, while decreasing the crypt depth in the duodenum. From these

results we can conclude that supplementation of birds with 500g/ton yeast extract rich in nucleotides for about 21 d may improve performance of birds by increasing absorptive surface area and enhancing barrier function in the intestine.

Key words: broiler chicken, gut health, nucleotides

P125 Effect of two sources of sodium butyrate on performance and gut morphology of post-weaned piglets.

C. Sol*¹, N. Hillis, M. Puyalto, P. Honrubia, and J. J. Mallo, NOREL SA, Madrid, Spain.

The present study evaluated the effect of GUSTOR N'RGY (70% of sodium butyrate protected with 30% of sodium salts of palm fatty acids distillates) and BUT (54% sodium butyrate protected with phosphate salts) on performance and gut morphology in piglets. A total of 72 piglets weaned at 21 d old (6.7 ± 0.13 Kg) were randomly divided into 3 groups with 6 replicates each (4 animals per replica): (CON) basal diet without additives; (N'RGY) basal diet with GUSTOR N'RGY (3kg/t in pre-starter phase and 1 kg/t in starter phase); and (BUT) basal diet with BUT (3.9kg/t in pre-starter phase and 1.3 kg/t in starter phase), achieving the same quantity of sodium butyrate in both treatments. Mash feed and water were offered *ad libitum*. The trial lasted 28 d, at the end BW, ADG, ADFI and FCR were recorded, and one piglet per replica was euthanized and samples from duodenum, jejunum and ileum were analyzed to evaluate gut development. Data were analyzed by one-way ANOVA using GLM procedure of SAS. Results of performance parameters were analyzed using initial BW as a covariable. There were no differences in performance parameters. Although piglets fed diets in N'RGY group achieved a higher final BW ($P > 0.05$). Piglets in BUT group were the lightest, with 850 g less than N'RGY group. The highest BW achieved by N'RGY group and the low ADFI, resulted in a better FCR at the end of the trial ($P > 0.05$). The reduction in FCR of N'RGY group accounted for a 12% compared with CON group and 19.4% respect to BUT group. At d 49, there were no differences in duodenum. In jejunum, crypt depth tended to be lower in BUT (314.2, 301.6 and 272.3 μ m, for CON, N'RGY and BUT treatments; $P = 0.089$). In ileum, villus height tended to be higher in N'RGY group (307.6, 385.9 and 320.1 μ m, for CON, N'RGY and BUT; $P = 0.095$) and the ratio of villus: crypts was higher in N'RGY group compared with CON group (1.18, 1.64 and 1.29, for CON, N'RGY and BUT; $P = 0.031$). It can be concluded that the protection of sodium butyrate with sodium salts of palm fatty acids (GUSTOR N'RGY) is more efficient than the protection with phosphates salts and for this reason in piglets fed with GUSTOR N'RGY more quantity of active ingredient can reach small intestine to increase absorption surface.

Key words: sodium butyrate, gut morphology, piglets

P126 Replacing enramycin by a probiotic, *Bacillus subtilis* PB 6, as a natural growth promoting agent in commercial broiler chickens (*Gallus gallus domesticus*).

C. P. Soon* and T. Nguyen, Kemin Industries (Asia) Pte Ltd., Singapore.

The use of beneficial microbes, or probiotics, as alternatives to antibiotics as growth promoter in poultry feed to prevent



necrotic enteritis in broiler chickens is gaining acceptance among poultry producers. CLOSTAT, a direct-fed microbial comprises a naturally occurring *Bacillus subtilis* strain isolated from healthy chicken gut, PB6 (ATCC-PTA 6737). PB6 produces several antimicrobial polypeptides called surfactins that have proven effect in inhibiting growth of *Clostridium perfringens* *in vitro* and *in vivo*. Two separate commercial trials were conducted in an integrated broiler farm of 18,500 birds in Vietnam in July 2016 to determine the ability of *Bacillus subtilis* PB6 in replacing enramycin, a commercially available growth promoter that was used to control the proliferation of *Clostridium perfringens* in the gut, and subsequently necrotic enteritis. In a first trial, birds in a control group were fed 20 ppm of enramycin while birds in the treatment group were fed with *Bacillus subtilis* PB6 at 2×10^{11} cfu per metric ton of feed. Birds from both groups were treated from day one to 37 d of age. At end of the trial, the treatment group had significant lower mortality rate by 0.28% (1.15%) as compared with the control group (1.43%). At the same time, the overall broiler performance index (European Efficiency Factor) for both the control and treatment groups were similar; 291 for the treatment group and 293 for the control group. A similar set up was carried out in another trial where birds in the control group were fed 20ppm of enramycin while birds in the treatment group were fed with *Bacillus subtilis* PB6 at 2×10^{11} cfu per metric ton of feed from day one to 38 d of age. In the second trial, birds in the treatment group had a notable average body weight that was 7.9% heavier than birds in the control group; 2.73kg in the treated group and 2.53kg in the control group. Overall broiler performance index for the treated group showed a numerically higher index than the control group; 407 in the treatment group and 289 in the control group. These 2 separate trials showed that *Bacillus subtilis* PB6 at 2×10^{11} cfu per metric ton of feed could be used as an alternative to replace enramycin in broiler production without compromising their performance.

Key words: *Bacillus subtilis* PB6, probiotic, broiler performance index

P127 Enterocyte protein tyrosine nitration in response to *Eimeria* infection in broilers.

T. Elsasser^{*1}, K. Miska¹, S. Kahl¹, R. Fetterer¹, and A. Martinez²,

¹USDA-ARS, Beltsville, MD, ²Center for Biomedical Research of La Rioja (CIBIR), La Rioja, Spain.

Activation of pathogen-sensing mechanisms in intestinal cells initiate the generation of pathway effectors that perturb normal nutritional enterocyte (ETC) functions. Among the conserved pathway mediator molecules generated are nitric oxide (NO) and superoxide anion (SOA) which are known to interact forming ONOO⁻ and ONO₂CO₂⁻, each of which can form 3'-nitrotyrosine proteins (NTp). Nitrotyrosine proteins are associated with significant inhibition of protein function, apoptosis and cell death. We undertook the present study to establish the temporal dynamics of potential nitration reactions in the ETCs of broiler chickens in response to infection with *Eimeria acervulina*. Ross Heritage broilers (n = 4/time point) were either maintained as noninfected (NI) or infected with 3×10^5 *E. acervulina* oocysts per bird. Duodenal tissue was harvested on days (D) 0, 1, 3, 6, 7 and 10 post-infection (PI) and fixed, embedded, and sectioned for analysis by quantitative immunohistochemistry with antibodies specific to NTp and the enzymes xanthine oxidase (XO, SOA generator) and inducible nitric oxide synthase (iNOS, NO generator). Photomicrographs were analyzed for specific pixel quantification using Media Cybernetics Image-Pro 9.2. Accumulation/content of the respective antigenic epitopes was specified by outlining and capturing areas of interest (AOIs) representative of ETCs. Data were statistically analyzed using a mixed models procedure in SAS. NTp, iNOS and XO increases were evident in intestinal villi as early one day PI ($P < 0.05$ v NI) and specific to enterocytes ($P < 0.02$). The increases in NT, iNOS, and XO (number of positive cells and pixel content/cell) occurred in a defined pattern, significant by villus location for day of infection, initiating in the distal villus region progressing down into the proximal villus, and further into the crypts largely mirroring the presence of *Eimeria* down the villus. Two NT patterns were observed in ETCs: a generalized increase (3-fold > NI) and the 8-fold higher levels associated with cells harboring nitrated parasites. The data suggest that the ETC NT response may mediate some of the cytotoxic processes that limit the progress of *Eimeria* infection.

Key words: broilers, *Eimeria*, enterocytes