Modulating the turkey host and its microbiome: Host-microbe interactions following antibiotic and probiotic administration. Timothy Johnson*1, Sally Noll1, Kent Reed1, Dan Knights1, Mike Kogut2, Ryan Arsenault1, Bonnie Youmans1, Jeanine Bramon1, Kristelle Mendoza1, and Tonya Ward1. 1University of Minnesota, St Paul, MN, 2USDA-ARS, College Station, TX, 3University of Delaware, Newark, DE.

Until recently, gut health and development in poultry was routinely managed through the use of low-dose levels of antibiotics in feed to prevent diseases, improve overall flock consistency, and enhance performance. However, worldwide efforts are underway to reduce and/or eliminate antibiotic use in animal agriculture. The elimination of this valuable management tool for use by farmers will leave a critical void that needs to be filled. The overarching goals of this work are to understand the mechanisms by which bacterial succession occurs in the avian gastrointestinal tract in coordination with the host, and to identify antibiotic-free ways to modulate the microbiome to prevent disease and improve bird performance. Caged performance experiments were conducted using turkey pouls, with 8 cage replicates per treatment group and 10 birds per cage (n = 400). Treatments included a negative control, GroGel carrier control, continuous subtherapeutic bacitracin methylenedisalicylate administration (50 g/ton) in feed, commercial probiotic (FM-B11) administered daily via GroGel carrier, and experimental 10-strain probiotic derived from turkey gastrointestinal bacteria administered daily via GroGel carrier. Tissues from birds were collected at d 3, 6, and 13 of age (spleen, ileum, cecum, trachea) and used for assessments of villi height and crypt depth, host gene expression via RNA-Seq, host signaling pathways via kinomic immune peptide arrays, and bacterial and fungal communities via amplicon sequencing. Body weights and feed consumption were measured throughout the experiment. Early significant differences were observed in average daily weight gain between treatment groups, with turkey-specific probiotic administration resulting in the best performance measurements throughout the experiment. Both antibiotic and probiotic administration shifted bacterial and fungal communities in the gut, with modulations from probiotic treatments peaking at d 6 of age and modulations from BMD treatment continuing throughout the trial. RNA-Seq revealed the largest effects at the gut mucosal level, with BMD administration in feed exerting the greatest impact on turkey host gene expression. Correlations were identified between specific microbial taxa and treatment type, as well as between microbial taxa and host gene expression. Overall, this work identifies key mechanistic differences between antibiotic and probiotic treatments relative to the host-microbiome continuum.

Key Words: Salmonella, laying hens, housing, stocking density, fecal shedding

Effect of butyrate release location on cecal microbiota composition of broilers. Pierre Moquet*1, Chuanlan Tang1, Lonneke Oursou2, and René Kwakkel1. 1Wageningen University, Wageningen, the Netherlands, 2Faculty of Veterinary Medicine, Gent, Belgium.

An experiment was conducted to investigate the effect of providing butyrate (Bt) to distinct gastrointestinal tract (GIT) segments on cecal microbiota composition of broilers. It was hypothesized that providing Bt to distinct GIT segments would lead to differential effects on cecal microbiota composition. A total of 320 male day-old Ross 308 broilers were randomly assigned to 5 dietary treatment groups: (1) control (no butyrate), (2) unprotected Bt (main activity in the crop and gastric regions), (3) tributyrin (main activity in the small intestine), (4) fat-coated Bt (main activity in whole GIT) and (5) unprotected Bt combined with tributyrin, each replicated 8 times. Bt was included at 1 g/kg, as fed basis. At 22–23 d of age, cecal contents of 5 birds per pen were collected. Total microbial DNA was extracted, V3-V5 regions of 16S rRNA genes were amplified by PCR and sequenced by Illumina HiSeq. Microbial profiling analysis was carried out using a bioinformatics pipeline. Additional factors that could have influenced cecal microbiota composition, such as SCFAs contents along the GIT, apparent ileal digestibility coefficients and mean retention time of digesta, were also quantified. Microbial diversity indicators were analyzed by GLM and contrasts were used to test the butyrate release location effect. Principal component analysis (PCA) was used to assess the contribution of dietary intervention and other factors to microbiota composition at the phyla.

Key Words: turkey, probiotic, antibiotic, microbiome, alternatives
level. Butyrate concentration varied significantly across GIT segments, indicating that dietary contrasts in Bt release location were successful. Elevated butyrate concentration in the crop, proventriculus and gizzard were associated with a decreased cecal microbiota richness (Chao1; \( P < 0.0001 \)) and phylogenetic diversity (\( P < 0.0001 \)). Elevated butyrate concentration in the colon and ceca were associated with an increase in cecal microbiota richness (Chao1; \( P < 0.0016 \)) and phylogenetic diversity (\( P < 0.0002 \)). The PCA indicated that butyrate and propionate concentration in the crop, proventriculus and gizzard explained 40.8\% of the variation in cecal microbiota composition at the phyla level. The PCA did not indicate a clear effect of cecal microbiota composition on growth performance. This study demonstrates that the effect of butyrate on cecal microbiota is conditioned by the GIT segment wherein the molecule is present.

**Key Words:** butyrate, release location, cecal microbiota, diversity

110 Application of a micro-aerosolized disinfectant system to control environmentally deposited poultry pathogens. Jeffrey Evans*, Scott Branton, Stephanie Collier, John Brooks, and Joseph Purswell, USDA-ARS, Mississippi State, MS.

Disinfectants are widely utilized in the poultry industry to limit encounters with avian pathogens and zoonotic agents. They are readily applied by a variety of means to both equipment and facilities to reduce pathogenic populations and minimize their associated risk. Further, the role of these agents may be of even more importance as the poultry industry reduces its reliance on antibiotics. While a variety of disinfectants and application means are currently available, the search for more efficacious products and technologies continues. Recently, technology has been developed which may be applicable to the poultry industry for pathogen reduction. The NebuPure disinfecting system utilizes a novel micro-aerosol forming dispersal unit to suspend a disinfecting solution in enclosed facilities and allows for largely automated decontamination. To test the disinfecting system for poultry-related applications, *Escherichia coli* and *Salmonella enterica*, were applied and dried on glass coupons. The coupons were then exposed to the NebuPure microaerosol in a sealed chamber and incubated for 1 or 4 h. To test the system against spores and viral agents, *Bacillus subtilis* spores and the MS2 bacteriophage was similarly evaluated. Results indicated significant reductions in all agents tested and the effect appeared independent of the GIT segment wherein the molecule is present.

**Key Words:** disinfectant, poultry sanitation, poultry disease, pathogen control, disease control

111 In vitro inhibition of *Enterococcus cecorum* by probiotic *Bacillus* strains. Alexandra Wealleans*, 1Marion Bernardeau, 1 and Marina Creteneau, 1 1Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, United Kingdom, 2Normandie Université, UNICAEN, Caen, France.

Enterococcal spondylitis, also referred to as “kinky back,” is associated with the translocation of *Enterococcus cecorum* from the intestinal tract to the articulating, freely movable thoracic vertebrae. *E. cecorum* pathogenesis is still poorly understood, though it is likely that improved bird gut barrier function and reduced gut burden would reduce disease incidence. This study aimed to compare the inhibitory potential of 3 *Bacillus* strains against 51 clinical *E. cecorum* isolates. Live cultures of *Bacillus* strains BS8, 15AP4, and BS2084 were centrifuged and filter-sterilized (0.2 \( \mu \)m) so as to obtain sterile pH-neutral CFSs. 96-well microtiter plates were incubated aerobically at 37°C for 14 h in a FlexStation 3 coupled with SoftMax Pro software to record absorbance every 15 min. Results are given as \% inhibition, comparing control at OD \( = 0.4 \) (pathogen alone) and treated (pathogen incubated with *Bacillus* CFS). When relevant, delay in growth was calculated as the difference in time to reach OD 0.4 between control and CFS-supplemented wells. All assays were conducted in duplicate. Means separation was conducted using Tukey’s HSD in JMP 11; differences were considered significant at \( P < 0.05 \). During the assays, untreated *E. cecorum* strains reached OD 0.4 (exponential phase) after between 8 to 10 h of incubation when grown in BHI at 37°C. All strains were capable of significantly inhibiting the growth of *Enterococcus cecorum*. The most effective strain overall was 15AP4, with an average inhibition of 88.79\%; no growth of CFS treated strains was observed after 14h for 59\% of the strains. BS2084 CFS produced an average growth inhibition of 88.60\%, with 43\% of tested *E. cecorum* strains showing no growth after 14h. BS8 provided the lowest average inhibition of 84.99\%, with no growth of CFS treated strains observed after 14h for only 19\% of the *E. cecorum* strains. There was no significant overall difference in inhibition between *E. cecorum* strains from Europe or America (\( P = 0.6611 \)), or between *Bacillus* strains (\( P = 0.4054 \)). In conclusion, *E. cecorum* isolates are sensitive to the antimicrobial compounds produced by the *Bacillus* tested, resulting in a lack of growth or delay in growth suggesting *Bacillus* may be able to delay gut colonization. However, further work is required to understand the effect of *Bacillus* on *E. cecorum* growth and translocation in vivo.

**Key Words:** *Enterococcus cecorum*, *Bacillus*, probiotics, in vitro

112 Effects of dietary eubiotics on *Salmonella enteritidis* excretion in layer hens. Diana Alvarez*, Claudia Torres3, Leticia Bitten-court1, and Carlos Lozano*, 11DSM Nutritional Products Brazil, São Paulo, Brazil, 2DSM Nutritional Products Colombia S.A, Bogotá, Colombia, 3Universidad Nacional de Colombia, Bogotá, Colombia.

Foodborne infection caused by *Salmonella enteritidis* (SE) is a major concern and has been associated with consumption of contaminated poultry products. The demand for reducing antibiotic usage has forced the poultry industry to find alternatives of SE control to ensure food safety. The objective of this study was to determine the in vivo efficacy of a probiotic containing *Enterococcus faecium* (NCIMB 10415; CYLAC-TIN ME 20 Plus; CY), a yeast culture plus enzymatically hydrolyzed yeast (EHY), and the combination of these 2 products (CY-EHY) on SE excretion of layers subjected to an experimental challenge with a field strain of SE. A total of 48 Lohman Brown layer hens at 26 weeks of age were placed in 16 isolation cages (3 birds each) and randomly allocated to 5 treatments with the same basal diet. Treatments were: birds not challenged (Negative control; NC); birds challenged (Positive control; PC); birds challenged and supplemented with CY (17.5 ppm; 2x10^10 cfu/g); birds challenged and supplemented with YW (500 ppm); birds challenged and supplemented with CY-YW. Groups PC, CY, CY-EHY were orally gavaged with 1 mL of 9x10^7 cfu of SE on d 13 of the experiment. The control groups had 2 replicates, and the experimental groups 4 replicates each. Microbiological cultures were made weekly for SE from individual hen cloacal swabs, fecal matter, egg shell membranes and yolk. At the end of 12 wk, birds were euthanized and samples of spleen, liver, ovarian follicles, and bone.
marrow were cultured. Daily egg production per treatment was recorded. Percentages of SE positivity were calculated by dividing the number of positive samples by the number of total samples. Data were analyzed by ANOVA. In comparison to PC, SE positivity was reduced by 92% (CY), 79% (EHY), and 78% (CY-EHY) in fecal samples ($P < 0.001$); by 100% (CY), 86% (EHY), and 84% (CY-EHY) in egg samples ($P < 0.0001$); by 50% (CY), 40% (EHY), and 50% (CY-EHY) in across tissue samples ($P < 0.1$). SE positivity was not detected in NC hens. No additive effects were seen between CY and EHY. The inclusion of these products effectively reduced SE fecal and egg excretion, as well as bird infection. The greatest reduction in SE excretion was found in CYLACTIN group followed by the EHY. This results support the potential use of eubiotics to reduce SE contamination in eggs of infected hens with this pathogen.

**Key Words:** *Salmonella*, probiotic, prebiotic, eubiotic, egg

**113 In-water supplementation of Trans-cinnamaldehyde nanoemulsion reduces *Campylobacter jejuni* colonization in broiler chickens.** Abhinav Upadhyay*2, Komala Arsi2, Basanta Raj Wagle2, Sandip Shrestha2, Indu Upadhyaya2, Kanika Bhargava1, Annie Donoghue1, and Dan Donoghue2, 1ARS, USDA, Fayetteville, AR, 2University of Arkansas, Fayetteville, AR, 3University of Central Oklahoma, Edmond, OK.

*Campylobacter jejuni* is a major foodborne pathogen that causes severe gastroenteritis in humans. Chickens act as the reservoir host for *C. jejuni*, wherein the pathogen colonizes the ceca thereby leading to contamination of the carcass during slaughter. Reducing *C. jejuni* cecal colonization could potentially reduce the risk of human infections. This study investigated the efficacy of in-water supplementation of Trans-cinnamaldehyde (TC; generally recognized as safe status compound from Cinnamom bark) nanoemulsion in reducing *C. jejuni* cecal colonization in 14-d-old broiler chickens. In addition, the effect of TC on colonization factors (motility, attachment to chicken enterocytes) was investigated using a motility bioassay and cell culture analysis. In 2 separate trials, day of hatch broiler chickens (Cobb 500; 10 birds/treatment/trial) were supplemented with TC (normal or nanoemulsion form) in drinking water at 0.0625, 0.125, 0.25, 0.5, and 1% level for 14 d. On d 7, the birds were challenged with a 4-strain cocktail of *C. jejuni* (~6 log cfu/bird) by oral gavage. On d 14, the birds were sacrificed and *C. jejuni* colonization in cecal contents were quantified by dilution and plating of cecal contents on Campylobacter Line agar. In addition, Ethidium monoazide based real-time PCR was employed to quantify bacterial viability in cecal contents. Data were analyzed using ANOVA with GraphPad ver 6. Differences between the means were considered significantly different at $P < 0.05$. Administration of TC nanoemulsion (Polydispersity index $< 0.3$; size ~100–200 nm; zeta potential $-0.35$ mV) in drinking water at 0.25% reduced *C. jejuni* colonization by ~1 or 2 logs cfu/mL in trial 1 or trial 2 as compared with respective controls ($P < 0.05$). Follow up mechanistic analysis revealed that TC reduced pathogen motility and attachment to primary chicken enterocytes ($P < 0.05$). No reduction in feed consumption, water intake or body weight gain was observed in 0.25% or lower concentration treatments as compared with controls ($P > 0.05$). Results suggest that TC nanoemulsion could potentially be used to control *C. jejuni* colonization in broiler chickens. Follow up analysis on the effect of TC on cecal microbiome and *C. jejuni* transcriptome and proteome in broiler chickens is currently underway.

**Key Words:** *Campylobacter jejuni*, trans-cinnamaldehyde, cecal colonization, virulence factors, nanoemulsion

**114 In vitro evaluation of the antimicrobial effects of a gram-negative control solution.** Chasity Pender*1, Antonia Tocconi2, Attila Kovacs2, and G. Raj Murugesan1, 1Biovet, S.A, Constanti, Spain, 2University of Navarra, Navarra, Spain.

Alquermold Natural (developed by Biovet, S.A.) is a natural formulation with bactericide and fungicide action, used to improve health status and reduce intestinal colonization with pathogens. It is based on cimenol ring and active against *E. coli, S. aureus, Salmonella, Clostridium*, and most fungal species, proven both in vitro and in vivo in poultry and swine. While its antimicrobial activity has been scientifically proven, the anti-virulence potential in the gut had never been tested. Hence, we designed a study in which the effect of sub inhibitory concentrations of the product on the ability of *Salmonella* to invade intestinal epithelial cells was measured (in vivo dose is 400 ppm). The strain used in this study was a wild type S. Enteritidis isolated from a patient diagnosed with salmonellosis, deposited in CECT (CECT 7236). Enterocytes used were IPECJ2 cells, cultured with Dulbecco’s modified Eagle medium, nutrient mixture F-12 (Ham) (1:1) with GlutaMAX-I (DMEM/F12)
supplemented with 20% fetal bovine serum. The experiment consisted of exposing confluent enterocyte monolayers to different concentrations of the product (0, 50 and 150 ppm) for periods of 30, 60 and 90 min (37°C, 5% CO₂, 86% relative humidity). Upon such first incubation, bacterial suspensions were added. Negative controls were exposed to plain culture medium and to medium containing only Alquermold Natural. Positive controls were exposed to bacteria only. Intracellular bacteria were quantified by plate counting after a treatment with 100 µg/mL gentamicin for eliminating bacterial cells that had not been able to invade the epithelium, and enterocyte lysis. Three independent studies were carried out and normality of obtained data were analyzed via Kolmogorov-Smirnov/Lilliefors test. After assessing normal distribution, an ANOVA test was performed followed by tukey’s range test as post-hoc analysis. As a result, we found out that exposure to 150 ppm Alquermold Natural correlated with a reduced ability of Salmonella to invade enterocytes by 34.3%. Such an effect was statistically significant when the treatment was applied for 90 min. Results at 90 min were 1.93E+05 for no treatment, 1.88E+05 at 50 ppm, 1.26E+05 at 150 ppm. Main conclusion from this study is that Alquermold Natural is able to significantly reduce Salmonella ability to invade intestinal epithelial cells. 

Key Words: natural preservative, Salmonella, bactericide, fungicide