Salmonella were challenged with 1 of 3 into 4 groups of 25 chicks each. On d 3, all chicks within one group chicks were obtained from a commercial hatchery and were divided intestines and ceca of young broiler chicks. One hundred male broilers peptide (liver expressed antimicrobial peptide-2, LEAP-2) in the small "b+AT; Excitatory amino acid transporter-3, EAAT3; Glucose trans-

**Key Words:** Salmonella Enteritidis, red seaweed, Chondrus crispus, Sarcodiotheca gaudichaudii

Effect on gene expression of nutrient transporters and anti-
microbial peptides in young chicks infected with Salmonella.

**Salmonella** is a major human foodborne pathogen that is able to colo-
nize the gastrointestinal tract of chickens. In this study, we evaluated the effect of a *Salmonella* infection on the gene expression of various nutrient transporters (Na+-independent neutral amino acid transporter, b6+AT; Excitatory amino acid transporter-3, EAAT3; Glucose transporter-2, GLUT-2; Zinc Transporter, ZnT1) and an antimicrobial peptide (liver expressed antimicrobial peptide-2, LEAP-2) in the small intestines and ceca of young broiler chicks. One hundred male broilers chicks were obtained from a commercial hatchery and were divided into 4 groups of 25 chicks each. On d 3, all chicks within one group were challenged with 1 of 3 *Salmonella* Typhimurium levels (10^6, 10^7, 10^8 cfu) while the control group was challenged with sterile saline. On d 7, 9 and 11, 6 chicks from each group were randomly selected and subjected to necropsy. Mucosa samples were taken from the duodenum, jejunum, ileum and ceca. Total RNA was extracted and gene expression was determined using real time PCR. No significant differences (P > 0.05) were observed between control and challenged groups except for expression of the basolateral zinc transporter in the duodenum, which was downregulated in challenged birds. The decrease in expression of the zinc transporter would increase intracellular zinc, which could be beneficial to the growth of *Salmonella* within enterocytes.

**Key Words:** *Salmonella*, transporters, antimicrobial peptide, broiler, gene expression

**Feed supplementation with the red seaweeds** Chondrus crispus and Sarcodiotheca gaudichaudi reduce *Salmonella Enteritidis* in layer hens. Garima Kulshreshtha*,1,2 Bruce Rathgeber1, Martine Boulanne1, Lehoux Brigitte1, Glenn Stratton2, Nikhil Thomas1, Alan Critchley4, Jeff Hafting4, and Balakrishnan Prithiviraj4, Dalhousie University, Halifax, Canada, 2Faculty of Agriculture, Truro, Canada, 3Université de Montréal, St-Hyacinthe, Canada, 4Acadian Seaplants Limited, Dartmouth, Canada.

Reduction of colonization by *Salmonella* Enteritidis (SE) in laying hens is important to provide safer eggs and minimize the spread of human salmonellosis. Antibiotics have been widely used to control bacterial diseases in broilers and laying hens. This use has been a major concern due to antibiotic resistance and adverse changes in antibiotic treatment on animal digestive flora. Thus, there is great interest in developing alternatives to antibiotics such as prebiotics. In the present study the effect of feed supplemented with the red seaweeds Chondrus crispus and Sarcodiotheca gaudichaudi were examined in late-phase White Leghorns. Ninety-six laying hens (in lay) were completely randomized to 6 treatment groups. Aureomycin (CTC) was used as positive control; basal layer diet was used as negative control; and 4 treatment groups were fed a diet containing one of the following; 2% and 4% Chondrus crispus (CC2, and CC4) and the same 2 levels of Sarcodiotheca gaudichaudi (SG2 and SG4). After 5 weeks of feed supplementation, 48 birds were challenged orally with 2 × 10^7 cfu/mL of *Salmonella* Enteritidis. Eggs and fecal samples were collected 1, 3, 5 and 7 d post inoculation. Birds were euthanized and organs (ceca, ovary) were evaluated for SE colonization 7 d after inoculation. Fecal enumeration showed that antibiotic (CTC) prevented SE colonization in the intestinal tract 3 d post inoculation. SG2, CC2 and CC4 treatments were significantly effective in reducing SE fecal colonization. Fecal samples from CC4 supplemented birds were SE negative on d 5 and 7 post inoculation. SE colonization of the ceca was also significantly reduced in birds fed 2% SG and 4% CC. No significant differences were observed in serum AST and sodium within the treatments. However, serum albumin levels were higher with SG2, CC2 and CC4 treatments. Results indicate that feeding diets containing 2% SG and 4% CC are effective in providing resistance to *Salmonella Enteritidis* colonization in laying hens.

**Key Words:** *Salmonella* Enteritidis, red seaweed, Chondrus crispus, Sarcodiotheca gaudichaudii

Development of PCR primer sets for *Heterakis gallinarum*, an important vector for *Histomonas meleagris*, the causative agent of Blackhead disease. Currently, the only way to diagnose *H. gallinarum* is via microscopic examination. While this method may be effective, the development of a PCR procedure would allow for a more rapid, specific methodology. Therefore, the objective of this study was to develop sets of PCR primers that allow for the detection of *H. gallinarum* DNA from different types of samples. A partial 18s ribosomal sequence for *H. gallinarum* was used to create 3 primers sets. Each primer set released a band fragment of different sizes ranging from 500 to 1000 base pairs. Thirty spent hens were sampled to gather *H. gallinarum* worms and roundworms. From these hens, 10 samples of *H. gallinarum* and 3 samples of roundworms were obtained. A sample of *H. gallinarum* worms was incubated in PBS at 42°C for 3 d to allow them to produce eggs. Roundworms were tested to determine the specificity of the primer sets. PCR results showed that *H. gallinarum* eggs were positive for *H. meleagris*. Furthermore, low annealing temperatures did not provide specificity for *H. gallinarum* worms because roundworm DNA also tested positive. A follow-up experiment was set up to determine the optimal annealing temperature to rule out roundworm DNA. PCR results showed that all 3 primer sets were specific for *H. gallinarum* at an annealing temperature of 70°C. Because all 3 primer sets were successful in detecting *H. gallinarum* DNA, a multiplex PCR diagnostic test may be developed to further increase the sensitivity of the primer sets. The significance of this research lies in that *H. meleagris* may be difficult to study because it is an anaerobic organism, so *H. meleagris* can only survive minutes in the environment. Using *H. gallinarum* as a surrogate organism for Blackhead disease would result in a better prediction of the Blackhead disease status of farms. Future works may focus on the study of the epidemiology of Blackhead disease in affected farms via *H. gallinarum* PCR diagnostic test.

**Key Words:** *Histomonas meleagris*, *Heterakis gallinarum*, Blackhead disease, turkey, broiler

Effect of environmental temperatures and nicarbazin levels on broilers challenged with *Eimeria* spp. Manuel J. Da Costa*,1, Ken W. Bafundo2, Hector M. Cervantes2, Emily A. Kimminau1, Lorraine Fuller1, and Gene M. Pest1,1University of Georgia, Athens, GA, 2Phibro Animal Health Corp., Teaneck, NJ.

Previous work has shown that reduction of environmental temperatures (ET) might be beneficial when raising broilers fed diets with nicarbazin (NIC). Two experiments were conducted to determine whether these

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favorable effects would be present when broilers were challenged with *Eimeria* spp. In each experiment, 1,920 d-old male Cobb 500 were placed in 48 pens divided between 2 rooms. ET treatments consisted of a standard (S) that followed breeder recommendations and a low (L) ET profile set 3°C lower after the first 2 d. There were 3 NIC inclusion levels (0, 100 and 125 ppm) in starter (0 to 18d) and grower feeds (19 to 28d). Cloacal temperatures (CT) were measured on 3 birds per pen every 7d. Birds were challenged on d12 with *Eimeria acervulina, Eimeria maxima,* and *Eimeria tenella.* Intestinal gross lesions scores (Johnson and Reid, 1970) were performed on 2 birds per pen 6 d post-challenge.

Data were analyzed as a CRBD with a 2 × 3 factorial arrangements of treatments with the 2 experiments as blocks. On d18 lowering ET (*P* = 0.021) or feeding 100 ppm of NIC (*P* < 0.001) increased BW gain. Conversely, on d 28 low ET (*P* < 0.001) resulted in lower BWG. NIC effects (*P* < 0.001) were consistent on d 28, where 100 and 125 ppm increased BW gain by 132 and 102 g, respectively, compared with birds not fed NIC. AdjFCR was improved (*P* = 0.038) by 0.03 g/g in birds exposed to low ET at 18 d. However, on d 28 lowering ET increased (*P* = 0.038) adjFCR by 0.03 g/g. Feeding 100 and 125 ppm decreased adjFCR by 0.09 and 0.08 g/g respectively, when compared with 0 ppm on d 28. Lowering ET resulted in (*P* < 0.001) lower CT across the experiment. Both 100 and 125 ppm NIC were shown to reduce CT after d 21 of age. As a consequence, lowering ET (*P* < 0.001) reduced mortality of birds fed NIC by 2.8 and 7.7% respectively comparing to S ET birds. Feeding NIC lowered (*P* < 0.001) gross lesions scores for the 3 *Eimeria* spp. In conclusion, low ET was shown to be beneficial for mortality reduction across the experiment and performance at 18 d; however, as the *Eimeria* infection progressed with age, lowering ET was detrimental to bird performance.

**Key Words:** nicarbazin, environmental temperature, *Eimeria,* broiler

77 **Avian β-defensin 10 expression is synergistically induced by butyrate and forskolin in chickens.** Kelsy J. Robinson*, Lakshmi T. Sunkara, and Glenn Zhang, *Oklahoma State University, Stillwater, OK.*

Public concern over antimicrobial resistance from routine use of in-feed antibiotics demands the development of antibiotic-free strategies for disease control and prevention. Several classes of dietary compounds are capable of inducing the synthesis of a large array of host defense peptides (HDP), which are critical components of innate immunity. Supplementation of some of these HDP-inducing compounds has led to enhanced bacterial clearance in chickens, suggesting their potential as a novel class of antibiotic alternatives for poultry applications. Butyrate is a major species of short-chain fatty acid produced naturally by bacterial fermentation in the chicken cecum, whereas forskolin is a natural labdane diterpene produced by the Indian Coleus plant. This study sought to evaluate the potential of butyrate and forskolin to augment chicken HDP synthesis and innate immunity. Chicken HD11 macrophage cells as well as live animals were treated with both compounds individually and in combination, and the expression of chicken HDP genes (as exemplified by avian β-defensin 10) was evaluated by real-time PCR. Our results indicated that the avian β-defensin 10 is significantly increased by up to 20-fold in HD11 cells in response to treatment with 1 to 2 mM butyrate and 5 to 10 μM forskolin for 24 h. Furthermore, stimulation of HD11 cells with 2 mM butyrate and 5 μM forskolin synergistically increased avian β-defensin 10 mRNA expression by at least another 3-fold relative to butyrate or forskolin alone. Importantly, a drastic synergy in HDP induction was observed between butyrate and forskolin in the jejunum of chickens 24 h after supplementation of 1 g/kg of sodium butyrate in combination with 5 or 10 mg/kg of forskolin in the feed. The ability of butyrate and forskolin to synergistically augment avian β-defensin 10 expression alludes to their viability as novel antibiotic alternatives for disease control and prevention.

**Key Words:** host defense peptide, butyrate, nutritional immunity, innate immunity, antibiotic alternatives

78 **Effect of in vitro 25-hydroxycholecalciferol treatment of lipopolysaccharide on heterophil nitrite production and in vivo effects of 25-hydroxycholecalciferol supplementation on turkey poultse during a coccidial challenge.** Antrion Morris* and Ramesh Selvaraj, *The Ohio State University, Wooster, OH.*

Two experiments were conducted to study the effects of 25-hydroxycholecalciferol 25(OH)D in vitro treatment on heterophil nitrite production post-lipopolysaccharide (LPS) challenge and the effects of 25(OH)D in vivo supplementation in turkey birds post-coccidial challenge. Heterophils were cultured in the presence of 0 or 200 nM of 25(OH)D and stimulated with 1 μg/mL of LPS for 24 h. 25(OH)D treatment increased heterophil nitrite production by approximately 3-fold (*P* = 0.02), compared with the control group with 0 nM of 25(OH)D. Turkey pouls were fed a basal diet supplemented with 25(OH)D at 27.5, 55, 82.5 or 110 μg/kg and at 21 d of age orally challenged with 1 × 10⁴ live coccidia oocysts. At 21 d of age, there was a trend in increasing the mean BW of birds supplemented with 25(OH)D at 82.5 μg/kg (*P* = 0.09). Compared with the control birds fed similar levels of 25(OH)D and unchallenged with the coccidia oocyst, birds challenged with the coccidia oocyst had 20, 13 and 24% increased (*P* = 0.01) BW gain in the groups supplemented with either 27.5, 55 and 82.5 μg/kg of 25(OH)D and a 3% reduced BW gain in birds fed 110 μg/kg of 25(OH)D (*P* = 0.01). At d 7 post coccidia challenge, in birds challenged with coccidia oocysts, birds fed 55, 82.5 and 110 μg/kg 25(OH)D had 1.71, 1.21 and 1.90 fold (*P* < 0.01) increase and those fed 27.5 μg/kg 25(OH)D had 1.34 fold decrease in IL-1β mRNA amounts in the cecal tonsils compared with control birds fed 27.5 μg/kg 25(OH)D and not challenged with coccidial oocyst. In conclusion, 25(OH)D treatment increased heterophils nitrite production post-LPS challenge and increased BW gain and cecal tonsil IL-1β mRNA post-coccidial challenge.

**Key Words:** heterophil, 25-hydroxycholecalciferol, coccidia, interleukin-1, turkey

79 **Immune response of layer chickens vaccinated in ovo for F-strain Mycoplasma gallisepticum.** Katie E. Collins*, Scott L. Branton², Jeff D. Evans², Sharon K. Womack¹, and Edgar D. Peebles³, ¹Department of Poultry Science, Mississippi State University, Mississippi State, MS, ²USDA-ARS, Mississippi State, MS.

*Mycoplasma gallisepticum* (MG) causes respiratory disease in poultry with a decrease in egg production in layer chickens. Layers are normally vaccinated for MG during pullet rearing. The objective of this research was to determine if in ovo vaccination for MG is a viable alternative vaccination route with the capability to induce immunity in layers by 6 wk of age (woa) post-hatch. Live embryonated eggs from an MG-clean Hy-Line W-36 breeder flock were either non-injected or administered a 50-µL injection volume of either diluent only (Poultvac Marek’s diluent) or high (1 × resuspended vaccine), medium (10⁻² dilution), low (10⁻⁴ dilution), or low-low (10⁻⁶ dilution) doses of an F-strain MG vaccine (Poultvac Myco F) at 18 d of incubation. The vaccine was resuspended and diluted in Poultvac Marek’s diluent. Hatched chicks (85) from each treatment were raised in 6 2.4 m × 2.4 m isolation chambers (1 per treatment). Mortality was recorded daily. At 6 woa, all birds were weighed, sexed, and bled for serum plate agglutination (SPA) and ELISA testing for the presence of antibodies against MG. The BW data were analyzed.
using the general linear model, distinguishing sex and treatment differences by least squared means comparisons (SAS 9.4). The SPA, ELISA, and mortality data were not analyzed due to lack of replication. For the remaining live birds at 6 woa (85 controls, 84 diluent, 82 low-low, 39 low, 23 medium, and 9 high), positive SPA results were 0%, 1.2%, 46.3%, 97.4%, 91.3%, and 88.9% in the control, diluent, low-low, low, medium, and high doses, respectively. Positive ELISA tests were 0%, 28.0%, 87.2%, 78.3%, and 88.9% in the control and diluent, the low-low, low, medium, and high doses, respectively. BW were lower in higher doses and in females ($P = 0.0014$). These results indicate that in ovo vaccination of MG does activate the humoral immune response of layer chickens by 6 woa with increasing stress (decreased BW of survivors) at higher doses. The low-low dose, the most practical dose (least mortality), would require further testing to determine if this immune response would be adequate against a field strain challenge.

**Key Words:** in ovo, vaccine, *Mycoplasma gallisepticum*, layer, embryo

**80 Epigenetic characterization of the effect of folic acid on chicken B cell receptors methylation patterns and mRNA expression.** Ori Elad*1, Juan Carlos Rodriguez-Lecompte1, Shayan Sharif2, and Patricia McKenna1, 1University of Prince Edward Island, Charlottetown, PE, Canada, 2University of Guelph, Guelph, ON, Canada.

Our aim was to characterize the effect of Folic acid (FA) on promoter methylation profiles and gene expression of toll-like receptors (TLR) 2b, TLR4, Igβ and Major histocompatibility complex II (MHC II) on chicken B cells. B cells were incubated with 0, 1.72 or 3.96 mM of folate (FA) for 4 h, and followed by a 0, 1, or 10 µg/mL of lipopolysaccharide (LPS) challenge for 4 more h. Genomic DNA methylation patterns and mRNA gene expression were analyzed by bisulfite conversion, sequencing, and real time PCR. Results show a positive association ($P < 0.001$) between FA concentration (conc.) and percent of methylation (%M) in Igβ promoter region at 4 and 8 h; and a negative association ($P < 0.001$) between FA conc. and %M of MHC II promoter region at 4 h. However there was only a positive association ($P < 0.03$) between %M and TLR4 gene expression at 4 h. Interestingly there was an interaction between FA conc. and time affecting TLR2b gene expression ($P < 0.03$). Incubation time affected the gene expression of all other genes except TLR2b. Under challenge conditions, there was an interaction between FA and LPS conc. in Igβ gene expression ($P < 0.03$); however, LPS concentration affected only TLR2b gene expression ($P < 0.001$). Real time PCR analysis shows that FA conc. upregulated TLR2b and MHC II at 4 h; conversely, at 8 h there was a downregulation of TLR2b and an upregulation of Igβ. Interestingly, low FA and LPS conc. upregulated Igβ; conversely at high FA and LPS conc. Igβ was downregulated. In conclusion, under T independent antigen stimulation, FA may act as an immune modulator, regulating the expression of B cell receptors. Those effects are FA concentration, time, and LPS dose dependent. For the first time, FA immune modulatory effects on chicken B cells are demonstrated, involving effects on both innate and adaptive immune responses.

**Key Words:** TLR2, RLR4, Igβ, MHCII, folate