

levels of fiber, moisture and protein on pellet manufacture variables. Treatments were arranged in a  $3 \times 3 \times 3$  factorial design consisting of 3 levels of added fiber (0, 2 and 4% oat hulls), added moisture (0, 2 and 4% tap water) and added protein (0, 2 and 4% soybean meal). Fiber, moisture and/or protein were added to a corn/soybean based basal diet that was formulated to Cobb specifications. Feed was manufactured at the West Virginia University pilot feed mill utilizing a randomized complete block design over a 4-week period. Steam conditioning temperature was held at  $80 \pm 2^\circ\text{C}$ . Steam pressure was held constant at 262kPa. Fiber decreased modified pellet durability index (MPDI), bulk density and production rate ( $P = 0.0001, 0.0001, \text{ and } 0.0524$ , respectively) and increased total fines ( $P = 0.0234$ ). Detrimental pellet manufacture effects of fiber were likely associated with particle size. Moisture increased MPDI and decreased bulk density and production rate ( $P = 0.0030, 0.0001, \text{ and } 0.0001$ , respectively). Protein increased MPDI ( $P = 0.0440$ ). Ingredient interactions (fiber|moisture|protein) were not significant ( $P > 0.05$ ). Results suggest that further research is warranted using increased levels of moisture and protein in practical diet formulations.

**Key Words:** pellet quality, feed manufacture, diet formulation

**169 Effects of diet preconditioning on digestive system morphology in roosters fed increasing levels of guar meal.** O. Gutierrez\*, A. Haq, and C. A. Bailey, *Texas A&M University, College Station.*

Several studies report adaptive morphological responses in the digestive system of gallinaceous birds fed high-fiber diets over an extended period of time. This often results in increased utilization of diets low in nutrient density. The purpose of the current study was to determine whether morphological adaptations could occur as a result of feeding relatively high levels of guar meal (GM) to adult Leghorn roosters over a 3-week period. Guar meal is a high fiber by-product of guar gum processing and contains approximately 18% residual gum, which is comprised of a  $\beta$ -1 $\rightarrow$ 4-linked D-mannopyranose chain with  $\alpha$ -1 $\rightarrow$ 6-linked D-galactopyranose branches. A total of 28 roosters were fed 1 of 4 diets containing differing amounts (0, 6, 12, and 24%) of GM for a period of 3 weeks. Following this preconditioning period, all birds were euthanized and evaluated for changes in villus height and incidence of intestinal mucosal damage as determined by light microscopy. Changes in relative organ weights (ventriculus, liver, heart, pancreas, spleen, and small intestine), cecum length, body weight, and feed consumption were also measured. Significant increases in cecum length, villus height and severity of intestinal mucosal injury were observed in birds consuming the 24% GM diet. Additionally, increased pancreas and liver weight and decreased heart and body weight was noted for this group. Birds consuming the 6% GM diet exhibited intestinal lesion scores and relative heart weights intermediate to those of the control and 24% GM groups. No significant differences were noted for any other parameters. These results indicate that a diet preconditioning period of 3 weeks is sufficient to induce morphological adaptations of the digestive system of chickens consuming a 24% GM diet. However, the effect of these adaptations on nutrient utilization is unknown due to factors such as the increase in intestinal mucosal damage associated with the 24% GM diet.

**Key Words:** diet preconditioning, digestive morphology, guar meal

## Pathology

**170 Effect of passage through laying hens on organ invasiveness and phenotypic heterogeneity of *Salmonella* Enteritidis.** R. K. Gast\*, J. Guard-Bouldin, R. Guraya, and P. S. Holt, *USDA-ARS, Egg Safety and Quality Research Unit, Athens, GA.*

Horizontal transmission within and between flocks is an important aspect of the epidemiology of *Salmonella* Enteritidis (SE) in poultry. Previously, a series of passages through infected laying hens increased the frequency at which an SE isolate was deposited inside eggs. The present study evaluated the effect of in vivo passage of an SE isolate on its ability to invade to internal tissues and its expression of a phenotypic property (biofilm production) associated with invasiveness and egg contamination. In each of 3 trials, a group of laying hens was infected orally with a PT13a strain of SE (prepared from a separate stock culture each time). After internal organ samples were removed for culturing at 7 days postinoculation, an SE isolate from the upper oviduct of an extensively infected hen was used to infect a second group of hens in each trial. In trial 1, the frequency of SE isolation from organs declined from 40 to 12% between the 2 rounds of infection, but the frequency of biofilm production by SE colonies obtained from round 2 organ samples (98%) was higher than for the original inoculum culture (59%). In trial 2, no colonies from either the inoculum strain or round 2 organ isolates were biofilm-positive, and the frequency of SE isolation from organs increased from 27 to 42% between rounds 1 and 2. In trial 3, the frequency of SE isolation from organs was similar in the 2 rounds

of infection (61 and 58%), but the frequency of biofilm production by round 2 organ isolates (58%) was lower than for the original inoculum strain (88%). Passage of SE through infected chickens did not always select for a higher ability to invade internal organs in the present study. Moreover, in vivo passage did not consistently select for either increased or decreased phenotypic diversity within the overall SE population. The characteristics of the original inoculum population, the selective pressure exerted in the tissues of infected chickens, and the exact proportions of relevant phenotypic subpopulations actually transferred to subsequently infected birds may combine to determine the outcome.

**Key Words:** *Salmonella* Enteritidis, in vivo passage

**171 Development and evaluation of candidate recombinant *Salmonella*-vectored *Salmonella* vaccines.** R. E. Wolfenden\*<sup>1</sup>, S. L. Layton<sup>1</sup>, A. D. Wolfenden<sup>1</sup>, A. Khatiwara<sup>1</sup>, G. Gaona-Ramírez<sup>1</sup>, N. R. Pumford<sup>1</sup>, K. Cole<sup>2</sup>, Y. M. Kwon<sup>1</sup>, G. Tellez<sup>1</sup>, and B. M. Hargis<sup>1</sup>, <sup>1</sup>*University of Arkansas, Fayetteville*, <sup>2</sup>*The Ohio State University, Columbus.*

Attenuated *Salmonella* Enteritidis ( $\Delta$ SE) recombinant vaccine vectors incorporating a *Salmonella* flagellar protein (*fliC*) subunit, a hydrophobic putative cell-mediated epitope, ( $\Delta$ SE/*fliC*) for LamB expression, with or

without co-expression of a putative immune-enhancing CD154 oligopeptide ( $\Delta$ SE*fliC*-CD154) were developed and compared to wild type SE (wtSE) as initial vaccine candidates against *Salmonella* infection. Two initial exp. were performed to assess the clearance of each construct and antibody response to the *fliC* peptide. Each construct was orally administered to broiler chicks at day-of-hatch by oral gavage ( $\sim 10^8$  cfu/chick). At d21 liver/spleen (LS) and cecal tonsils (CT) were removed aseptically for recovery of SE or  $\Delta$ SE mutants. For exp. 1 vector recovery from LS and CT was as follows: Control (nonvaccinated) LS-0/7 (0%)<sup>b</sup>, CT-0/7 (0%)<sup>c</sup>; wtSE LS-5/8 (63%)<sup>a</sup>, CT-8/8(100%)<sup>a</sup>;  $\Delta$ SE*fliC* LS-0/9 (0%)<sup>b</sup>, CT 0/9 (0%)<sup>c</sup>;  $\Delta$ SE*fliC*-CD154 LS-4/8 (50%)<sup>a</sup>, CT-4/8 (50%)<sup>b</sup>. In exp. 2 vector recovery was as follows: Control (nonvaccinated) LS-1/10 (10%)<sup>ab</sup>, CT-3/10 (30%)<sup>bc</sup>; wtSE LS-5/10 (50%)<sup>a</sup>, CT-8/10(80%)<sup>a</sup>;  $\Delta$ SE*fliC* LS-0/10 (0%)<sup>b</sup>, CT 2/10 (20%)<sup>c</sup>;  $\Delta$ SE*fliC*-CD154 LS-4/10 (50%)<sup>a</sup>, CT-7/10 (70%)<sup>ab</sup>. A significant difference in antibody response to the *fliC* peptide was not detected, which may be anticipated for a hydrophobic epitope. These preliminary exp. suggest that cell surface expression of *fliC* alone markedly increased clearance rate at 21 d postvaccination. Although more total exogenous peptide sequence is expressed by the  $\Delta$ SE*fliC*-CD154 than  $\Delta$ SE*fliC*, clearance was enhanced by the *fliC* sequence alone, suggesting that insertion of this candidate epitope was responsible for enhanced immune response in these preliminary experiments. Since a significantly different antibody response against *fliC* was not found, we suspect that  $\Delta$ SE*fliC* induces a cell-mediated response.  $\Delta$ SE*fliC*-CD154 was less effective, possibly due to steric hindrance or production of anti-CD154 antibody. Ongoing studies will evaluate the effect of immunization with these vectors on protection from wt challenge and further characterization of the immune response to  $\Delta$ SE*fliC*.

**Key Words:** *Salmonella* vaccine, *fliC*, *Salmonella* vector

**172 Comparison of vitamin U, Bio-Mos and BMD on *Salmonella* control and intestinal measures.** A. L. Shaw\*, K. S. Macklin, and J. P. Blake, Auburn University, Auburn, AL.

This study evaluated the effectiveness of Vitamin U (DL-methionine methylsulfonium chloride), Bio-Mos (mannan-oligosaccharide), and BMD (bacitracin methylene disalicylate) on cecal loads of *Salmonella* and characteristics of the small intestine villi in broilers challenged with *S. Typhimurium*. Two-hundred day-old broilers of mixed sex were randomly allotted to treatments (3 reps/trt) employing a corn-soy basal diet (21.5% CP, 3,142 kcal/kg). Dietary treatments were: 1) Control, 2) Mos (Ctl + 0.095% Bio-Mos), 3) BMD (Ctl + 0.005% BMD), 4) LVU (Ctl + 0.03% Vitamin U), and 5) HVU (Ctl + 0.3% Vitamin U). All birds were orally gavaged with 1 mL *S. Typhimurium* ( $10^8$  cfu/mL) at placement. On a weekly basis cecal contents were obtained for derivation of colonization and liver samples collected to verify septicemia in 6 birds/trt. Intestinal samples were also gathered from the duodenum, jejunum, and ileum for measurement of villi length and crypt depth. Cecal colonization remained constant across all treatments throughout the experiment, except during week 3, where HVU birds were found to have lower colonization ( $P < 0.05$ ) as compared with all other treatments. A greater number of birds ( $P < 0.05$ ) exhibited septicemia for BMD, LVU, and HVU groups during week 2 when compared with Ctl and Mos birds. No differences were found during the prior or subsequent weeks.

Mos birds were found to have greater jejunal and ileal length ( $P < 0.01$ ) than HVU birds during the first week, though similar crypt depths were found in both treatments. During the second week, Mos provided improvements ( $P < 0.05$ ) over BMD in duodenal villi length as well as duodenal and jejunal crypt depth. Week 3 intestinal differences were inconclusive for the measured tissue sections. During the final week, Mos birds showed deeper crypts ( $P < 0.05$ ) in the duodenum and jejunum than HVU birds, while both BMD and LVU birds had greater duodenal villi length ( $P < 0.01$ ) than HVU birds.

Overall effects of Vitamin U on *Salmonella* colonization and septicemia were comparable to Bio-Mos and BMD. BMD and LVU treatments effected intestinal villi measures similarly, with Mos affecting the same measures to a different extent.

**Key Words:** vitamin U, Bio-Mos, BMD

**173 Control of necrotic enteritis and reduction of environmental level of *Salmonella* with the natural feed additives NatuStat and Bio-Mos.** G. Mathis\*<sup>1</sup>, C. Hofacre<sup>2</sup>, and S. Heintzelman<sup>3</sup>, <sup>1</sup>*Southern Poultry Research, Inc., Athens, GA*, <sup>2</sup>*University of Georgia, Athens*, <sup>3</sup>*Alltech, Inc, Lexington, KY*.

The objective of the study was to determine if a NatuStat, starter/grower, Bio-Mos, finisher program would reduce Necrotic Enteritis (NE) and *Salmonella* environmental contamination when fed to coccidial vaccinated broiler chickens. The treatments were nonmedicated (NM), no *Clostridium perfringens* (CP) challenge; nonmedicated, CP challenge; and NatuStat (2 kg/mt) in the starter/grower diets and Bio-Mos (1 kg/mt) in the finisher, CP challenged (NatuStat/Bio-Mos). A complete randomized block design was used with 6 replications of each treatment. Sixty male broiler chickens were placed into each pen. Prior to placement all birds were vaccinated with the coccidial vaccine, Coccivac-B. Half of the birds from each pen were tagged and dosed with *Salmonella* Heidelberg. On Days 20 and 21, NM CP challenged and NatuStat/Bio-Mos treatment birds were dosed with CP. On Day 22, ten birds per pen were Necrotic Enteritis lesion scored.

The birds fed NatuStat/Bio-Mos had significantly lower NE lesion score and NE mortality compared to NM, CP challenged birds. NM, CP challenged birds had significantly poorer performance on Days 22 and 42 compared to the both NM, no CP challenge and NatuStat/Bio-Mos treatments. NatuStat/Bio-Mos birds' performance, both feed conversions and weight gains, were not significantly different from the birds that were not dosed with CP. *Salmonella* drag swab samples on Day 14 showed that *Salmonella* was detectable in all pens, confirming the validity of the disease model. *Salmonella* drag swab samples on Day 42 showed significantly lower number of positive samples in the NatuStat/Bio-Mos compared to the both control treatment pens. This indicates a reduction in environmental *Salmonella* contamination. This study demonstrated the benefits of feeding NatuStat and Bio-Mos to coccidial vaccinated broilers exposed to *Clostridium perfringens* and *Salmonella*. The study showed a significant improvement in performance, less severe Necrotic Enteritis development, and a reduction in environmental contamination of *Salmonella*.

**Key Words:** necrotic enteritis, Bio-Mos, NatuStat

**174 Gene expression profiling within the spleen of *Clostridium perfringens*-infected Broilers fed antibiotic-medicated and non-medicated diets.** A. J. Sarson<sup>\*1</sup>, Y. Wang<sup>2</sup>, Z. Kang<sup>1</sup>, H. Yu<sup>1</sup>, Y. Han<sup>3</sup>, H. Zhou<sup>2</sup>, S. Sharif<sup>4</sup>, and J. Gong<sup>1</sup>, <sup>1</sup>Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada, <sup>2</sup>Texas A&M University, College Station, <sup>3</sup>Nutreco Canada Agresearch, Guelph, ON, Canada, <sup>4</sup>University of Guelph, Guelph, ON, Canada.

*Clostridium perfringens* (CP) is an anaerobic bacterium causing necrotic enteritis in chickens. It is a major target of dietary antibiotics to reduce flock mortality; however, this practice is facing a restriction worldwide. Thus, developing alternatives to improve immunity against CP has become important. Since little is known about molecular mechanisms of host response to CP infection, determination of mechanisms that may lead to such alternatives has been pursued by this study. Gene expression profiles were examined with a chicken 44K Agilent microarray, comparing RNA from spleen tissues of antibiotic-medicated (bacitracin, 55 ppm) and nonmedicated birds prior to, and following CP inoculation. At hatch, 600 Ross broilers were divided into 6 pens fed medicated Starter diets and 6 pens fed nonmedicated Starter diets. At 18 days of age, birds were challenged with CP. Spleens were collected from 12 birds per group at day 18 (before infection), 19, 20, and 22. cDNA was prepared from splenic total RNA for microarray hybridizations. LOWESS-normalized signal intensity was analyzed using a mixed model to identify significant differentially expressed genes between treatments and time points. Expression profiles indicated up-regulation of genes encoding members of the Toll-like receptor pathway, antibody response, T-cell markers, and inflammatory cytokines in nonmedicated, CP-infected chickens compared to infected chickens fed a medicated diet. Moreover, when expression profiles of day 19, 20, and 22 of CP infected, nonmedicated birds were compared to the day 18 baseline, the majority of immune-related genes were up-regulated. Overall, CP infection appeared to have a more robust effect on inducing gene expression in the spleen than did antibiotics; however, host response factors that were differentially expressed upon infection were very similar between the medicated and nonmedicated groups. Further analysis of the highlighted immune mechanism is underway to better understand the role of antibiotics in the host response to CP infection in chickens.

**Key Words:** *Clostridium perfringens*, necrotic enteritis, microarray

**175 *Bacillus licheniformis* (GalliPro Tect) prevent necrotic enteritis in broiler chicken.** I. Knap<sup>\*1</sup>, B. T. Lund<sup>1</sup>, and G. F. Mathis<sup>2</sup>, <sup>1</sup>Chr. Hansen A/S, Hoersholm, Denmark, <sup>2</sup>Southern Poultry Research Inc., GA.

Purpose of study: Evaluate the dose effect of *B. licheniformis* to prevent necrotic enteritis (NE) in *Clostridia perfringens* challenge studies and to understand mode of action of the NE preventing effect of *B. licheniformis*.

Trial design: Three *Clostridium perfringens* challenge studies were carried out at Southern Poultry Research, Inc. Two studies were cage studies and one study was performed as floor pen study. In the studies were different doses of Bacillus spores tested from  $8 \times 10^5$  CFU/G to  $8 \times 10^7$  CFU/G. In all studies were a nonchallenged group, a negative control; challenged group without additive; and a positive control with Virginamycin 15 g/t. Unmedicated commercial chicken feeds commonly used in the United States were used in all studies. Feed and water were available ad libitum throughout all trials. The *Clostridium* challenge was made by fresh *C. perfringens* broth culture given to the birds daily

in 2 or 3 days. Weight gain, feed consumption, feed conversion, lesion scores, and mortality were calculated.

Results: In all trials a significant effect was seen of using *B. licheniformis* with regards to lesion score, mortality, weight gain, and FCR. There was no significant difference between the *B. licheniformis* treatments and the Virginamycin treatment with regards to mortality and lesion score. A dose of  $1.6 \times 10^6$  CFU/G feed seems to be optimal to prevent necrotic enteritis and gave the same performance (live weight, mortality) as the nonchallenged group

Conclusion: *B. licheniformis* used as a direct-fed microbial could prevent necrotic enteritis in broiler chicken.

**Key Words:** *B. licheniformis*, necrotic enteritis, *C. perfringens*

**176 Utility of probiotics as part of an integrated control strategy against coccidiosis in broiler chickens.** J. L. McPherson Komorowski<sup>\*</sup> and J. R. Barta, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada.

Coccidiosis is a major parasitic disease of poultry caused by protistan parasites that invade and inhabit the gut. Probiotics (defined or undefined commensal enteric bacteria, e.g., lactobacilli) could contribute to successful coccidiosis control because microflora are an important first line of defence against enteric infections. To assess this, groups of chickens were orally challenged with *E. tenella* and were either administered a probiotic or sham inoculated and/or vaccinated or not vaccinated. Growth rate and food conversion efficacy of the birds was calculated over the challenge period and lesions resulting from the parasite were scored blindly using a qualitative scale. Messenger RNA was isolated from cecal tonsils to detect differences in cytokine gene expression to characterize the nature and intensity of any immune response. Lastly, chickens were bled and ELISAs were performed to detect the level of antibodies against sporozoites to further characterize any immune response. These experiments examined the complex interactions among protistan pathogens, beneficial gut microflora and the immune system of the chicken and may lead to more successful and widespread use of live coccidiosis vaccines in the broiler industry, thereby reducing the industry's reliance on in-feed prophylactic medications.

**Key Words:** coccidiosis, probiotics, broilers

**177 Effect of some chemical pollutants on the response of chickens to vaccination.** A. A. El Meleigy<sup>\*1</sup>, S. E. Mesalhy<sup>1</sup>, M. M. El Hammamy<sup>1</sup>, N. A. Shalaby<sup>2</sup>, and S. F. El Hadad<sup>2</sup>, <sup>1</sup>Suez Canal University, Ismailia, Egypt, <sup>2</sup>Animal Health Research Institute, Gharbia, Egypt.

Based on the water analyses in the field study, 180 one-day-old broiler chickens were used in the experiment and divided into 12 equal groups. All birds were vaccinated against Newcastle and Gumboro diseases except the negative control group (gp). The groups were treated daily at the age between 1 and 42 days; gp. 1 (potassium nitrite normal, 0.005 mg/L), gp. 2 (potassium nitrite high, 0.0083 mg/L), gp. 3 (potassium nitrite low, 0.003 mg/L), gp. 4 (potassium nitrite high with Bio-v-mix, 0.005 + 0.5 mL/L), gp. 5 (lead acetate normal, 0.1 mg/L), gp. 6 (lead acetate high, 0.381 mg/L), gp. 7 (lead acetate low, 0.05 mg/L), gp. 8 (lead acetate high + Bio-v-mix, 0.381 + 0.5 mg/L), gp. 9 (calcium hypochloride, 14 mg/L), and gp. 10 (calcium hypochloride + Bio-v-mix,

14 + 0.5 mg/L), gp. 11 served as control + ve (vaccinated), while gp. 12 served as control – ve (nonvaccinated).

In this study, the mean values for cobalt, copper, lead, cadmium, chloride, nitrite, nitrate, and phosphate were  $0.16 \pm 0.05$ ,  $0.07 \pm 0.02$ ,  $0.26 \pm 0.47$ ,  $0.02 \pm 5.95$ ,  $89 \pm 11.66$ ,  $0.02 \pm 0.01$ ,  $4.14 \pm 274$ , and  $0.04 \pm 0.01$ , respectively. The comparison of these chemical values with their permissible limit in the water revealed only cobalt, lead, cadmium, and nitrite were exceeded the permissible limit. Bio- v-mix, is a catalyst and vaccine stabilizer, contains yucca extract, castor oil, calcium chloride, and magnesium sulfate dissolved with water. It neutralizes chlorine which reduces or damages the vaccine present in the drinking.

Body weight was determined. Blood samples were collected 3 times, at days 14, 28, and 42 days for determination of antibody titers against Newcastle and Gumboro diseases. Chickens from each group were slaughtered at days 14, 28 and 42 day of ages and examined for postmortem lesions and specimens from thymus, bursa, brain, liver, kidney, heart, lung, spleen, and intestine were collected and examined microscopically.

The mean values of antibody titers against Newcastle vaccine of chickens significantly ( $P < 0.001$ ) decreased at 14 days of experiment in gps. 6 and 7, at 28 days in gps. 1, 4, 6, 7, and 8 and at 42 days in gps. 1 and 7. The antibody titer against Gumboro vaccine revealed significant decrease in gp. 4 at 28 days using the neutralizing antibody test and in gps. 3, 5, 6, 7, and 9 using ELISA. The antibody titer against Gumboro vaccine revealed significant decrease in gp. 4 at 42 days using the neutralizing antibody test and in gps 5 and 6 using ELISA. The body weight, growth performance and histopathological examination revealed variable changes depending on type, dose and period of pollutant application.

**Key Words:** pollutants, vaccine response, pathology

**178 Evidence for *Clostridium septicum* as a primary cause of gangrenous dermatitis (cellulitis) in commercial turkeys.** G. Tellez\*, M. J. Marion, N. Pumford, A. Wolfenden, S. Shivaramaiah, E. V. Eskridge, S. L. Layton, R. Wolfenden, G. Gaona-Ramirez, R. L. Brewer, N. Neighbor, C. Lester, and B. M. Hargis, *University of Arkansas, Fayetteville, AR*.

Recently, we have investigated the etiology and methods of immunoprophylaxis against common field gangrenous dermatitis (cellulitis), in commercial turkeys. In Exp. 1, 9 *Clostridium* isolates from cellulitis lesions were purified, grown to high titer, and evaluated for ability to produce lesions in apparently susceptible culls from young (10–16 week) breeder hens. Five *C. perfringens* (CP) isolates, and 3 *C. septicum* (CS) isolates did not induce cellulitis lesions following IV administration of  $\sim 10^8$  cfu. One CS isolate consistently reproduced lesions, often associated with bruised areas, when administered IV as a single dose. CS and CP were isolated from turkeys that died acutely with the lesions of cellulitis. In Exp. 2, both isolates were grown in Cooked Meat Medium (CMM) and injected IV into turkeys ( $\sim 10^8$  cfu), singly, and in mixed culture. Turkeys receiving CP alone failed to develop clinical lesions of cellulitis. Turkeys inoculated with CS alone or mixed with CP developed lesions of cellulitis. CS was recovered again from the cellulitis lesions. In Exp. 3, CMM cultures of the CS isolate was centrifuged to remove cells. The supernatant was injected IV into turkeys. The turkeys became ill but survived. Lesions of cellulitis were not observed. In Exp. 4, an ELISA was developed for measuring antibody titer against the known CS etiology. This assay has allow to predict susceptibility to infection. When turkeys were selected from flocks without detectable antibody, they were susceptible and vice versa. In Exp. 5, an experimental

formalin-killed bacterin was produced from the challenge strain of CS to yield maximum toxin and  $\sim 10^8$  cells per mL. This bacterin (SQ, day of hatch) generates rapid and persistent antibody response against the homologous CS to the time of move (6 wk of age). The ability of this vaccine to protect birds in the field as well as the evaluation of unvaccinated flocks to establish the time frame for sero-conversion and the relationship to clinical disease is currently under evaluation.

**Key Words:** turkeys, cellulitis, bacterin

**179 Antibiotic treatment, small group size and strict biosecurity as a method to eradicate *Mycoplasma* and *Salmonella* from novel turkey breeding stock.** B. J. Wood\*, N. Buddiger, H. van der Hoef, C. J. Kostal, and A. Ferenz, *Hybrid Turkeys, Kitchener, ON, Canada*.

The commercial turkey industry requires the supply of breeding stock guaranteed free of major pathogens. To maintain the supply of breeding stock, new genetic lines may occasionally be incorporated into a primary breeding program. The source of that material must be of comparable or higher health status than the current program. The Orlopp Turkey Farms pure lines were acquired in 2005 with knowledge of the stock being positive for a number of *Mycoplasma* and *Salmonella* spp., consequently, a program was initiated to eradicate both types of pathogens from subsequent generations.

First generation eggs were temperature and pressure differential dipped and injected with a sensitive antibiotic solution. Treatment had a negative effect on hatchability with decreases of 20–45% depending on line. Male lines were more susceptible than female. Male lines were injected with larger amounts of total antibiotic to compensate for greater egg size but both had similar total egg concentration. An explanation for the greater susceptibility other than total dose and line were not identified. First generation poults were mass placed with fortnightly screening (cloacal and tracheal swab) for pathogens with all but *M. meleagridis* eradicated in generation one. Second generation eggs were again dipped and injected with a sensitive antibiotic but in this generation poults were placed into small brooding groups of between 250 and 300 birds. Converted tractor-trailers proved suitable brooding areas with each having positive ventilation and individual bio-secure entry points. Brooding groups returning positive cultures were removed leaving other brooding groups uncontaminated. Generation 3 remained negative for both pathogens. *M. meleagridis* appears less susceptible to egg treatment than other pathogens with the key to eradication, the identification of an effective antibiotic, biosecurity and the use of small groups in the anticipation that the pathogen will remain viable in a select number of eggs.

**Key Words:** disease eradication, mycoplasma, turkeys

**180 Evaluation of an inactivated autogenous vaccine for turkey bordetellosis.** N. R. Pumford\*, G. Tellez, M. J. Morgan, A. D. Wolfenden, and B. M. Hargis, *University of Arkansas, Fayetteville*.

Bordetellosis is a highly infectious respiratory disease that causes millions of dollars in annual losses to the turkey industry. The disease is caused by the bacterium *Bordetella avium*, frequently in combination with other opportunistic pathogens. Commercially available vaccines are thought by some to have marginal efficacy, possibly due to vaccine delivery or strain specificity. *B. avium* isolated from a turkey farm with

persistent clinical bordetellosis in sequential flocks was used to develop an autogenous vaccine. Day-of-hatch poultts were vaccinated subcutaneously with decreasing concentrations of this bacterin. Vaccinated turkeys had significantly higher antibody levels (sample to positive ratios, S/P ratio) compared to controls. Since *B. avium* tends to persist on infected premises, poultts raised in a typical turkey farm would probably be continuously exposed to *B. avium*. Potentially, continuously exposed vaccinated poultts would experience an anamnestic response. To test this hypothesis, we vaccinated poultts delivered to a farm with a history consistent with a diagnosis of bordetellosis (60–80%) in sequential flocks. Poultts were subcutaneously vaccinated on day-of-hatch with *B. avium* inactivated with formaldehyde and aluminum hydroxide was added as an adjuvant. This vaccinated flock had an antibody response twice the level found in a facility that had no history of outbreaks of bordetellosis. Continual exposure to *B. avium* on this premise may have stimulated an anamnestic response to the *Bordetella*. Not only did the vaccinated birds have an elevated immune response, the turkeys did not break with bordetellosis. Although not evaluated in the present study, day-of-hatch bacterin administration may have reduced shedding of *B. avium*, thereby reducing challenge, while accelerating the acquired secondary immune response. Ongoing studies will evaluate the potential for day-of-hatch autogenous *B. avium* bacterin administration for reducing the incidence and severity of bordetellosis in commercial turkey flocks.

**Key Words:** turkey, vaccine, *Bordetella avium*

**181 Bone development and leg problems in four strains of turkeys.** P. E. Eusebio-Balcazar\*<sup>1</sup>, E. O. Oviedo-Rondón<sup>1</sup>, J. Small<sup>1</sup>, J. Grimes<sup>1</sup>, P. Valdivia<sup>2</sup>, and A. Tercero<sup>2</sup>, <sup>1</sup>North Carolina State University, Raleigh, <sup>2</sup>Escuela Agrícola Panamericana Zamorano, Tegucigalpa, Honduras.

Leg problems are a concern for turkey production and a poultry welfare issue. Leg health is affected by genetics, nutrition and management. The objective of this experiment was to evaluate the leg bone development of 4 strains of turkeys. Fertile eggs from each strain identified as A, B, C and D were selected and incubated under the same conditions and placed in the same house divided in floor pens. Random samples of turkeys from each strain were taken from day of hatch until 20 wk on a biweekly basis. At day 1, body weights (BW) and residual yolk weights were obtained, both legs were dissected and shank, tibia, and femur weights, lengths, and thicknesses were recorded. Relative asymmetry (RA) and weight relative to BW without yolk (BWY) of each leg section were calculated. Leg problems were observed at 11 d. The BW and leg traits of birds with twisted legs were recorded at 16 d. Percentages of incidence of leg disorders were obtained at 33 d. At day 1, strain A had higher BW ( $P < 0.001$ ), BWY, and residual yolk % ( $P < 0.01$ ) compared with C and D strains. Strain B had higher BW ( $P < 0.001$ ) and longer femurs ( $P < 0.05$ ) compared with C, but was not different from A and D. Strain A had heavier ( $P < 0.01$ ) right tibias and femurs compared with strain C. The strains A and B had heavier ( $P < 0.01$ ) shanks and left tibias and longer shanks compared with C. No significant differences among strains were observed in relative weights (%) of bones and RA, except for relative weight of shanks ( $P = 0.06$ ) at 1 d and right

tibia ( $P = 0.09$ ) at 16 d. At 16 d of age, strain B had longer left shanks, but higher RA of tibia weights ( $P < 0.05$ ) compared with C, but was not different from the strain A. The incidence of twisted legs at 16 d of age was 5.1, 2.7, 5.0 and 0% for A, B, C, and D strains, respectively. At 33 d, varus, valgus, twisted legs, crooked toes and slipped tendons were observed. The total leg problem incidence until 33 d was 10.8, 14.4, 14.6, and 5.7% for A, B, C, and D strains. These 4 genetic strains differed in bone development and leg disorder incidence. However, to draw final conclusions, it is necessary to consider the effects of yolk absorption and initial body weight on leg problems.

**Key Words:** bone development, leg problems, turkeys

**182 Leg defects and gait patterns on turkey bone biomechanical properties.** E. O. Oviedo-Rondón\*<sup>1</sup>, P. L. Mente<sup>2</sup>, B. D. X. Lascelles<sup>3</sup>, J. Grimes<sup>1</sup>, P. Ferket<sup>1</sup>, and A. Mitchell<sup>4</sup>, <sup>1</sup>Department of Poultry Science, CALS, North Carolina State University, Raleigh, <sup>2</sup>Biomedical Program, COE, North Carolina State University, Raleigh, <sup>3</sup>Department of Clinical Sciences, CVM, North Carolina State University, Raleigh, <sup>4</sup>USDA-ARS, BARC, Beltsville, MD.

Bone fractures have become more frequent in fast growing turkeys. It has been difficult to determine the origin of these spontaneous fractures. Bone strength has been related to bone mineral density (BMD) and forces applied to bone during daily physical activity. The objective of this experiment was to evaluate the effects of common leg defects and gait patterns on bone biomechanical properties. Sixty male-Nicholas turkeys were selected from a large flock raised in floor pens. Turkeys were clustered into 4 categories: normal (N) valgus (V), crooked toes (C) and shaky legs (S) according to visual evaluations performed between 9 and 12 wk of age. Turkeys were trained to walk on a force plate walkway. Data of gait analyses were collected at 13, 16, and 20 wk of age. Data from the pressure sensitive mat was analyzed to obtain the peak vertical force, vertical impulse, peak contact area, foot contact time, and stride length. At 20 wk of age, all turkeys were euthanized, legs collected and frozen for analyses. Weights and morphologic measurements of femur, tibia and shanks were recorded. The BMD and bone mineral content (BMC) were obtained with Dexa. Tibia strength was evaluated by 4-point bending test. There were no significant differences for morphological measurements or bone strength of tibias among the 4 groups evaluated. The BMD and BMC of both tibias from C turkeys were lower ( $P = 0.07$ ) than in the other groups. The tarso-metatarsus BMC in C and S turkeys was lower ( $P < 0.05$ ) than in the N and V groups. There were no differences ( $P > 0.05$ ) on femora BMD and BMC among groups. The BMD of proximal tibia epiphyses varied between medial and lateral sections. No significant differences were observed in femur lengths, diameters and the diaphyseal curvature. However, the relative asymmetry of femur length was lower ( $P < 0.05$ ) in N and V turkeys than in C and S turkeys. Differences observed in gait parameters and effects on bone biomechanics will be discussed. In summary, leg defects such C and S that occur during early development affect the BMD of turkey bones at 20 wk of age and this may affect fracture incidence.

**Key Words:** leg problems, bone strength, gait