

3 hens per cage). The birds were housed in an open hen house with curtains; feed & water were provided ad libitum. Treatment 1 consisted of a sorghum-SBM diet formulated to fulfill at 100% the Bovans white nutritional requirements, then treatments 2 to 3 were added with 111 ppm of PLP with the following nutrient equivalent values (01% Ca & Av. P, 40 kcal/kg ME, 148 ppm Lys, 102 ppm Met, & 148 ppm Thr) also, 300 ppm of RB25, were added to these 3 treatments, however, a different RB25 feed formulation matrix was applied to each diet, T2 RB25 nutrient equivalent values (NEV) were 35 kcal/kg ME; Lys, 252 ppm; Met, 123 ppm; Thr, 159 ppm; & Arg, 291 ppm (50% ME 100% AA). T3 RB25 NEV were 70 kcal/kg ME & AA values the same as T2 (100% ME 100% AA), T4 NEV were: 70 kcal/kg & 50% of AA values

assigned to T2(100% ME 50% AA). The trial lasted 9 weeks; the data was analyzed as a CRD. When the ANOVA showed a statistically significant response the differences between means were analyzed by the LSD procedure. Egg production (%) T1, 89.1; T2, 89.1; T3, 87.3; & T4, 90.2, feed intake, feed conversion T1, 1.893; T2, 1.909; T3, 1.892; & T4, 1.869, & egg mass, were not statistically ($P > 0.05$) affected by treatment. However, relative feed egg production cost (%), T1, 100b; T2, 91b; T3, 86a; & T4, 86a was lower for treatments 3 & 4 compared to T1 & T2 ($P < 0.001$). Under the experimental conditions of the present trial the combination of *Peniophora lycii* phytase and Ronozyme Blend 25 allowed to keep the hen's productive performance along with a more competitive relative egg production cost.

Key Words: hens, carbohydrase enzymes, *Peniophora lycii*, phytase

Pathology

193 Chemotherapy of coccidiosis in gamebirds. L. R. McDougald* and R. W. Gerhold, *University of Georgia, Athens.*

Anticoccidial drugs commonly available for other poultry were tested against recent field isolates of *Eimeria* in Chinese ringneck pheasants, the chukar partridges, and bobwhite quail. Recent field isolates of coccidia from each of the bird species were used for challenge of 2-3 week old birds in battery cages. Each treatment was replicated in 3 cages, with 10 birds/cage. The anticoccidial drugs were obtained from the manufacturer as premixes, and were mixed into game bird starter mash. The experimental design included an unmedicated, uninfected control and an unmedicated, infected control. In four battery tests with pheasants, 3 with chukars and 2 with quail we evaluated zoalene (125 or 150 ppm), diclazuril (1 or 2 ppm), decoquinate (33 ppm), salinomycin (66 ppm), lasalocid (125 ppm), robenidine (33 ppm), Rofenaid (125/75 ppm for sulfadimethoxine and ormetoprim) and semduramicin (25 ppm). Also, in bobwhite quail, we tested amprolium (250 ppm). The endpoints for efficacy were 1) weight gain 0-6 days postinoculation, 2) reduction in lesion scores in comparison with infected controls, and 3) fecal scores (diarrhea) in comparison with controls. Drugs with good efficacy in pheasants were rofenaid, robenz, decoquinate and lasalocid. Semduramicin and zoalene had moderate activity. In the chukar, rofenaid, robenidine, decoquinate, diclazuril and lasalocid had good efficacy. In the bobwhite quail, rofenaid and diclazuril (2 ppm) had good activity, while lasalocid, decoquinate and robenidine had moderate activity. Amprolium had no detectible activity in comparison with infected controls. There was no noticeable toxicity with any of the treatments in any bird species. These results showed generally poorer efficacy of some drugs, in comparison with earlier published tests, suggesting the possible emergence of drug resistance after extensive field usage.

Key Words: pheasant, chukar, quail, coccidia, anticoccidial

194 The effects of different *Eimeria* challenge methods on weight gain and lesion formation in broilers. S. Pohl*¹, J. Lee¹, S. Anderson¹, S. Fitz-Coy², and D. Caldwell¹, ¹Texas A&M University, College Station, ²Intervet/Schering-Plough Animal Health, Summit, NJ.

The objective of this study was to examine the effects of direct or indirect routes of *Eimeria* exposure on body weight gain and intestinal lesion development in broilers. Challenge methods were administered on day of hatch (d0) or d7 post-placement. Broilers were challenged with 5,000 (d0) or 50,000 (d7) sporulated oocysts per bird of a mixed species inocu-

lum of field strain *Eimeria*. In addition to a negative (non-challenged) control group, four challenge methods were evaluated on d0: litter spray, oral gavage, feed administration, and a seeder:contact challenge model where 20% of placed broilers were inoculated. Methods evaluated on d7 were litter spray, oral gavage, and feed administration. Birds challenged on d0 were subjected to necropsy on d9, 16, and 23 while birds challenged at d7 were subjected to necropsy on d16 and 23 to assess gross and microscopic intestinal lesions. Body weights were obtained weekly and at each necropsy. Of broilers challenged on d0, lesions were more pronounced and consistent at necropsy on d9, 16, and 23 in oral and feed challenged groups, with peak lesion development occurring on d16. Body weights were not different among d0 challenged broilers on d9 or 16, but reduced body weight in all d0 challenged broilers was observed at d23. For d7 challenge methods, feed challenge and oral gavage resulted in higher and more consistent intestinal lesions on d16 and 23. Differences in broiler body weight among d7 challenge groups were not observed on d16, but on d23, depression in body weight was observed in feed challenge and oral gavage groups. These data suggest that selected *Eimeria* challenge methods may have a more pronounced effect on body weight gain and lesion development in broilers thereby allowing researchers to select the most appropriate methods for different experimental objectives.

Key Words: *Eimeria*, challenge method, broilers, lesion development, coccidiosis

195 Which *Eimeria* species most affects the production of necrotic enteritis in broiler chickens. G. F. Mathis*¹, C. Hofacre², and J. Fricke³, ¹Southern Poultry Research, Inc., Athens, GA, ²University of Georgia PDRC, Athens, ³University of Georgia PDRC, Athens.

Coccidiosis damage to the intestinal mucosa often leads to proliferation of *Clostridium perfringens* resulting in Necrotic Enteritis (NE). The objective of this study was to examine the association of various species of *Eimeria* to the production of NE. A battery study using 3 replications of 8 birds per cage was conducted. The treatments were no coccidia (NC), *E. acervulina* (EA), *E. maxima* (EM), *E. tenella* (ET), and *E. praecox* (EP). Birds were coccidia challenged at 14 days of age. Each treatment was divided into *Clostridium perfringens* (CP) challenged on days 4, 5, 6, and 7 post coccidia challenge or no CP. The parameters measured were feed conversion, weight gain, necrotic enteritis (NE) mortality, and coccidiosis (0-4) and NE lesion scores (0-3). A significant

coccidiosis infection occurred with EA and ET with over 3.0 avg. lesion score. EM infection level was moderate (1.6 lesion score). *E. praecox* did significantly change the appearance of the intestinal tract (more fluid and white mucus) producing an avg. lesion score of 1.2. Except EP, all species significantly decreased weight gain. The EA, EM, and EP treatments plus CP had significantly higher feed conversions and lower weight gains (except ET) than related treatments without CP. EM, CP treatment had 22 % NE mortality. A much lower level of NE mortality occurred in the EA birds (6%). No birds died from NE in the No Cocci, ET, and EP, CP challenged treatments. These results imply that damage to the intestinal tract from EM, EA, and EP exacerbates the pathogenic effects of CP, leading to NE. Infections with EM greatly increased the probability of Necrotic Enteritis over the other species. This data does demonstrate that NE can occur but to a lesser degree with *E. acervulina*. No NE mortality occurred with *E. praecox* but performance was affected with CP. Even though this species is classified as a non-pathogenic *Eimeria* species, sub-clinical NE appears to be possible with *E. praecox* and under certain circumstance this can relate to performance losses.

Key Words: necrotic enteritis, *Eimeria*, *Clostridium perfringens*, coccidiosis, chicken

196 *Bacillus licheniformis* (GalliPro Tect) prevents necrotic enteritis and improves performance in broiler chickens. I. Knap*, B. T. Lund, and M. M. Jensen, *Chr. Hansen A/S, Hoersholm, Denmark.*

Bacillus licheniformis can prevent necrotic enteritis in *C. perfringens* challenge studies. To understand both the mode of action of the NE preventing effect of *Bacillus licheniformis* and the performance under non- challenge condition, new studies were performed.

Three *C. perfringens* challenge studies were carried out at Southern Poultry Research, Inc.; two cage studies and one floor pen study. In the studies different doses of *Bacillus* spores were tested from 8E+5 CFU/G to 8E+7 CFU/G. All challenge studies included a non challenged control group, 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 *Clostridia* challenge was made using fresh *C. perfringens* broth culture given to the birds in 2 or 3 days. Weight gain, feed consumption, feed conversion, lesion scores, intestinal *Clostridia* counts and mortality were reported. In the non challenged study weight gain and FCR were measured.

In all challenged trials a significant effect was seen of using *Bacillus licheniformis* with regards to lesion score, mortality, weight gain and FCR. There was no significant difference between the *Bacillus licheniformis* treatments and the Virginamycin treatment with regards to mortality and lesion score. A dose of 1.6E+6 CFU/G feed seems to be optimal to prevent necrotic enteritis. In the non challenged trial a significant effect on feed conversion was seen. GalliProTect in starter diet significantly improves live weight by 3.4 and 3.8% at slaughter in non challenged birds.

In laboratory studies growing *Bacillus licheniformis* together with an alpha-toxin producing strain of *C. perfringens* resulted in a significant reduced the level of alpha-toxin.

Bacillus licheniformis used as a DFM improves production performance and can prevent necrotic enteritis in challenged broiler chickens.

Key Words: *Bacillus licheniformis*, necrotic enteritis, *Clostridium perfringens*, DFM

197 Gene expression in chicken and turkey tibia growth plates is affected by oxygen concentrations during the plateau stage of incubation. E. O. Oviedo-Rondón*, M. Ashwell², and M. J. Wineland¹, ¹North Carolina State University, Department of Poultry Science, Raleigh, ²North Carolina State University, Department of Animal Science, Raleigh.

Long bone development is altered by elevated temperatures (T) and by low oxygen (O₂) concentrations during the last four days of incubation. These developmental changes have a negative impact in post-hatch leg disorders of broilers and turkeys. Genomics can help to understand these effects and determine markers for genetic selection of birds with lower susceptibility to leg problems. Proximal tibias collected from chickens and turkeys that were exposed to two O₂ (17 and 23%) and elevated T (39°C) conditions during the plateau stage of incubation were analyzed for gene expression. Two strains of chickens with low (LG) and high (HG) eggshell conductance (G), and one turkey strain were evaluated. Tibia growth plates frozen in liquid nitrogen at time of collection were sectioned to obtain the prehypertrophic and hypertrophic zones. Chondrocyte RNA was isolated and quantitative real-time-PCR used to evaluate expression of 12 genes related to development of tibial dyschondroplasia (TD). These genes are *Vegfr1*, *Vegfr2*, *Vdr*, *Tgfb3*, *Pthrp*, *Mmp13*, *Mmp9*, *Ihh*, *Dio2*, *ColX*, *ColII*, and *Hif1a*. Results indicated that the expression of the matrix metalloproteinases *Mmp13* and *Mmp9* were downregulated approximately 2-fold (P<0.06) in chickens and turkeys exposed to 17% O₂ compared to 23% O₂. The expression of collagen type X (*ColX*) was down-regulated 2.5-fold (P<0.05) in chickens of the LG strain. Hypoxia caused 1.3 fold down-regulation (P<0.07) in the gene expression of the vascular endothelial growth factor receptor 1 (*Vegfr1*) only in the LG chickens. In turkeys, the expression of *Vegfr1*, *Vegfr2*, and *ColX* tended to be down-regulated (P<0.08) in 17% O₂. All genes affected by hypoxia during last 4 d of incubation are essential for the formation of the organic matrix of bones and vascularization prior to ossification. The expression of the remaining genes evaluated did not appear to be influenced by O₂ and were not different (P>0.1) between these 2 strains of chickens. These findings confirm that incubation conditions have an impact on bone development and incidence of bone disorders such as TD.

Key Words: broilers, turkeys, gene expression, bone development

198 Proteomic changes in tibial head cartilage of chickens with tibial dyschondroplasia, a metabolic skeletal disease. K. S. Rasaputra*^{1,2}, R. Liyanage³, J. O. Lay³, and N. C. Rath², ¹University of Arkansas, Department of Poultry Science, Fayetteville, ²USDA/ARS, Fayetteville, AR, ³University of Arkansas, Statewide Mass Spectrometry Lab, Fayetteville.

Tibial dyschondroplasia (TD) is a leg problem in meat type poultry that affects the proximal growth plate cartilage of tibia and tibio-tarsus and prevents their transition to bone. Identifying the birds susceptible to TD and understanding its mechanism is of interest in order to control the disease. Proteins form the basic structural and functional components of the cells and are likely to be affected by the disease. Therefore, the objective of this study was to find whether there are proteomic changes which occur as the result of the disease process. We used an experimental model of TD, feeding one week-old broiler chicks with thiram. Individual growth plate cartilages from 3 birds in each control and thiram fed groups, were used for tissue protein extraction and conditioned media preparation. The later was done to find any secreted proteins that could form the basis of identifying downstream biomarkers. Proteins from tissue and media were extracted using a guanidine hydrochloride

buffer and analyzed by two-dimensional gel electrophoresis. Comparative analysis of Coomassie blue stained protein spots from individual samples from both the groups using a Melanie software, revealed 7 and 3 differentially expressed proteins in tissue and conditioned media respectively. Tryptic digests of the protein spots were analyzed by automated matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) followed by a MASCOT data base search. Five of the tissue proteins were identified as calumenin, actin, chondroitin sulphate, matrilin, and one predicted protein of *Gallus gallus*. The conditioned media proteins corresponded to collagen II, triose phosphate isomerase alpha chain, and an unidentified protein. All these proteins were down regulated in TD except calumenin. Functional relevance of these proteins indicates that they may be involved in the maintenance of cartilage integrity such as their survival, maturation, hypertrophy, and mineralization. The compromise of these functions is likely to affect endochondral bone formation leading to the development of TD.

Key Words: tibial dyschondroplasia, 2D gel electrophoresis, proteomics

199 Astrovirus infection induces sodium malabsorption and redistributes sodium hydrogen exchanger expression. P. K. Nighot*, A. J. Moeser, R. A. Ali, A. T. Bliklager, and M. D. Koci, *North Carolina State University, Raleigh.*

Turkey astrovirus type 2 (TAsV-2) is recognized as a major cause of enteritis in poult in the US and around the world; however our understanding of how it induces disease is limited. Previous studies have demonstrated TAsV-2 infection induces severe watery diarrhea and growth suppression with no significant change in intestinal morphology. More recently, other groups have reported the capsid virus of human astroviruses can induce changes to intestinal cell barrier function *in vitro*. To determine the patho-physiology of TAsV-2-mediated diarrhea we assayed for changes in barrier function and ion transport of control and infected poult jejunum by mounting either intact or stripped intestinal mucosa from poult at 4 days post-infection (peak of clinical signs) on Ussing chambers. Isotopic flux studies demonstrated impaired Na⁺ absorption with reduced short circuit current and increased conductance

in astrovirus-infected jejunum. Electron microscopic examinations of these tissues revealed the terminal web region displayed frequent dense aggregations in TAsV-2 infected intestine. Rearrangement of apical F-actin in form of thick, dense aggregations, particularly in the areas of viral localization, was seen in TAsV-2-infected intestine. The actin rearrangement was limited to morphological alterations as total actin content and ratio of G: F actin in intestinal mucosa was not altered after infection with TAsV-2. Although the total protein expression for the major apical membrane Sodium Hydrogen Exchanger, NHE3, was not changed in western blot analyses of astrovirus-infected jejunum, a significant shift was observed for expression of NHE3 from detergent insoluble fractions to detergent soluble fractions of TAsV-2 infected jejunum. Alternatively, total expression of NHE2 was up regulated in infected jejunum. Taken together, astrovirus TAsV-2 induces malabsorptive diarrhea associated with actin re-arrangement and redistribution of apical membrane NHE3.

Key Words: astrovirus, diarrhea, NHE3

200 Removed

Immunology II

201 Synergistic effect of dietary *Curcuma*, *Capsicum*, and *Lentinus* on enhancing local protective immunity against *Eimeria acervulina* infection. H. Lillehoj*¹, S. Jang¹, D. Kim¹, C. Ionescu², D. Bravo², and S-H. Lee¹, ¹*Animal and Natural Resources Institute, Agricultural Research Service-U.S. Department of Agriculture, Beltsville, MD*, ²*Pancosma S.A., Geneva, Switzerland.*

The protective effect of orally administered *Curcuma longa* (turmeric), *Capsicum annum* and *C. frutescens* (hot pepper), and *Lentinus edodes* (shiitake mushroom) on avian coccidiosis was evaluated in young broilers. Broiler chickens were continuously fed with a standard diet or standard diet supplemented with *Curcuma*, *Capsicum/Lentinus* or *Curcuma/Capsicum/Lentinus* from hatch. Body weight gain, fecal oocyst shedding, antibody titers, and pro-inflammatory cytokine gene expression were measured as parameters of protective immunity following challenge with *E. acervulina*. Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet showed significantly improved body weight gain

compared with birds on the standard diet alone or birds given *Capsicum/Lentinus*-supplemented diet following challenge with *E. acervulina*. Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet shed significantly reduced fecal oocysts and produced higher serum antibody titers compared to the groups fed the standard diet alone or fed *Curcuma* or *Capsicum/Lentinus*. Finally, the levels of local cytokine transcripts of IL-1 β , IL-6, IL-15, and IFN- γ were consistently higher in the *Curcuma/Capsicum/Lentinus*-fed group compared to the controls fed only the standard diet, *Curcuma*, or *Capsicum/Lentinus* groups. This study provides first immunological evidence that dietary supplementation of turmeric, pepper, and shiitake work synergistically to enhance local innate immunity and provide higher protective immunity against *E. acervulina* infection.

Key Words: broiler, coccidiosis, *Curcuma*, *Capsicum*, cytokine