124 Effect of anticoccidial medication upon broilers infected with eimeria species during the starter or grower phase of production. P. M. Matles*1, H. D. Chapman1, and M. W. Lavorgna2.1 Department of Poultry Science, University of Arkansas, Fayetteville, 2 Alpharma.

The effect of salinomycin and roxarsone upon broilers infected with Eimeria species during the starter phase (18 days of age), grower phase (35 days of age) or production was investigated. Birds medicated for 6 weeks and infected during the grower phase produced fewer oocysts than birds medicated for 4 or 5 weeks whether infected during the starter or grower phase. Feed conversion at 6.7 and 8 weeks of birds infected during the starter phase was lower if medicated for 6 rather than 4 or 5 weeks. Feed conversion of birds infected during the grower phase was less if medicated for 6 rather than 5 weeks. It is concluded that medication beyond 5 weeks may be advantageous whether birds are exposed to infection during the starter or grower phase of production.

Key Words: Anticoccidial Drug, Eimeria, Broiler

125 Evaluation of an exogenous enzyme (Avizyme®) as feed additive to enhance immunity against Eimeria spp and replace antibiotics and ionophores in broiler diets. J. Parker*,1 S. Clemente-Hernandez1, J. Remus2, E. Pierson1, B. Clack1, and E. O. Oviedo-Rondón1.1 Stephen F. Austin State University, Nacogdoches, TX, 2DANISCO Animal Nutrition, St. Louis, MO.

Changes in gut microflora and crude protein levels have been linked to enhance immunity against coccidia. The objective of this trial was to evaluate the utilization of a combination of amyrase, protease, and xylanase designed for corn-soybean meal diets (Avizyme® 1502) as a feed additive to enhance immunity against coccidia, and replace antibiotics and ionophores in broiler starter diets with different levels of crude protein. This trial was conducted in Petersime brooding units with 504 day-old male Cobb-500 chickens distributed in 72 cages. Twelve treatments as a result of a 3 x 3 factorial, plus 3 negative controls (No additives-No challenge) within each protein level were distributed. Crude protein levels (19, 21, 23%) and anticoccidial control program (Cocci-Vaccine, Antibiotic + Ionophore, and Vaccine+Enzyme) were evaluated as main effects in the factorial. All chickens, but those in the negative and positive control (antibiotic + ionophore) treatments were vaccinated at 1 d of age with Adven® vaccine. All chickens, but those in negative control treatments were challenged at 17 d of age with a mixed oral inoculum of E. acervulina, E. maxima, and E. tenella. Lesion scores and oocyst counts were evaluated at 24 d of age. Body weight and feed intake were recorded at 17 and 24 d. Feed conversion ratio was determined on days 12, 19, and 25. Lesions scores were taken on day 25. Significant differences (P < 0.05) due to the interaction crude protein x anticoccidial program were observed in post-challenge FI and BWG. Crude protein levels affected (P < 0.01) BW, FCR in pre- and post- challenge periods. Anticoccidial programs affected (P < 0.05) BW at 17 d, BW, FI and FCR at 24 d. The enzyme product improved BW in vaccinated birds at 19 and 21% crude protein diets, but not at 23% CP. Lesion scores were affected (P < 0.05) by anticoccidial programs. Vaccinated chickens fed diets with enzyme had the lowest lesion scores in the caecum, while the antibiotic+ionophore group had the lowest lesion scores in midgut. Enzyme may help in responses against coccidiosis, especially by E. tenella, and this response is dependent upon dietary protein level.

Key Words: Enzyme, Coccidia, Protein

126 Enhancement of homologous vaccination to Eimeria acervulina via CpG-ODNs. K. Ameiss*,1 A. M. Rose,1, B. D. Lively1, N. V. Clack1, and E. O. Oviedo-Rondón1.1 Stephen F. Austin State University, Texas A&M University, College Station, 2Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg.

Synthetic oligodeoxynucleotides containing CpG motifs (CpG-ODN) have been demonstrated to be effective oral mucosal adjuvants in mice. Recent studies in chickens have shown that CpG ODNs can improve the immune response to protein antigens as well as pathogens. The goal of this study was to determine whether oral administration of a single dose of CpG-ODNs could enhance the efficacy of trickle vaccination to a homologous challenge with Eimeria acervulina (EA). Chicks were immunized with 500 oocysts of EA strain #12 per day from days 1 to 5 post hatch with and without 50 μg of CpG-ODN or a control non-CpG ODN. They were then challenged with 1.25 X 109 oocysts of the same strain on day 19 post hatch. Body weight and feed consumption were measured on days 12, 19, and 25. Lesions scores were taken on day 25. Mean feed conversion ratios (grams feed consumed/gram gain) during the immunization period, days 1-12, revealed that both the CpG and non-CpG treated groups had significantly lower (P < 0.001) feed conversion ratios (2.9 ± 0.14 and 3.1 ± 0.14, respectively) than the vaccination only group (3.5 ± 0.12). Mean feed conversion ratios during the challenge period, days 19-26, revealed that all immunized groups, CpG, non-CpG and vaccination only (2.05 ± 0.06, 2.08 ± 0.10, and 2.19 ± 0.05, respectively), were significantly lower (P < 0.001) than the unimmunized birds (2.4 ± 0.09). Mean lesion scores also revealed a significant difference (P < 0.001) between groups receiving either CpGs or non-CpGs (0.40 ± 0.09 and 0.52 ± 0.09, respectively), immunization only (0.81 ± 0.07), and unimmunized (2.21 ± 0.12) birds. These findings indicate that a single oral administration of CpG ODNs can improve protection from a homologous challenge of Eimeria acervulina, improving feed conversion ratio during immunization and reducing lesion score upon challenge.

Key Words: Eimeria acervulina, Immunization, CpG-ODNs

127 Egg characteristics of commercial egg laying hens between 20 and 58 weeks of age when inoculated with the S6-strain of Mycoplasma gallisepticum 10, 22, or 45 weeks of age. E. Y. Basenko1, S. W. Park#1, E. D. Peebles1, S. L. Branton2, D. V. Maurice3, S. K. Whitmarsh1, and P. D. Gerard4.1, 2Department of Poultry Science, Mississippi State University, Mississippi State, 3 USDA-ARS, SCPRL, Mississippi State, MS, 4 Clemson University, Clemson, SC, 5Experimental Statistics Unit, Mississippi State University, Mississippi State.

The effects of S6-strain Mycoplasma gallisepticum (S6MG) inoculation and its timing on egg characteristics were investigated using a total of 100 Hy-Line W36 hens in each of two trials. In each of 16 biological isolation units 10 birds were housed, with four replicate units in each of four treatments. Control birds received sham inoculations at 10 wk of age and treated birds received S6MG inoculations at 10, 22, or 45 wk. Egg weight (EW) was determined weekly between 21 and 60 wk in Trial 1, and between 21 and 53 wk in Trial 2. Eggshell weight per unit of surface area (SWUSA), percentage egg yolk (PY), albumen (PA), and shell (PSW) weights, and yolk moisture (YM) and lipid (YL) contents were determined at 24, 32, 43, 47, and 58 wk, whereas yolk cholesterol (YCHOL) and fatty acid (YFA) contents were determined at 47 and 58 wk in both trials. Hen age influenced EW, PA, PY, PSW, YM, YL and YFA in both trials, but influenced SWUSA only in Trial 2. EW was increased across 46-60 wk by S6MG inoculation at either 10, 22, or 45 wk in Trial 1, and was decreased across 22-45 wk by S6MG inoculation at 22 wk in Trial 2. PSW in W and SWUSA were decreased across 47-58 wk by S6MG inoculation at 45 wk. YL was increased at 58 wk in Trial 1 by 45 wk S6MG inoculation, and was decreased at 24 wk in Trial 2 by 22 wk S6MG inoculation. Across 47-58 wk, the 22 and 45 wk inoculations decreased yolk myristic and arachidonic acid concentrations in both trials. S6MG inoculation during production may increase egg weight, decrease eggshell quality, and influence yolk total lipid and fatty acid contents during post-peak production.

Key Words: Egg Characteristics, Lipid, Mycoplasma gallisepticum

128 Reduction of Salmonella enteritidis infection by therapeutic administration of Lactobacillus probiotic culture. G. M. Nava1, C. M. Pioley1, R. L. Jarquin1, C. D. Sartor1, J. L. Vélez1, G. Tellez1, B. M. Donoghue2, and B. M. Harig3.1 Center of Excellence for Poultry Science, University of Arkansas, 2PPPSRU, ARS, USDA, Fayetteville, AR.

These studies evaluated the therapeutic effect of a Lactobacillus probiotic culture (LPC) producing bacteriocin-like inhibitory substances on Salmonella enteritidis PT-13a (SE) infected chicks. All chicks were challenged at day of hatch with approximately 108 CFU of SE orally, and 2 hr later were randomly assigned to floor pens (n=40) and treated.
Treatments were control group (CG), Lactobacillus casei probiotic culture (LCa), Lactobacillus cellobiosus probiotic culture (LCo), combination of L. casei-L. cellobiosus (LCC) and probiotic culture that contained 11 Lactobacillus spp isolates (L11). Groups LCa, LCo, LCC and L11 received 10⁶ cfu/ml of drinking water; CG received tap water only. Twenty chicks per group were killed at 24 and 72 h post-challenge and cecal tonsils were aseptically collected for SE isolation. Administration of LPC significantly (p<0.05) reduced SE recovery from chicks when compared to the CG (LCa, 59% and 62% reduction; L11, 94% and 63% reduction, 24 or 72 h, respectively). However, LCC reduced SE recovery by 82% only at 24 h. The design of EXP II was similar with CG, LCa, LCo-10x (107 cfu/ml), L11, and L11-10x. LPC treatment significantly (p<0.05) reduced SE recovery when compared to the CG (LCa, 53% and 41%; LCa-10x, 100% and 100%; L11, 81% and 85%; L11-10x, 82% and 100% reduction at 24 and 72 h respectively). In EXP III, CG, LCa-10x and L11-10x were compared and samples obtained only at 72 h. LPC administration significantly (P<0.05) reduced SE recovery when compared to the CG (LCa-10x 81% and L11-10x 81% reduction). These studies suggest that in addition to their better known prophylactic effects, appropriately selected defined Lactobacilli cultures may be useful for actually displacing Salmonella infections.

Key Words: Salmonella, Probiotic, Lactobacilli


Tibial dyschondroplasia (TD) is characterized by the presence of a plug of unresolved cartilage in the growth plate that fails to form bone. Because the etiology of the naturally occurring TD is unknown, it has been difficult to determine the mechanisms of its pathogenesis. Naturally occurring lesions have limited potential to provide insight into the initiation and the progression of TD. The objective of our study was to develop an experimental model that can help understand the mechanisms of TD and should be amenable to screen for the factors that may protect against the disease. To address this objective we tested whether it is feasible to induce TD using a short duration of feeding with thiram, a fungicide. One-week-old broiler chicks were placed on a diet containing 0.1 ppm of thiram for 24-48 h and the growth plates were examined for the presence of TD at the end of treatment or 5, 12, and 17 days after feeding. The lesions were given an evaluation score ranging from 0 (none) to 1 (moderate), and 2 (severe) based on growth plate width. The results showed that as little as 10-20 ppm of thiram fed for only a 24 h period increased the incidence of TD which was augmented as the concentration was raised up to 100 ppm where most chickens were affected with severe TD. The TD index (severity x incidence) showed to plateau with100 ppm of thiram fed for 48 h. A dose of thiram between 80-100 ppm appeared reasonable to study the early changes leading to TD. Under this condition the growth plate showed little visual demarcation at 48 h after feed treatment but contained a progressive pathogenesis of TD with subsequent periods with both morphological and histological changes in spite of the fact that the birds no longer received thiram. There was no cell division in the maturing zone cartilage but an accumulation of chondrocytes with many cells showing nuclear blebbing, and shrinkage was accompanied by an extensive degeneration of blood capillaries. We conclude that this optimized protocol to induce TD by short feeding of thiram or similar compounds may be valuable to understand the initiation and progression of TD and to develop screening methods for its control.

Key Words: Tibial Dyschondroplasia, Thiram, Chicken

130 Salmonella typhimurium Felix-O1 and P22 bacteriophage host range and viability under gastrointestinal conditions. P. Herrera*, E. M. Kozhina, and S. C. Riche, Texas A&M University, College Station.

Salmonella typhimurium is both a human and veterinary health problem. The traditional method of control is the use of antibiotics as a feed supplement. However the increased rate of antibiotic resistance has for alternative methods. Bacteriophage have several advantages over antibiotics including being host specific and self replicating. However in order to use phage, it must be able to survive potential denaturation in the gastrointestinal tract. The purpose of these experiments are to test the stability of two phage specific to Salmonella typhimurium under conditions they would encounter in the gastrointestinal tract. Felix-O1, a lytic bacteriophage that infects most smooth phenotypes of Salmonella, was tested along with P22, a temperate Salmonella phage. Phage lysates (10 μl) with concentrations of 10⁶ PFU/ml were spotted on lawns of 10 strains of Salmonella typhimurium. Felix lysed 7 of the 10 strains as compared to P22 which only lysed 4 strains. Felix was able to infect Salmonella typhimurium strains ATCC 14028, ATCC 13311, UK-1 and serovars Javiana, Muenchen, Rubislaw, and Texas. To test the stability of phage under environmental conditions seen in the cecum of a chicken, 1 ml of a Felix-O1 suspension (10⁵ PFU/ml) was dispensed in tubes containing 5 ml of 1.10x dilution of cecal contents and 0.25 g of ground alfalfa. The tubes were incubated at 37°C under anaerobic conditions for 1, 3, 6, 12, and 24 hours at which times phage concentration was titrated using the soft agar overlay method. Within one hour of incubation, the phage was reduced below measurable levels. Felix-O1 suspension (1 ml) was added to 1 ml of a pepsin solution (32,100 units/ml) and incubated with agitation at 37°C for three hours. Addition to the acidic proteinase solution produced an decrease in phage viability of six orders of magnitude. Pepsin produced only a modest decrease in phage numbers over the 3 h period. These results suggest that Felix-O1 might be an effective anti-Salmonella typhimurium agent if its stability under gastrointestinal condition can be improved.

Key Words: Salmonella, Bacteriophage, Gastrointestinal

131 Rapid detection of infectious bursal disease virus using one-step RT-PCR in clinical samples in Pakistan. M. A. Zahoor*, I. Hussain1, M. K. Mansoor1, S. Masood1, and Q. M. Khan2, 1University of Agriculture, Faisalabad, Pakistan, 2NIBGE, Faisalabad, Pakistan.

A SuperScript One-Step reverse transcriptase polymerase chain reaction (RT-PCR) technique was used for the detection of infectious bursal disease virus in clinically affected bursal tissue specimens. The viral RNA was isolated using TRIzol One-step RNA isolation kit (LS Reagent, Life Technologies). A set of primers was used that amplified a 743-bp fragment of VP2 gene hypervariable region from the nucleotides 701 to 1444. An amplified fragment was found in 21 samples out of 26 whereas none was obtained in case of unrelated virus or IBDV negative samples. Results showed that SuperScript One-Step RT-Taq system can be used for the rapid detection of IBDV in tissue specimens.

Key Words: One STEP RT-PCR, IBVD, Pakistan

132 Very virulent strains of infectious bursal disease virus in Pakistan. M. A. Zahoor1, I. Hussain1, M. K. Mansoor1, S. Masood1, and Q. M. Khan2, 1Faculty of Veterinary Science University of Agriculture, Faisalabad, Pakistan, 2NIBGE, Faisalabad, Pakistan.

Nine bursa samples were collected from a severe outbreak of infectious bursal disease in two commercially reared broiler farms in Oct 2003. The gross lesions particular of IBD and the presence of high mortality speculated the presence of very virulent strains of infectious bursal disease virus (IBDV) in Pakistan. The bursa samples were subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) and restriction fragment length polymorphism analysis. A primer pair that amplified a 743-bp fragment from the nucleotides 701-1444 was used. Seven bursa samples were found to contain the IBDV. The PCR product was further digested with SspI restriction enzyme to identify the very virulent phenotype. Results indicated that three of the tested bursa samples were SspI positive i.e. vvIBDV. This first report describes that there exist vvIBDV strains in Pakistan and SspI restriction enzyme could be used as virulent marker.

Key Words: Very Virulent Strains, IBDV, Pakistan

133 RT-PCR Based diagnosis of infectious bursal disease virus in Pakistan. M. A. Zahoor*, I. Hussain1, M. K. Mansoor1, S. Masood1, and Q. M. Khan2, 1University of Agriculture, Faisalabad, Pakistan, 2NIBGE, Faisalabad, Pakistan.

A total of 26 bursa samples collected from different outbreaks of infectious specific and sold were tested at Molecular Virology Lab (EBD, NIBGE, Faisalabad) for the presence of infectious bursal disease virus using a highly sensitive method i.e. reverse transcriptase polymerase
134 Compromised liver mitochondrial function and complex activity in low feed efficient broilers associated with higher oxidative stress and differential expression of proteins within a single male line. M. Iqbal1, N. R. Pumford2, Z. X. Tang1, K. Lasisset1, W. Bottje1, T. Wing2, and M. Cooper2, 1University of Arkansas, Fayetteville, 2Cobb-Vantress, Inc., Siloam Springs, AR.

Two experiments were carried out to determine the relationships of: a) mitochondrial function and activities of various complexes, b) production of reactive oxygen species (ROS) and its subsequent effect on protein oxidation, and c) protein expression in liver of male broilers with low and high feed efficiency (FE, n =5-8 per groups). Mitochondrial function and complex activities were measured polarographically and spectrophotometrically, respectively. Hydrogen peroxide (H2O2) was measured fluorometrically, while oxidized protein (carboxyls) and immuno-reactive mitochondrial proteins were analyzed using Western blots. Mitochondrial function (ETC coupling) and activities of all respiratory complexes (I, II, III, IV) were higher in high FE compared to low FE broilers. H2O2 and protein carbonyls were higher in the liver of low compared to high FE broilers. Whereas the expression of four immunoreactive proteins [NAD3 (Complex I), QPC (Complex III), COX II and COX IVb (Complex IV)] were higher in low FE liver mitochondria and two proteins [70S (Complex II) and alpha-ATPase (Complex V)] were higher in high FE birds, there were no differences between groups in the expression of 18 other respiratory chain proteins. SDS-PAGE revealed several proteins (ranging from 20 to 180 kDa) that were differentially expressed between the low and high FE groups. In conclusion, taken together with our previous findings in breast muscle (Iqbal et al., 2004, Poult. Sci. 83:474-484), the differential expression of certain mitochondrial proteins might be a compensatory response aimed to maintain the compromised respiratory chain activity and/or to overcome the increased protein oxidation in low FE birds. Funded in part by USDA-NRI grant (#2001-03444).

Key Words: Feed Efficiency, Mitochondrial Complex Activity, Oxidative Stress

135 Membrane potential and hydrogen peroxide production in duodenal mitochondria in broilers with low and high feed efficiency. C. Ojano-Darin1, N. Tinsley1, M. Iqbal1, T. Wing2, M. Cooper2, and W. Bottje1, 1University of Arkansas, Fayetteville, 2Cobb Vantress Inc., Siloam Springs, AR.

Increased hydrogen peroxide (H2O2) production was observed in duodenal mitochondria obtained from broilers with low feed efficiency (FE). As a decrease in mitochondrial membrane potential (MMP) due to stimulation of uncoupling of oxidative phosphorylation reduces reactive oxygen species (e.g., H2O2) production, this study was conducted to evaluate the effect of uncoupling on MMP and H2O2 production in duodenal mitochondria from broiler breeder males with low and high FE. Duodenal mitochondria were isolated from broilers with low (0.48 ± 0.02, n = 8) and high (0.68 ± 0.01, n = 7) FE. H2O2 production and membrane potential were measured fluorometrically using dichlorofluorescin and tetramethylrhodamine methyl ester probe, respectively, in the presence of different levels (0, 200, 400, 600, 800 and 1,000 nM) of an uncoupler, carbonylcyanide p-trifluoromethoxyphenylhydrazone (FCCP). The MMP was higher (P < 0.05) in the high FE mitochondria at 0 to 600 nM FCCP. A decrease in MMP was observed at 600 and 800 nM FCCP for the low and high FE groups, respectively. H2O2 generation was higher in the low FE mitochondria at all FCCP levels except at 200 nM. Adding 200 to 800 nM FCCP caused a decrease in H2O2 production in low but not in high FE mitochondria. The results indicate that FCCP-induced uncoupling lowered H2O2 production in low FE but not in high FE duodenal mitochondria and indicate that mitochondrial membrane potential may influence reactive oxygen species production in broilers with low FE. Supported in part by USDA-NRI #2001-03444.

Key Words: Feed Efficiency, Duodenal Mitochondria, Membrane Potential and H2O2 Production

136 Evidence of protein oxidation in mitochondrial respiratory complexes in broilers with low and high feed efficiency. J. P. Higgins1, N. R. Pumford2, M. Iqbal1, T. Wing2, M. Cooper1, and W. G. Bottje1, 1Department of Poultry Science, University of Arkansas, Fayetteville, 2Cobb-Vantress, Inc., Siloam Springs, AR.

We have observed increased mitochondrial radical production, increased protein oxidation and lower activities of respiratory chain complexes in broilers with low feed efficiency (FE) compared to broiler breeders with high FE. As radical-mediated protein damage could cause the reduction in complex activities, the purpose of this experiment was to determine if there is evidence of protein oxidation that is specifically associated with respiratory chain complexes. Mitochondria were isolated by differential centrifugation from breast muscle obtained from breeder males identified as either low (0.62 ± 0.01, n = 8) or high (0.80 ± 0.01, n = 8) FE. Respiratory chain complexes were separated using a non-denaturing gradient polyacrylamide blue native gel. Protein carbonyl levels were measured with an immunochemical assay in which proteins are reacted with 2,4 dinitrophenylhydrazine to produce a corresponding hydrazone. The hydrazone is then detected by Western analysis using anti-dinitrophenyl antisera. Preliminary results indicate that Low FE mitochondria exhibited higher levels of protein carbonyls (indicating increased protein oxidation) in Complex III compared to levels in High FE mitochondria. These findings suggest that increased protein oxidation may be responsible in part for the lower respiratory chain activities observed in broilers with Low FE. Further research is being conducted to ascertain protein oxidation in other mitochondrial complexes and other mitochondrial proteins in relation to the phenotypic expression of feed efficiency in broilers. Supported in part by USDA-NRI (#2001-03444).

Key Words: Feed Efficiency, Protein Oxidation, Blue Native Gel Electrophoresis

137 Dietary phytates may noncompetitively inhibit intestinal mucosa phytase in broiler chicks. E. M. Onyango1, E. K. Asen2, J. S. Sands3, and O. Adeola1, 1Department of Animal Sciences, Purdue University, West Lafayette, IN, 2Basic Medical Sciences, Purdue University, West Lafayette, IN, 3Danisco Animal Nutrition, Marlborough, Wiltshire, UK.

The role of dietary phytate in the regulation of intestinal mucosa phytase was investigated in broiler chicks. One hundred and eight 7-d-old male broiler chicks were group by weight into six blocks of 3 cages with six birds per cage. Three chemically defined diets were randomly assigned to cages within each block. The three diets were a chemically defined casein diet; chemically defined casein diet plus sodium phytate (20 g/kg diet), and a chemically defined casein diet plus sodium phytate (20 g/kg diet) and microbial phytase (1000 U/kg diet). The chicks were fed experimental diets from 8 to 22 d of age. At the end of the study, chicks were weighed, killed, and duodenal mucosa and left tibiae removed. Tibiae were defatted and ashed overnight at 600 °C. Phytase activity in brush border vesicles prepared from the duodenal mucosa was determined by measuring the amount of inorganic phosphorus released from sodium phytate. Addition of phytate to the chemically defined diet reduced (P < 0.05) weight gain, feed intake, feed efficiency and percentage ash. However, addition of microbial phytase fully restored (P < 0.05) feed efficiency but weight gain was only partially restored (P