
Bordetellosis, caused by Bordetella avium infection, is characterized by tracheal inflammation, epithelial cell degeneration, and tracheal distortion. The inflammation results from dermonecrotic toxins produced by B. avium. Inflammatory reactions are accompanied by generation of reactive oxygen metabolites (ROM) that kill cells. Antioxidant enzyme activity associated with either sodium selenite (SS) or organic selenium (SP, Sel-Plex, Alltech, Inc., Nicholasville, KY) elevates cellular redox status to reduce ROM. Aspirin (AS) can be an antioxidant because it inhibits synthesis of oxidative prostacyclins that induce ROM. The objective of this work was to examine the influence of AS with either SS or SP on development of bordetellosis in female poults. Experiments were conducted with day of hatch poults given intranasally 107 cfu of W strain B. avium or no challenge and monitored for 21st post challenge. Challenged and non-challenged poults were given 6 experimental diets made with poult starter: 1. SS (0.3 ppm), 2. SP (0.3 ppm), 3. no selenium or AS, 4. SS (0.3 ppm)+AS (0.35%), 5. SP (0.3 ppm)+AS (0.35%), 6. AS (0.35%). At 9 and 14, 5 poulets (1/rep of 7 poulets/rep) were killed and necropsied. Regardless of dietary treatment, lower BW (P<0.05) was observed in infected groups. In one experiment, AS prevented epithelial cell degeneration in infected poults. In control groups, SP alone and SP+AS increased thymus weight (g/100 g BW) over other treatments (P<0.06). With infection, SP only was associated with a smaller thymus, but addition of AS maintained thymus wet weight to the other treatments. Bursa and spleen wt were not altered significantly by selenium source, AS, or infection. Abdominal exudate macrophage IL-1 was elevated significantly (P<0.0001) by SS but not by SP or AS, and infection elevated IL-1 (P<0.001). It was concluded that SP and AS can alter the course of developing bordetellosis.

Key Words: Selenium, Aspirin, Bordetellosis

T88 Silymarin PHYTOSOME against AFB1 in broilers: effects on serum biochemistry. D. Tedesco*, Galletti E, K. Ameiss*, L. Ravarotto1, and D. Caldwell1, 1Department of Veterinary Sciences and Technologies for Food Safety, Milan, Italy, 2Instituto Zooprofilattico Sperimentale delle Venezie Via dell’Università, Legnano, Italy, 3Indena S.p.A., Milan, Italy.

Silymarin, the bioactive extract of Silybum marianum, is a natural hepatoprotector and a potent anti-hepatotoxic agent. In poultry, silymarin has shown to be effective against toxic effects of AFB1, preventing the negative repercussions on performance of broilers. This study focused on the effects of silymarin on serum biochemistry in AFB1-in-toxicated broiler chickens. Twenty-four 14-d-old male commercial broilers (377±34g body weight) were randomly allotted into 3 groups and treated for 35 days as follows: control group on a basal diet alone; AFB1 at 0.8mg/kg of feed; AFB1 at 0.8mg/kg of feed plus silymarin PHYTOSOME®, a silymarin complexed form with phospholipids from Silybum marianum, as well as healing following mucosal damage resulting from luminal exposure to deleterious compounds or parasites, is dependent on sustained supplies of putrescine, spermidine and spermine. An experiment was conducted with one hundred and twenty eight, day-old male broiler chickens fed a corn and soybean meal based control diet. Experimental diets were formulated by adding putrescine (0.0, 0.15, 0.30 and 0.45%). Experimental diets and water were offered ad libitum for 21 days. Birds were housed at the Isolation Unit of the University of Guelph, in four rooms with 8 battery cages each with external drinker and feeder, with 4 birds/cage (4 diets with 2 cages per room each for a total of 8 birds/diet/room). On day 11, in each room 16 birds (4 cages/4 birds/diet) were inoculated with Eimeria acervulina Guelph strain, with a dose of 1,000,000 oocysts per ml per bird. At day 17, and at day 21, the end of the experiment, 12 birds randomly selected by wing band number per diet were killed by cervical dislocation. Excreta were collected per pen in both rooms from days 11-13 for determination of fat and energy. Lesions in the small intestine were scored (0 to +4 scale). There was a significant quadratic increase in excreta fat content with increasing dietary putrescine in control birds. There was significant effect of diet on excreta energy content. Overall, the infection did influence growth rate and feed intake of broiler chicks, these levels of putrescine supplementation were inadequate to overcome the protozoal challenge.

Key Words: Polyamines, Putrescine, Coccidia

T90 Development of an antigen specific indirect ELISA for Eimeria using recombinant antigen EASZ240 from Eimeria acervulina. K. Ames1,2, M. Jenkins1, H. Danforth1, A. Barri1, and D. Caldwell1, 1Texas A&M University, College Station, 2USDA/ARS, Beltsville, MD.

The etiological agents of chicken coccidiosis are a number of Eimeria species including E. acervulina, E. tenella and E. maxima. While a cellular response to these parasites has been shown to be important, our laboratory is investigating the mucosal humoral response after both immunization and infection. In order to perform these studies an indirect ELISA was developed based upon the recombinant antigen EASZ240. EASZ220 was isolated from E. acervulina strain #12 and inserted into an expression vector containing both an arabinoose promoter and a His tag for purification. This clone represents an immunodominant sporozoite surface antigen which also has high sequence homology to antigens from both E. maxima and E. tenella. The goal of this study was to develop and verify an indirect ELISA assay for the detection of antibodies to this antigen. The clone pBADES220 was grown using standard microbiological methods and then the protein purified using low pressure chromatography with a Ni-NTA column. Purity was confirmed using SDS-PAGE. Positive control serum was obtained by hyper-immunizing chickens with the antigen and collecting and pooling serum. Mean absorbance of positive control serum as compared to normal chicken serum and pre-immune serum revealed a more than 20 fold increase when diluted at 1:2560. Validation of the assay was done by testing serum from birds challenged with both E. acervulina strain #12 and E. tenella WLR-1 strain. Mean absorbance revealed that, at six days post challenge, challenged birds (0.437 ± 0.03) were significantly (P<0.001) higher than non-challenged birds (0.15 ± 0.03). In addition birds immunized with Clostridium-B showed significantly higher (P<0.001) mean absorbance (0.33 ± 0.03) as compared to non-immunized birds (0.05 ± 0.01). These finding indicate that this indirect ELISA may be useful in examining the humoral response to Eimeria species.

Key Words: Indirect ELISA, Eimeria, Recombinant Antigen
Dietary lutein and fat interact to modify macrophage nitric oxide production in chicks hatched from carotenoid depleted eggs. R. K. Selvaraj* and K. C. Klasing, University of California, Davis.

An experiment was conducted to study the interaction between dietary lutein and fat in broiler chicks hatched from lutein depleted eggs. Six dietary treatments in a 2 X 3 factorial (fat at 3 and 6% and lutein at 0, 25 and 50 mg /Kg feed) were led to 18 birds per treatment (in 3 replications) for 23 days. Anti-DNP-KLH serum antibodies were measured on d 22 by ELISA. Peripheral blood monocytes were collected on d 17 for macrophage phagocytosis assay. On d 19, blood lymphocytes were stimulated with either Con-A or PHA-P and proliferation index was measured. On d 23, peripheral blood macrophages were stimulated with lipopolysaccharide in vitro and nitric oxide (NO) production was measured at 16 and 40 hours of stimulation. The anti-DNP-KLH serum antibody titer and macrophage phagocytic index did not differ among treatment groups (P>0.05). The Con A and PHA-P proliferation index was increased (P<0.05) in birds fed 50 mg of lutein and 3% fat compared to all other treatments. Macrophage NO production at 16 hours was increased (P<0.05) in birds fed lutein compared to the birds fed no lutein, independent of the level of dietary fat. Among the birds fed lutein at 25 or 50 mg/Kg feed, birds in the 3% fat group had increased NO production compared to the birds fed 6% fat group. At 40 hours, macrophage NO production in birds fed 25 and 50 mg lutein with 3% fat was increased (P<0.05) compared to all other treatments. Thus, in birds hatched from lutein depleted eggs, modulation of macrophage NO production by lutein is dependent on the level of dietary fat.

Key Words: Lutein, Fat level, Macrophage

Construction of a naïve chicken antibody library using phage display. Daad Abi-Ghanem*1, Suryakant D. Waghela1,2, and Luc R. Berghman2, 1Department of Poultry Science, Texas A&M University, College Station, 2Department of Veterinary Pathobiology, Texas A&M University, College Station.

Phage display technology has emerged in recent years as a powerful tool for the creation of antibody libraries. It is based on the possibility of linking the displayed antibody phenotype on the phage surface to the encapsulated genotype. This approach was used to create a naïve chicken combinatorial antibody library. Total RNA was extracted from the bursa of Fabricius of ten 9-week old Leghorn layers. Messenger RNA was then used in a primary PCR to amplify VH and VL genes. The VH forward primer and the VL reverse primer included complementary sequences to the VH (400 bp) and VL (350 bp) fragments, thus creating a single chain Fv fragment (scFv, 700-800 bp). The primers used in the overlap PCR incorporated the cloning sites of the restriction endonuclease Sfi I, thus allowing for subsequent cloning into the phagemid pComb3X vector (provided by Dr. C. Barbas, The Scripps Research Institute, CA). Vector and scFv were digested with Sfi I. Digests were loaded on a 1% agarose gel, and bands corresponding to the double-cut vector (3,400 bp), the stuffer fragment (1,600 bp), and the digested scFv (700 bp) were excised. Three ligation reactions using T4 DNA ligase were then carried out, including either insert and vector, vector and stuffer, or vector alone. Ligation products were incubated overnight at room temperature, followed by transformation into XL1-Blue Supercompetent cells. Cells were plated onto LB/carbenicillin plates and incubated overnight at 37°C. About 5X10^7 transformants/µg of vector DNA were obtained from the vector-stuffer ligation, indicating good vector quality. Background ligation was determined by self-ligation of the vector, and was found to be minimal (< 5%). The scFv antibody library thus produced will be used to select for antibodies that bind to surface antigens of chicken enteric pathogens.

Key Words: Phage Display, scFv, Chicken

Valine needs for immune responses in male broilers from day 21 to 42. S. A. Thornton*, G. T. Pharr1, A. Corzo3, S. L. Branton2, and M. T. Kidd1, 1Mississippi State University, Mississippi State, 2United States Department of Agriculture.

A number of studies have evaluated the impact of Met, Arg, Lys, and Thr on immune responses of broilers. Although a limited amount of research exists evaluating Val needs for immunity in broilers, these studies point to its adequacy as being important to support normal immunity. Because Val can become limiting in broiler diets, especially those based on vegetable protein sources, two experiments were conducted to evaluate Val needs for cellular and humoral immunity in Ross 508 male broilers from 21 to 42 d. Birds in Experiment 1 were fed 3 dietary treatments with Val levels at 0.72%, 0.82% (accomplished by adding L-Val), and 0.82% (corn and soybean meal control equaling NRC (1994)). Experiment 1 measurements included: a hypersensitivity test to phytohemagglutinin-P (PHA-P) on d 35 and 36 (2 birds/pen); relative immune organ weights at d 42 (1 bird/pen); and a primary antibody response to SRBC on d 34 and 41 (2 birds/pen); relative immune organ weights at d 42 (1 bird/pen); a primary antibody response to SRBC on d 34 and 41 (2 birds/pen). Cell quantification of BU1 receptors on splenic cells at d 42 (1 bird/pen) was measured in treatments with 0.64% and 0.78% Val. In Experiment 1 there was a decrease (P = 0.08) for relative spleen weight in birds fed the 0.72% Val diet compared to birds fed the 0.82% corn and soybean meal control diet; however, significant differences did not occur for relative bursa and thymus weights. There were no significant differences in SRBC or PHA-P responses in Experiment 1. In Experiment 2, significant differences did not occur for any parameter tested. The results indicate that as CP is reduced in broiler diets to the extent of a marginal Val deficiency, the immune system of the birds should not be compromised.

Key Words: Broiler, Immunity, Valine

Tulathromycin (Draxxin®) is the first of a new class of veterinary macrolide antimicrobial, the triamikidols. This study explored the accumulation of tulathromycin by bovine and swine phagocytes in vitro. Bovine blood polymorphonucleocytes (PMNs) incubated in a range of tulathromycin concentrations from 0.5 to 5 µg/ml concentrated the antibiotic to an internal/external (I/E) ratio of approximately 28. These antibiotic concentrations are within the plasma and lung tissue concentration ranges achieved in tulathromycin-treated animals. When tulathromycin-loaded bovine PMNs were transferred to antibiotic-free media, by 1 h 50% of the intracellular tulathromycin remained, and by 4 h 40% of the intracellular antibiotic remained intracellular. Bovine alveolar macrophages (MΦ) accumulated tulathromycin to a lesser extent (I/E = 19.3), as did porcine blood PMNs (I/E = 16.6) and porcine alveolar MΦ (I/E = 8.1). This pattern of accumulation is distinct from that of other macrolides. Recruitment of tulathromycin-loaded phagocytes to infected lungs could contribute to in vivo efficacy observed for this antibiotic in livestock. Accumulation of tulathromycin by bovine PMNs was influenced by time, temperature, pH, and formalin fixation, but was insensitive to metabolic inhibitors (NaF, KCN). Efflux of tulathromycin was slower than for erythromycin. An active carrier system has been proposed for macrolides and its molecular identity is under active investigation; for the tribasic tulathromycin reversible protonation of amine is probably also important in membrane penetration. In summary, the triamikidol tulathromycin represents a new class of macrolide antibiotics that accumulates within phagocytes from cattle and swine; this may contribute to in vivo efficacy for this compound.

Key Words: Tulathromycin, Neutrophil, Macrophage
T95 Anti-rbosCD14 monoclonal antibodies (mAb) inhibits in vitro production of tumor necrosis factor-α (TNF-α) by bovine monocytes following stimulation with LPS. E. J. Sohn*, M. J. Paape, R. R. Peters, and D. D. Bannerman. 1Department of Animal and Avian Sciences, University of Maryland, College Park, 2Bovine Functional Genomic Laboratory, USDA-ARS, Beltsville, MD.

Endotoxin (lipopolysaccharide, LPS) from Gram-negative bacteria activates host innate immune responses that promote bacterial clearance. Paradoxically, such a response also contributes to septic shock, a clinical problem occurring with high frequency during Gram-negative sepsis. CD14 is a glycoprotein which binds LPS and initiates cell activation. The production of TNF-α by monocytes stimulated with LPS is mediated, in part, by the interaction of LPS with CD14 on monocytes. The objective of the present study was to determine whether anti-rbosCD14 mAb could block the production of TNF-α by bovine monocytes stimulated with LPS. The concentration of LPS used ranged from 1 to 100 ng/ml. The inhibition of anti-rbosCD14 mAb on the LPS induced production of TNF-α by adherent monocytes during a 24 h incubation was dose dependent. The greatest inhibition (61%) was achieved when 100 ng/ml of LPS was used to stimulate the monocytes. With 1 and 10 ng of LPS/ml, inhibition averaged 37% and 54%, respectively. The inhibition of TNF-α secretion by anti-rbosCD14 mAbs can be explained by the binding of mAb to membrane bound CD14 on the monocytes, thus blocking interaction of LPS with mCD14. The anti-rbosCD14 mAb has the potential for neutralization of TNF-α during acute coliform mastitis.

Key Words: Endotoxin, CD14, TNF-α

T96 Serum and urine indice comparisons between llamas and alpacas fed three forages. M. Sharp*, C. Horey, T. F. Robinson, and B. L. Roeder, Brigham Young University, Provo, UT.

The objective of this experiment was to determine species differences for serum and urine indices between llamas and alpacas fed three forages of differing quality. Four llamas (115 kg BW; 3 yrs old) and four alpacas (58 kg BW; 3 yrs old) were housed in metabolic crates, given ad libitum water and grass hay initially. Three forage treatments of alfalfa (AH), barley (BH) and grass (GH) were fed in random order to each animal. Treatment periods were for 14 d; d 1 to d14 for forage adjustment and d13 to d14 for collection. Four male alpacas (62 kg BW, 3 yrs old) were housed in metabolism crates, given ad libitum water and grass hay. The llamas were fitted with a urine collection harness and a jugular catheter. Urine in take differed between species and forage (P < 0.01) for lamas, 7.7 to 7.2 mmol/L. Total serum protein was higher for llamas than alpacas (P < 0.01) for forage (P < 0.01) forage: 7.8, 4.7 and 3.9 for AH, BH and GH. Urine results are expressed on a metabolic weight basis (kg 0.75). AH urine excretion was highest (P < 0.005) at 70.9, and 42.2 and 32.8 ml/d/mwt for BH and GH. Urine electrolyte excretion differed for forage (P < 0.05) and for urine osm (P < 0.005). Total protein, urea N and creatinine were not different. It has been hypothesized that llamas and alpacas are similar in physiology and respond to diets in a similar fashion. This data demonstrate that the two species responded similarly to the three forages, with the exception of serum glucose and total protein and urine urea N. Diet did affect urine indices.

Key Words: Alpaca, Llama, Forages


Four male alpaca (62 kg BW, 3 yrs old) were housed in metabolism crates and given ad libitum water and grass hay. The alpacas were fitted with a urine collection harness and a jugular catheter. Urine and plasma were collected at 4-h intervals during the 14 d baseline, 120-hr water dehydration (Dh) and 160-hr rehydration (Rh) periods. Plasma and urine were analyzed for electrolytes (Na, Cl, K, Ca and P), urea N, creatinine, feed intake, feed intake differed between species and forage (P < 0.01), alfalfa the highest (P < 0.05), alfalfa, barley, and grass hay. Urine analytes of alpacas.

Comparison Animals

T98 Affect of water deprivation on plasma and urine analytes of llamas. J. A. Anderson*, A. Petersen, T. F. Robinson, and B. L. Roeder, Brigham Young University, Provo, UT.

Four male llama (123 kg BW, 3 yrs old) were housed in metabolism crates and given ad libitum water and grass hay. The llamas were fitted with a urine collection harness and a jugular catheter. Urine and plasma were collected at 4-h intervals during the 14 d baseline, 120-hr water dehydration (Dh) and 160-hr rehydration (Rh) periods. Plasma and urine were analyzed for electrolytes (Na, Cl, K and Ca), total protein, urea N and creatinine. Data are presented as a mean of the baseline samples, while the Dh and Rh data were analyzed as repeated measures using SAS Proc Mixed. Water intake was 17.8 ml/d/kg 0.75 while urine output was 3.7 ml/d/kg 0.75. Pack cell volume increased (P < 0.001) during the Dh period from 24 to 26%, returning to 23% for Rh. Plasma osm increased (P < 0.001) from 315 to 354 mmol/kg with the Dh/Rh, while COP increased (P < 0.001) from 21 to 24 mmHg. Plasma electrolytes increased (P < 0.001) from 22 to 24 mmHg. Plasma electrolytes increased (P < 0.001) with the exception of K. Na and Cl increased from 151 and 255 mmol/L to 166 and 133 mmol/L, respectively. Glucose increased (P < 0.001) from 6.6 to 7.7 mmol/L, with PUN increasing from 4.3 to 7.8 mmol/L (P < 0.001). Creatinine was not affected and TPP increased (P < 0.01). Glucose may have been elevated from endogenous glucocorticoid release during Dh and during Rh rebounded due to realimentation. Rh elicited a diuresis affect with increased Na and very large Cl excretion.

Key Words: Alpaca, Dehydration, Analytes

Key Words: Llama, Dehydration, Analytes