

results of this study indicate that heel erosion is a common affliction, especially for mid-lactation multiparous cows.

Key Words: Heel Erosion, Dairy Cattle, Hoof

PSA - Pathology

T83 Identification of a c-reactive protein gene in cardiomyopathic turkeys: A possible genetic marker for turkey cardiomyopathy. A. E. Hauser* and M. M. Corley, *Tuskegee University, Tuskegee, AL.*

C-reactive protein (CRP) is an inflammatory protein released by the body in response to infection and injury. Elevation of this serum protein has been linked to increased risks of heart disease through inflammation that is believed to play a key role in the hardening of arteries resulting in a heart attack or stroke. Consequently, CRP could serve as a genetic marker for an eminent cardiac event. Thus far, the poultry industry has experienced considerable loss due to turkey cardiomyopathy. Furthermore, the gross and microscopic lesions in tissue from cardiomyopathic turkeys, have been shown to be very similar to human cardiomyopathic heart tissue. Therefore investigation of those genes involved in turkey cardiomyopathy can lead to further insight of cardiovascular disease and thus benefit both the poultry industry and the human population. In this study, we attempted to identify a crp gene from turkeys that carry a genetic trait (unknown) that renders them susceptible to cardiomyopathy. The identification and expression of this gene as it relates to heart disease in these turkeys has not been investigated. The reverse transcriptase polymerase chain reaction (RT-PCR) was used to target this gene. Total RNA, extracted from frozen heart tissue (0.1g) of a cardiomyopathic turkey was used to generate turkey cDNA, and oligonucleotide primers were designed from a partial crp gene sequence derived from a chicken liver cDNA library to amplify the crp gene. The expected 504 bp RT-PCR product was successfully amplified. Identification and analysis of the gene that codes for CRP in turkeys will lend further insight into the etiology of turkey cardiomyopathy.

Key Words: Cardiomyopathic, C-reactive Protein, Turkeys

T84 Identification and analysis of an apolipoprotein- A gene in cardiomyopathic turkeys. T. A. Dugger* and M. M. Corley, *Tuskegee University, Tuskegee, AL.*

Apolipoprotein A is a major constituent of high-density lipoproteins, which aids in the regulation of high cholesterol levels in the blood and peripheral tissues. If this process is defective, cholesterol molecules can accumulate in arteries, result in arterial blockage, thereby leading to a cardiac event (heart attack). The turkey cardiomyopathic heart resembles that of humans in gross and microscopic morphology. Therefore analysis of genes implicated in turkey cardiovascular disease may be beneficial to both poultry and human health. Heart tissue from cardiomyopathic turkeys was used to identify an apoA gene and its possible involvement in cardiovascular disease. The role of this gene as it relates to turkey cardiomyopathy has not been investigated. To isolate this gene, total RNA was isolated from heart tissue samples and purified. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed using primers designed from the chicken apoA-1 gene. The RT-PCR product was visualized via agarose gel electrophoresis (1.5% w/0.5 µg/ml ethidium bromide). The expected 519 bp RT-PCR product was observed, indicating successful amplification of the apoA gene from cardiomyopathic turkey heart tissue. Identification and analysis of the turkey apoA gene will lead to further knowledge of the etiology of turkey cardiomyopathy.

Key Words: Apolipoprotein, Cardiomyopathy, Turkeys

T85 Isolation and characterization of an anti-Salmonella phage collection for use as antibacterial agents. E. Kozhina*, P. Herrera, and S. Ricke, *Texas A&M University, College Station.*

Interest in phage therapy has increased in the past several years due to the rise in the antibiotic resistance. There are several problems to overcome before bacteriophages can be routinely used as antibacterial agents. One problem is that most bacteriophages are highly specific

infecting only a few host strains of bacteria. However some bacteriophages exhibit a wider host range. Another problem is the maintenance of the stability and viability of a phage collection. Our objective is to isolate a stable phage collection capable of lysing a number of different *Salmonella* strains. First, we isolated phages from the environmental water samples. This was followed by five consecutive step propagation procedures in a mixture of *Salmonella* strains and in four different *Salmonella* strains. A number of plaques grew on a bacterial lawn containing the 4 *Salmonella* strains. The largest and clearest plaques were chosen for further study. We investigated stabilizer (YT media supplemented with 10 mM MgSO₄, 1% BSA and 5% sucrose) for storage of phage collection. We found that it result in 100% recovery of newly isolated phage collection stored for a month in 4°C (compare to 98% of recovery in SM buffer (100 mM NaCl, 8 mM MgSO₄, 50 mM Tris-Cl, pH 7.5). The DNA from these phages were isolated, digested with EcoRI restriction enzyme, and the banding patterns analyzed by agarose electrophoresis. In order to select for the most stable phage isolates, the mixtures of primary phage isolates were stored for 5 months at 4°C. Phages isolated from these mixtures were tested for their ability to survive while incubated in non-sterile bovine rumen fluid at 37°C. Stabilizers, such as 1M MgSO₄ and 1% gelatin, were added to determine if they could enhance the bacteriophages survival. In all cases phages were isolated from rumen fluid after 3 days of incubation. The presence of stabilizer did not influence phage stability in rumen fluid. Initial results indicate that these procedures, with minor modification, can be used routinely to generate a useful and stable anti-*Salmonella* phage collection.

Key Words: *Salmonella*, Phage, Isolation

T86 Pathology of listeriosis resulting from respiratory infection of turkey poults with *Listeria monocytogenes* Scott A. G. R. Huff*¹, W. E. Huff*¹, J. N. Beasley², M. G. Johnson², R. Nannapaneni², J. M. Balog¹, and N. C. Rath¹, ¹USDA/ARS/PPPSRU, ²University of Arkansas.

The pathogenesis of a human epidemic strain of *Listeria monocytogenes*, Scott A, was studied by challenging day-old turkey poults with air sac inoculation of 10⁰ (Control), 10⁴, 10⁵, 10⁶, 10⁷, or 10⁸ cfu. Respiratory challenge with all levels resulted in listeriosis. Mortality at 2 wk post-infection ranged from 25-100% and was directly correlated with level of challenge. Gross pathology included enlarged gall bladders and pale livers, some of which were also yellowish, mottled, or cooked in appearance. Ruptured yolk sacs were common. Lungs were necrotic and hearts were swollen and surrounded by fluid. Sections of liver, heart, spleen, bursa, lung, and brain were fixed in 10% buffered formalin. Paraffin-embedded sections were cut at 5µ, stained with hematoxylin and eosin as well as Gram stain and were examined for histological lesions. Lesions were observed in liver, heart, spleen, bursae, and lung, however no significant changes were present in brain. The myocardial lesions consisted of large infiltrations of mononuclear cells deep in the myocardium and were associated with Gram positive rods. In the liver, focal infiltrations of mononuclear cells were small and scattered and were also associated with Gram positive rods. Congestion and reticuloendothelial hyperplasia were prominent in the spleen and there was necrosis of scattered cells. Lymphocytes and mononuclear cells infiltrated areas surrounding bronchi in the lung. In the bursae there was depletion of lymphocytes in bursal follicles. *Listeria* challenge also resulted in significantly decreased relative weight of the bursa of Fabricius and increased relative weight of the spleen. *L. monocytogenes* was isolated by direct plating of liver, gall bladder, pericardium, brain, yolk sac, lung, cecal tonsil, and both left and right knee synovium cultures on UVM *Listeria* selective agar. These results suggest that respiratory infection with *L. monocytogenes* can be invasive in young turkeys and may be responsible for some unexplained cases of early poult mortality as well as the initiation of chronic infection leading to product contamination.

Key Words: *Listeria Monocytogenes*, Respiratory Infection, Turkeys

T87 Aspirin and selenium influence on bordetellosis in turkey poults. A. M. Tyner*, F. W. Edens, and G. H. Luginbuhl, *College of Agriculture & Life Sciences, Dept. of Poultry Science, North Carolina State University, Raleigh.*

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 21d 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 (.05%) 5. SP (0.3 ppm)+AS (0.05%), 6. AS (0.05%). At 9 and 14d, 5 poults (1/rep of 7 poults/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 wt equal 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^{*1}, S. Galletti¹, L. Ravarotto², M. Tameni¹, S. Steidler¹, and P. Morazzoni³, ¹Department of Veterinary Sciences and Technologies for Food Safety, Milan, Italy, ²Istituto Zooprofilattico Sperimentale delle Venezie Via dell'Università, Legnaro, 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 intoxicated broiler chickens. Twentyone 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 of 0.8mg/kg of feed plus silymarin PHYTOSOME*, a silymarin complexed form with phospholipids from

soy, at 600 mg/kg body weight. Before slaughter, blood samples were collected from the brachial vein and total protein, albumin, globulin, glucose, urea, total bilirubin, direct bilirubin, indirect bilirubin, aspartate amino transferase (AST), gamma glutamil transferase (GGT), alanine amino transferase (ALT), calcium, and phosphorus were analyzed on sera. No differences were evidenced in any of the biochemical parameters evaluated, except for the level of ALT. The serum ALT activity was lower ($P<.05$) in AFB₁ treated animals with respect to control (6.16 vs 18.65 U/L, SEM=3.12). In animals receiving AFB₁ plus silymarin PHYTOSOME there was no difference in ALT serum activity in respect to control (26.14 vs 18.65 U/L, SEM=3.12). The decrease in serum ALT content is due to a recognized hepatotoxic effect of AFB₁. We can affirm that silymarin PHYTOSOME prevented these change in ALT activity, supporting the suggestion that these compound may achieve a protection against toxic effects of AFB₁. *PHYTOSOME is a trademark of Indena S.p.A.

Key Words: Aflatoxin B1, Silymarin PHYTOSOME, Serum Biochemistry

T89 Supplemental dietary 1,4-diaminobutane (putrescine) on growth and development of small intestine in broiler chicks challenge with *E. acervulina*. F. A. Santoyo¹, T. K. Smith^{*2}, and J. R. Barta², ¹Universidad Autonoma de Nuevo Leon, Monterrey, Mexico, ²University of Guelph, Guelph, ON, Canada.

Dietary putrescine (1,4-diaminobutane, DAB) the mammalian polyamine has been shown to play a regulatory role in growth, cellular development, division and anabolic processes including synthesis of DNA, RNA and protein. The high turn over rate of intestinal epithelium, 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 (4cages/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

PSA - Immunology

T90 Development of an antigen specific indirect ELISA for *Eimeria* using recombinant antigen EASZ240 from *Eimeria acervulina*. K. Ameiss^{*1}, M. Jenkins², H. Danforth², 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. EASZ240 was isolated from *E. acervulina* strain #12 and inserted into an expression vector containing both an arabinose 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 pBADZ240 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 Coccivac[®]-B revealed 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