Pathology

168 Comparative pathogenicity of PEMS associatedastroand reoviruses in the presence or absence of E. coli in turkey poults. L. Macalintal1, C. L. Heggen-Peay2, R. A. Ali3, K. A. Schat3, and M. A. Qureshi1, 1 NC State University, 2Embrex Inc., 3Cornell University.

Poults enteritis and mortality syndrome (PEMS) is an acute, contagious disease with high mortality and morbidity for poults between 2-4 wk of age. The current study was conducted to compare the relative pathogenicity of various organisms (viruses and bacteria) associated with PEMS, given alone or in combination through oral gavage, to turkey poults raised in controlled environment. Determinants for clinical/pathological changes were BW, liver, bursa, spleen and thymus wt relative to BW. The first study included 4 challenged groups: E. coli (2mL, 1 x 10^8), PEMS reovirus (ARV-CU98)(2mL, 10^7 TCID50), reovirus + E. coli and a sham control. The results showed that starting at 7 DPI the reovirus inoculated poults had decreased wt gain overtime (P < 0.05) and at 14 DPI the spleen in challenged groups was enlarged (P < 0.05). In the 2nd study, poults were challenged with the PEMS astrovirus (Tast-OSU)(2mL, 10^6 EID50), Tast-OSU + E. coli (2mL, 1 x 10^8) and E. coli alone. The results of this study showed that in comparison to the sham-exposed poults, the 7-day post-challenge BW gain was significantly less in all treatment groups with Tast-OSU group exhibiting the poorest wt gain. Thymic (P < 0.05) and bursal atrophy was also observed in Tast-OSU group. In the 3rd study, poults were challenged with ARV-CU98 + Tast-OSU and ARV-CU98 + Tast-OSU given at d1 followed 3d later with E. coli challenge and a sham-exposed control group. The results showed a progressive and significant decrease in BW in both challenged groups when compared to the unchallenged poults. However, the virus + E. coli groups showed the highest reduction in BW at 6DPI. These poults exhibited severe watery, foul smelling, foamy, diarrheea with thin-walled, gaseous distended intestines. Bursal and thymic atrophy was also observed at 9 DPI without any difference in the liver and spleen wt. Taken together, these studies showed that while no mortalities occurred, the astro and reovirus can reduce BW and induce bursal and thymic atrophy. However, with the addition of E. coli to these viruses, wt loss and associated PEMS signs get more pronounced, supporting the previous descriptions that PEMS can be induced by certain novel viruses with a strong bacterial component responsible for the severity of PEMS.

Key Words: Poults enteritis and mortality syndrome, Reovirus, Astrovirus, E. coli, Poults


Salmonella is an important human enteric pathogen responsible for causing severe gastroenteritis. It is believed to be the source of between 2 and 4 million cases of foodborne illness each year in the US. Bacterial virulence is regulated in response to a combination of environmental stimuli and involves a coordinated genetic response; therefore, it is important to understand the relevant features of the host that affect development of virulence in vivo. The Hyper Invasive Locus (hilA) gene is involved in the expression of virulence in Salmonella Typhimurium (ST). It is a transcriptional activator encoded on Salmonella pathogenicity island 1 (SPI1), which has been found to be a required gene component for the intestinal phase of infection. The Maillard reaction (MR) is a browning reaction common to the food and poultry industry found to take place during heating, processing, and storage of food products as well as processed poultry feeds. These Maillard reaction products (MRP) are one of the major contributors to the volatile content, which compile the aroma and taste normally associated with roasted poultry products and processed foods. The objective of this research was to study the effect of MRP on growth and virulence expression in ST. Laboratory generated MRP was added to liquid medium to assess growth rates and hilA expression of ST E6658 hilA virulence gene response of the Salmonella fusion strain, a β-galactosidase enzyme assay was conducted in microtiter plates. MRP glucose derivatives of arginine, histidine, or lysine did not significantly affect the growth rates of ST at 0.5 or 1.0 % v/v (0.360.00 to 0.440.01 h-1). However, 1.0% histidine and arginine MRP derivatives increased expression of ST hilA (six fold and seven fold respectively) while lysine MRP complexes did not.

Key Words: Salmonella, Maillard Reaction Products, hilA, Virulence


The gram-negative enterobacterium S. Typhimurium (Salmonella enterica serovar Typhimurium) is one of the leading causes of intestinal infections in developed countries. During infection of their hosts, salmonellae enter intestinal epithelial cells. When S. Typhimurium is present in the intestinal lumen, the expression of several invasion factors is required for bacterial entry into host cells. The hilA gene, encoded on Salmonella pathogenicity island 1 (SPI-1), is a transcriptional activator which plays a central role in invasion and whose expression is modulated by oxygen, pH, and osmolarity. The objective of this study is to develop a rapid PCR based detection system for the hilA gene. Specific primers were generated by GeneFisher against S. Typhimurium hilA promoter and coding region. The forward primer was 5’-TGG TTC TGA TGG TGT -3’ and the reverse primer was 5’- TCG ATA CAG GAA CAG-3’. DNA was isolated from wild type Salmonella Typhimurium by conventional techniques and subjected to a modified version of PCR using the specific primers. The program was carried out at a denaturation temperature of 95°C, annealing temperature of 45°C and polymerizing temperature of 70°C for 30 cycles. The PCR reaction was optimized to contain 2 mM MgCl2 and 20µg/µl glycerol. Agarose gel electrophoresis was carried out to confirm the amplification of the hilA promoter. A positive PCR product of a predicted 500 bp size was visualized and confirmed the presence of the hilA sequence in the preparation. This detection technique has potential for use in a one-step RT-PCR analysis for rapid detection of virulence state in S. Typhimurium.

Key Words: PCR, Salmonella, hilA, Virulence


Hemorrhagic enteritis virus (HE) a member of genus Aviadenovirinae, family Adenoviridae, induces an acute disease syndrome in turkeys 4 wks of age or older. The viral infection is characterized by depression, bloody droppings and death. The clinical disease lasts for a period of 7-10 days if uncomplicated by secondary bacterial challenge. Marble Spleen Disease Virus (MSD) of pheasants belongs to the same taxonomical group and as such can function as a heterologous vaccine for protection against HE field challenge in turkeys. Preparations consisting of spleen homogenates from turkeys inoculated with MSD make excellent vaccines. Splenic homogenates of MSD isolates were passed through susceptible turkeys to acquire vaccine stocks tested in these experiments. The immune response of the turkeys to each separate vaccine stock was observed at day 4 and 14 post I.V. challenge. Physical evaluations included body weight and spleen weight. Spleen and liver samples were collected at necropsy and virus titer was determined using an agar gel immunodiffusion test (AGID). Histological evaluations of infected tissues were performed utilizing standard H&E staining methodology. Tissues were further evaluated for the presence of virus utilizing a goat anti-human adenovirus hexon protein conjugated to biotin. Whole blood samples were evaluated for cell population parameters utilizing a Cell-Dyne. Results indicate significant increases in splenic weight in proportion to body weight for challenged groups only. AGID tests showed the presence of virus in spleen and liver homogenates, while histological tests observed a diffuse medullary infiltration of lymphocytic cells in the spleen, and nodular infiltration of lymphocytes in the liver. The presence of virus was confirmed by fluorescence of infected tissues using the hexon antibody. An increase of white blood cells, specifically lymphocytes, was observed among the treated groups as compared to the negative controls.

Key Words: Hemorrhagic enteritis virus, Marble spleen disease, Turkeys


Hemorrhagic enteritis virus (HE) a member of genus Aviadenovirinae, family Adenoviridae, induces an acute disease syndrome in turkeys 4 wks of age or older. The viral infection is characterized by depression, bloody droppings and death. The clinical disease lasts for a period of 7-10 days if uncomplicated by secondary bacterial challenge. Marble Spleen Disease Virus (MSD) of pheasants belongs to the same taxonomical group and as such can function as a heterologous vaccine for protection against HE field challenge in turkeys. Preparations consisting of spleen homogenates from turkeys inoculated with MSD make excellent vaccines. Splenic homogenates of MSD isolates were passed through susceptible turkeys to acquire vaccine stocks tested in these experiments. The immune response of the turkeys to each separate vaccine stock was observed at day 4 and 14 post I.V. challenge. Physical evaluations included body weight and spleen weight. Spleen and liver samples were collected at necropsy and virus titer was determined using an agar gel immunodiffusion test (AGID). Histological evaluations of infected tissues were performed utilizing standard H&E staining methodology. Tissues were further evaluated for the presence of virus utilizing a goat anti-human adenovirus hexon protein conjugated to biotin. Whole blood samples were evaluated for cell population parameters utilizing a Cell-Dyne. Results indicate significant increases in splenic weight in proportion to body weight for challenged groups only. AGID tests showed the presence of virus in spleen and liver homogenates, while histological tests observed a diffuse medullary infiltration of lymphocytic cells in the spleen, and nodular infiltration of lymphocytes in the liver. The presence of virus was confirmed by fluorescence of infected tissues using the hexon antibody. An increase of white blood cells, specifically lymphocytes, was observed among the treated groups as compared to the negative controls.

Key Words: Hemorrhagic enteritis virus, Marble spleen disease, Turkeys
172 Pathogenicity of proventricular homogenates containing IBDV. T.V. Dormitório1, J.J. Giambone1, and F. J. Hoem2.1 Auburn University, Auburn AL, 2Alabama State Veterinary Diagnostic Laboratory, Auburn AL.

Proventriculitis has become a major problem in the broiler industry. Agents that have been implicated are: IBDV, Reovirus, Adenovirus, Copper Sulfate, Clostridium or a combination of these agents.

To investigate the involvement of IBDV in causing proventriculitis, proventricular homogenates each containing a different strain of IBDV as tested by PCR were inoculated into day-old SPF broilers. At 3, 7 and 14 days post infection birds were weighed, bled, and killed. Organs were examined, weighed and collected for PCR and histopathological tests.

At 3 days post-infection (PI), body, bursa and proventricular weights of the birds were not significantly different from each other, however, 14% and 28% of the birds inoculated with culture, phage titer (pfu) was re- spectively, had proventricular lesions. All inoculated groups tested positive for IBDV by PCR, but none showed antibodies against it. At 7 days PI, body and bursa weights of the infected groups were significantly smaller than that of control birds. Proventricular weights were not signifi- cantly different, however, 43% of the 707B and 2054 inoculated groups and 57% of the ARK group had proventricular lesions. ARK group tested weakly positive for antibodies to IBD while the rest of the other groups tested negative. Bursae of 2054 did not undergo significant at- trophy compared to the other 2 inoculated groups at 14 days PI, but the proventricular weight was significantly larger and had the highest percentage (89%) of lesions. Serum from Group 707B and Group ARK tested strongly positive for IBD antibodies while Group 2054 was found to be negative at 14 days PI.

The 3 strains of IBDV had effects on body weight, bursa weight, proventriculus weights, and cause proventricular lesions when inoculated into SPF broilers. These effects started at 7 days PI and were significantly evident 14 days after infection.

Key Words: IBDV, Proventriculitis, Pathogenicity

173 Determination of Salmonella host range of selected bacteriophages which exhibit increased host specificity. L. R. Bielek1, S. E. Higgins1, K. L. Gunther1, G. M. Nava1, G. I. Tellez1, D. J. Donoghue1, A. M. Donoghue2, and B. M. Hargis1, 1University of Arkansas, 2USDA-ARS-PPRSU.

Conventionally, bacteriophages (phages) are presented as viruses capable of amplification only in a narrow range of closely-related bacteria. We selected phages with the ability to infect more than one bacterial genus. Initially, wild-type phages were selected by ability to form plaques in Salmonella enteritidis (SE) agar overlays. For determination of host specificity, a pool of 44 combined isolates of phages were evaluated for ability to propagate in individual non-pathogenic enteric bacterial iso- lates. % of the cultures incubated with culture, phage titer (pfu) was de- termined by incubation of serial dilutions of the phage mixture in tryptic soy agar (TSA) overlay with SE. In 2 separate experiments phage was combined with each bacterial isolate and tryptic soy broth (1:3:5, re- spectively). This mixture was incubated, sterile filtered, and recombined using the above ratio with fresh bacterial culture and media for 4 se- quential passes, and the resulting phage titer was determined using SE, One Klebsiella and 3 different Escherichia isolates, successfully ampli- fied some phage(s) from the SE-selected phage pool. Resulting plaques were then reisolated and passed in their respective enteric bacterial iso- late. Amplification in each species was confirmed by the formation of increased pfu’s in a TSA overlay with the enteric (alternative host) bac- teria. In a subsequent study, 13 selected wide-host-range phages were evaluated for ability to amplify in 10 different Salmonella isolates (dif- ferent serovars) by amplification in broth culture. Phage A had the ability to amplify in 8 different Salmonella serovars and Phage B had the ability to amplify in 2 different Salmonella serovars. These experiments suggest that phage host range is not always genera-restricted, and that selection of subpopulations of phages, capable of amplification in alterna- tive genera, may provide a tool for selection of broad host-range phages for the pathogen of interest. Ongoing studies are evaluating the poten- tial for more phylogenetically distant non-pathogenic isolates to support replication of Salmonella phages, which may allow improved safety for bacteriophage application to poultry.

Key Words: Bacteriophage, Poultry, Salmonella, Host range


A study was conducted to determine the efficacy of multiple versus sin- gle intramuscular (im) injections of phage to treat an E. coli respiratory infection. The birds were challenged at 7 d of age by injecting 106 cfu E. coli into the thoracic air sac followed by an im injection into the thigh with either heat killed or active phage. There were 16 treatments with 3 replicate pens of 10 birds. There were 4 control treatments, untreated birds, birds injected with either heat killed or active phage, and birds only challenged with E. coli. In the remaining treatments, birds were either injected with heat killed or active phage once immediately after E. coli challenge or immediately after challenge and 8 and 9 d of age, once at 8 d of age or 8, 9, and 10 d of age, and once at 9 d of age or 9, 10, and 11 d of age. Mortality was significantly decreased from 57 to 13% in the birds given a single im injection of phage immediately after E. coli challenge, and there was complete recovery in birds treated immediately after challenge, and at 8, and 9 d of age, which was significantly different from the single injection treatment. There was a significant reduction in mortality from 57 to 10% in the birds treated with phage once at 8 d of age and treated at 8, 9, and 10 d of age, with no difference between single or multiple treatments. The mortality in the single or multiple phage treated birds that started at 9 d of age was reduced from 57 to 28 and 27%, respectively, but was not statistically different from the con- trol. These data suggest that phage can be an effective treatment when administered early in this E. coli induced respiratory disease, and that multiple treatments are better than a single treatment. The efficacy of phage treatment diminishes as it is delayed with no difference between single or multiple treatments. Bacteriophage may provide an effective alternative to antibiotics, but like antibiotic therapy, the effectiveness of phage to rescue animals decreases the longer treatment is delayed.

Key Words: Bacteriophage, Escherichia Coli, Poultry

175 Prion degradation by a feather-degrading keratinase. J.C.H. Shih1, J.P.M. Langeveld2, and A. Bossers2. 1North Carolina State University, USA, 2CIDC-Lelystad, The Netherlands.

Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of humans and animals. In clinical cases, infectivity is accompanied by the conversion of the normal cellular prion protein PrPc to a structurally altered form, PrPSc. No differences in primary structure or in post-translational chemical modifications have been found between PrPc and PrPSc. PrPSc has a high content of -sheet conformation, resistant to common proteases, and is believed to exist as an aggregate. Methods to inactivate TSE agent are highly desirable. They would be especially useful for the treatment of valuable medical instruments, laboratory equipment, and processed animal by-product, such as meat and bone meal. Recently, a feather-degrading keratinase was tested for its degradative activity on PrPSc in brain stem tissue from animals suffering from bovine spongiform encephalopathy (BSE) and scrapie. The detection of PrPSc in tissues was performed according to routine diagnostic Western blotting. It was found that pre-heating tissue homogenates in the presence of detergents at 115 °C allows the keratinase to break down PrPSc from infected brain stem homogenates to a state where it is immunochemically undetectable. The conditions, such as enzyme levels, reaction time, detergent requirement, etc., for prion degradation were studied and will be reported. (Supported by a grant from USDA-IFAFS)

Key Words: Prion, Prion diseases, Keratinase, Prion degradation, Animal by-products


Specific-Pathogen-Free (SPF) flocks are maintained at the Avian Disease and Oncology Laboratory (ADOL). Strict bio-security measures and cleaning protocols are effective in maintaining flocks free of twelve common avian pathogens. During four year survey (1999 - 2002), ADOL SPF flocks were tested for antibodies to Chicken Anemia Virus (CAV) using Enzyme-linked immunosorbent assay. At 18 weeks of age, CAV anti- bodies were detected in 37% to 56% in Class I breeders maintained in plastic canopy isolators with filtered air positive pressure (FAPP) from...

Transovarian transmission of paratyphoid Salmonella is well documented and occurs at a low incidence in chickens. However, the exact mechanism of follicular invasion is not well understood. The following study investigates the ability of Salmonella to invade ovarian follicles at different stages of fertility and fecundity. Ovarian follicles were collected from Leghorn hens and separated into three stages of maturity: 1) large yellow follicles or F follicles (LYF), 2) small yellow follicles (SYF), and 3) small white follicles (SWF). All follicles were incubated at 37°C in RPMI 1640 medium. Initially, follicles were incubated with 1x10^6 cfu/mL of Salmonella Typhimurium (ST) sensitive to gentamicin for 0.5, 1, or 2 h. Follicles were then removed from the ST culture, rinsed in fresh medium, and placed in medium containing gentamicin sulfate for 5 h to kill any ST which had not invaded the follicular membrane. After the 5 h incubation, follicles were rinsed in fresh medium and stomached in phosphate buffered saline. Serial dilutions were made of each follicle and viable ST cells were enumerated on brilliant green agar. Two identical trials were conducted. In trial 1, a numerical increase in ST invasion was observed in the SWF as compared to the LYF and SYF (at 2 h, SWF=6667±3865, SYF=67±33, and LYF=1865±365 cfu ST/BL). Additionally, a numerical increase in ST invasion was observed in the LYF as compared to the SYF. In trial two, it was observed that ST invasion of SWF was significantly greater than in SYF or LYF (at 2 h, SWF=9233±2895, SYF=100±100, and LYF=550±250 cfu ST/BL). A numeric increase in recoverable ST was also observed in the LYF as compared to the SYF in trial two. These data suggest that ST may differentially invade ovarian follicles depending on maturity of the follicle, and that SWF may be more susceptible to ST invasion than either the SYF or the LYF.

Key Words: Salmonella, Invasion, Ovary, Follicle

178 Efficacy of bacitracin methylene disalicylate for prevention and control of necrotic enteritis and subsequent effects on carcass yield. J. Brennan1, R. McKay2, and J. Skinner3.

A floor pen study evaluated the effects of bacitracin methylene disalicylate (BMD3) for prevention and control of necrotic enteritis (NE) in combination with salinomycin (SAL) and Roxarsone (ROX). The effects of BMD on intestinal breaking strength and carcass yield were evaluated, under conditions of a NE challenge. The treatments included: a control (non-medicated and non-infected), an infected control (non-medicated), SAL with ROX (infected), SAL with BMD and 55 ppm (infected), and SAL with ROX and 220 ppm BMD added for 7 days after signs of NE were observed. SAL and ROX were included in feeds at 60 and 50 ppm, respectively. Each of the 5 treatments appeared once within each of 8 blocks (40 pens with 50 male broilers each). On days 14, 15 and 16 inoculum containing Clostridium perfringens was provided. Live weights (days 22 and 43) and rate of weight gains by birds treated with BMD for NE prevention or control were greater (P <0.01) than those of birds in the other treatments. Birds medicated with BMD, whether for NE prevention or control, had lower (P <0.01) NE lesion scores on Day 17 than the infected control birds. NE mortality was affected (P <0.01) by treatment, and no birds treated with BMD died from NE. Birds medicated with SAL and ROX regardless of BMD treatment had heavier fat pads compared to the control. Birds medicated with BMD tended to have stronger guts compared to those birds not medicated with BMD (P=0.7). Control birds had heavier gut weights. Carcass weights of the medicated birds were greater than the control; however, these birds had heavier live weights. Dressing percent tended to be greater when BMD was fed (P=0.6). Birds medicated with BMD for NE prevention had a greater (P <0.01) percent breast meat than did the controls. Control birds had a higher percent leg quarters compared to the medicated treatments. The benefits of NE prevention and control could be observed at processing.

Key Words: Chickens, Necrotic enteritis, Bacitracin methylene disalicylate, Intestinal strength, Carcass yield

179 Comparative incidence of pathological conditions in clinically normal broiler chickens from 3 regions of the USA. H. Cervantes*, Phibro Animal Health.

Data regarding incidence of 24 pathological conditions was collected during 13 months from routine broiler chicken health surveys conducted in 3 regions of the United States. Incidence data was averaged and compared by region. Region 1 (R1) included 3 companies from the southeast, region 2 (R2) included 3 companies from the northeast and region 3 (R3) included 2 companies from the west. Average bird age was 30.30, 33.96 and 28.57 days respectively.

Percentage incidence of moribund chickens was highest in R1 (2.50) and lowest in R3 (1.11). Active IBD was found only in R1 (0.25); pododermatitis was higher in R1 & R2 (3.88 & 3.55) than in R3 (6.31); oral lesions were much higher in R1 (36%) than in R2 & R3 (8.15 & 9.13); tracheitis was highest in R1 (6.51) and lowest in R3 (2.17); inflammatory process was found only in R1 & R2 (0.48 & 0.52); bursal dysplasia was highest in R1 (6.01) and lowest in R2 (3.23); synovitis was found only in R1 & R3 (0.55 & 0.85); femoral head necrosis was found only in R1 & R3 (0.42 & 0.85); airsacculitis was highest in R2 (12.32) and lowest in R3 (1.55), colisepithicemia was highest in R3 (1.30) and lowest in R1 (0.55); ascitis was similar for all regions (1.88, 1.57 & 1.96); retained yolk sacs were also similar for all regions (12.55, 11.83 & 11.66); proventriculitis was highest for R2 (5.70) and lowest for R3 (3.83); litter eaters were highest for R3 (2.43) and lowest for R2 (1.55); gizzard erosions for R2 (20.81) were higher than R1 & R3 (11.54 & 10.96); coccidiosis was highest in R1 (30.05) and lowest in R3 (21.29); enteritis was higher in R2 (27.41) than in R1 & R3 (18.87 & 18.42); intestinal debris was highest in R3 (12.56) and lowest in R1 (4.74); tapeworms were found only in R1 (3.43); feed passage was highest in R3 (9.15) and lowest in R2 (2.51). Bursa scores were 4.06, 4.15 and 4.44 respectively. Dehydration, ricketts and roundworms were not detected in birds from any region.

Overall incidence in decreasing order was: pododermatitis, coccidiosis, enteritis, oral lesions, gizzard erosions, retained yolk sacs, intestinal debris, airsacculitis, proventriculitis, feed passage, bursal dysplasia, tracheitis, litter eaters, ascitis, stunning, tapeworms, coccidiosis, synovitis, femoral head necrosis, inflammatory process and active IBD.

180 Myopathies in broiler chickens: The roles of oxidative damage and vitamin E. D. A. Sandercock* and M. A. Mitchell, Roslin Institute, Midlothian, UK EH25 9PS.

Recent studies have shown that disruptions in intracellular calcium (Ca^{2+}) homeostasis play a major role in the development of skeletal muscle damage (myopathy) in broilers. Elevations of intracellular free calcium result in Ca^{2+}-activation of phospholipase A2 (PLA2) and the subsequent initiation of a cascade of pro-inflammatory mediators and oxygen-derived free radical generation. The ultimate consequence of free radical induced membrane lipid peroxidation is altered cell membrane integrity and thus pro-oxidative mechanisms may be central in the pathogenesis of myopathy. Vitamin E (a-tocopherol) plays an important role as a naturally occurring lipid soluble antioxidant acting as a free radical scavenger and may therefore prevent myopathy mediated by the above mechanisms. This study employed an isolated in vitro chicken skeletal muscle preparation to examine the potential role of vitamin E in Ca^{2+}-mediated oxidative membrane damage. Muscle damage was assessed by the measurement of CK in the incubation medium. Tissue calcium accumulation was estimated by 45Ca uptake. Membrane lipid peroxidation was determined by malondialdehyde (MDA) measurement. Incubation with Ca^{2+}-ionophore significantly (p<0.001) increased muscle 45Ca uptake (2.2-fold). MDA concentration (2.5-fold) and CK efflux (12-fold). Incubation with α-tocopherol (250 μM) significantly reduced MDA concentration (26%) and CK efflux (37%) but not 45Ca accumulation (18%; p=0.001). In a related in vivo study, broilers from fast (FG)
and slow growing (SG) lines were reared with a dietary supplementation of vitamin E (500mg/kg) and subjected to acute oxidative stress (high thermal load—31°C/75%RH, 2h). SG birds exhibited a marked myopathic response, which was significantly reduced by vitamin E supplementation (p<0.05). It is suggested that vitamin E may exert protective actions in birds during spontaneous and stress induced myopathies. Its mechanism of action may involve inhibition of lipid peroxidation and/or PLA2 activity.

Key Words: Myopathy, Vitamin E, Lipid peroxidation, PLA2, Broiler

**Physiology - Reproduction**

**181 Effect of electrolytic lesions of the lateral septal organ on gonadal development in male broiler chicks (Gallus domesticus).** R. Thilaka and W. Kuenzel, University of Arkansas, Fayetteville, AR 72701.

Most species of birds in temperate zones respond to long photoperiods by showing recrudescence of gonads. Compelling evidence shows that non-photoreceptive birds exist in the ventral forebrain. Within the ventral forebrain are specialized neurons found in the medial portion of a structure called the lateral septal organ (LSO). The LSO contains Va-soactive intestinal polypeptide (VIP)-like-immunoreactive cerebrospinal fluid (CSF)-contacting neurons, which may act as receptors that respond to light. The objective of this study was to examine the effect of electrolytic lesioning of the LSO region on gonadal development in male broiler chicks (Gallus domesticus). Birds were placed in an environmentally controlled chamber on a short photoperiod (LD 8:16) and were given chick starter and water ad libitum until two weeks of age, at which time surgical procedures occurred. Birds were anesthetized, placed in a stereotaxic instrument and the target approached from the dorsal portion of the skull. An electrode was directed to brain loci determined by the stereotaxic atlas of chick brain, and 1mA current was passed for 12 seconds. All treatment groups had their respective sham-operated groups, where birds underwent the surgical procedure, but did not receive the current. After surgery, birds were kept on a long photoperiod (LD 16:8) and received chick starter ration mixed with 0.2% sul-famethazine (SMZ), a compound known to advance sexual maturation in male chicks. Sham-operated groups either received chick starter, or chick starter mixed with 0.2% SMZ. At the end of three weeks, brains were perfused in situ with 4% paraformaldehyde and collected for histology, and testes were weighed. Standard histology and immunocytochemical techniques were used to confirm complete ablation of VIP-like immunoreactive neurons and the viability of GnRH neurons. In three separate experiments conducted to date, bilateral lesions directed to the LSO resulted in a significant decrease in testes weight, compared to the sham-operated controls (p<0.05). Results suggest that cerebrospinal fluid-contacting neurons in the LSO may be directly involved in photoreception and play a significant role in gonadal development in male chicks.

Key Words: Photoperiod, Lateral septal organ, Encephalic photoceptor

**182 Time-dependent c-fos expression as a neuronal activation marker following electrical stimulation in the turkey hypothalamus.** S. W. Kang*, O. M. Youngren, and M. E. El Halawani, University of Minnesota, St. Paul, MN, USA.

It is well accepted that electrical stimulation (ES) within the medial preoptic area (POA) of the turkey hypothalamus results in the secretion of prolactin (PRL) from the anterior pituitary gland. However, the neuronal connections activated by ES which lead to PRL secretion have not yet been adequately investigated. To clarify the pattern of neuronal connections activated by ES which lead to PRL release have not been adequately investigated. To clarify the pattern of neuronal connections activated by ES which lead to PRL release, D2 DA and VIP receptor mRNA was determined by the stereotaxic atlas of chick brain, and 1 mA current was passed in a stereotaxic instrument and the target approached from the dorsal portion of the skull. An electrode was directed to brain loci determined by the stereotaxic atlas of chick brain, and 1 mA current was passed for 12 seconds. All treatment groups had their respective sham-operated groups, where birds underwent the surgical procedure, but did not receive the current. After surgery, birds were kept on a long photoperiod (LD 16:8) and received chick starter ration mixed with 0.2% sul-famethazine (SMZ), a compound known to advance sexual maturation in male chicks. Sham-operated groups either received chick starter, or chick starter mixed with 0.2% SMZ. At the end of three weeks, brains were perfused in situ with 4% paraformaldehyde and collected for histology, and testes were weighed. Standard histology and immunocytochemical techniques were used to confirm complete ablation of VIP-like immunoreactive neurons and the viability of GnRH neurons. In three separate experiments conducted to date, bilateral lesions directed to the LSO resulted in a significant decrease in testes weight, compared to the sham-operated controls (p<0.05). Results suggest that cerebrospinal fluid-contacting neurons in the LSO may be directly involved in photoreception and play a significant role in gonadal development in male chicks.

Key Words: Photoperiod, Lateral septal organ, Encephalic photoceptor

**183 WITHDRAWN. . .**

**184 Coexpression of dopamine or vasoactive intestinal peptide receptors with prolactin in the turkey pituitary.** Y. Chaiseha, O. M. Youngren, and M. E. El Halawani, 1 School of Biology, Institute of Science, Suranaree University of Technology, Thailand, 2 Department of Animal Science, University of Minnesota, St. Paul, MN.

Avian prolactin (PRL) secretion and PRL expression are regulated by vasoactive intestinal peptide (VIP) neurons residing in the infundibular nuclear complex (INF) of the hypothalamus. Dynorphin, serotonin, dopamine (DA), and VIP appear to stimulate PRL secretion along a common pathway, with VIP as the final mediator. Recent evidence indicates that DA and VIP receptors play a pivotal role in VIP and PRL secretion. The differential expression of DA and VIP receptors on anterior pituitary cells may regulate the prolactinemia observed during the turkey reproductive cycle. PRL mRNA gene expression was quantitated utilizing in situ hybridization histochemistry. Coexpression of D1D or D2 DA receptor mRNA or VIP receptor mRNA within pituitary cells expressing PRL mRNA was quantitated utilizing double in situ hybridization histochemistry. PRL mRNA, while found throughout the hypothalamus, was predominantly expressed within the pituitary. Pituitary PRL mRNA was 1.9-fold greater in laying hens and 6.5-fold greater in incubating hens than that of non-photostimulated hens. When hens shifted from incubation to photorefractoriness, pituitary PRL mRNA levels decreased to the same levels as that of non-photostimulated birds. Double in situ hybridization revealed that D1D and D2 DA receptor mRNA and VIP receptor mRNA expression was scattered on cells expressing PRL mRNA across the reproductive cycle. However, D1D and D2 DA receptor mRNA was more highly expressed than that of D1D DA receptor mRNA. The expression of D2 DA receptor mRNA on PRL-expressing cells was greatest in photorefractory and non-photostimulated birds, as compared to that of laying and incubating birds. The most dense VIP receptor mRNA found on cells expressing PRL mRNA was observed in incubating birds, followed by laying birds, when compared to non-photostimulated birds. When hens stopped incubating and became photorefractory, PRL VIP receptor coexpression became the same as that of non-photostimulated birds. The present findings clearly demonstrate that, in birds, DA and VIP receptors play a central role in VIP and PRL secretion, reinforces the evidence that VIP is the PRL-releasing factor, and suggest that PRL secretion is principally regulated by DA and VIP receptors at the pituitary level. USDA Grant No. 00-35203-9157

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**185 Tyrosine hydroxylase mRNA expression in the turkey hypothalamus.** A. Thayanunaphat*, S. W. Kang, K. Al-Zaiaie, O. M. Youngren, and M. E. El Halawani, Department of Animal Science, University of Minnesota, St. Paul, MN.

Avian prolactin (PRL) secretion is regulated by vasoactive intestinal peptide (VIP) neurons residing in the infundibular nuclear complex (INF) of the hypothalamus. This VIPergic activity is modulated by stimulatory dopamine (DA) inputs acting through D1 DA receptors located in INF. At the level of the anterior pituitary DA activates inhibitory D2 DA receptors and subsequently prevents VIP from stimulating the