

## Pathology: Genetics/Pathology

**55 Joint analysis of health and production traits.** R. L. Sapp\*<sup>1</sup>, R. Rekaya<sup>2</sup>, and T. Wing<sup>3</sup>, <sup>1</sup>USDA-ARS, Miles City, Montana, <sup>2</sup>The University of Georgia, Athens, <sup>3</sup>Cobb-Vantress, Inc., Siloam Springs, Arkansas.

The objective of the study was to investigate the relationship between health and production traits in chickens. The data were obtained from a closed, fully pedigreed, commercial broiler line. Records included measurements of body weight (BW), residual feed intake (RFI), percent breast meat (PBM), and two health traits (H1 and H2) from 13,665 birds and 1,524 hens with progeny records. Production traits were recorded as continuous and health traits were recorded as binary (1 or 0). After data editing, 13,632 records remained for analysis. For the health traits there were no extreme case problems observed in the 28 contemporary groups. Two analyses were conducted: 1) a threshold-linear mixed model for the joint analysis of BW, RFI, PBM, and H1; and 2) a threshold mixed model for analysis of H2. The mixed model included fixed effects of contemporary group (flock-week of hatch-parent's flock) and sex (not included in the model for H2); and random additive effects of bird and residual. A separate analysis for H2 was required since BW, RFI, PBM, and H1 were considered missing for these birds. In analysis 1, each bird had a valid observation for BW and H1, however, a bird which had RFI did not have PBM and vice versa. Thus a large number of birds had at least one missing observation in the first analysis and missing records for RFI and PBM were predicted based on the model parameters. Heritability estimates obtained for BW, PBM, RFI, H1, and H2 were 0.38, 0.55, 0.30, 0.14, and 0.12, respectively. Genetic correlations obtained from analysis 1 for BW with PBM, RFI and H1 were 0.08, 0.14 and -0.20, respectively; PBM with RFI and H1 was 0.38 and 0.08, respectively; and RFI with H1 was 0.03. The results suggest that as RFI is increased, BW and PBM were also increased. However, it would appear that as BW is increased, the health of the bird is decreased for H1. Residual correlations around -0.20 were reported between H1 with PBM and BW with RFI and 0.57 between BW with PBM. Given that H2 was analyzed separately, breeding values for birds in both analyses were correlated in order to obtain an approximation of the genetic correlation between BW, RFI, PBM, and H1 with H2. Correlations between H2 with BW, PBM, and RFI ranged from -0.03 to -0.04. A positive correlation of 0.05 was obtained for H2 with H1. The correlations were close to zero indicating that no definitive conclusions could be drawn for the relationship of H2 with the other traits using breeding values. In conclusion, the heritability estimates for the health and production traits of interest suggest that genetic improvement could be made. However, the relationship between these traits varies with the health traits having little to no relationship with the production traits. The fluctuation in these relationships could be due to the large number of missing records for PBM and RFI.

**Key Words:** body weight, breast meat, feed conversion

**56 Effects of the Rfp-Y gene complex on the development of rous sarcoma tumors in the arkansas progressor and regressor chicken lines.** M. V. Spanakos\*<sup>1</sup>, H. O. Pavlidis<sup>1</sup>, S. M. Sullivan<sup>1</sup>, L. K. Stamps<sup>1</sup>, R. Kopolos<sup>2</sup>, G. F. Erf<sup>1</sup>, and N. B. Anthony<sup>1</sup>, <sup>1</sup>University of Arkansas, Fayetteville, <sup>2</sup>Northern Illinois University, DeKalb.

The Arkansas Progressor (AP) and Regressor (AR) lines of chickens have been developed based on their respective ability to cope with a

tumor induced at the site of Rous sarcoma virus (RSV) injection (wing web). It has been demonstrated that RSV regression or progression can be influenced by the MHC; specifically the B complex. Another locus, the Rfp-Y also contains MHC genes in the chicken. The objective of this study was to characterize AP and AR chicken lines for Rfp-Y haplotype, and to determine whether the Rfp-Y significantly influences the development of RSV-induced tumors in the pure lines and respective crosses between them. Also, complementation between the Y types and the B types was evaluated. The AP is fixed for the B13 haplotype while the AR has both the B13 and B221. Nine Rfp-Y haplotypes, defined for class I MHC-like loci, were identified between the three pure sublines (AP 13, AR 13, and AR 221) and the three crosses from these sublines. Only three Y haplotypes (Y1Y1, Y5Y5, and Y5Y5) out of those identified were used in this study due to those haplotypes having high enough frequencies for analysis, and being present in all sublines and crosses for comparison. Pattern of tumor response to the tumor was evaluated using tumor score, tumor profile index, and mortality. Regardless of B background it was determined that the Y1Y5 haplotype was less efficient in tumor regression than the other two Rfp-Y haplotypes studied. When examined on a common B background, the Y1Y5 haplotype was less effective in regressing tumors only in the AP 13 subline, and AP 13/AR 13 cross. The four other B backgrounds resulted in no significant differences in tumor development based on Y type. Data were generated that indicate interactions between MHC and non-MHC loci for tumor regression.

**Key Words:** MHC, Rfp-Y, rous sarcoma

**57 Growth performance and immune response of naked neck and normally feathered genotypes of chicken.** M. M. Fathi\*, A. H. El-Attar, and A. Nazmi, *Ain Sham University, Cairo, Egypt.*

The favorable effect of naked neck (Na) gene on productive performance under high ambient temperatures has been documented by numerous researchers. While the immunocompetence measurements for naked neck birds are still under investigation. In this concern, an experiment was undertaken to evaluate the effect of Na gene in a single or double state on the growth performance and immune response in chickens. Two hundred and twenty (NaNa, Nana and nana) one day old chicks were used in this study. They were reared under similar environmental, managerial and hygienic conditions from hatching to 16 weeks of age. The current results revealed that the Nana genotype had significantly heavier body weight compared to nana one. However, there was no significant difference between NaNa and nana genotypes for body weight. The presence of Na gene in a single or double state significantly improved feed conversion ratio compared to nana sibs. With respect to cutaneous basophil hypersensitivity (CBH) response, the NaNa genotype significantly increased toe-web swelling at 48 and 72 hrs after PHA-P injection compared to Nana and nana genotypes.

There were no significant differences among genotypes for relative weight of both spleen and thymus. However, the Nana genotype significantly increased relative bursa weight compared to both NaNa and nana genotypes. Also, the Nana genotype significantly increased hematocrit level compared to nana and NaNa genotypes. Concerning the phagocytic activity, the Nana genotype had significantly lower levels of carbon in their circulation as compared to nana genotype. The homozygous (NaNa) birds recorded a significantly higher H/L ratio compared to the other two genotypes. The presence of Na gene either in a single or in a double manner (NaNa, Nana) increased

total antibody level for sheep red blood cells compared to normal case (nana).

We concluded that the homozygous (NaNa) genotype had a better cell-mediated immunity, while the heterozygous (Nana) genotype had better phagocytic ability and H/L ratio compared to normal (nana) genotype.

**Key Words:** immunocompetence, naked neck, growth performance

**58 Use of real-time reverse transcriptase polymerase chain reaction assay for surveillance of influenza viruses in wild waterfowl populations.** T. Dormitorio\*, J. Giambrone, and K. Guo, *Auburn University, Auburn, Alabama.*

Wild aquatic birds are considered the natural reservoirs of Influenza A, from which viruses can be transmitted to domestic poultry, pigs and other animals including humans. Through surveillance of wild birds, prevalence of avian influenza viruses (AIVs) can be monitored and the pathogenic and antigenic properties of the circulating viruses can be determined.

Real-time reverse transcriptase polymerase chain reaction (RRT-PCR) assay developed by the Southeast Poultry Research Laboratory in Georgia was optimized with modifications and used to detect AIVs from cloacal swabs of wild ducks from Southeastern United States. Viral RNA was extracted using a commercial kit and used as template in a one step protocol with specific primers and hydrolysis probes. The Roche LightCycler® instrument and software were used to run and analyze the test. In vitro-transcribed matrix, H5 or H7 gene RNA and live virus A/turkey/W/68 (H5N9) were used as positive controls.

alone of the swab samples tested to date, including those that agglutinated red blood cells was positive by RRT-PCR. Further testing is being conducted on allantoic fluid collected from embryonated chicken eggs inoculated with swab samples. The RRT-PCR test is a more sensitive, cost-effective and rapid assay compared to the standard virus isolation in embryonating eggs procedure.

**Key Words:** Avian Influenza, real-time RT-PCR, virus isolation

**59 Over-supplementation of vitamin D and the risk of sudden death syndrome in fast growing commercial broilers.** S. Nain\*, B. Larveld, and A. A. Olkowski, *University of Saskatchewan, Saskatoon, SK, Canada.*

Broiler diets are frequently fortified with Vitamin D (D3) above the recommended levels in an attempt to prevent commonly occurring leg problems. Since the basal levels of dietary D3 are rarely known, there is a risk of over-supplementation. Excessive intake of D3 has been shown to have detrimental effects on the heart. In order to evaluate the risk associated with D3 oversupplementation on cardiac health in fast growing broilers, we evaluated the effects of excess dietary D3 in commercial male broilers. The birds were randomly divided into two dietary groups, control and treatment. Respective groups were offered either commercial diet or commercial diet fortified with D3 at a level 16x NRC recommendation. The birds were housed in pens with raised perforated floors in an environmentally controlled room. During the first 7 days the temperature was maintained at 34°C followed by a gradual decrease to a level approximately 30% lower than that set for normo-thermal brooding. Feed and water were provided ad-lib. All birds were monitored several times a day for overt signs of disease and periodically electrocardiographic measurements were obtained.

Morbidity and mortality data were collected daily.

The risk of sudden death (SDS) was approximately 2.5 fold higher ( $P<0.05$ ) in birds fed a D3 enriched diet. The incidence of SDS mortality in the control group was 5.3 %, whereas 13.5% of broilers fed D3 fortified diet succumbed to SDS. Electrocardiographic examination showed a significantly higher ( $P<0.01$ ) rate of cardiac arrhythmia in birds fed the D3 enriched diet (46.4%), in comparison to those fed the control diet (17.7%). The present findings indicate that over-supplementation of vitamin D may increase the risk of SDS in broilers.

**Key Words:** broiler, SDS, vitamin D

**60 Effects of egg antibody to phospholipase A<sub>2</sub> (aPLA<sub>2</sub>) on preventing coccidia induced growth depression.** M. Schwartz\*, M. Yang, and M. Cook, *University of Wisconsin, Madison.*

Reduced animal performance of conventionally raised animals as compared to animals raised in germ-free environments has mainly been associated with immune induced growth suppression. Models for systemic immune regulation of growth involving injected lipopolysaccharide are well described however models where inflammation is localized in the gut are not well studied. Previous work has shown that egg yolk antibody to phospholipase A<sub>2</sub> (aPLA<sub>2</sub>) improved the growth rate and feed efficiency of healthy broilers. Since fed antibodies have their effects at the gut lumen/mucosal interface, it was hypothesized that improved growth from aPLA<sub>2</sub> feeding was due to anti-inflammatory effects in the gut. The objective of these experiments was to determine if aPLA<sub>2</sub> improves growth and feed efficiency during coccidia induced gastrointestinal inflammation. In two experiments, 5 pens of 5 broiler chicks were assigned to 4 dietary treatments with or without immune stimulation in a 2x4 factorial design. Birds received either water or a 10X dose of a commercial coccidiosis vaccine by oral gavage on days 0, 7, and 14. The dietary treatments consisted of egg yolk containing aPLA<sub>2</sub> (0.5 g/kg diet, exp 1 and 0.5, 1.0, or 1.5 g/kg diet, exp 2) or normal egg yolk (0.5 or 1.5 g/kg diet for respective exp controls). Immune stimulation decreased body weight gain 15.8% exp 1 ( $p<0.001$ ) to 8.4% exp 2 (ns). Intestinal weight (g/kg body weight) was increased by 14.7% exp 1 ( $p<0.001$ ) to 12.2% exp 2 ( $p<0.01$ ). Immune induced growth suppression and intestinal weight increase was not consistently affected by dietary aPLA<sub>2</sub>. This model fails to explain the mechanism by which feeding aPLA<sub>2</sub> improved broiler performance.

**Key Words:** gastrointestinal inflammation, egg yolk antibodies, coccidia

**61 Use of a lactobacillus-based probiotic culture to reduce Salmonella in day of hatch broilers.** J. P. Higgins\*<sup>1</sup>, S. E. Higgins<sup>1</sup>, A. Torres-Rodriguez<sup>1</sup>, V. Salvador<sup>1</sup>, S. N. Henderson<sup>1</sup>, A. D. Wolfenden<sup>1</sup>, C. M. Pixley<sup>1</sup>, A. M. Donoghue<sup>2</sup>, G. Tellez<sup>1</sup>, W. G. Bottje<sup>1</sup>, and B. M. Hargis<sup>1</sup>, <sup>1</sup>University of Arkansas, Fayetteville, <sup>2</sup>PP&PSRU, ARS, USDA, Fayetteville, Arkansas.

A commercially available *Lactobacillus* probiotic (FM-B11™ Ivesco LLC) (B11) significantly reduced *Salmonella* recovery from day-of-hatch chicks in several studies. For all experiments, day-of-hatch male broiler chicks (n=40 per pen) were challenged with approximately 10<sup>4</sup> cfu per chick of *Salmonella enteritidis* (SE) or *Salmonella typhimurium* (ST) and 1 h later treated (as below). Cecal tonsils or cecae were harvested for *Salmonella* detection 24 h following treatment. In

experiment 1, oral administration of  $10^6$  or  $10^8$  cfu per chick of B11 significantly reduced SE recovery by 82% ( $p < 0.05$ ) as compared to untreated controls whereas  $10^4$  cfu of B11 had no effect. In experiment 2, oral administration of B11 killed with penicillin or heat caused no reduction in SE recovery. In experiments 3, 4 and 5, B11 significantly reduced SE or ST recovery as compared to controls. Additionally, there was a 3  $\log_{10}$  reduction in cfu of SE recovered from the cecae of B11 treated chicks as compared to controls in all three experiments. In experiments 6 & 7, the recovery of SE from the control and treated groups at 6 and 12 h following B11 treatment was not different, but at 24 h B11 a significant reduction in SE was observed as compared to controls. In experiment 8, again at 6 and 12 h following B11 treatment, SE recovered from the control and treated groups were similar, but at 12 h B11 caused a significant 1  $\log_{10}$  reduction in cfu of SE from the cecae as compared to controls. This difference continued to increase over time as significant 1.5 and 2.5  $\log_{10}$  reductions were observed at 19 and 24 h respectively. Sufficient concentrations of live B11 significantly reduce *Salmonella* recovery in day-of-hatch chicks.

**Key Words:** probiotic, *Salmonella*

**62 Characterization of avian pathogenic *E. coli* in a layer production facility.** T. Baltzley\* and T. Rehberger, *Agtech Products, Inc.*

Avian pathogenic *Escherichia coli* (APEC) include a subset of pathogenic *E. coli* that are linked to extraintestinal diseases in poultry. The objective of this study was to gain a thorough understanding of the diversity of APEC isolates present in pullets and layer hens from several production sites within one company. Two thousand seven hundred twenty three isolates were collected for this study. Of the isolates collected 1,920 were from the gastrointestinal tract, 517 from ceca, ovaries, oviducts, tracheas, air sacs, and 286 from air and manure. Multiplex PCR was used to identify APEC isolates based on the presence of four virulence genes *iss*, *tsh*, *iucC*, and *cvaC*. Isolates were considered to be APEC only if they contained at least two of the genes. Of the four virulence-associated genes, the *cvaC* gene was the most prevalent detected in 75% of the isolates. The *iss* gene was the next most prevalent at 57%, and the *tsh* gene was detected 39% of the time. The *iucC* gene was the least prevalent, detected only 23% of the time. Only 12% of the isolates had none of the four genes present. The combination of the *iss/cvaC* genes was the most prevalent pathotype found 28% of the time. *Iss/tsh/cvaC* was the next most common pathotype occurring 11% of the time. RAPD PCR analysis grouped the APEC isolates into 197 clusters with a coefficient of similarity of greater than 80%. The largest cluster consisted of 207 members and was 12% of the APEC population. Thirty five of the families contained ten or more isolates and made up 72% of the population. Forty five clusters contained only one member and constituted 2.7% of the population. The location of the farm, age, health or organ of the bird did not appear to have an impact on clustering pattern of isolates collected from layer hens. The isolates collected from pullets did appear to cluster more closely and were only related to a small percentage of isolates collected from the layer hens. A better understanding of the diversity of APEC isolates in a production system is a prerequisite for a more efficacious and less costly treatment for APEC related diseases.

**Key Words:** *E.coli*, APEC, multiplex

**63 Additional studies on idiopathic hepatic and splenic necrosis associated with vaccine administration in White Leghorn pullets.** R. Porter\*<sup>1,3</sup>, E. Gingerich<sup>2</sup>, K. Toohey-Kurth<sup>1,3</sup>, C. Radi<sup>1</sup>, and D. Zoromski<sup>1</sup>, <sup>1</sup>*Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin*, <sup>2</sup>*University of Pennsylvania School of Veterinary Medicine, Kennett Square, Pennsylvania*, <sup>3</sup>*University of Wisconsin School of Veterinary Medicine, Madison*.

Two companies (A and B) in different states reported mortality in 14 to 15-week-old pullets at approximately 28 days after the birds had received an inactivated *Salmonella*/Newcastle/Infectious bronchitis vaccine (0.5 ml, intramuscular injection). The mortality comprised 1-3% over 2 weeks. Gross lesions in both flocks consisted of mild subcutaneous edema, occasional intracoelomic hemorrhage originating from hepatic subcapsular hematomas, free blood in the small intestines (primarily duodenum and jejunum), enlarged, friable livers, and enlarged, pale and friable spleens. Histopathological lesions consisted of hepatocellular necrosis with accumulation of fibrin or blood in the sinusoids and space of Disse. There was coagulative necrosis of the spleen and variable mucosal and luminal hemorrhage in the small intestine. Marked lymphoid depletion was observed in the bursa of Fabricius. Electron microscopy also revealed necrosis in liver and spleen with no observable viruses. Liver and spleen were negative for avian hepatitis E virus by PCR. There were no significant bacteriology and virology findings. Comparisons between two trivalent vaccines (1 and 2) used by Company A linked the hepatopathy to a specific vaccine 1. Endotoxin activity was greater in vaccine 2 (176,300 EU/ml) compared to vaccine 1 (2446.41 EU/ml) as measured by limulus amoebocyte lysate assay. Mass spectroscopy revealed no potentially toxic compounds in vaccines 1 and 2.

The gross lesions are similar to hepatitis-splenomegaly syndrome described in adult laying hens infected with avian hepatitis E virus. In the case presented here no virus has been identified and an immune-mediated process is suspected. There is one previous report of hepatitis-splenomegaly occurring in layer flocks receiving repeated administration of oil emulsion vaccines.

**Key Words:** hepatic necrosis, splenic necrosis, vaccine administration

**64 Comparison of *S. enterica* serovar Enteritidis levels in crops of fed or fasted infected hens.** P. S. Holt\*, L. E. Vaughn, R. W. Moore, and R. K. Gast, *USDA/ARS Egg Safety & Quality Research Unit, Athens, Georgia*.

Long term feed withdrawal has been shown to increase ileocecal intestinal colonization and fecal shedding of *S. enterica* serovar Enteritidis (SE) in challenged hens. Less information is available regarding effects of fasting on crop colonization. Two trials were conducted to compare effects of 14-day feed withdrawal vs full feed on crop colonization in hens challenged with SE. The levels of SE in the crop of fasted hens were significantly higher than in nonfasted hens on days 3 and 10 and days 3, 9 and 16 post-infection (PI) in trials 1 and 2, respectively. Fecal shedding of SE was significantly increased in the fasted hens on day 10 PI in trial 1. Analysis of crop IgA anti-SE lipopolysaccharide levels in crop lavage samples of hens in trial 1 revealed a humoral response post-infection in both treatment groups with no significant differences, although peak response for fasted hens occurred one week later. Histologic evaluation of H & E crop sections from trial 1 birds revealed mild to moderate heterophilic infiltration within the crop LP or LP and epithelium of nonfasted infected hens at 24 and 96 hours PI. In comparison, heterophils in crops of fasted

hens infected at this time point were sparse, indicating a possible diminished heterophil response in the fasted birds. Multifocal areas of tissue inflammation, as indicated by marked heterophil infiltration, with necrosis and sloughing of epithelium, were observed in crops from fasted hens at day 11 PI (14th day of feed withdrawal) but not in the fed groups. This severe heterophilic inflammation was observed in both challenged and non-challenged fasted hens, suggesting some factor other than SE was responsible. These results indicate that feed withdrawal can have a dramatic effect on the integrity of the crop and its ultimate response to infection.

**Key Words:** fasting, induced molt, *Salmonella*

**65 Effects of F-strain *Mycoplasma gallisepticum* inoculation at twelve or twenty two weeks of age and diet supplementation on the digestive and reproductive organ characteristics of commercial egg laying hens housed in a caged layer facility.** S. W. Park<sup>1</sup>, E. D. Peebles\*<sup>1</sup>, S. L. Branton<sup>2</sup>, M. T. Kidd<sup>1</sup>, S. K. Whitmarsh<sup>1</sup>, and P. D. Gerard<sup>1</sup>, <sup>1</sup>Mississippi State University, Mississippi State, <sup>2</sup>USDA, ARS, Poultry Research Unit, Mississippi State, Mississippi.

Dietary poultry fat (PF), phytase, and 1, 25 dihydroxycholecalciferol have been reported to alleviate decreases in egg production in *Mycoplasma gallisepticum* (MG)-infected laying hens. Also, layers inoculated with F-strain MG (FMG) have been shown to perform less adequately when housed in a commercial caged facility rather than in biological isolation units, and compared to 22 wk FMG inoculations, 12 wk inoculations have affected egg size and reduced pre- and post-peak egg production. The effects of FMG inoculation at 12 and 22 wk of age and various dietary supplementations on the digestive and reproductive organ characteristics of commercial layers housed in a caged layer facility were investigated. The following isocaloric diets were provided to birds at 20 wk: 1) Control basal (CB) formulated to contain 0.50 % PF; 2) CB formulated to contain 1.25 % PF; 3) CB formulated to contain 2.00 % PF; 4) CB formulated to contain 2.00 % PF, 0.013 % phytase, and 0.025 % 25-hydroxycholecalciferol (25-D<sub>3</sub>). Parameters assessed at 58 wk of age were liver weight, liver moisture and lipid concentration, incidence of fatty liver hemorrhagic syndrome, ovary weight, ovarian mature follicle numbers, weights and lengths of the oviduct and oviductal regions, and weights and lengths of the small intestine and small intestinal regions. In birds inoculated with FMG (12 or 22 wk), decreases in liver moisture and lipid content occurred in response to diets containing 2.00 % and 1.25 % PF, respectively. Furthermore, in FMG-infected birds (12 or 22 wk), the PF, phytase, and 25-D<sub>3</sub> supplemented diet increased jejunum weight as a percentage of total small intestinal weight, whereas in birds inoculated with FMG at 12 wk, the 2.00 % PF diet led to an increase in the percentage of birds possessing 3 mature ovarian follicles. The potentiation of performance in FMG-infected layers by diets containing PF (2.00 %) may be mediated through metabolic effects in the liver and ovary. Supplemental phytase and 25-D<sub>3</sub> may also exert effects in the digestive system during FMG infection.

**Key Words:** layer, *Mycoplasma gallisepticum*, poultry fat

**66 Effects of pre-lay 6/85 strain *Mycoplasma gallisepticum* inoculation on the digestive and reproductive organ characteristics of commercial laying hens when given alone or in conjunction with F-strain *Mycoplasma gallisepticum* inoculations during lay.** K. A. Viscione\*<sup>1</sup>, E. D. Peebles<sup>1</sup>, S. L. Branton<sup>2</sup>, A. M. Vance<sup>2</sup>, S. K. Whitmarsh<sup>1</sup>, R. W. Keirs<sup>1</sup>, and P. D. Gerard<sup>1</sup>, <sup>1</sup>Mississippi State University, Mississippi State, <sup>2</sup>USDA, ARS, Poultry Research Unit, Mississippi State, Mississippi.

Performance in layers has been shown not to be adversely affected by a pre-lay 6/85-strain of *Mycoplasma gallisepticum* (6/85MG) inoculation when given alone or in conjunction with subsequent overlay inoculations of F-strain of *Mycoplasma gallisepticum* (FMG) during lay. However, other reports have demonstrated that the reproductive tract of layers infected by MG and maintained under ideal conditions can be altered without any subsequent effects on overall performance. Two trials were conducted in this study, using layers housed in isolation units under controlled conditions, to compare the effects of the following inoculation treatments on their digestive and reproductive organ characteristics: sham inoculation at 10 wk of age, 6/85MG at 10 wk of age, 6/85MG at 10 wk overlaid by a subsequent FMG inoculation at 22 wk, and 6/85MG at 10 wk overlaid by a subsequent FMG inoculation at 45 wk. Parameters assessed in each trial at necropsy were body, liver, ovary, oviduct and small intestine weight; oviduct and small intestine length; regional lengths and weights of the oviduct and small intestine; liver moisture and lipid contents; incidence of fatty liver hemorrhagic syndrome; and follicular hierarchy. Total and regional organ weights were expressed as percentages of total organ and BW. There were no significant inoculation treatment effects on any of the parameters examined except for liver moisture content. The moisture content of livers from birds inoculated at 10 wk with 6/85MG or inoculated with 6/85MG at 10 wk followed by an overlay inoculation of FMG at 45 wk was higher compared to livers from birds that were sham inoculated at 10 wk or inoculated with 6/85MG at 10 wk with an FMG inoculation overlay at 22 wk. Pre-lay 6/85MG inoculations either alone or in conjunction with subsequent overlay inoculations of FMG during lay have no adverse impact on reproductive organ characteristics as well as the subsequent performance of commercial laying hens. The inoculation regimens used in this study may be effective for the protection of commercial layer flocks from field strain MG infections.

**Key Words:** layer, *Mycoplasma gallisepticum*, reproductive organ

**67 Natural history of lesions development in chickens succumbing to necrotic enteritis.** A. Olkowski\*, C. Wojnarowicz, C. Chirino-Trejo, and B. Laarveld, *University of Saskatchewan, Saskatoon, SK, Canada.*

The objectives of this work were to investigate the natural history of lesions development in broilers succumbing to necrotic enteritis (NE). The experimental approach was based on evaluation of morphological changes in the intestinal tissue of broiler chickens diagnosed with NE and in chickens challenged with *C. perfringens* freshly isolated from field cases of NE. Microscopic examination of sections representing various levels of pathological changes in clinical cases of NE revealed progressive features of lesions development. The pathogens were seen in large numbers in the lumen (tissue debris), but were not found to be attached to the mucosal epithelial cells, and never penetrated the epithelium even in the areas showing clear evidence of advanced necrosis. Overall, the evolution of the pathological process appears

to follow morphologically distinctive initial phases affecting first the connective tissue underlying the epithelium, then progressively invading the inner structures of the lamina propria. These initial changes are followed by the pathological process spreading throughout the entire lamina propria, as well as the adjacent epithelial cells, which showed all features of necrotic cell death. Upon electron microscopic examination of the mucosal epithelium, significant ultra-structural changes were apparent at a level of the connective tissue at the interface of lamina propria and mucosal epithelium, and in the lateral domain of enterocytes, but the overall structure of the apical domain of these cells (plasma membrane and microvilli) appeared intact, even in enterocytes that showed clear signs of advanced necrotic process. The birds challenged with *C. perfringens* experimentally showed histological and ultra-structural changes similar to those seen in NE specimens, but of lesser magnitude. Our findings suggest that, in addition to alpha toxin, other factors may be involved in the initiation of necrotic enteritis.

**Key Words:** clostridium perfringens, necrotic enteritis, morphological changes

#### 68 Automatic counting with differential and selective media.

Z. Williams and Y. Vizzier-Thaxton\*, *Mississippi State University, Mississippi State.*

The need to estimate bacterial diversity and identify certain organisms led to the development of chromatogenic media. These media are

expensive and only effective with certain groups of organisms. In order to determine if utilization of a computer-operated color colony counter could be "trained" to do the same thing using common differential and selective media, a series of tests were run. The primary focus was on coliform bacteria of concern in poultry production. The Spiral Biotechnology Color Q was used for the counts and the media selected included eosin methylene blue, brilliant green, McConkey, *Salmonella-Shigella*, Hektoen Enteric and Rambach.

First the counter was "trained" using known cultures on the various media to establish the color for each genus. Organisms chosen represented the target group as well as others. These included *Enterobacter aerogenes*, *Salmonella typhimurium*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, *Proteus mirabilis* and *Escherichia coli*. Next mixed known cultures were inoculated on the various media and the system was allowed to do all counts according to the colors established in the training session.

The system is capable of differentiating among the colonies and thus speeding both the counting process and the selection of colonies for further testing resulting in significant savings of time, labor and expendables.

**Key Words:** bacteria, automatic counting, chromatogenic medium

## Physiology, Endocrinology, and Reproduction: Endocrinology

**69 Evaluation of the serotonin receptor blockers ketanserin and methiothepin on the pulmonary hypertensive responses of broilers to intravenously infused serotonin.** M. E. Chapman\* and R. F. Wideman, *University of Arkansas, Fayetteville.*

The pathogenesis of pulmonary hypertension remains incompletely understood. Many factors have been implicated, however there has been great interest in the potent pulmonary vasoconstrictor serotonin (5-HT) due to episodes of primary pulmonary hypertension in humans triggered by serotonergic appetite suppressant drugs. Pulmonary hypertensive patients have elevated blood 5-HT levels, and pulmonary vasoconstriction induced by 5-HT is believed to be mediated through 5-HT<sub>1B/1D</sub> and 5-HT<sub>2A</sub> receptors expressed by pulmonary smooth muscle cells. The vascular remodeling associated with pulmonary hypertension also appears to require the serotonin transporter (5-HTT). We investigated the roles of 5-HT receptor blockers on the development of pulmonary hypertension induced by infusing 5-HT i.v. in broilers. For this purpose, we treated broilers with the selective 5-HT<sub>2A</sub> receptor antagonist ketanserin (5 mg/Kg BW) or with the nonselective 5-HT<sub>1/2</sub> receptor antagonist methiothepin (3 mg/Kg BW). Receptor blockade was followed by infusion of 5-HT while recording pulmonary arterial pressure and pulmonary arterial blood flow. The results demonstrate that methiothepin but not ketanserin eliminated the 5-HT-induced pulmonary hypertensive responses in broilers. The 5-HT<sub>2A</sub> receptor does not, therefore, appear to play a role in the 5-HT-induced pulmonary hypertensive responses in broilers. Methiothepin did not inhibit pulmonary vascular contractility per se, as the pulmonary hypertensive response to the thromboxane A<sub>2</sub> mimetic U44069 remained intact

in methiothepin-treated broilers. Methiothepin will be a useful tool for evaluating the role of 5-HT in the pathogenesis of pulmonary hypertension syndrome (PHS, ascites) as well as the onset of pulmonary hypertension triggered by inflammatory stimuli such as bacterial lipopolysaccharide.

**Key Words:** hypertension, ketanserin, methiothepin

**70 Evaluation of the serotonin receptor blocker methiothepin in broilers injected intravenously lipopolysaccharide and microparticles.** M. E. Chapman\* and R. F. Wideman, *University of Arkansas, Fayetteville.*

There has been considerable interest in the role of serotonin (5-hydroxytryptamine, 5-HT) in the pathogenesis of pulmonary hypertension due to episodes of primary pulmonary hypertension (PPH) in humans linked to serotonergic appetite suppressant drugs. In this study, we investigated the impact of serotonin on the development of pulmonary hypertension induced by injecting bacterial lipopolysaccharide (LPS, endotoxin) and cellulose micro-particles intravenously using the non-selective 5-HT<sub>1/2</sub> receptor antagonist methiothepin. In experiment 1 broilers selected for ascites susceptibility or resistance under conditions of hypobaric hypoxia were treated with methiothepin or saline followed by injection of LPS while recording PAP. In experiment 2 ascites-susceptible broilers were treated with methiothepin or saline followed by injection of cellulose micro-