The NRC requirement for chlorine (0.15%) appears to be too low for 0 to 4-week-old poults.

Key Words: Chlorine, Poult, Roundheart

557 Influence of supplemental citric acid and sodium and potassium citrate on phytate-phosphorus utilization in broiler chicks fed phosphorus-deficient diets from one to 42 days of age. Ahmed Metwally*, *Animal Prod. Dept., Fac. of Agric., Assiut University, Assiut-Egypt.*.

Experiments were conducted to determine if the addition of citric acid to a phosphorus (P) deficient corn-soybean meal (SBM) diet would enhance phytate P utilization in broilers. Citric acid (C), Citric acid and Sodium citrate(1:1 mixture,CS) and Citric acid and Sodium citrate and Potassium citrate (1:1:1 mixture, CSP) were added at levels of 0, 4.5 and 6% to a P-deficient diet containing 0.91 Ca and 0.22% available phosphorus. Each of three dietary treatments was fed to replicate groups of eight chicks from one to 42 days of age. Increasing levels of dietary citrate to 4.5% increases performance traits. Carcass quality and tibia weight and ash improved for chicks fed 4.5% mixture of CSP. The bone ash response to the mixture of CSP was much greater than the bone ash response to the mixture of CS. The results of this study indicate that the mixture of CSP at level of 4.5% enhances phytate P utilization in broiler diets from one to 42 days of age.

Key Words: Citrate, Performance, Phytate-phosphorus utilization, chicks

PSA Pathology Session I

558 Influence of IBDV on the immune system and incidence of proventriculitis in SPF leghorns. T.V. Dormitorio*[1], J.J. Giambrone[1], and K. Cookson[2], [1]*Auburn University, Auburn, Alabama,* [2]*Ft. Dodge Animal Health, Lawrenceville, Georgia.*

Infectious bursal disease virus (IBDV) isolates have been linked to cases of proventriculitis in commercial broilers. These isolates appear to be variant in nature as they do not cause much edema, but mostly atrophy of the bursae. Some are very pathogenic and associated with immune suppressed flocks.
Fourteen-day old Specific Pathogen Free (SPF) white leghorns received four different IBDV vaccines. At 28 days, chicks were challenged with 707B IBDV isolate by eye and nose routes. At 34 days of age, pullets were tested for cell-mediated immune (CMI) response using a skin test. At 36 days of age all birds were killed and weighed, and the bursa were weighed and examined post mortem for gross lesions. Bursa weight to body weight ratios were also determined and the means of all groups analyzed for differences using SAS system.
SPF white leghorns were susceptible to the 707B IBDV field isolate at 26 days of age. Nearly 100% of these birds had severe gross lesions in bursa (atrophy) and proventriculus (glandular enlargement and edema), and numerically reduced final body weight. All commercial live intermediate IBDV vaccines produced excellent protection against proventricular lesions induced by this isolate. This IBDV isolate also cause a reduced CMI response. These data provide more evidence that this IBDV isolate had a major role in causing immunosuppression and proventriculitis in chickens.

Key Words: IBDV, Proventiculitis, CMI

559 *In ovo* administration of experimental reovirus vaccines[b]. Z.Y. Guo* and J.J. Giambrone, *Auburn University, Auburn AL* .

Avian reoviruses are an important cause of poultry diseases worldwide and can induce various diseases in chickens. *In ovo* administration of viral vaccines is a new technique used in preventing diseases. It is commonly used for the administration of Marek's Disease and infectious bursal disease viruses (IBDV) vaccines in commercial broilers. According to our preliminary experiments, the current reovirus vaccine, Enterovax, complexed (combined) with specific antibody against reovirus was too pathogenic for *in ovo* use. Therefore, a milder reovirus vaccine, Chick Syno-vac, was employed. This vaccine, complexed with antibody, was able to induce immunity against reovirus challenge and was less pathogenic than Enterovax. We are currently examining an even milder vaccine (VA Chick Vac) combined with antibody to determine its pathogenicity and immunogenicity, when given by *in ovo* route.

Key Words: Reovirus , *In ovo*, Vaccination

560 Changes in serum levels of ovotransferrin during experimental inflammation and diseases in chickens. H. Xie*[1,2], N. Rath[1], F. Clark[2], L. Newberry[2], W. Huff[1], G. Huff[1], and J. Balog[1], [1]*PPPSRU, ARS, USDA,* [2]*Department of Poultry Science, University of Arkansas.*

We have identified serum ovotransferrin as an avian acute phase protein. To measure the changes in serum levels of ovotransferrin during inflammation and poultry diseases, we developed a solid phase competitive enzyme immunoassay using rabbit anti-chicken serum transferrin antibody and biotinylated ovotransferrin. Serum ovotransferrin competes with biotinylated ovotransferrin to bind to anti-chicken transferrin antibody. The residual biotinylated ovotransferrin bound to anti-chicken transferrin is then detected using streptavidin-horseradish peroxidase followed by a colorimetric detection step. Serum levels of ovotransferrin are then determined according to a standard curve generated using known concentrations of ovotransferrin. Inflammation was experimentally induced in 4-wk-old male broiler chickens by subcutaneous injection of croton oil, and specific pathogens free (SPF) chickens were challenged with various bacteria or viruses to induce specific disease. In the experimental inflammation model with croton oil, the serum levels of ovotransferrin increased by 16 h post-injection, reached a peak by 72 h, remained high through 5 days, and returned to the basal level of olive oil-injected sham-controls by 10 days. Similarly, compared to the control birds, SPF chickens challenged with *E.coli*, pox virus, *reovirus*, infectious bursal disease virus, or laryngotracheitis virus had significantly higher levels of ovotransferrin in serum ($P<0.05$). Our results demonstrate that ovotransferrin can be utilized as a clinical marker for inflammation associated with certain infectious avian diseases.

Key Words: Ovotransferrin, Inflammation, Enzyme immunoassay

561 Differential intestinal response to *Eimeria acervulina* challenge in broiler chickens. B.C. Morris*[1], H.D. Danforth[2], D.J. Caldwell[3], and A.P. McElroy[1], [1]*Virginia Tech, Blacksburg, VA,* [2]*USDA/ARS/LPSI/PBEL, Beltsville, MD,* [3]*Texas A&M University, College Station, TX.*

Immunovariability between coccidial species in vaccines and those found in poultry rearing facilities has emerged as a potential complication associated with vaccination. The host pathogen interaction and immune response in the intestine must be further investigated to understand immunity and pathophysiology to *Eimeria* in chickens. Experiments were conducted comparing two isolates of *Eimeria acervulina* (EA), EA1 and EA2. In three experiments, commercial broilers chicks were divided into control (non-challenged) and EA1 or EA2 challenged (14 days of age) groups. In all 3 experiments, EA1 resulted in significantly ($P<0.05$) higher lesion scores than EA2, however, weight gain of EA1 challenged birds was not significantly different from controls. EA2 challenged birds had significantly higher lesion scores than control birds in Expts 1 and 3, with no lesions characteristic of classical EA infection in Expt 2. EA2 resulted in significantly decreased weight gain as compared to EA1 or control in Expt 3. While EA1 resulted in classical EA lesions with no significant difference in weight gain, EA2 resulted in few classical lesions with significant depression of weight gain. Subjective observation of intestines from EA2 challenged birds was suggestive of a severe secretory intestinal response and weakened intestinal strength. In Expt 4, EA2 oocysts were cleaned with 5.25% sodium hypochlorite to evaluate the possibility of an external bacterial factor contributing to the observed detrimental affects in the presence of few lesions. Birds were challenged with bleached or non-bleached EA2. Although there was no significant difference in lesion scores between EA2 challenged groups, non-bleached EA2 resulted in significantly decreased weight gain

as compared to bleached. These data are indicative of immunovariability between different isolates of the same coccidial species and are suggestive of differences in the host response that may contribute to the pathogenicity.

Key Words: *Eimeria*, Immunovariability, Broilers

562 Digesitve and Reproductive Organ Characteristics in Commercial Laying Hens as affected by F-Strain *Mycoplasma gallisepticum*. M. R. Burnham*[2], S. L. Branton[1], E. D. Peebles[2], M. S. Jones[2], B. D. Lott[1], J. B. Yeatman[2], S. K. Whitmarsh[2], and P. D. Gerard[3], [1]*USDA, ARS, South Central Poultry Research Laboratory,* [2]*Department of Poultry Science, Mississippi State University, Mississippi State, MS 39762,* [3]*Agricultural Information Science, Mississippi State University, Mississippi State, MS 39762.*

The effects of F-strain *Mycoplasma gallisepticum* (FMG) on digestive and reproductive organ characteristics in commercial laying hens were investigated. Ten hens were assigned to each of sixteen negative pressure fiberglass biological isolation units. Birds in eight units served as uninoculated controls and those in eight other units were inoculated (treated) with FMG at 12 wk of age. At 20, 36, 44, 46, and 48 wk of age, two birds from each of two units and at 60 wk of age, four birds from each of six units designated as either control or treated were euthanized by cervical dislocation and their organs removed. Variables examined included liver, duodenum, jejunum, ileum, infundibulum, magnum, isthmus, uterus, vagina, and ovarian weights, percentage liver moisture and lipid contents, and ovarian follicular hierarchal numbers. Organ weights were expressed as percentages of bird weight. Main effects due to bird age were observed for all parameters. Hierarchal follicle number decreased in FMG-treated hens relative to controls. Relative vagina weight was decreased at 20, 36, 44, and 48 wk by FMG; whereas, the effects of FMG on percentage liver moisture and lipid contents were inconsistent throughout the 20 to 60 wk period. These data suggest that changes in egg production in response to FMG infection in commercial layers, as noted in previous reports, may be associated with changes in liver and reproductive organ characteristics in commercial layer hens.

Key Words: *Mycoplasma gallisepticum*, necropsy, liver, intestine, ovary, oviduct, layer hen

563 Virulence Response of a *Salmonella* Typhimurium *hilA:lacZY* Fusion Strain to Spent Media From a *Salmonella* Typhimurium Poultry Isolate and Non-*Salmonella* Bacteria. J. D. Nutt*[1], L. F. Kubena[2], D. J. Nisbet[2], and S. C. Ricke[1], [1]*Texas A&M University, College Station, TX USA,* [2]*USDA-ARS Food and Feed Safety Research Unit, College Station, TX USA.*

Salmonella invasion into host epithelial cells requires genes located on *Salmonella* pathogenicity island 1 (SP1). HilA, a transcriptional activator encoded on SP1, is necessary for maximum expression of SP1 genes and invasion into epithelial cells. Certain environmental factors stimulate the expression of SP1 genes, specifically *hilA*, which can used be used as an indicator of overall level virulence expression. Influential factors may include changes in the gastrointestinal environment of birds during different dietary regimes. The objective of this study was to determine if growth of specific microorganisms alters the environmental conditions sufficiently to signal *S.* Typhimurium virulence response. Spent media was obtained from a *hilA:lacZY S.* Typhimurium strain, a poultry *S.* Typhimurium strain and *Escherichia coli* K12 after 2 and 23 hrs incubation in brain heart infusion broth (BHI). Cells were removed by centrifugation and the supernatant was filter sterilized. Spent media samples (1.5ml) were each inoculated with 0.120ml of a *hilA:lacZY* fusion strain of *S.* Typhimurium inoculum and incubated for two hours. After incubation, β-galactosidase assays were performed on the samples to determine virulence expression. Although no significant differences were seen among the virulence responses of the three separate bacteria strains, in general responses were 5 to 10-fold higher ($p<0.05$) than uninoculated BHI controls (40 to 50 Miller units) and more than doubled when exposed to 23 h spent media versus 2 h spent media for *S.* Typhimurium (481 to 507 versus 217 to 221 average Miller units). Based on these results, it appears that growth of similar bacterial species may alter the composition of rich media sufficiently to influence virulence.

Key Words: *Salmonella*, spent media, virulence

564 Viral disinfectant efficacy assay for duck hepatitis B virus using PCR. Chi-Young Wang*[1] and Joseph Giambrone[1], [1]*auburn university.*

The risk of transmission of human hepatitis B virus (HBV) by plasma concentrates has been reduced by use of virus inactivation procedures. However, serious concern persists over nosocomial infections that could be acquired through the use of inadequately disinfected equipment or accidental exposure to blood or other infectious body fluids from HBV-positive individuals. Duck hepatitis B virus (DHBV) is closely related to HBV and shares its general biological and structural properties. With the duck embryonic hepatocyte culture system and PCR, the evaluation of efficacy of disinfectants for DHBV was made more plausible and accurate. Nested PCR was also developed and could increase the limits of detection one hundred fold. In our study, when the concentrations of disinfectants (Quat-stat) were below 600ppm, they caused only a one log10 reduction in titer(titer=5 X 10 to 3.5 TCID50/ml). If we increased the concentrations above 2400 ppm, it caused a reduction in titer$\geq$4 log10. No cytotoxicity due to the disinfectants was observed on uninfected indicator cells. The U.S. EPA requires that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by efficacy test. According to this, the optimal concentration of a compound is virucidal reduces the viral titer by 3 logarithm units. Therefore, the optimal concentration of "Quat-Stat" for DHBV was 2400ppm.

Key Words: Duck hepatitis B virus, nested polymerase chain reaction (nested PCR), viral disinfectant efficacy assay

565 Water-soluble tylosin tartrate (Tylan Soluble Powder) for treatment of necrotic enteritis in broiler chickens. J.J. Brennan*[1], R.B. Bagg[2], G. Vessie[2], J. Wilson[3], D.A. Barnum[3], G. Moore[4], A. Zimmermann[4], P. Dick[2], and S. Poe[4], [1]*Shur-Gain Agresearch, RR#3, Burford, ON N0E 1A0,* [2]*Elanco Animal Health, Eli Lilly Canada Inc., Research Park Centre, 150 Research Lane, Guelph, ON N1G,* [3]*Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1,* [4] *Elanco Animal Health, 2001 West Main Street, POB 708,Greenfield, Indiana 46140.*

The effects of tylosin tartrate (Tylan® Soluble Powder) on mortality, intestinal lesion scores and growth performance of *Clostridium perfringens*-challenged broiler chickens were evaluated in a floor pen study. A randomized complete block design was used to study the effects of administration of 0, 50, 100 or 150 ppm tylosin tartrate in drinking water following confirmation of a necrotic enteritis (NE) outbreak. Each floor pen contained 25 male and 25 female birds on day of placement (Day 0). The pen was considered the experimental unit. There were ten replicate location blocks in the study. Challenge was administered via feed on days 14 and 15. An NE outbreak was confirmed based on NE mortality on Day 16. Medicated drinking water was introduced on Day 16 and provided for five consecutive days. On Day 17, three birds per pen were randomly selected, sacrificed and scored for necrotic enteritis lesions on a scale of 0 (normal) to 4 (extensive necrosis). Necrotic enteritis (NE) mortality (%) for the four respective treatments was 2.4a, 1.6ab, 1.4ab and 0.4b ($P<0.05$) during Day 16 to 28. Mean small intestinal lesion scores of sacrificed birds were 1.27a, 0.20b, 0.17b and 0.20b on Day 17 for 0, 50, 100 and 150 ppm treatments, respectively ($P<0.05$). Final (Day 28) bodyweight was 1.001a, 1.124b, 1.129b and 1.128b ($P<0.05$) kg for the four respective treatments. The results of this study indicate that tylosin tartrate is effective for treatment of necrotic enteritis when administered in drinking water at a concentration of 50 to 150 ppm.

Key Words: Broiler chicken, Tylosin phosphate, Necrotic enteritis