POSTERS: Monday, John Q. Hammons Hall IV, IMMUNOLOGY

167 Effect of mutations in Campylobacter jejuni genes on cecal colonization, and liver invasion, when given in ovo or on day-of-hatch. R. L. Ziprin*1, M. E. Konkel2, M. E. Hume1, L. H. Stanker1, S. A. Gray2, B. J. Kim3, and C. R. Young1

Campylobacter jejuni (CJ) is a commensal of chickens. The objective of the present work was a determination of CJ genes involved in cecal colonization and organ invasion. Chicks were treated either by direct injection of the embryo in ovo or by i.p. injection on day-of-hatch. Chicks were treated with either the parental CJ strain, F38011, or with strains containing mutations in genes dnaJ, cadF, ciaB or pldA. The in ovo challenge dose was 10^9 CFUs and the i.p. dose was 10^8 CFUs. Liver and cecal samples were examined for CJ when the chicks were 14 days old. CJ were recovered from the ceca of all birds challenged with the parental strain, regardless of challenge route. Livers of birds challenged in ovo with CJ F38011 contained CJ. In contrast, the CJ ciaB mutant colonized cecas but not the livers. The other strains were poor colonizers of both ceca and liver. None of the strains caused morbidity on i.p. challenge though the doses were high. Mutations in cadF, dnaJ impair the ability of CJ to colonize the cecum or infect the liver. CJ ciaB retains an ability to colonize the cecum after in ovo challenge, though it was seldom recovered from the liver. pldA colonized ceca after i.p. challenge. By determining the role of these genes in colonization we hope to find some means to prevent CJ from establishing in the gastrointestinal tract of chickens.

Key Words: Campylobacter, Chicks, in ovo, avirulent, mutant

169 Immune response in broilers to an Aflatoxin B1 mucosal vaccine. J. Wilkinson*1, D. Rood1, D. Minior2, K. Guillard3, M. Darre1, and L. Silbart1

We tested the hypothesis that a serum IgG and intestinal secretory IgA antibody response to the mycotoxic aflatoxin B1 (AFB), using BSA as a carrier protein, could be elicited in broiler chickens utilizing a mucosal vaccine protocol. One hundred and twenty, seven-day-old male broiler chicks were divided into groups of ten and received AFB-BSA (1) conjugate alone, (2) coupled to the recombinant heat-labile toxin B subunit (rLTB), (3) mixed with rLTB or (4) mixed with cholera toxin (CT). Vaccine preparations were administered via the oral, intrarectal and intraperitoneal routes resulting in twelve treatment groups. Primary immunizations were given at the onset of the study followed by a booster immunization two weeks later. Sera and fecal samples were collected weekly and assayed for anti-AFB antibodies. The anti-AFB serum IgG response to AFB-BSA, AFB-BSA-rLTB or AFB-BSA+CT, was low, however, co-administration of AFB-BSA and rLTB resulted in an enhanced AFB specific serum IgG response. Intraperitoneal immunization elicited a stronger serum IgG response compared to oral and intrarectal routes, regardless of vaccine preparation. CT did not enhance the immune response to AFB, however, anti-CT antibodies were detected in fecal supernatants. We conclude that CT is not an adjuvant in chickens when administered by the oral, intrarectal or intraperitoneal routes. However, rLTB is an adjuvant when co-administered with antigen in an immunogenic form. Also, the mucosal unresponsiveness to AFB is hapten specific and not accompanied by systemic tolerance when administered in an immunogenic form.

Key Words: Aflatoxin, Mucosal vaccine, Immunity, Cholera toxin, Broilers

170 Effect of Dietary Zinc on Immune Response of Heat-distressed Broilers. J. R. Bartlett*1 and M. O. Smith, The University of Tennessee, Knoxville, TN

Male broilers were used to evaluate the effect of Zinc status on growth performance and immune competence of heat distressed broilers. Chicks were raised to day 21 in brooder batteries and fed diets containing no added zinc (low Zn), Zn added to meet NRC recommendations (control), and control + 100 mg/Kg Zn (high Zn). Birds were transferred to wire cages in two environmental chambers on day 22. One chamber was maintained at 23.9 C (thermonutral, TN) while the other cycled between 23.9 and 35 C (heat distress, HD). Humoral immunity was assessed by injecting birds i.v. with 7% SRBC suspension and evaluating serum for total, Mercaptoethanol Resistant (ME-R) and ME-sensitive (ME-S) antibody titers. Cell mediated immunity was assessed by using Sephadex stimulation to recruit and collect abdominal exudate cells (AEC) and phagocytic activity of macrophages determined. Heat distressed birds consumed 11% less feed and gained 23% less weight than TN, but Zn did not impact performance. AEC count was not affected by HD, however, HD increased (P < 0.05) macrophage count (opsonized and unopsonized). Unopsonized phagocytic macrophage numbers were increased (P<0.05) by high Zn. Total antibody titers tended to be depressed (P<0.06) in HD but were not affected by Zn. Spleen, bursa of Fabricius and thymus weights as a percentage of live weight, were depressed by HD, but were unaffected by Zn. Results indicate HD influences immune competence of male broilers but the effect of Zn status is unclear.

Key Words: Heat-distress, Broilers, Zinc, Immune response

171 Experimental factors that impact in vitro lymphocyte proliferation. T. V. Leshchinsky* and K. C. Klasing, Department of Animal Science, University of California, Davis, CA 95616

The purpose of this work was to determine if mitogen-induced lymphocyte proliferation (LP) can be significantly affected by factors traditionally not considered such as stress related to experimental procedures and sufficient genetic variation exists to effectively select for reduction of SE burden in poultry.

Key Words: Resistance, Genetics, Salmonella enteritidis, Layer chicks, Disease
time required for sample processing. Effects of stress and blood processing time on whole blood LP induced by phytohemagglutinin (PHA) at 5, 25 mg/mL and concanavalin A (Con A) at 20, 100 mg/mL were evaluated in four-week-old broilers (12 chicks per treatment). Stress was caused by noise created by moving, bleeding and killing chicks located in the same room but not used for this experiment for two hours. Blood samples were either placed into the assay plates immediately after collection or were left on ice for two hours (delayed processing; DP). LP (PHA at 5, 25 mg/mL and Con A at 20, 100 mg/mL) in individual whole blood samples was compared to a pool of the samples (7 chicks). Both stress and DP increased proliferation of non-stimulated cells (P<0.001) and cells stimulated with PHA 5 mg/mL (P<0.001). Only stress increased whole blood LP at Con A 20 mg/mL (P=0.001). Effects of stress and DP were not additive. LP in the pooled whole blood did not represent the average LP of the individual samples. LP in a pool of samples was higher for PHA 5 mg/mL (P=0.001) and lower for Con A 100 mg/mL (P=0.001) than the average of the LP of the individual samples. Using LP assay to measure the lymphocyte activation capacity in a large group of experimental animals one should control for the effect of stress and sample processing time. We found that using the whole blood LP assay to minimize the time of sample processing, proper blocking of animal groups, timing the experiments, and controlling the effect of animal stress can make evaluation of an experimental treatment on LP possible more precise.

Key Words: Lymphocyte proliferation, Stress, Experimental design, Chickens

172 Proportions among lymphocyte subsets in chicken blood cell suspensions differ with isolation method. T. K. Bersi* and G. F. Erf, University of Arkansas, Dept. of Poultry Science, Fayetteville, AR 72701, USA.

Peripheral blood lymphocyte cell suspensions can be obtained from chicken blood by slow-speed centrifugation or Ficoll density gradient separation. The resulting cell preparations are referred to as peripheral blood lymphocyte (PBL) and peripheral blood mononuclear cell (PMNC) suspensions, respectively. To compare the proportions among various lymphocyte populations in PBL and PMNC suspensions, blood was obtained from two lines of adult broiler breeders (40 birds per line, equal numbers of males and females). PBL and PMNC suspensions were prepared from each blood sample. Cells were immunofluorescence stained using mouse-monomoclonal antibodies (mAb) specific for B cells and various T cell surface molecules (i.e., CD4, CD8-alpha chain, CD8-beta chain, gamma-delta T cell receptor (TCR1) and alpha-beta TCRs (TCR2 or TCR3). The percentage of total lymphocytes within a sample (PBL or PMNC) was estimated with a chicken lymphocyte-specific mAb (K55). One-, two- and three-color cell population analyses were carried out using a Becton Dickinson flow cytometer (FACSort). Results were expressed as percentage of lymphocytes. The proportions among lymphocyte subsets in PBL suspensions (slow-speed centrifugation) were significantly different from those observed in PMNC suspensions (Ficoll density gradient separation). Differences included higher percentages of lymphocytes that are CD4+CD8+, TCR1+ or TCR2+ in PBL compared to PMNC suspensions. Additionally, the ratio between CD4+CD8- and CD4-CD8+ T lymphocytes was higher (P<0.001) in PBL compared to PMNC suspensions. The higher percentage of CD4+ lymphocytes in PBL suspensions was due to increased levels of TCR2+CD4- and TCR3+CD4- T cells. Cell isolation method did, however, not influence trends in lymphocyte profiles attributed to line of chicken or gender. These observations explain some of the discrepancies in the primary literature on studies, both descriptive and functional, involving immune cells isolated from chicken blood.

Key Words: Chickens, lymphocyte subsets, PBL, PMNC, cell isolation method

173 Non-MHC alloantigen systems L and P influence phagocytic function independent of the B system in chickens. M. A. Qureshi1, R. A. Ali2, L. N. Thomas3, R. N. Baloch4, and W. E. Bries5, 1North Carolina State University, Raleigh, NC, USA, 2Northern Illinois University, DeKalb, IL USA.

Chicks obtained from B19/B19 females and B19/B21 male parents were blood typed into groups carrying A-E, C, D, H, I, L and P alloantigen systems with the objective to determine which, if any, of the eight alloantigen systems may influence or interact with the B system genotypes for blood monocyte phagocytic activity. Leukocytes obtained from whole blood at 2 and 4 wk, were separated on a Ficoll gradient and allowed to adhere to glass coverslips. The resulting adherent monocyte monolayers were incubated with viable E. coli for one hour, stained with Leukostat, and the phagocytic monocytes as well as the numbers of internalized bacilli were enumerated. Phagocytic monocytes were scored microscopically. The combined results from two separate trials demonstrated that the genotypes of the A-E, C, D, H, and I systems did not differ in percent of monocytes exhibiting phagocytosis, while significant differences were noted relative to B system genotype at 2 wk of age B19/B21 higher: p=0.0494, L at 4 wk L1/L1 > L1/L2: p=0.0088, and P at 4 wk (P4/P4 > P1/P1, P1/P4 > P2/P2, p=0.0172). The data were further analyzed to determine any interactions of P and L alloantigen systems with the B system. No such interaction was observed. These studies suggest that L and P non-MHC alloantigen systems have the potential to influence immune responses by modulating phagocytic system function in chickens. Furthermore, this modulation seems to be independent of the B (Mhc) system.

Key Words: Alloantigen Systems, Phagocytosis, Chickens

174 Cellular changes in the blood and the spleen during the development of the Cutaneous hypersensitivity reaction in chickens. A. E. Gehad1, H. S. Lillehoj2, and M. M. Mashaly, 1The Pennsylvania State University,University Park, PA, 2 USDA, LPS1, IDRL,Beltsville, MD.

Cutaneous hypersensitivity to T-cell dependent mitogens like Phytohemagglutinin (PHA) has been used as a measure of cellular mediated immunity and in vivo T-cell reactivity in different species. In chickens, injection of PHA into the wing web or the wattle results in a visible edema, an increase in the thickness of the injected tissue, and marked cellular infiltration 24 hr after PHA injection. However, mechanisms involved in this cellular infiltration is not clear. Therefore, the present study was conducted to investigate the different T-cell subtypes profiles in both the blood and the spleen at the early (2 hr), intermediate (6hr), and late (24 hr) stages after the local injection of PHA in the wattle. Nine-wk-old immature male leghorn K-strain chickens were injected with 100μg PHA/0ml saline in the wattle and another group was injected with 0.1 ml saline and served as a control. At 2, 6, 24 hr following injection, blood and splenic lymphocytes were collected from the two different groups and the percentage of CD4+ cells (T-helper cells), CD8+ cells (T-cytotoxic cells), and CD3+ cells (Total T-cells) were determined. At 2hr following PHA injection there was a drop in the percentage of circulating CD4+ and CD8+ cells followed by an increase at 6hr. However, in the spleen, at 2hr following PHA injection there was an increase in the percentage of CD4+ and CD8+ cells followed by a decrease at 6hr. Similar results were not found following saline injection. The percentage of CD3+ cells showed no difference at any time following PHA or saline injection either in the blood or the spleen. Our results indicate that the reported local cellular infiltration that develops following PHA injection may be in part due to the homing of splenic and circulating T-cells to the site of injection.

Key Words: Cutaneous hypersensitivity, Phytohemagglutinin, T-Cells

175 Macrophage function in Arkansas Rous Sarcoma Regressor and Progressor chickens prior to, and throughout, tumor development, regression, or progression. G. F. Erf1, T. K. Bersi, N. B. Anthony, J. W. Brandebura, and O. Dikensoy, Dept. of Poultry Science, University of Arkansas, Fayetteville, AR, 72701.

Approximately 80% of chickens from the Arkansas Regressor (AR) line are able to reject tumors induced by Rous sarcoma virus (RSV). In contrast, nearly 80% of chickens from the Arkansas Progressor (AP) line are not able to reject RSV-induced tumors. To examine macrophage function in AR and AP chickens prior to and following RSV-injection, 9-week-old AR and AP chickens were assigned to three groups: pre-injection, vehicle injection (PBS), and RSV-injection. Nitric oxide (NO) production and cytotoxicity of macrophage-rich abdominal excudate cells (AEC) and tumor macrophages (when available) were examined (1) prior to RSV or vehicle injection, (2) during tumor development (Day 10 post-injection), and (3) during tumor regression (AR) or tumor suppression (AP). Macrophage (AEC and tumor) NO production was assessed indirectly by nitrate assay. Cytotoxicity was assessed by the ability of macrophage supernatant fluid to kill cells from the RP9 tumor cell line. Both aspects of macrophage function were examined with and without in vitro activation of macrophages with lipopolysaccharide (LPS). Overall, AEC from AP chickens exhibited higher NO production and cytotoxicity than AEC from AR chickens at all time points examined. During
tumor development. NO production by LPS activated AEC from RSV- injected AR and AP chickens was higher than NO production by LPS activated AEC from age-matched, vehicle-injected AP and AR controls. Macrophages isolated from regressing tumors had higher NO production and exhibited greater cytotoxicity than macrophages isolated from progressing tumors. Based on these data, the inability of AP chickens to regress RSV-induced tumors is not due to a general impairment of macrophage function. Functional differences between macrophages obtained from regressing or progressing tumors suggests a local, tumor-specific impairment of macrophage function in AP chickens.

**Key Words:** Rous sarcoma, Macrophage, Chicken, Nitric oxide, Cytotoxicity

176 Polymorphism of turkey CD8α molecule did not appear to be associated with body weight. Z. Li1, K. E. Nester3, Y. M. Sаi1, Z. Fan2, M. Luhtala3, and O. Vainio3, 1Dept. of Animal Sciences, 2Food Animal Health Research Program, the Ohio State University, 3Dept. of Medical Microbiology, Turku University, Turku, Finland.

A panel of mouse anti-chicken CD4 and CD8α monoclonal antibodies (mAb) was tested by flow cytometric analysis for their cross-reactivity with turkey peripheral blood lymphocytes (PBL) from individuals in five turkey lines. The PBL were isolated and stained with single or dual colors. There were large line differences in detecting turkey CD8 molecule with the commercial CT8 mAb (anti-chicken CD8α). However, other anti-chicken CD8α mAb (3-298, 3-292, and 11-39) had good cross-reactivity with the turkey PBL. The present data suggested the presence of polymorphism of turkey CD8α molecule. Most anti-chicken CD4 mAb (CT4, 2-46, 2-35, and 7-125) were also cross-reactive with the turkey PBL, and consistent results were obtained with these monoclonal antibodies. There was no difference in the body weight at 8, 16, and 20 wk, and in the Shank parameters (shank width, length, and depth) at 16 wk between the CT8 and CT8α turkey groups as characterized by the cross-reaction with the CT8 mAb. These results suggested that the polymorphism of the turkey CD8α molecule did not appear to be associated with growth rate, but might be related to disease resistance.

**Key Words:** Turkey, CD4 molecule, CD8 molecule, Monoclonal antibodies, Polymorphism

178 Behavioral and carcass quality responses to feed cueing for broilers. R. H. McGovern1, M. J. Zuidhof1, J. R. J. Feddes2, and E. J. Emmanuel1, 1Alberta Agriculture, 2University of Alberta.

The effects of feed cueing on broiler behavior and carcass characteristics of broiler chickens were evaluated using 12000 commercial female broilers studied over two 6-wk periods. Sixteen pens out of a total of 32 pens of broilers were subjected to cueing in each period. The feeding cue consisted of lifting the feeders for a one-hour period for five consecutive days prior to slaughter. The behavior of 80 birds, in two weight range categories (heavy, 15% above and light, 15% below the mean), was monitored in four pens to determine the effect of the cue on feeding patterns. The number of birds feeding at two-minute intervals and the number of feeding bouts (period of feed consumption) was recorded. The effect of the feeding cue on bird quality (carcass scratches, trimmed, condemnation, and contamination) at the time of processing was determined for all 12000 broilers. Feed consumption patterns were monitored electronically in four pens, two pens receiving the cue and two pens not receiving the feeding cue. The post-cue time period had the greatest percentage of birds feeding. On day 5, there was a 19% decrease in the number of birds feeding post-cue compared to day 4. After 2 days of feed cueing, broilers in the cued treatment had a greater number of birds feeding pre-cue compared to the non-cued treatment. There was a greater number of feeding bouts for light broilers (1.54 bouts/bird) compared to heavy broilers (1.23 bouts/bird) before the feed cue. After the feed cue, however, there was no difference between the number of birds feeding in either the heavy or light weight range. The number of old scratches and severe scratches that occurred prior to shipping was reduced with the feeding. The greatest feed consumption occurred in the morning 55.85g compared to the afternoon, evening, and night.

**Key Words:** Feed cueing, Carcass scratches, Broiler, Feed consumption

177 The Use of Image Processing and Statistical Modeling Techniques for Automated Recognition of Bacterial Cells in Chicken Carcass Wash Water. O. Tujiila1, M. Slavik1, Y. Li1, and C. Griffis1, 1University of Arkansas, Fayetteville, AR /USA.

The objective of this research was to develop an automated method for identification and enumeration of bacteria using image processing on slides containing poultry carcass wash water. This study focused on two bacteria, Salmonella typhimurium and Campylobacter jejuni. In this paper, a multi-plane focus algorithm was developed based on image processing techniques. A composite image was obtained from sliced images of the sample, scanned one-micron of distance between each other in the Z plane of a single field of view. Pictures of bacterial cells were acquired using a CCD camera attached to a motorized fluorescence microscope. In addition, the performance of a shape boundary modeling technique based on the use of circular autoregressive (CAR) model parameters was investigated. The boundary representation consisted of an ordered sequence of lengths of radii projections from the boundary centroid to twenty angularly equispaced points along the boundary contour of the bacterial shapes. The parameters of the model were estimated using the standard least square method and varying the number of past terms (or model order) between 1 and 3. In this investigation we used a minimum-distance classifier known as the feature weighting (FW) technique. The FW classifier was trained using ten images pertaining to each shape class. The images were chosen in such way that each of the bacterial cells had a different size and orientation within the same class. The classification accuracy was tested on seven test images belonging to each bacterial class. The experimental results showed that the model parameters can be used as a descriptor of shape boundaries detected in digitized binary images of bacterial cells. The identification technique presented in this paper is independent of bacterial size and orientation. Recognition accuracy from 85 to 100 percent was obtained.

**Key Words:** Autoregressive model, Image processing, Multi-plane focus, Immuno-fluorescence microscopy, Bacteria

179 Familiar odors attract domestic chicks. R. B. Jones* and N. L. Carmichael.

Chickens are reluctant to enter novel or exposed areas (including free range), to accept new food or to approach unfamiliar people. If they became attracted to artificial odors during rearing their subsequent incorporation in otherwise unfamiliar situations might reduce fear and avoidance. We know that chicks of a line maintained at Roslin formed such attachments to litter treated with geranium and orange oils, that perforated tubes of clove oil attracted Sussex x Warren birds, but that garlic did not attract Australorps. These results might reflect differences in the type of odorant, experimental situation, or genetic background. In Experiment 1, we found that vanilla was neither attractive nor aversive upon its first presentation to 11-day-old ISA Brown chicks. Therefore, in Experiment 2 we presented vanillin in dishes (replenished daily) below the grid floor of group-housed chicks' home cages during rearing. When tested individually at 9 days of age, the chicks moved first toward and spent longer (both P < .001) in that half of a novel cage containing a dish of vanilla rather than similarly coloured water. These results confirm the existence of olfactory memories in domestic chicks; they also suggest that olfactory attachment generalizes across diverse genetic strains, odorants, and methods of presentation.

**Key Words:** Domestic chicks, Familiar odor, Olfactory attachment

180 Early T-maze behavior predicted growth, sociality and stress responsiveness in broiler chickens. R. B. Jones* and R. H. Marin2, Roslin Institute, Scotland, 2CRILAR (CONICET), Argentina.

We measured the time taken by 2 or 3-day-old broiler chicks to traverse a runway and thereby reestablish visual contact with their companions. They were assigned to high (HP) or low (LP) performance groups if the