

agar for 48 h for *Campylobacter* enumeration. Aeration of the semen by either method did not reduce the amount of *C. jejuni* in semen compared to controls at all temperatures. Similarly, *C. jejuni* growth after storage of semen at 4 or 24C was not different for any of the sample times. *C. jejuni* was significantly reduced, however, when stored for 24h at 42C (4 log reduction). Aeration and reduced temperatures, typical procedures used to maintain sperm viability before insemination did not reduce *Campylobacter* concentrations in vitro. Studies are currently underway to determine if these treatments have similar effects on turkey semen, however it appears that alternative methods will be needed to reduce *Campylobacter* contamination of poultry semen. Funded in part by U.S. Poultry and Egg Association #394 and the USDA Food Safety Consortium.

Key Words: *Campylobacter*, Semen, Aeration

24 Broiler Breeder roosters' ability to naturally mate after utilizing ultrasound as a non-destructive means to measure testicular size. L. J. Richardson^{*1}, J. L. Wilson¹, E. R. Bowling¹, A. B. Caudle², and K. C. Powell³, ¹*Poultry Science Department, University of Georgia*, ²*Veterinary Medicine, University of Georgia*, ³*Roche Animal Nutrition and Health*.

The use of an Ultrasound machine (Aloka SSD-900V) fitted with a linear surgical probe (UST-5526L-7.5 laproscopic transducer) has been shown to be a non-destructive means to internally measure testicular size in broiler breeder roosters without effecting semen ejaculation. However, the effect of the ultrasound procedure on the roosters ability to naturally mate needed to be investigated. The objective of this trial was too; evaluate the effect of the ultrasound procedure on the natural mating frequency of the broiler breeder male and subsequent fertility of the flock. Thirty-seven week old broiler breeders were housed in 16 pens (60 females and 6 males/pen) that were designed to industry standards [2/3rd slats, 1/3rd scratch; nipple drinkers; mechanical trough (females), and pan feeder system (males)]. Eight of the pens were randomly designated as the control, while the remaining pens were randomly designated as the treatment. At 37 weeks of age, 4 mating observations were taken over six days prior to the ultrasound procedure and eggs incubated and eggs were candled to estimate fertility at 14 days of incubation. At 38 weeks of age, the roosters in the treatment pens were ultrasounded. Following ultrasound, three mating observations were taken over a three-day period. Seven days following ultrasound, one final mating observation was performed. Eggs were collected and incubated weekly over a four-week period following the ultrasound procedure. There were no significant differences in mating frequency between the control pens when compared to the treatment pens. The mean fertility in the control pens was 97.79 percent and not found to be significantly different ($P=0.05$) compared to the treatment pens fertility of 97.87 percent. The natural mating ability of the broiler breeder rooster was not affected after an ultrasound procedure to measure testicular size in broiler breeder rooster. These observations support the use of ultrasound measurements as a non-destructive means of assessing testis size in breeder males.

Key Words: Broiler breeder, Ultrasound, Fertility, Testis

25 The optimum semen dilution for the Sperm Quality Index that is most predictive of fertility when inseminating with a constant volume of semen. H. M. Parker^{*} and C. D. McDaniel, *Mississippi State University*.

The Sperm Quality Index (SQI) predicts semen quality and fertility by measuring the deflections created in a light path by sperm movement.

The objective of this study was to establish if a semen dilution rate below 10-fold could improve the ability of the SQI to predict semen quality and fertility when hens are inseminated with a constant volume of semen. Once a week for 3 wk, semen was collected from 28 Cobb males, and ejaculates were analyzed for sperm concentration, viability, and the SQI. After obtaining an SQI for neat semen, semen was diluted 2-, 4-, 8-, 10-, and 25-fold prior to analysis for the SQI, and 15 hens/male were inseminated with 20 μ L of 1:4 diluted semen. To determine which semen dilution rate yielded an SQI that was most predictive of fertility, Pearson's correlation coefficients were obtained for the SQI at each dilution rate with fertility and also with different sperm characteristics. Correlation coefficients for the SQI at each dilution rate with fertility and sperm viability were statistically similar. The coefficients for the correlation of the SQI with fertility were 0.75, 0.72, 0.72, 0.66, and 0.61, for the 4-, 8-, 10-, 25-, and 2-fold dilutions, respectively. The correlation coefficients for the SQI with sperm viability were 0.68, 0.65, 0.65, 0.63, and 0.49 for the 2-, 4-, 10-, 8-, and 25-fold SQI dilutions. The SQI from 25-, 10-, and 8-fold dilutions produced a statistically stronger correlation with total sperm concentration ($r=0.85, 0.82, \text{ and } 0.80$, respectively) when compared to the 4-fold SQI dilution ($r=0.68$) correlation. Correlation coefficients for live sperm concentration were also statistically higher for 25-, 10-, 8-fold, and 4-fold SQI dilutions ($r=0.86, 0.86, 0.83, \text{ and } 0.73$, respectively) as compared to the 2-fold SQI dilution which had a non-significant relationship. It appears that the 10-fold SQI dilution is the most consistent at predicting fertility and semen quality.

Key Words: Sperm Quality Index, Fertility, Broiler breeder, Semen, Artificial insemination

26 Effect of organic selenium (Selplex[®]) and male comb size on initial broiler breeder fertility. H. Romero-Sanchez^{*}, P. Plumstead, B. A. Lenfestey, C. V. Williams, and J. Brake, *North Carolina State University, Raleigh, NC USA*.

An experiment was conducted to test the effect of organic selenium (Selplex[®]) on fertility of male broiler breeders subjected to the same cumulative nutrition to photostimulation (20 wk of age) but with evidence of different stage of sexual maturity as indicated by comb size. From a group of 96 Ross 344 males, the males with the largest and smallest comb size were divided into two groups that were then randomized into 16 pens (6 males per pen) with 55 Ross 308 females. Pens received a diet with or without organic selenium in a 2 x 2 factorial design with respect to male treatment with 4 replicates per interaction cell. BW, comb size, and shank length of males were measured at 20, 22, 24, 25, 27, 29, and 32 wk. Analysis of percentage egg fertility was done weekly from 28 to 34 wk with arcsine transformation carried out before statistical analysis. Males were fed the same diet as that of the female and separation of sexes was insured by special grills on the feeders. The male feed allocations were intended to be restrictive. Differences in male BW and shank length were positively associated with comb size although the small comb size group exhibited a significantly higher comb growth rate. The large comb males did experience a plateau in BW gain at 24 wk of age while the small comb males continued to gain BW. Grouping by comb size did not affect fertility. However, there were significant differences in fertility due to organic selenium and interaction of comb size and organic selenium. Organic selenium improved fertility of large comb males by up to 4%, but did not affect small comb males. The significance of this effect decreased with age from $P < 0.004$ to $P < 0.1$ from 28 to 34 wk of age. These data suggest that when early maturing males (large comb) were slightly underfed after photostimulation organic selenium had a nutrient sparing effect that allowed them to maintain high fertility even when they did not gain BW in a consistent manner.

Key Words: Broiler Breeders, Fertility, Organic Selenium, Comb Size

Immunology

27 Determination of cytokine activity in crude and fractionated supernatants from the chicken Harderian gland. N. H. Noblet, M. D. Owens, A. B. Bodine, and T. R. Scott, *Clemson University*.

The Harderian gland (HG) of chickens, as in many other avian species, is unique in that it is populated with a large number of plasma cells and serves as a peripheral lymphoid organ that is responsible for protecting the ocular region and the upper respiratory tract. Previous work in our

laboratory demonstrated that a soluble factor(s) present in the processed supernatant (SPNT) of the gland positively influences bursa of Fabricius-lymphocyte proliferation, as evaluated using a co-mitogenic bioassay with phorbol-12,13-dibutyrate used as the stimulating mitogen. The goals for this experimentation were to corroborate previous findings, identify (glyco)protein candidates with cytokine activity, and develop an antibody source capable of inhibiting the proliferative influence of the HG-SPNT, as evaluated by the bursal cell co-mitogenic bioassay. SDS-PAGE was performed to visualize the low molecular weight proteins (10-30 kDa)

of the HG-SPNT, in the range of known cytokines, and the chromatofocusing technique was used to separate HG-SPNT protein constituents based on their isoelectric points. Three prominent, low molecular weight proteins (13.5, 14, and 28 kDa) of the HG-SPNT were identified. Chromatofocusing fractions (pH 8.6 to 7.4) containing the three proteins, along with some additional proteins, were pooled and found to positively influence bursal cell proliferation. An antibody source developed against the proteins of the fractionated HG-SPNT effectively inhibited the proliferative influence of the HG-SPNT proteins on bursal-cell proliferation as evaluated using the bioassay. Additional bioassay inhibition experimentation with separate monoclonal antibodies that bind to the 14 and 28 kDa (glycol) proteins, respectively, revealed that both of these (glycol) proteins contribute to the cytokine activity observed in the co-mitogenic bioassay. Further work to identify these and other cytokines is on-going.

Key Words: Harderian gland, Cytokines, Bursa of Fabricius, Phorbol-12,13-dibutyrate, Co-mitogenic bioassay

28 Structural and functional characterization of chicken interleukin-16 and interleukin-17 cDNAs. W. Min* and H. S. Lillehoj, USDA-ARS, Beltsville, MD, USA.

In this study, we isolated and initially characterized cDNAs encoding IL-16 and IL-17 using a chicken intestinal library. IL-16 and IL-17 are pro-inflammatory cytokines produced by activated lymphocyte cells. Chicken IL-16 gene contained the entire open reading frame (ORF) of pro-IL-16 and encoded the mature IL-16 protein which consists of 607 amino acids. Chicken IL-16 showed 86% sequence homology to duck pro-IL-16 and 49-52% to various mammalian IL-16s. By Northern blot analysis, IL-16 transcripts showed a restricted expression to the lymphoid tissues. Recombinant chicken IL-16 consisting of 149 C-terminal amino acids of pro-IL-16 was biologically active when expressed in COS-7 cells and showed chemoattractant property for lymphocytes. IL-17 contained a 507 bp open reading frame predicted to encode a protein of 169 amino acids with a molecular mass of 18.9 kDa. Chicken IL-17 shared 37%-46% amino acid sequence identity with mammalian IL-17 and homologous to the ORF 13 of Herpesvirus saimiri. By Northern blot analysis, IL-17 transcripts were identified in a reticuloendotheliosis virus-transformed chicken lymphoblast cell line (CU205) and Con A-stimulated splenic lymphocytes, but not Cu91, RP9, RP13 cell lines or kidney, bursa, heart, spleen, cecal tonsils and thymus. Conditioned medium from COS-7 cells transfected with chicken IL-17 cDNA induced the production of IL-6 by embryonic fibroblasts.

Key Words: Chicken, Interleukin-16, Interleukin-17

29 In ovo immunomodulatory effects of CpG-containing oligodeoxynucleotides in *Eimeria*-infected chickens. R. A. Dalloul^{1,2}, H. S. Lillehoj¹, U. S. Babu³, R. B. Raybourne³, D. M. Klinman⁴, and R. A. Heckert², ¹Parasite Biology, Epidemiology and Systematics Laboratory, ANRI, USDA-ARS, Beltsville, MD, 20705, ²VA-MD Regional College of Veterinary Medicine, Univ. of Maryland, College Park, MD, 20742, ³Center for Food Safety and Applied Nutrition, FDA, Laurel, MD 20708, ⁴Center for Biologics Evaluation and Research, FDA, Bethesda, MD, 20892.

Short synthetic oligodeoxynucleotides (ODN) containing CpG motifs (CpG ODN) have been shown to be effective immunoprotective agents in murine models. Recently, CpG ODN were reported to have both in vitro and in vivo immunostimulatory effects in domestic animals including poultry. The objective of this study was to investigate the immunomodulatory effects of four CpG ODN on disease susceptibility in *Eimeria acervulina*-infected chickens. On day 18 of incubation, specific pathogen-free embryos were injected (air cell) with either 25 or 50 micrograms of CpG ODN (CpG-1, CpG-2, CpG-3 and CpG-4), a non CpG-containing control (CpG-0), or a negative control (PBS, phosphate buffered saline). At one week of age, birds were orally inoculated with 10^4 *E. acervulina* oocysts. Body weights, oocyst shedding and antibody response were measured. Body weights and body weight gains were not affected by any of the treatments. CpG-2 and CpG-4 significantly reduced oocyst shedding at 25 and 50 micrograms, respectively. Compared to PBS control, antibody response of any group was not significantly different. However, among CpG ODN treatments, a differential antibody response was observed with the lowest response in CpG-2 birds and highest in CpG-4

group. This study demonstrates that CpG ODN were effective immunoprotective agents in chickens, and further research into their action as vaccine adjuvant is needed.

Key Words: CpG, Immunomodulation, Oligodeoxynucleotides, *Eimeria*, Chicken

30 Safety and efficacy of a coccidiosis vaccine delivered in ovo to commercial broilers. R. M. Poston¹, C. L. Heggen-Peay^{*1}, L. M. Charniga¹, A. Martin¹, G. R. Mathis², and V. W. Doelling¹, ¹Embrex, Inc., ²Southern Poultry Research.

Broiler embryonated eggs were vaccinated *in ovo* at E18 with live sporulated oocysts of *E. acervulina*, *E. tenella* and two strains of *E. maxima*. Vaccine safety was demonstrated by comparable percent hatch and chick quality for vaccinates and non-vaccinated controls. Male chicks were selected and placed in five floor pens per treatment with 45 birds per pen. To assess bird performance, mortality, average live weight, and adjusted feed conversion were monitored for both groups throughout the study. Birds appeared healthy and there were no significant differences in performance parameters between non-vaccinated and vaccinated birds. To assess efficacy, a subset of birds was weighed and challenged with homologous strains on days 14, 22, 28 and 35. Six days post challenge, birds were individually weighed and lesion scored for upper, mid, and cecal regions of the intestines. Efficacy data showed a progression in development of immunity. Some improvements in weight gain and lower lesion scores in response to challenge were observed as early as day 14. Good weight gain combined with low lesion scores post-challenge indicated significant immunity by day 22. These data indicate that *in ovo* vaccination with live oocysts is safe and efficacious in broiler chickens.

Key Words: In ovo, Coccidiosis, Vaccine

31 B complex and alloantigen system L effects on resistance and immunity to cecal coccidiosis. Z. O. Medarova¹, W. E. Briles², and R. L. Taylor, Jr.^{*1}, ¹Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH 03824, ²Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

This study examined alloantigen system L effects on resistance to initial infection and acquired immunity to *Eimeria tenella* infection in three B complex genotypes. Experimental progeny segregating for B and L genotypes were produced from pedigree matings of $B^2B^5 L^1L^2$ sires and dams. Chicks were weighed and inoculated with 30,000 *E. tenella* oocysts at 6 weeks of age to evaluate resistance in four trials (n = 262). Immunity was studied in four additional trials (n = 244) by immunizing progeny with 500 *E. tenella* oocysts per day for five days beginning at 5 weeks of age. Two weeks after the last immunization dose, the birds were weighed and challenged with 30,000 *E. tenella* oocysts. All birds were weighed again and scored for cecal lesion six days after the 30,000 oocyst dose challenge. Weight gain and cecal lesion scores were evaluated by ANOVA. Major histocompatibility (B) complex genotypes B^2B^2 and B^5B^5 did not affect resistance to initial challenge with *E. tenella* based on lesion score and weight gain. However, after immunization, the B^5B^5 and B^2B^5 genotypes had significantly lower cecal scores than the B^2B^2 genotype when the birds were rechallenged. Weight gain was not affected among immunized birds. No significant L system effects with or without immunization were detected. These results are consistent with previous research demonstrating B complex effects on immunity to cecal coccidiosis.

Key Words: B complex, L system, Alloantigen, *Eimeria tenella*, Cecal coccidiosis

32 Antibody response against sheep red blood cells in lines congenic for major histocompatibility B complex recombinant haplotypes. E. S. Schulten^{*1}, W. E. Briles², and R. L. Taylor, Jr.¹, ¹Department of Animal and Nutritional Sciences, University of New Hampshire, Durham, NH 03824, ²Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115.

Line UCD 003, ($B^{17}B^{17}$), is the genetic background for congenic lines containing six different B complex recombinants. The recombinant types are: $R^1 = B-F/B-L^{24}$, $B-G^{23}$, $R^5 = B-F/B-L^{21}$, $B-G^{19}$, and $R^6 = B-F/B-L^{21}$, $B-G^{23}$. R^2 , R^3 , and R^4 arose from independent recombinational events but have $B-F/B-L^2$, $B-G^{23}$. Each recombinant was crossed

to Line UCD 003 followed by ten backcross generations. Heterozygotes were then mated to produce *B* complex recombinant homozygous lines having 99.9% background gene uniformity. Four-week-old birds of each line were injected intravenously with 1 mL of 2.5% SRBC to induce a primary antibody response. Blood samples were collected 7 days post-injection. Microtiter methods were used to assay total anti-SRBC and mercaptoethanol-resistant (MER) serum antibody. Titer was expressed as the log₂ of the reciprocal of the highest dilution having visible agglutination. The same birds were injected at 11 weeks of age to produce a secondary antibody response. Antibody titers for the primary and secondary response were evaluated by least squares ANOVA with hatch and *B* recombinant genotype as main effects. Fisher's protected LSD separated significant means. Genotypes R⁵R⁵ and R⁶R⁶ had significantly higher primary total antibody titer to SRBC compared with R¹R¹, R²R², R³R³, and R⁴R⁴. Both R⁵ and R⁶ have *B-F*²¹, a haplotype known for high antibody response to SRBC. Primary MER antibody response did not differ significantly among the genotypes. Both total and MER-resistant secondary antibody titers of R⁵R⁵ chickens were significantly higher than the other five recombinants. These results indicate that congenic lines carrying six *B* complex recombinants differ in their primary and secondary antibody responses to SRBC.

Key Words: *B* complex, SRBC, Congenic, Antibody, Immune response

33 Somatostatin and its receptor are expressed in the thymus of the chicken. Xiaodong Zhang* and Luc Berghman, Texas A&M University.

Various neuropeptides have been shown to act as immunomodulators within the microenvironment of the immune organs. One of the neuropeptides that has received a lot of attention for its immunomodulatory activities in the mammalian thymus is somatostatin (a.k.a. somatotropin release-inhibiting factor, or SRIF), a neuropeptide mainly produced in the brain. In this study, we have examined the thymus of the chicken with respect to the expression of somatostatin, and somatostatin receptor (SSTR) using immunocytochemistry for the peptide and reversed transcription PCR (RT-PCR) for the receptor. Numerous somatostatin-positive cells (stained with a rabbit anti-human somatostatin antiserum) were readily observed in chicken thymus. These cells were localized predominantly in the cortex of the thymic lobules, and displayed diverse morphologies. Most cells had a stellate appearance with numerous slim extensions. Immunofluorescent double staining for somatostatin and chromogranin A (CgA), a marker for neuroendocrine cells, showed a small overlap between these two cell populations, but the majority of cells were clearly single-stained. Based on the partial cDNA sequence of chicken pituitary SSTR2, RT-PCR results suggest that the chicken thymus expresses at least one somatostatin receptor subtype, identical to the one that was recently demonstrated in the chicken pituitary. This study demonstrates that somatostatin is a candidate mediator of immuno-neuro-endocrine interactions in the immune system of the chicken. Our current experiments are focusing on the further characterization of the cell types in chicken thymus that express somatostatin and/or its receptor as well as their biological functions in T-cell immunity.

Key Words: Somatostatin, SSTR, Thymus, Chicken

34 TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) induces apoptosis-related gene expression in chicken B lymphocytes in vitro. N. Puebla-Osorio*¹, K. S. Ramos², J. J. Delrow³, and L. R. Berghman¹, ¹Poultry Science Department, Texas A&M University, College Station, TX, 77843, ²Center for Environmental and Rural Health, Texas A&M University, College Station, TX, 77843, ³Genomics Resource, Fred Hutchinson Cancer Research Center, Seattle, WA 98109.

In this study, we used specific chicken immune cDNA arrays to identify the transcriptional profile induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in vitro. The arrays were constructed at the Fred Hutchinson Cancer Research Center (FHCRC) from three different libraries, containing cDNAs from a DT40 cell line library, from a chicken activated T-cell cDNA library and from a normal bursal library (DFKZ426). The complete array contained 3,011 chicken lymphocyte cDNA spots representing about 2,200 genes. Cultures of v-rel immortalized chicken B lymphocytes (DT40) were exposed to two different concentrations of TCDD (1 and 10 nM) for 6 and 12 h. Non-exposed cells challenged with an equivalent volume of DMSO, were used as controls. Total RNA was extracted

from each sample using the Trizol™ method. Sample preparation, performed at the FHCRC, included labeling of cDNA samples using total RNA in reverse-transcription reactions and subsequent coupling to either Cyanine-3 (Cy3) or Cyanine-5 (Cy5) fluorophores. The labeled cDNA samples were combined and co-hybridized to the array. Scanning and image processing involved a GenePix 4000 Microarray scanner (Axon Instruments, Inc., Union City, CA). Two 16-bit tiff images, corresponding to 532 nm (Cy3) and 635 nm (Cy5), respectively, were collected for each array. Several up- and down-regulated genes were identified as major TCDD responsive genes in the DT40 B cell line, including genes involved in apoptosis: Bcl-2, SRY, homologous to BCL-6, TNF, TALL-1 member of the TNF family, MHC6, actin binding protein-280, caspase 8, LaminA, PKC-α; oxidative stress: Cytochromes B-561, p450, C, B5, and C; FAS, protein A170 and chick GTM2; and DNA repair: RAD 51 and RAD 52, RAD3, and Cdc25 B-type tyrosine phosphatase. Specific primers were designed for each of these genes and Real time PCR will be used for validation of the microarray data.

Key Words: B lymphocytes, Dioxin, Transcription, Apoptosis, Microarray

35 CD14 and TLR4 expression on transformed T cell lines and interaction with macrophages in inducing iNOS activity. M. A. Qureshi*¹, R. A. Ali¹, and K. A. Schat², ¹North Carolina State University, ²Cornell University.

Both T-cells and macrophages interact at several levels of immune response. One such interaction is manifested at the level of nitric oxide induction. In the current study we examined several Marek's disease herpes virus and reticuloendotheliosis (REV) virus transformed T-cell lines for their ability to express CD14 and TLR4 (LPS binding and signaling molecules). Furthermore, the nitrite production by these T-cell lines (with or without LPS) as well as their macrophage activation potential was examined. All REV transformed cell lines (CU91, CU205 and CU210); MDCC cell lines (CU115, CU133, CU141); and tumor-derived (MSB-1, Levine and MSB-1, Solvay) cell lines were positive for CD14 and TLR4 expression but in various percentages (range 40 - 79% for CD14 and 43-92 % for TLR4) as determined by flow cytometry using anti-human CD14 and TLR4 antibodies. LPS (1 μg / 1 x 10⁶ cells) stimulation did not increase the CD14 or TLR4 expression. No nitric oxide synthase activity was seen in these T-cell lines when their conditioned medium was tested for nitrite levels with or without LPS stimulation. However, when conditioned medium taken from these T-cell lines was added to macrophages (MQ-NCSU cell Line), all three REV cell lines (CU91, CU205 and CU210) and one tumor-derived line (MSB-1, Solvay) exhibited nitric oxide synthase activity by producing nitric oxide in various μM concentrations. The supernatants from CU115, CU133, CU141 and MSB-1, Levine did not stimulate nitrite production in macrophages. To eliminate the possibility of REV in the supernatants of CU91 and CU205, the supernatants were filtered through .45 and .1 μm filters before treating macrophages with them. This filtration did not inhibit the macrophage activation potential of these cell lines. In fact, the addition of LPS to supernatants plus macrophage culture systems increased the nitrite activity by 3 to 8 folds. Furthermore, supernatants from CU115, CU141, MSB-1 L and MSB-1 S after LPS stimulation also exhibited significant macrophage activation potential. These findings suggest that while all T-cell lines express CD14 and TLR4, they do not produce significant amounts of nitric oxide after LPS stimulation. However, their supernatants are fully capable of inducing macrophage activation perhaps mediated via γ interferon.

Key Words: T-cell lines, CD14, TLR4, Macrophages, Nitrite

36 The role of macrophages in the pathogenesis of Marek's Disease in chickens. R. C. Robbins*¹, R. A. Ali¹, K. A. Schat², and M. A. Qureshi¹, ¹NC State University, ²Cornell University.

Marek's Disease Virus (MDV) is a cell-associated, tumor-inducing herpesvirus that causes significant economic losses to susceptible commercial chicken flocks. The first interaction the virus has with the immune system is still unidentified. The assumption is that macrophages may serve as "carriers" of MDV by being the first cells to get exposed/infected by the virus. In this study, chick kidney cells (CKC) were infected with JM16 and RK1 strains of MDV. This infected CKC stock was used to infect splenic cells (lymphocytes). Infected splenic cells were repeatedly incubated with fresh splenic cells to establish infected splenic cells stock

for co-culture experiments with macrophages. To examine if MDV infects macrophages, Sephadex-elicited macrophages, from 2-3 wk old K-strain chickens, were co-cultured with infected CKC and splenic cells. The infection status of cells was tested by fluorescence antibody staining technique using MDV-specific pp38 and gb antibodies. The effect of MDV co-incubation on macrophage viability was also tested by incubating MQ-NCSU macrophages with MDV and quantitating cell viability by MTT assay. The results of these experiments showed that both RK1 and JM16 successfully infected the CKC as well as the splenic lymphocytes. However, no MDV-specific antigen could be detected in macrophages after 24, 48 or 72h cultures. Both MDV virus strains failed to induce any significant macrophage cytotoxicity; i.e., in two separate experiments no more than 13% of the macrophages were found to be either dead or metabolically inactive. The findings of these studies, which showed no macrophage infection, imply that macrophages may not serve as the "carrier" cells during the initial stage of the MDV infection.

Key Words: Marek's Disease, RK1, JM16, Macrophages

37 Tissue fatty acid composition and immune response of broiler chickens fed diets containing conjugated linoleic or n-3 or n-6 polyunsaturated fatty acids. R. K Selvaraj* and G. Cherian, *Oregon State University, Corvallis, OR, USA.*

The tissue fatty acid profile and immune response of broiler chickens were investigated. One hundred and twenty day-old broiler chicks were distributed randomly to four treatments (3 replications of 10 birds per replication) and were fed diets containing conjugated linoleic acid (CLA) (Diet I), sunflower oil (Diet II), linseed oil (Diet III) or fish oil (Diet IV). The total lipid content of the diets was 3.5%. The body weight and feed intake was higher ($p < 0.05$) in Diet IV compared to Diets I, II and III. Birds fed Diets III and IV had higher n-3 fatty acids than Diets I and II in all the tissues studied. A preferential incorporation of CLA was observed in splenocytes over other tissues. The thiobarbituric acid reactive substances (TBARS) were higher in the breast and thigh muscle of birds fed Diet IV than other diets. The serum anti-BSA immunoglobulin content was higher ($P < 0.05$) in birds fed Diets III and IV when compared to Diets I and II. Delayed type hypersensitivity response, measured as the wing web skin thickness, increased from 0.71 and 0.98 in Diets IV and III to 1.19 and 1.44 in Diets I and II respectively ($P < 0.05$). The number of peripheral blood lymphocyte $CD4^+$ and $CD8^+$ cells and splenocytes $CD4^+$, $CD8^+$ and IgM^+ cells did not differ ($P > 0.05$) between treatment groups.

Key Words: Polyunsaturated fatty acids, Delayed type hypersensitivity, Immunoglobulins

38 Organic selenium affects broiler responses to immunostimulation. K. M. Gowdy* and F. W. Edens, *North Carolina State University, Raleigh, NC USA.*

Selenium maximizes immunological responses of poultry, but the inorganic and organic forms have not been compared. Organic selenium from selenized yeast (Sel Plex, Alltech, Inc., Nicholasville, KY 40356) is now approved for poultry. We have conducted experiments with broilers given diets with no supplemental selenium (NS), sodium selenite (SE), or Sel Plex (SP) and investigated their responses after they were challenged with either an enteropathogenic *E. coli* (EPEC, 1,000,000 cells at hatch to 4d post hatch) or *Salmonella* (natural infection). Four groups (NS, SP, NS+EPEC, and SP+EPEC) were in the 2 x 2 factorially arranged EPEC study. Broilers were given either NS (0 ppm supplemental selenium) or SP (0.2 ppm) broiler diets for 6 wk. BW was increased ($P < 0.05$) by SP compared with NS. In EPEC groups, BW was reduced significantly with NS, but SP prevented the large losses in BW. Mortality due to EPEC was reduced ($P < 0.05$) by SP. In a second trial to evaluate the influence of NS (0 ppm), SP (0.3, 0.6, or 1.2 ppm) or SE (0.3, 0.6, or 1.2 ppm) on lymphoid organ weight in 3 wk old broilers, 2 of 3 trials were complicated by a natural *Salmonella* infection. BW was reduced significantly in NS, SE and SP infected groups, but SP supplemented broilers in the infected groups grew better. With no infection, only the 1.2 ppm SP group had a greater BW than SE. Bursa of Fabricius relative weight was increased by 0.6 ppm SP in the uninfected group, and all groups showed decreased bursa weight with infection. Spleen weights were not affected by selenium feeding but were increased by infection. Thymus weights

were increased by SE (0.3 and 0.6 ppm) in the uninfected groups, but with infection, NS and SE thymus weights were depressed as compared with SP. Wingweb response to PHAP was significantly smaller with SP in the uninfected group. The results suggest that both SE and SP have a positive influence on the immune system, but they may not affect the immune system the same way. SP appeared to be more beneficial for the broiler.

Key Words: Organic Selenium, Immunity, Infection, Broiler

39 Effect of the acute phase response and lysine deficiency on cationic amino acid transporter (CAT) expression and organ weights in broiler chicks. B. D. Humphrey*, C. C. Calvert, and K. C. Klasing, *University of California, Davis.*

Cationic amino acid transporters (CAT) mediate cellular uptake of arginine and lysine. Two experiments were conducted to determine the effect of the acute phase response and lysine deficiency on CAT tissue expression. In experiment one, male broiler chicks (77 day of age) were fed a lysine deficient diet and were injected s.q. with lipopolysaccharide (LPS; 1mg/kg BW). Brain, heart, gastrocnemius, pectoralis, spleen, thymus, and bursa were collected 4, 8, and 16-hrs later for quantitative PCR for CAT 1, 2, & 3 mRNA. Tissues from uninjected birds served as controls. Thymus had the greatest decline in CAT expression due to LPS, with CAT-1 mRNA decreasing ($P < 0.05$) 75% by 8-hrs. Liver CAT-3 mRNA levels increased by 112% at 4-hrs ($P < 0.05$) and remained elevated at 8 and 16-hrs ($P < 0.05$). Spleen CAT-1 mRNA was induced ($P < 0.05$) at 4-hrs while CAT-2 mRNA was increased ($P < 0.05$) by 276% at 4-hrs. Of all the tissues examined, bursa was affected the most by LPS; CAT-1 mRNA increased ($P < 0.05$) 664% at 4-hrs while CAT-3 increased ($P < 0.05$) 384% at 16-hrs. In experiment two, broiler chicks (14 d) were fed a lysine adequate (1.2%) or deficient (0.7%) diet from hatch and at 14 d half were injected s.q. with LPS (1mg/kg BW; half remained uninjected). Bursa, spleen, thymus, liver, and pectoralis weights were determined at 6, 12, and 24-hrs and expressed as % BW post-injection. Tissues from uninjected chicks from each diet served as controls at each time point. Lysine deficient chicks had lower ($P < 0.05$) organ weights compared to lysine adequate fed chicks. LPS increased ($P < 0.05$) spleen and liver weights and tended ($P = 0.08$) to increase bursa weight. LPS did not result in any change in thymus or pectoralis weights ($P > 0.05$). The combination of increased expression of CAT isoforms and organ weights in secondary immune tissue may enhance its ability to compete for limiting nutrients and use them for protective functions.

Key Words: Cationic amino acid transport, Lysine, Acute phase response

40 Effect of dietary supplementation with bacterial cell powders prepared from Gram-positive and Gram-negative bacteria on lymphocyte profiles in broilers. G. F. Erf* and T. K. Bersi, *University of Arkansas, Fayetteville, Arkansas, USA.*

Optimal development and function of the immune system is central to poultry health. More recent strategies to optimize health in fast growing commercial broilers and turkeys have focused on enhancing the immunofunctional abilities of the birds through nutrition and immunostimulants. We have previously shown that dietary supplementation with dead bacterial cell powders (DBCP) prepared from *Brevibacterium lactofermentum* and *Escherichia coli* (Ajinomoto Co., Inc) enhanced macrophage function in broilers. Additionally, we observed that *E. coli* DBCP added to the diet of broilers increased the clearance of orally administered *Salmonella* bacteria from organs. To further examine the immunomodulatory effects of dietary supplementation with DBCP, broilers were fed a control diet or diets supplemented with either 100 ppm of *B. lactofermentum* DBCP (BL-DBCP diet) or 100 ppm *E. coli* DBCP (EC-DBCP diet). At 4 and 7 weeks of age, heparinized blood, thymus, bursa, and spleen were collected. Peripheral blood mononuclear cell suspensions and cell suspensions from thymus, bursa and spleen were prepared and immunofluorescently stained using a panel of chicken lymphocyte-specific monoclonal antibodies. One- two- and three-color cell population analyses were carried out using flow cytometry. At 7 weeks of age, immune cell profiles in the bursa were not affected by these treatments. In spleens of 7-week-old DBCP-treated broilers, the proportions of T cells with gamma-delta T cell receptors (TCR) were lower ($P = 0.007$) and the proportions of T cells with alpha-beta TCR were higher ($P = 0.001$) than

in broilers fed the control diet. Within the alpha-beta TCR-expressing T cell population, the ratio of T helper cells (CD4+CD8-) to cytotoxic T cells (CD4-CD8+) was higher ($P = 0.02$) in broilers fed the EC-DBCP diet compared to broilers fed the control diet. These changes in the proportions among TCR-defined T cells with DBCP supplementation were not observed in the thymus. Although these observations suggest

immunopotentiating effects of dietary supplementation with DBCP, the immunofunctional consequences of the observed shift in splenic T cell populations need to be examined.

Key Words: Lymphocyte, Broiler chickens, *E. coli*, *B. lactofermentum*, Lymphoid organs

Nutrition

41 In ovo feeding increases glycogen content in the liver and muscle size in broiler hatchlings. Z. Uni*¹ and P.R. Ferket², ¹*Department of Animal Sciences, Faculty of Agricultural, Food and Environmental Quality Sciences, Hebrew University of Jerusalem, Israel*, ²*Department of Poultry Science, College of Agriculture and Life Sciences, NCSU, Raleigh, NC 27695*.

The few days pre and post hatch are critical for the development of the broiler hatchlings. During this period, the bird utilizes their energy reserves to meet the high demands for glucose, which fuels hatchlings activities. The sources for glucose include glycogen, stored mainly in the liver, and muscle proteins and amino acids via gluconeogenesis. At day of hatch glycogen stores decrease substantially, and remain low until the newly hatched chick has full access to oxygen, necessary to mobilize and utilize body fat reserves, and its developing gut can digest and assimilate external dietary. When energy status is limited, hatchlings may lose weight, and the development of critical tissues is restricted. These limitations in early energy status may be alleviated by administering refined carbohydrates into the amnion (in ovo feeding) at 18 d of incubation. This hypothesis was tested by examining the effect of feeding broiler embryos (with 1 ml containing about 20% dextrin, 3% maltose and sucrose) and measuring liver glycogen and muscle size, from 3 d before hatch until 7 d post hatch. The in ovo feeding treatment increased body size at hatch through 7 days of age by 3% over controls, ($P < .05$) liver glycogen content in embryos and hatchlings from in-ovo fed birds was significantly ($P < .05$) higher than controls (13.62.7 mg/g vs 7.81.1 at 20 d of incubation, and 6.90.5 vs 4.80.5 at day of hatch). Moreover, relative breast muscle size (% of hatchling BW), was significantly ($P < .05$) higher in the in ovo fed birds than controls (3.2% \pm 0.1 vs 2.5% \pm 0.08 on d 3 and 6.4% \pm 0.3 vs 5.20.2 on d 7). The results indicate that in ovo feeding may improve the energy status of the hatchlings by increasing glycogen stores and preventing mobilization of muscle protein reserves for gluconeogenesis.

Key Words: Broilers, In ovo feeding, Liver glycogen, Breast muscle size

42 The effect of in ovo feeding of carbohydrates and beta-methyl-beta hydroxybutyrate (HMB) on the development of the digestive tract. E. Tako*¹, R.P. Ferket², and Z. Uni¹, ¹*Department of Animal Sciences, Agriculture Faculty, Hebrew University of Jerusalem Israel*, ²*Department of Poultry Science, College of Agriculture and Life Sciences, NCSU, Raleigh, NC 27695-7608*.

Early function of the digestive tract is crucial for achieving the maximal growth and development of broilers. Studies showed that early feeding cause an acceleration of the intestine development during the first days of the chick's life. Therefore, in ovo feeding (inserting a nutrient solution into the amniotic fluid), might increase the digestive system development and enhance the growth of the chick. This research examined the effect of in ovo feeding solutions, which contains different amounts of carbohydrates and HMB, on the broiler embryos and chicks digestive system from d 18 of incubation until 4d posthatch. The following parameters were examined: 1) intestine epithelium morphological changes; 2) the ratio between yolk sac and embryo body weight; 3) brush border aminopeptidase (AP) and sucrase isomaltase (SI) activities and gene expression. The in ovo-fed birds exhibited greater villus surface area than controls (27² vs. 13² at d 20 of incubation and 500² vs 350² at 4 d posthatch, $P < 0.05$) and greater yolk sac to BW ratio (36% vs 18% at d 20 of incubation and 13% vs 9% on hatch, $P < 0.05$). Low SI and AP activities were observed at 18 d of incubation in both groups (0.07 mM glucose/g and 0.011 u/mg respectively, in the in ovo group; 0.05 mM glucose/g and 0.08 u/mg, respectively for the controls). SI and AP activities increased significantly at hatch, with the in ovo fed group exceeding the controls (0.23 mM glucose/g SI and 0.015 u/mg AP vs 0.20 mM glucose/g SI and 0.012 u/mg AP, $P < 0.05$). SI and AP gene expression increased at 19 d of incubation in both groups, with the in ovo fed birds exhibiting higher expression than controls (13au SI and 0.35au AP vs 8au SI and

0.18au AP, $P < 0.05$). In ovo feeding increases intestinal morphological development, brush border enzymes gene expression and activity.

Key Words: Broilers, In ovo feeding, Brush border enzymes

43 The effects of In-Ovo feeding of protein and beta-methyl-beta-hydroxybutyrate (HMB) on early growth and glycogen status of turkey poults. O. T. Foye*¹, Z. Uni², and P. R. Ferket¹, ¹*North Carolina State University, Raleigh, NC 27608*, ²*Hebrew University of Jerusalem, Israel*.

In-ovo (IO) feeding, injecting dietary components into the amnion prior to internal pipping, may enhance early growth performance by altering glycogen status. Two experiments studied the effect of IO feeding protein and HMB on bodyweights, organ weights, total liver and pectoralis major muscle glycogen of poults. At 23 d of incubation, 100 eggs were each injected with 1.5 ml of a .4% saline solutions containing 4 nutritional treatments consisting of a factorial arrangement of two levels of protein (P) (0% or 18% egg albumen) and two levels of HMB (0% and .1%). All poults fed ad libitum within 24 hours after hatch. In exp. 1, all IO-fed poults (P, HMB, P-HMB) weighed significantly more than controls at hatch (6.1%, 3.4%, and 2.7%, respectively). P and P-HMB treated birds weighed significantly more than controls at 3 d (3.9% and 2.9%, respectively) and 7 d (3.9% and 7.1%, respectively). At hatch the pectoralis major muscle was larger in all IO-fed poults than controls. In comparison to controls pectoralis major (PM) muscle weight at 7 d was 7% and 5% greater among poults IO-fed HMB and P-HMB, respectively. At hatch, total liver glycogen in the P IO-fed poults was increased by 38% over the controls, total glycogen in PM muscle was 20.2% greater in HMB IO-fed poults than controls ($P < .05$). At 7 d, total liver glycogen was not affected by IO feeding, but HMB increased total PM muscle glycogen by 12.3% over controls ($P = .05$). In exp 2, total glycogen in PM muscle at hatch was improved by HMB IO-feeding ($P = .05$). At 7d, all IO-fed poults had greater total glycogen in the PM muscle than the controls ($P < 0.05$). These results imply that IO feeding of protein or HMB may improve glycogen status posthatch, which may enhance early growth and development of poults.

Key Words: Poults, In-ovo feeding, Liver glycogen, Muscle glycogen, Bodyweights

44 Quantitative Computed Tomography as a tool for assessing bone quality in poultry. J. Saunders-Blades*, K. Nadeau, and D. Korver, *University of Alberta, Edmonton, Canada*.

Quantitative Computed Tomography (QCT) is used to measure bone density in humans, but its use in poultry research has not been validated. The present research was conducted to validate QCT as a tool for the determination of bone quality in poultry. In Experiment 1, breeder hens, accidentally fed a low Ca diet, were replenished with Ca, and subsequent QCT measurements at 7 regions of interest (ROI) along the length of the tibia, bone weight, bone ash, and ash and bone Ca were obtained on tibias at 0, 7 and 14 d after repletion. Most bone traits were affected by time ($P < 0.05$). Bone weight (r^2 of 0.08 to 0.34) and ash weight (r^2 of 0.16 to 0.30) were linearly related with density ($P < 0.05$) at all ROI. In Experiment 2, tibias from laying hens used in studying the effect of mid-night feeding on egg traits were examined for density by QCT at 7 ROI, ash and breaking strength. Treatment did not affect bone measurements ($P > 0.05$). Ash weight (r^2 of 0.14 to 0.29), % ash (r^2 of 0.07 to 0.23), breaking strength (r^2 of 0.20 to 0.66) and bone Ca (r^2 of 0.07 to 0.23) were linearly related to bone density at the proximal end and middle of the bone ($P < 0.05$). In Experiment 3, femurs from broilers fed varying dietary levels of vitamin D₃ or 25-OH vitamin D₃ were measured for density at the midpoint by QCT, breaking strength, bone ash and bone Ca at 42 d of age. Diet did not affect bone traits ($P > 0.05$). Density