

**54 Differential neural activation in the septo-hypothalamic region following sexual and agonistic behavior in male broiler breeders.** J. Xie\*, W. J. Kuenzel, D. L. Allen, and A. Jurkevich, *University of Arkansas, Fayetteville.*

Sexual and agonistic behaviors are important social variables affecting fertility in male broiler breeders. Studies in birds and mammals have shown that the same brain nuclei, such as the medial bed nucleus of stria terminalis (BSTM) and paraventricular nucleus (PVN), are activated by both copulatory and agonistic behaviors. Our goal was to determine anatomical specificity of neural circuits within the septo-hypothalamic region following activation by copulatory or agonistic behavior. Mature male broiler breeders ( $n = 19$ ) with sexual experience were randomly allocated to 4 groups. Each male was transferred to a testing pen for 20 min and placed either with a sexually mature female (FEM), a taxidermy adult female in a crouching position (DUMMY), a male (MALE), or alone (EMPT). One hour after observation, birds were perfused with a fixative and an immediate early gene product, Fos protein, a marker of cell activation was visualized in brain sections using immunocytochemistry. Number of Fos-immunoreactive cells, stained cell areas and optical density were determined in the BSTM1, BSTM2, PVN, and NCPa (bed nucleus of the pallial commissure). In the BSTM1, the FEM group had significantly more Fos-ir cells than the MALE group. No differences were found in the area and optical density of Fos immunoreactivity of this subnucleus. In contrast, no overall significant effect of treatment was found for Fos cell counts, their area and optical density in the BSTM2. In the PVN, the MALE group had significantly higher Fos-ir cell counts than the other 3 groups. In the NCPa, more Fos-ir cells were found in MALE and DUMMY groups than the FEM birds. Area of Fos-ir cells in the NCPa were significantly larger in the MALE group compared to the remaining ones. In summary, the BSTM1 appears to be activated following copulation, while stimulation of the PVN and NCPa is associated with agonistic behavior or stress. Supported by NRI grant 2005-35203-15850 from USDA, CSREES.

**Key Words:** Fos, stress, image analysis

**55 Investigating the effects of estrogen on Zebra Finch eggshell thickness and eggshell morphology.** S.L. Westmoreland\*<sup>1</sup>, H. Pourarsalan<sup>1</sup>, K. A. Schug<sup>3</sup>, and J. R. Millam<sup>2</sup>, <sup>1</sup>*Department of Biology, University of Texas, Arlington,* <sup>2</sup>*Department of Animal Science, University of California, Davis,* <sup>3</sup>*Department of Chemistry, University of Texas, Arlington.*

Female Zebra finch chicks are exposed to estrogen in their daily consumption of seeds. We hypothesized that estrogen causes eggshell thinning in Zebra Finch and eggshells treated with estrogen show a higher mammillary cone density. We then hypothesized that the eggshells treated with estradiol benzoate show a higher concentration of magnesium. Jim Millam at UC Davis administered the experimental female chicks with estradiol benzoate and canola oil for 7 days when they were 5–11 days old. Control females were administered canola oil only. They were allowed to form mating pairs with males at 110 days and 25 eggs were collected: 15 control and 10 experimental. Three samples were removed from each eggshell and digital electron micrographs of the eggs were taken at 500 $\times$ . Thickness of the eggshells was determined using Image Pro Plus. It was found that the treated eggshells were significantly thinner than the control ( $P = 0.02$ ). We then investigated the relationship between eggshell thickness and mammillary cone density. We marked the cone tips on the photos and counted the tips. The mean for treated eggshells was 65.97 (SD = 11.2) while the mean for control was 63 (SD = 14.6). This difference was not significant ( $P = 0.228$ ). We then asked the eggshells to make 8 control and 8 experimental eggshells and measured their magnesium content using the atomic mass spectrometer. There was no significant difference between the treated and control groups ( $P = 0.48$ ). We concluded that estradiol causes eggshell thinning in Zebra Finch, but the mammillary cone and magnesium content are not directly related to thickness.

**Key Words:** eggshell, estrogen, morphology

## Immunology

**56 Natural source vitamin E (RRR- $\alpha$ -Tocopheryl Acetate) level in broiler diets modulates expression of some cytokines.** S. S. Block\*<sup>1</sup>, M. G. Kaiser<sup>2</sup>, E. Beach<sup>2</sup>, M. Sifri<sup>3</sup>, and S. J. Lamont<sup>2</sup>, <sup>1</sup>*ADM Research, Decatur, IN,* <sup>2</sup>*Iowa State University, Ames,* <sup>3</sup>*ADM Alliance Nutrition, Quincy, IL.*

Level of vitamin E in the broiler diet has been reported to impact both growth and immune function. The objective of the current study was to determine the effects of varied levels of natural-source vitamin E (Nova-E<sup>TM</sup>) on growth and on mRNA expression level of 13 cytokines in the broiler chicken, with or without immune activation. Broilers, in a randomized block design, were fed 1 of 3 diets. Diets were industry standard, enhanced (standard + 10 $\times$  Natural Source Vitamin E) and reduced (reduced Zn, Mn, Se, and vitamins A and D) for 23 days and a common diet days 24–36. Birds were given either a saline or lipopolysaccharide (LPS) injection on day 23, 3 hours before collecting blood for isolation of white blood cells for cytokine gene expression assays. Cytokines are natural immunomodulators produced in response to immune activation. The LPS source for immune activation was *E. coli*. Diet had a significant

effect on body weight, with birds on the reduced diet being smaller and having a higher death rate than the other 2 diets from 2 weeks of age through trial completion. Birds fed the enhanced vitamin E diet had less reduction in average daily gain after immune activation by LPS than did the industry standard diet group. LPS treatment decreased mRNA expression of interferon-gamma and the major histocompatibility complex IIB; and increased transforming growth factor  $\beta$ -4 and inducible nitric oxide synthase and interleukin-6. Interferon-gamma and transforming growth factor B4 gene expression was greater for birds on the enhanced diet than the standard. Blood vitamin E was numerically lower after LPS injection in birds receiving the industry standard (4.6 vs. 3.9  $\mu\text{g}/\text{mL}$ ;  $P > 0.1$ ) and reduced diets (5.4 vs. 3.9  $\mu\text{g}/\text{mL}$ ;  $P > 0.1$ ). Collectively, the results indicate that standard and enhanced vitamin E levels support growth performance, level of vitamin E modulates the expression of interferon-gamma, and enhanced levels of Natural Source Vitamin E may protect broilers against the reduction in gain that occurs with immune activation.

**Key Words:** natural source vitamin E, immunity, broiler

**57 Cytokine gene expression in CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets of chickens infected with Marek's disease virus.** P. Parvizi\*, L. Read, M. F. Abdul-Careem, C. Lusty, and S. Sharif, *University of Guelph, Guelph, ON, Canada.*

Immunity to Marek's disease virus (MDV) is dependent on T-cell-mediated immune response. However, little information is available on the cytokines produced by T-cell subsets in response to MDV infection. To assess cytokine gene expression in CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets of MDV-infected birds, chickens were infected with the RB1B strain of MDV. Subsequently, T cells from the spleens of infected as well as age-matched controls were fractionated by flow cytometry at 4, 10, and 21 days postinfection (d.p.i.), RNA was extracted and subjected to real-time RT-PCR to assess the level of cytokine transcripts as well as viral genes including meq and glycoprotein B (gB) in fractionated T-cell subsets. There was an increase in the number of CD4<sup>+</sup> T cells as well as a significant increase in the expression of viral meq gene in CD4<sup>+</sup> T cells, which coincided with the presence of tumours in various organs. It was also observed that there was an up-regulation of IL-18 and IL-6 expression in both T-cell subsets at 4 and 21 d.p.i. The expression of IL-10 was up-regulated in both subsets at all time points, except for 10 d.p.i. when the expression of this gene was down-regulated in CD8<sup>+</sup> T cells. IL-4 was also up-regulated in both subsets at 4 and 21 d.p.i. Overall, there was a tendency in T-cell subsets of MDV-infected birds toward the expression of type II cytokines. Our study indicates that MDV-associated cytokine profiles vary in CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets and that cytokines including IL-4 and IL-10 may play a role as a part of host responses to MDV.

**Key Words:** T-cell subsets, Marek's disease, real-time RT-PCR

**58 Expression of inflammatory mediators in lungs of ascites-resistant and susceptible broilers in response to microparticles entrapped in the pulmonary vasculature.** K. R. Hamal\*, R. F. Wideman, N. B. Anthony, and G. F. Erf, *University of Arkansas, Fayetteville.*

Intravenous microparticle (MP) injection is a patented method used to select broilers that are resistant to pulmonary hypertension syndrome (PHS, ascites). In addition to occluding pulmonary arterioles, MP cause mononuclear cell infiltration and local inflammatory activity in the perivascular region. PHS-resistant broilers (R line) are able to resolve the vascular occlusion and inflammation, whereas PHS-susceptible broilers (S line) tend to progress to PHS. The objective of this study was to examine the expression of inflammatory mediators in lungs from 4-wk-old R and S line broilers before (0 h, no MP) and 2, 6, 12, 24, and 48 h post-i.v. MP injection (6 birds/line/time point) by real-time quantitative RT-PCR. Data were expressed as the fold change in target gene expression relative to 0-h levels. In lungs from both broiler lines, the expression of IL-1 $\beta$ , IL-6, IL-8, IL-12, and K60 increased ( $P < 0.05$ ) from 0 to 6 h, reached peak levels at 6 and 12 h, and decreased thereafter, whereas IFN- $\gamma$  and iNOS expression increased consistently from 0 to 48 h post-i.v. MP injection. The pattern of IL-4 expression differed in lungs from R vs. S line broilers; in the R-line IL-4 was elevated by 6 h and continued to increase thereafter, whereas in the S line IL-4 was elevated by 2 h and remained at that level. Compared to lungs from S line broilers, lungs from R line broilers had higher expression ( $P < 0.05$ ) of IL-1 $\beta$  and IL-6 at 2, 6, and 12 h; higher IL-4 at 12, 24, and 48 h; higher IL-8 at 6 and 12 h; higher K60 at 6, 12, and 24 h; higher IFN- $\gamma$  at 6 and 48 h and higher iNOS at 12, 24, and 48 h post-i.v. MP injection. The lines did not differ in IL-12 expression. Higher expression of

pro-inflammatory mediators and iNOS in R compared to S line lungs following i.v. MP injection may explain the ability of R line broilers to effectively counteract the MP-induced pulmonary hypertension and resolve the vascular occlusion.

**Key Words:** ascites, cytokines, inflammation

**59 Development and initial evaluation of a candidate *Clostridium perfringens*  $\alpha$  toxin-based necrotic enteritis vaccine utilizing recombinant *Salmonella* as a vaccine vector.** A. Khatiwara\*<sup>1</sup>, S. L. Layton<sup>1</sup>, G. Tellez<sup>1</sup>, L. R. Berghman<sup>2</sup>, W. J. Bottje<sup>1</sup>, B. M. Hargis<sup>1</sup>, and Y. M. Kwon<sup>1</sup>, <sup>1</sup>*Department of Poultry Science, University of Arkansas, Fayetteville,* <sup>2</sup>*Department of Poultry Science, Texas A&M University, College Station.*

Necrotic enteritis (NE) is a common disease of poultry associated with poor performance and mortality. *C. perfringens* (CP) is the primary causative agent of NE. Social pressures are demanding alternatives to antibiotics to prevent the incidence of NE. Among the alternatives to control NE in poultry, vaccination is a promising approach. Previous studies have shown that attenuated *Salmonella*-vectored vaccines presenting foreign immuno-protective epitopes, along with an immune-enhancing oligopeptide, on the cell surface is a promising and versatile strategy to fight infectious diseases in poultry. The objective of this study was to develop and evaluate a candidate CP  $\alpha$  toxin-based NE vaccine utilizing recombinant *Salmonella* as a vaccine vector. Attenuated *S. Enteritidis* (SE) genome was manipulated to display CP  $\alpha$  toxin epitope (ARGFAK) as a fusion to the outer membrane protein LamB. In addition, a short 10 aa sequence of CD154 (WAEKGYTMS) was inserted to enhance immune response (SE vaccine candidate). Day of hatch chicks were randomly divided into 3 groups (saline control; SE vector only; SE vaccine candidate, 65/group). Saline or 107 cfu/chick of the SE vector or the vaccine candidate were administered by oral gavage (0.25 mL) to the respective groups. At d 3, 10, 21, and 30, liver/spleen and cecal tonsils were removed to determine invasion and colonization by the vector. Both the vector and the vaccine candidate showed significant invasion and cecal colonization ( $P > 0.001$ ). Saline control birds were negative for SE at all time points. Blood samples were collected to measure  $\alpha$  toxin-specific IgG antibody titers at d 10, 21, and 30. Results demonstrated that chicks, which received the vaccine candidate exhibited significantly higher levels of  $\alpha$  toxin-specific IgG antibody at d 21 and 30 when compared to the saline control or SE vector groups. These preliminary data suggest that effective  $\alpha$  toxin seroconversion is possible with this *Salmonella*-vectored vaccine candidate and may provide an effective tool to combat NE in poultry.

**Key Words:** *Salmonella*, *Clostridium perfringens*, vaccine vector

**60 Smyth line chicken model for autoimmune disease.** G. F. Erf\*, K. D. Bateman, F. Shi, C. T. Trovillion, and B. L. Plumlee, *University of Arkansas, Fayetteville.*

Vitiligo is a common acquired hypopigmentary disorder characterized by a loss of epidermal pigment cells (melanocytes). The Smyth line (SL) chicken is the only animal model for autoimmune vitiligo that recapitulates the entire spectrum of clinical and biological manifestations of the human disease. SL vitiligo (SLV) is a multifactorial disorder involving an inherent melanocyte defect (abnormal melanosomes), an

immune system component (primarily melanocyte-specific CMI), and environmental factors (e.g., herpesvirus of turkey; HVT). Hence, SL chickens offer unique opportunities to examine the interplay between genetic susceptibility, immune system activity, and environmental factors. Moreover, as pigment cells are located in the feather rather than the skin, the target tissue is easily accessible and can be sampled prior to and throughout the development of vitiligo. Another advantage of this animal model is that the incidence of SLV by 20 wk of age is predictably high (>90%) and low (<20%) in HVT-vaccinated and HVT-negative SL chicks, respectively. Lastly, this animal model includes 2 MHC-matched control lines: the parental Brown Line (BL, vitiligo-susceptible, but <2% incidence) and the Light Brown Leghorn (LBL, similar plumage colors, vitiligo resistant). Previous studies showed that melanocyte destruction in the growing feather is associated with mononuclear cell infiltration (primarily CD8+ T cells), MHC class II expression, melanocyte apoptosis, and the presence of melanocyte-specific cell mediated immunity. Current studies using targeted gene expression analyses revealed that the onset of SLV is associated with increased expression of pro-inflammatory cytokines (IL-1 $\beta$ , IL-8, IL-12, IFN- $\gamma$ ) and NALP-1, but not the Th2 cytokine IL-4. These findings support a role of inflammation and Th1 mediated immune activity in SL melanocytes destruction. Using HVT-negative chicks, studies are underway to further examine the role of these inflammatory mediators as well as LPS and muramyl dipeptide in triggering the expression of SLV in susceptible individuals. Taken together, the Smyth line chicken model is an excellent model to study chronic inflammatory and autoimmune disease processes.

**Key Words:** cytokines, Smyth chicken, vitiligo

**61 Proteomics of host responses in spleen of chickens infected with Marek's disease virus.** N. Thantrige-Don\*<sup>1</sup>, M. F. Abdul-Careem<sup>1</sup>, S. C. Burgess<sup>2</sup>, and S. Sharif<sup>1</sup>, <sup>1</sup>Ontario Veterinary College, Guelph, ON, Canada, <sup>2</sup>Mississippi State University, Mississippi State.

In this study, differential protein expression in spleen in response to Marek's disease virus (MDV) infection was studied at various time points postinfection, which coincided with phases of MDV life cycle. Although several studies have been performed to investigate host responses to MDV at the transcriptional level, there are limited studies on the proteomic changes in host tissues during the course of Marek's disease. To achieve the objective of the study, 5-day-old specific pathogen free chickens were infected with 750 plaque forming units of a very virulent strain of MDV, RB1B, and spleens were harvested at 7, 14, and 21 days postinfection (dpi) to study the protein expression using 2-dimensional gel electrophoresis (2DE). Identities of differentially expressed proteins were obtained using liquid chromatography/mass spectrometry. We were able to resolve more than 550 spots between 158 and 9 kDa and pI of 3–10 pH from each spleen. 65 proteins were identified as significantly ( $P \leq 0.01$  and FDR  $\leq 0.05$ ) differentially expressed at least once out of 3 sampling points. The highest number of differentially expressed proteins was detected on 7 dpi whereas the number declined by 14 dpi and increased again by 21 dpi. Among those proteins that were expressed only in infected birds at 7 dpi, predicted immunoglobulin superfamily member 3, heat-shock protein 70 and triosephosphate isomerase 1 are of note. A predicted protein similar to NK cell enhancement factor isoform 4 was significantly induced at both 7 and 14 dpi. Cathepsin D, major vault protein, aldolase b fructose biphosphate and eukaryotic translation elongation factor proteins were among those that were significantly induced in all infected birds regardless of the sampling time point. Among down-regulated proteins, a predicted

hypothetical protein homologous to MHC class II $\alpha$  chain was noted. Overall, differential protein expression in spleen in response to MDV infection was successfully studied using 2DE and mass spectrometry. The present findings provide a basis for further studies aimed at elucidation of the role of these proteins in MDV interactions with its host.

**Key Words:** Marek's disease virus, host response, proteomic

**62 Immunostimulatory and antitumor effects of plant extracts demonstrated by in vitro culture systems.** S-H Lee\*<sup>1</sup>, H. S. Lillehoj<sup>1</sup>, Y. H. Hong<sup>1</sup>, S-I Jang<sup>1</sup>, C. Ionescu<sup>2</sup>, and D. Bravo<sup>2</sup>, <sup>1</sup>Animal and Natural Resources Institute, Agricultural Research Service, USDA, Beltsville, MD, <sup>2</sup>Pancosma, Le Grand, Saconnex, Switzerland.

Traditionally, the application of prophylactic antibiotics has been successful in reducing infection-related morbidity and mortality in animal production. However, with increasing concerns over the widespread use of feed-added chemicals in animal production and the emergence of antibiotic resistant microbial pathogens in the fields, there is much interest to develop alternative methods of disease control in poultry production. In this regard, rapid progress has been achieved on the identification and commercial application of novel plant phytonutrients to boost innate immunity against infectious diseases and tumors in clinical medicine. In this study, we evaluated several phytonutrients isolated from various plant sources for their immunostimulatory activities using in vitro cultures of splenocytes, macrophages and tumors. Extracts from 4 different medicinal plants (cinnamaldehyde, capsicum, shiitake, and curcuma) were tested for their effects on various in vitro parameters of innate immunity such as lymphocyte proliferation, macrophage activation, cytokine secretion and the inhibition of tumor cell growth. All plant extracts tested stimulated lymphocyte proliferation and inhibited tumor cell growth compared to the medium control. Co-culture of macrophages with cinnamaldehyde, capsicum, and shiitake induced nitric oxide production with shiitake significantly inducing the expression of IL-1 $\beta$  and IL-6 transcripts. Stimulation of macrophages with cinnamaldehyde and curcuma significantly induced IL-15 and IL-12 transcripts, respectively. This report documents the immunological basis of health-promoting effects of these plants in poultry for the first time. Further studies on the nature of the immunomodulating activities of these phytonutrients will facilitate the development of novel dietary strategies to reduce economic losses due to infectious diseases in poultry.

**Key Words:** immunomodulation, lymphocyte, macrophage

**63 Lysis of human and rabbit erythrocytes by duck sera: Differentiating the alternate from the classic complement pathway.** P. F. Cotter\*<sup>1</sup> and R. Murdoch<sup>2</sup>, <sup>1</sup>Cotter Laboratory, Arlington, MA, <sup>2</sup>Maple Leaf Farms, Milford, IN.

Duck immune system peculiarities include: true lymph nodes, intestinal lymphoid bands, and a truncated antibody, IgY( $\Delta F_c$ ). The latter might explain the inability of duck antibody to participate in secondary reactions; and duck immunity may illustrate a compromise between protection and injury. Market age ducks possess high titers of Rabbit RBC (Rb) agglutinins but generally lack human type O (Hu<sup>O</sup>) agglutinins. In 42 d drakes the anti-Rb titer was 1:1,024, but Hu<sup>O</sup> were not agglutinated by sera diluted to 1:10. Both cell types were lysed. Rb were rapidly agglu-

tinated and lysed, but Hu<sup>O</sup> were not agglutinated and lysed only after prolonged incubation. Hu<sup>O</sup> lytic titers were typically lower than those for Rb in the same sera. In 91 commercial drakes complete Hu<sup>O</sup> lysis occurred in 14/91 (15%),  $\geq 50\%$  lysis in 51/91 (56%), but 7 (8%) sera were negative. Heating sera reduced lytic capacity for both Hu<sup>O</sup> and Rb but not to the same degree. Nearly all Hu<sup>O</sup> lytic capacity was removed after 10 min exposure to 57°C while the same treatment reduced Rb lytic capacity by only 50%. Chelation of Ca<sup>++</sup> by EGTA interfered with Rb lysis suggesting inhibition of the classic complement pathway (CP) perhaps by interference at the C1 q-r-s levels. EGTA had little inhibitory effect on Hu<sup>O</sup> lysis, supporting an alternate pathway (AP) mechanism. EGTA did not affect Rb agglutination.

The addition of carbohydrates, especially melibiose and galactose, to diluents reduces both agglutination and lysis of Rb but has little effect on Hu<sup>O</sup> lysis. This supports a role for anti-Gal as the antibody providing the substrate for CP lysis of Rb and tends rule out an antibody requirement for Hu<sup>O</sup> lysis. Collectively these 2 systems seem to provide a convenient means for investigating innate and acquired immunity of ducks. Moreover the heterogeneity in lytic capacity for Hu<sup>O</sup> of market age ducks may offer a screening tool for identifying those with a weak immunity.

**Key Words:** ducks, alternate and classic complement pathways, erythrocyte agglutination, lysis, immunity

**64 Importance of in ovo environment on subsequent immune function and health risks.** R. R. Dietert\*, *Cornell University, Ithaca, NY.*

The embryonic environment supporting immune development plays a crucial role in defining posthatch immune function as well as potential health risks of the chick. Because landmark immune maturational events occur in ovo and set the template for host defenses of the chick, management of immune maturation is a critical first step in overall management of poultry health. Much of our knowledge of both immune development and embryonic immunomodulation has occurred through the use of in ovo environmental manipulation and/or intervention. Through these techniques a wide array of intended and unintended immune modifiers have been identified and studied. These include feed and water contaminants such as mycotoxins and heavy metals as well as dietary factors, cytokines and various adjuvants. This presentation will consider the role that in ovo manipulation and intervention has played toward improv-

ing immune function and reducing health risks of the chick. Research supported in part by USDA Regional Grant 1016.

**Key Words:** embryonic development, immune maturation, health risks

**65 The role of photoperiod and melatonin injection on alleviation of inflammation induced by lipopolysaccharide injection in broiler chickens.** A. O. Abbas\*<sup>1</sup>, G. M. K. Mehaisen<sup>1</sup>, G. L. Hendricks<sup>3</sup>, A. E. Gehad<sup>2</sup>, and M. M. Mashaly<sup>1,4</sup>, <sup>1</sup>*Cairo University, Cairo, Egypt*, <sup>2</sup>*National Research Centre, Cairo, Egypt*, <sup>3</sup>*Pennsylvania State University, State College*, <sup>4</sup>*Kuwait Institute for Scientific Research, Safat, Kuwait.*

Two experiments were conducted with male broilers to study the effect of different light schedules and melatonin (mel) injection in enhancing the immune response and reducing the inflammatory response induced by lipopolysaccharide (LPS) injection. In the first experiment, 160 one-day-old broiler chicks were divided into 2 groups: the first group was exposed to continuous light (CL) [23L:1D], whereas the other group was exposed to intermittent light [1L:3D]. At 6 wk of age, within each light treatment, 15 broiler chicks were injected intravenously (i.v.) 3 mg/kg BW LPS, and another 15 broiler chicks were injected with saline. B-lymphocyte proliferation and antibody production were significantly higher in the IL-LPS group. However, body temperature and plasma corticosterone concentration, at 3 and 12-h postinjection, were significantly lower in the IL-LPS group, compared to the CL-LPS group. Furthermore, plasma IL-1-like activity, plasma IL-6 concentration, and H/L ratio were significantly lower in the IL-LPS group compared to the CL-LPS group, at 3, 12, and 24 h postinjection. In the second experiment, 160 one-day-old broiler chicks were reared under continuous light (23L:1D). At 6 wk of age, 45 broiler chickens were divided into 3 groups. The first group was injected i.v. with 40 mg/Kg BW mel, followed by 3 mg/Kg BW mel 1 h later. The second group was injected with saline followed by 3 mg/kg BW LPS 1 h later. The third group received 2 saline injections 1 h apart. T and B lymphocyte proliferation and antibody production were significantly higher in the mel-LPS group (mel-LPS) compared to the saline-LPS group (s-LPS). Plasma corticosterone concentration and body temperature were significantly lower in the mel-LPS group compared to the s-LPS group at 3 h postinjection. Plasma IL-1-like activity was significantly higher in the s-LPS group compared to the other 2 groups at 0, 3, 12, and 24 h postinjection, while plasma IL-6 concentration was significantly lower in the mel-LPS group compared to the s-LPS group at 3 and 12 h postinjection.

**Key Words:** broilers, LPS, light, melatonin, immunity

## Environment and Management I

**66 Low protein, hydroxy- and keto-amino acid analog supplemented diets for broiler chickens: 1. Performance and carcass weight.** A. Adrizal<sup>1</sup>, P. Patterson\*<sup>1</sup>, C. Angel<sup>2</sup>, and A. Markant<sup>3</sup>, <sup>1</sup>*Department of Poultry Science, Penn State University, University Park*, <sup>2</sup>*Department of Animal & Avian Science, University of Maryland, College Park*, <sup>3</sup>*Fresenius Kabi Deutschland, Germany.*

This study evaluated the potential of reduced dietary CP and amino acid analogs to sustain growth and carcass weight (wt) in broilers (0 to 21

d). Six dietary treatments with 3 levels of CP (22.5, 19.5, and 16.5%) and 2 sources of amino acids (AA): synthetic AA (SA) vs. OH- or keto-AA analogs (KA) were assigned to 36 pens (8 chicks/pen) in a 3 × 2 design. DL-Met in all SA diets was replaced by MHA in all KA diets. L-Ile and L-Val in the 16.5 and 19.5% SA diets were replaced by keto-Ile and keto-Val in the 16.5 and 19.5% KA diets. The 16.5% KA diet was made by replacing L-Leu and L-Phe in the 16.5% SA diet with keto-Leu and keto-Phe. All isocaloric diets (3,050 kcal) were given ad libitum. Lowering CP to 16.5% reduced BW at 7, 14, and 21 d (P