industry. Poultry growers hear or read conflicting sound bites and stories on these issues. An educational program was conducted to provide factual information on these topics to poultry producers. Ten local educational sessions were conducted for poultry producers and company support personnel from seven different poultry companies. In-depth discussions on the topics listed above were presented. Over five hundred producers have attended these programs. The attendees indicated that these programs improved their understanding of these issues. As a follow-up, a statewide one-day educational program for poultry growers was held that provided additional information on these issues. Funding for these educational efforts was provided by the Kentucky Department of Agriculture and the Kentucky Poultry Federation.

Key Words: grower education, issues education


In order to strengthen the youth programs in poultry a training workshop and resource manual was developed for teachers and extension agents. A focus group of six people that were representative of our target audience were invited to participate in an all day discussion on educational materials and activities. From this discussion a resource manual and program was developed. This program used the egg as the basis for providing information on nutrition, food safety, meal planning, science and agriculture. Educational resources and worksheets were developed. The workshops were designed to include a mixture of lectures, activities and hands-on demonstrations. The resource materials were distributed to 180 educators and extension professionals. The training was well received and the program has been implemented in numerous schools and county programs across the state. This program was developed in cooperation with the Kentucky Poultry Federation/Kentucky Egg Council with funding provided by a grant from the US Poultry and Egg Association.

Key Words: youth programs, 4-H, resource manual

Immunology


Chicken major histocompatibility complex (MHC) genes include B-F, MHC class I; B-L, MHC class II; and B-G, MHC class IV. The genes are closely linked on chromosome 16 in the chicken genome. Recombinants between the MHC B-F and B-G regions have been useful to define genetic control of immune responses more precisely. Inbred Line UCD 003, (B17B17) is the genetic base for six congenic lines each containing a single unique MHC recombinant. These congenic lines have undergone ten backcrosses to Line UCD 003 and thus have 99.9% genetic uniformity. A new recombinant, designated R13 (BF17-BG23), was found in a single male from the tenth BC generation for R1 (BF24-BG23). The number of R13 birds was increased through an additional backcross to Line UCD 003 and thus have 99.9% genetic uniformity. A new recombinant, designated R13 (BF17-BG23), was found in a single male from the tenth BC generation for R1 (BF24-BG23). The number of R13 birds was increased through an additional backcross to Line UCD 003 and thus have 99.9% genetic uniformity.

Key Words: immunity, antibody, recombination

89 Identification and characterization of thymosin β4 in chicken macrophages. L. Kannan*1,2, R. Liyanage1, J. Lay1, and N. Rath2, 1University of Arkansas, Fayetteville, 2USDA-ARS, Poultry Production and Product Safety Research Unit, Fayetteville, Arkansas.

Molecular markers are important to understand the developmental, physiological, metabolic, and pathology-related changes in the cells and tissues. Low molecular weight proteins and peptides play pivotal roles in signal transduction and cellular regulation. To prospect for such markers we compared the differences between monocytes and granulocytes isolated from chicken peripheral blood. Using whole cell MALDI/TOF mass spectrometry in the mass range of 1–20 kDa, the results showed association of several minor and few major peaks with each population of cells. One major peak corresponding to mass 4963 was uniquely associated with monocytes but not with the granulocytes. To characterize 4963 we developed a purification procedure using reverse phase liquid chromatography and electrospray ionization mass spectrometry. We purified this peptide from the lysates of two transformed macrophage cell lines HD11 and HTC. Attempts to sequence this peptide using Edman degradation showed it to be N-terminally blocked. Trypsinization and peptide mass finger printing followed by MASCOT database search yielded it to be N-acetylated thymosin β4 (Tβ4). It was further confirmed by collision-induced dissociation. RT-PCR also showed the expression of Tβ4 mRNA by the macrophages. Though originally discovered in thymus gland, Tβ4
appears to be present in many different cells and sequesters G-actin, a cytoskeletal protein. Many mammalian studies have reported Tβ4 to promote wound healing and angiogenesis. We hypothesize that the release of Tβ4 in macrophages may be crucial to post inflammatory tissue repair and resolution of inflammation. Our future research will focus on the modes and modulations of Tβ4 secretion by macrophages.

**Key Words:** macrophage, mass spectrometry, thymosin β4

### 90 Plasma nitric oxide concentrations in broilers after i.v. injections of lipopolysaccharide or microparticles.


Nitric oxide (NO) is a potent vasodilator synthesized from L-arginine by nitric oxide synthase (NOS). Constitutive nitric oxide synthase in endothelial cells (eNOS) produces transient bursts of NO in low but physiologically effective levels. Activated monocytes/macrophages express inducible nitric oxide synthase (iNOS) which produces copious quantities of NO. Previous studies showed that NO attenuates pulmonary hypertensive responses induced by i.v. injections of lipopolysaccharide (LPS) or cellulose microparticles (MP). The present study was designed to determine whether changes in plasma NO concentrations can be used to assess the time course of iNOS expression and NO production in response to LPS or MP injections. Broilers (120 per group) were injected i.v. with 1 mL of PBS (control), 1 mL of LPS (1 mg/mL), or 0.4 mL of MPs (0.02 g/mL). Plasma samples were collected from 10 broilers per group at 15, 30, 45 and 60 min, and at 2, 3, 4, 5, 6, 8, 10 and 12 h post-injection. Total plasma NO concentrations were analyzed by nitrate + nitrite assay. After PBS or MP injection plasma NO did not change throughout the 12 h period. In LPS-injected broilers plasma NO increased from non-detectable levels at 15 min to 26.3 ± 4.0 µM by 3 h post-injection, reached peak levels of 85.1 ± 10.6 µM at 5 h, and returned to baseline levels similar to MP- and PBS-injected broilers by 12 h post-injection. We conclude that LPS triggered widespread iNOS expression by circulating monocytes/macrophages, resulting in copious NO production as reflected by significant increases in total plasma NO. Proportionally few monocytes/macrophages responded to MPs entrapped in pulmonary arterioles, consequently the quantities of NO produced by iNOS in these activated leukocytes or by eNOS in the pulmonary vasculature had a minimal impact on total plasma NO concentrations. Total plasma NO levels in broilers did reflect the time course of massive iNOS activation in response to LPS, but biologically relevant quantities of NO produced by iNOS and eNOS in response to entrapped MPs were too low to affect total plasma NO.

**Key Words:** nitric oxide, lipopolysaccharide, broiler

### 91 Time-course of expression of inducible nitric oxide synthase in lungs following intravenous cellulose microparticle injection in three broilers lines.


Injection of microparticles (MP) has been used to select broilers with sufficiently robust pulmonary vascular capacity to resist onset of pulmonary hypertension (PH). Injected MP (i.v.) become lodged in the pulmonary vasculature where they increase the resistance to blood flow and trigger PH. MP lodged in vessels initiate aggregation of mononuclear leukocytes within the surrounding lung parenchyma. Activated avian macrophages express inducible nitric oxide synthase (iNOS), which produces large quantities of nitric oxide (NO). NO attenuates PH through its role as a vasodilator and its ability to modulate the release of key vasoconstrictors. The objective of this study was to establish the time-course over which i.v. MP injections induce the responding macrophages to express iNOS. Broilers from Control (C), PHS-Resistant (R), and PHS-Susceptible (S) lines were injected with MP (10 birds/line/time point) and their left lung was collected at 0 h (no MP) and 2, 24 and 48 h post-injection. The lungs were snap frozen and stored at -80°C for gene expression and immunohistochemical studies. iNOS expression was studied by quantitative real time 2-step RT-PCR (Taqman) and was quantified by relative standard curve method and expressed as fold change in iNOS mRNA levels. At 2 h, there was no line difference in the iNOS expression (P = 0.190). At 24 h, iNOS expression in line R (15.8 ± 6.4) was higher (P = 0.074) compared to lines C (5.4 ± 1.0) and S (2.4 ± 0.6). At 48 h, lines R (24.9 ± 6.4) and S (26.2 ± 4.4) had higher (P = 0.014) iNOS expression than line C (5.4 ± 1.5). Immunohistochemistry of NO-producing cells by NADPH-diaphorase histochemical staining revealed the presence of macrophages in broilers (KUL01 mAb) and NO, respectively, in the large mononuclear aggregates that had formed around the MP-occluded vessels by 24 h onwards. These observations suggest that the macrophages recruited to the site of entrapped MP were activated and expressed iNOS to attenuate the MP-induced PH via NO production.

**Key Words:** iNOS, macrophage, broiler lung

### 92 Enumeration of macrophages in the cecae following challenge with Salmonella enteritidis and treatment with a probiotic culture.


During previous studies with neonatal chicks, we have found consistent reduction of Salmonella enteritidis (SE) within 24 h of infection when a probiotic culture (FM-B11) was administered one hour after challenge. Two studies were performed to determine whether the observed reduction in SE could be associated with the numbers of macrophages present in cecal tissue. Briefly, 100 day of hatch chicks were randomly assigned to four groups. Two groups were challenged by oral gavage with approximately 10⁶ cfu of SE, and two groups received sterile saline by oral gavage (n = 25 per group). One hour later, one challenged group and one un-challenged group were treated by oral gavage with approximately 10⁶ cfu of FM-B11. The other two groups received the vehicle, skim milk. Twenty-four hours after treatment, the birds were humanely killed, and the cecal tonsils removed aseptically and enriched for detection of SE. Also, one cecum was removed and snap-frozen in liquid nitrogen. The frozen cecae were embedded in OCT medium and cut into 8um thick sections. Immunohistochemistry was performed on these tissues using a monoclonal antibody specific for chicken monocytes and macrophages (KUL01). In both experiments there was a significant reduction of SE in challenged chicks receiving probiotic treatment compared to untreated controls (Exp 1, 67% reduction; Exp 2, 59% reduction). Enumeration of macrophages in cecal tissue in experiment one showed an increase (p<0.02) in macrophages in the cecae of chicks receiving only probiotic treatment compared to chicks receiving neither treatment nor challenge (3.32 cells/10,000 μm² and 3.66 cells/10,000 μm²). However, in experiment two, there were no significant differences in the numbers of macrophages between any groups. These data do not suggest that
there is a biologically meaningful relationship between the number of macrophages in the cecae of chicks and reduction of SE due to probiotic treatment.

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

93 Lycopene and α-tocopherol incorporation into egg yolks and their effects on laying hen immune function. J. Olson* and E. Koutsos, California Polytechnic State University, San Luis Obispo.

Lycopene is a carotenoid that has several biological functions associated with decreased cancer risk. The purpose of these trials was to examine the ability of the laying hen (Hyline W36) to deposit dietary lycopene and/or α-tocopherol into the egg yolk and to investigate their effects on immune function. All birds were housed in commercial cages, had ad libitum access to water, and were fed 100 g/bird*day for 15 days in trial 1 and 17 days in trial 2. Trial 1 consisted of four dietary levels of lycopene (0, 65, 257, 650 mg lycopene/kg diet). HPLC analysis revealed that dietary lycopene was incorporated into egg yolks (p<0.01 versus 0 mg lycopene). Regression analysis shows that maximum incorporation of lycopene into egg yolk occurs at 420 mg lycopene/kg diet resulted in 0.09 mg lycopene per egg yolk. Trial 2 was designed as a 3 x 2 factorial, with three levels of lycopene (0, 420, 840 mg lycopene/kg diet), and two levels of α-tocopherol (0, 200mg α-tocopherol/kg diet). Inflammatory response was measured following LPS administration (subcutaneously at 1 mg LPS/kg BW). LPS increased liver and spleen weights for all diets (p<0.01), although body weight was not affected by LPS (p=0.82). There was an interaction between α-tocopherol and lycopene (p<0.01); at 24 h post-LPS, birds fed 200 mg α-tocopherol had greater liver weights compared to control diets. LPS also increased plasma haptoglobin (p<0.01), but neither lycopene nor α-tocopherol affected haptoglobin concentrations (p>0.39 for each). Cutaneous basophil hypersensitivity was measured following PHA administration (injected in the toe web at 0.2 mg/bird). Toe web swelling was increased by PHA (p<0.01) and birds fed diets with 420 mg lycopene/kg had greater swelling at six hours compared to the control diets (p<0.01). These data indicate that lycopene can be incorporated into egg yolks and that α-tocopherol and lycopene may not have beneficial effects to the laying hen’s immune system at some dietary levels.

**Key Words:** lycopene, α-tocopherol, laying hen

94 A novel formula for predicting infectious bursal disease vaccination time on chick weight rather than age. A. Vaziry*1, D. Venne2, D. Frenette1, and A. Silim1, 1Faculté de Médecine Vétérinaire, Université de Montréal, St. Hyacinthe, QB, Canada, 2Couvoir Scott, Scott Jonction, QB, Canada.

Growth rate in broiler birds has increased substantially in the last decade due to improvement in genetics, feed formulation, cleaner environment and bio-security. As a result, it has become necessary to review and revise prediction method for vaccination in chicks. This study was undertaken to determine the possible use of weight-gain rate rather than age in predicting vaccination time. Two groups of one-day-old broilers originating from old and young breeders respectively and with different levels of maternal antibodies against infectious bursal disease virus (IBDV) were used in this study. Both groups were divided into 2 sub-groups and subjected to two feed regiments: groups Ia and Ia were fed broiler feed for normal growth rate and groups Ib and Iib were fed breeder feed for slower growth rate. At 1, 4, 8, 12, 16, 22, 29 and 36 days of age, 22 chicks in each group were weighed and blood samples collected. Serum samples were tested for antibodies against IBDV by ELISA (Synbiotics Corporation) and virus neutralization test (VNT). The maternal antibody decline curves for each group were plotted out according to chick age and chick weight. Fast-growing birds in groups Ia and Ia showed a faster rate of antibody decline whereas slow-growing birds in groups Ib and Iib had a slower rate of antibody decline. No difference in antibody decline rate was observed between chicks from younger versus older breeders. Based on the effect of weight-gain on maternal antibody decline, a new way of predicting vaccination time for IBDV based on measuring maternal antibody titers at 4 days of age was proposed and tested. The predicted antibody decline was shown to correspond to the real ELISA titers measured in our experiments (R2 = 0.9812) whereas a lower correlation (R2 = 0.5853) between real ELISA titers and the titers predicted by current method using age-based Deventer formula was observed.

**Key Words:** maternal antibody, vaccination formula, infectious bursal disease

95 Evaluation of Coccivac-B® or Bio-Cox® (salinomycin) for control of field strain *Eimeria* in broilers on two different feeding programs. J. T. Lee*1, C. Broussard2, S. Fitz-Coy2, P. Burke2, N. Ecket1, S. Stevens1, P. Anderson1, and D. J. Caldwell1, 1Texas A&M University, College Station, 2Schering-Plough Animal Health, Union, New Jersey.

The objective of this study was to compare Coccivac-B® or Bio-Cox® (salinomycin) for controlling field strain *Eimeria* in broilers reared on two different dietary rations varying mostly in protein concentration. Each treatment included 10 replicate pens for a total of 40 pens with 43 broilers placed per pen. Broilers were reared to 50 days on a four-phase feeding program: starter (Day 1-14), grower (Day 15-29), finisher (Day 30-40), and withdrawal (Day 41-50). Both diets evaluated in this study, Diet A (control) or Diet B (test diet), were formulated to simulate a local commercial integrator’s diets by season. The dietary protein profile for Diet A was 21.5% (starter), 20% (grower), 16.5% (finisher), and 15.75% (withdrawal), and Diet B was 22% (starter), 19.6% (grower), 17.8% (finisher), and 17.5% (withdrawal). On day 14 of grow-out, *Eimeria* collected from commercial broiler farms in Texas were spray applied to the litter in all pens. Broilers reared on Diet B were heavier (P<0.05) at Day 40 while body weights at day 50 were similar (P>.05) for all groups. Broilers fed Diet B had lower (P<0.05) mortality corrected feed conversions ratios (FCR) during the starter and finisher diets. Broilers fed salinomycin had lower (P<0.05) mortality corrected FCR for the starter and grower diets while vaccinated broilers had lower (P<0.05) mortality corrected FCR during the withdrawal period. Cumulative FCR for the entire grow out period were similar (P>0.05) for all groups. The results of this investigation suggest that feeding an appropriately formulated diet while vaccinating broilers with Coccivac-B® as an alternative to the use of salinomycin yields at least equivalent if not elevated performance in the presence of field-strain *Eimeria* during grow-out.

**Key Words:** dietary protein, vaccination, *Eimeria*
96 Evaluation of heterophil function in molting hens fed an alfalfa diet. J. L. McReynolds*, 1, K. J. Genovese1, H. He1, J. A. Byrd3, S. C. Ricke2, D. J. Nisbet1, and M. H. Kogut1, 1USDA-ARS-SPARC, Food & Feed Safety Research Unit, College Station, Texas, 2University of Arkansas, Fayetteville.

Currently in the U.S. feed deprivation is used to induce molting and stimulate multiple egg-laying cycles in laying hens for commercial egg production. Previous reports show that alfalfa is effective in inducing a molt as well as producing protection against Salmonella enteritidis (SE) organ invasion. Our laboratory has also shown that immune function is significantly reduced during molting. The present investigation was performed to evaluate heterophil function during an induced molt in hens fed alfalfa. Two replicate experiments utilized hens over 65 wk of age that were divided into 6 groups of 12 hens each and placed in individual laying cages. Two wk prior to dietary changes, hens were placed on an 8-h light and 16 h-dark photoperiod that continued for the 12-day experiment. Blood samples were taken from the hens during three sampling periods, 1-2d, 5-6d, and 11-12d. Treatments groups consisted of non-fed hens (NF), full-fed hens (FF) and alfalfa-fed hens (AF). Heterophil function was measured using several in vitro assays. To evaluate the oxidative burst of heterophils, phorbol 12-myristate acetate was used to stimulate collected cells from each treatment group. Both the FF and AF birds oxidative response was significantly (P<0.05) higher than the NF birds for all three sampling periods. During the degranulation assay heterophils were stimulated with opsonized SE for one hour. Results showed a significant (P<0.05) increase in degranulation during all three sampling periods when the AF birds were compared to the NF controls. These results confirm that heterophil function is significantly reduced in NF birds during an induced molt. More importantly AF birds showed an increased immune response during a 12d molting period. The commercial layer industry should consider using alfalfa as a diet when developing commercial molting programs.

Key Words: chicken, heterophil, molting

97 Selenium source affects the properties of the anti-Gal natural antibody system in laying hens. P. Cotter*1, A. Pescatore1, A. Cantor1, M. Ford1, T. Ao2, and J. Pierce2, 1Framingham State College, Framingham, Massachusetts, 2Alltech, Inc., Nicholasville, Kentucky, 3University of Kentucky, Lexington.

Anti-Gal, a naturally occurring agglutinin found in many avian species, represents a useful parameter in assessing immune status. In chickens younger than 2 wk titers are low but these soon rise and in older chickens they are present at very high levels. The detection of anti-Gal is facilitated because rabbit erythrocytes expressing the α-Gal epitope are agglutinated. Both IgM and IgG anti-Gal isotypes occur and because IgM fixes complement agglutinated rabbit cells are lysed at low serum dilutions. Agglutination coupled with lysis allows estimation of 3 parameters from a single microtiter test. HA1 measures strongly agglutinated cells at low serum dilutions, HA2 measures weaker agglutination at higher dilutions. The degree of lysis representing the activity of complement is measurable in the first two or three serum dilutions.

Selenium has been shown to interact with vitamin E in affecting the maturation and function of lymphocyte subpopulations and so it influences immunity. Mature Brown (BL) and White (WL) commercial hens were raised on basal, selenite sodium, or organic selenium diets. Serum anti-Gal levels and lysis were measured by a standard microtiter procedure. HA1 titers were higher in BL vs. WL (log2, 4.6 vs. 4.3, P<0.03) but the highest titers occurred in WL fed organic selenium. Lysis was higher in WL vs. BL (P < 0.01) and lower in hens fed either selenite or organic selenium. HA2 titers, presumably representing agglutination predominated by IgG averaged (log2) 9.4 across treatments were unaffected by diet. There were clear differences in agglutinin quality reflecting both hen type and diet. The results indicate that selenium source affects two aspects of humoral immunity, IgM and complement. They extend the utility of the anti-Gal system as a convenient assessment tool with a capacity for measuring the importance of nutritional status on humoral immunity.

Key Words: selenium, humoral immunity, anti-Gal

98 Developing chicken lymphocytes increase glucose metabolism after hatch. S. Rudrappa and B. Humphrey*, University of Maryland, College Park.

Coordinated regulation of energy metabolism in lymphocytes is vital for their development. Three experiments examined glucose, glutamine and fatty acid metabolism in developing lymphocytes from broiler chickens during embryogenesis (e) and posthatch (d). In experiment one, bursacyte and thymocytes were sampled (n=5) on e17, e20, d1, d7 and d14. Thymocyte glucose transporter-3 (Glut-3) and hexokinase (HK) mRNA abundance increased 10-fold from e17 to d14 (P<0.05) and Glut-1 mRNA abundance increased 3-fold from e17 to d14 (P<0.05). Bursacyte Glut-3 mRNA abundance increased 2.5-fold from e17 to d14 while Glut-1 mRNA abundance decreased 2.5-fold from e17 to d14 (P<0.05). Bursacyte HK mRNA abundance increased 2.5-fold from e17 to e20 (P=0.05). Thymocyte glutamine transporter (SNAT-1), SNAT-2 and glutaminase (GA) mRNA abundance increased at least 5-fold from e17 to d7 (P<0.05). Bursacyte SNAT-1 and GA mRNA abundance increased 3-fold from e17 to d7 (P<0.05). Carnitine palmitoyl transferase-1 (CPT-1) mRNA abundance decreased 5-fold from e17 to e20 in bursacytes and increased 5-fold from e17 to d1 in thymocytes (P<0.05). In experiment two, bursacytes, thymocytes and serum were sampled (n=6) on e20, d1, d3 and d7. HK enzyme activity increased to maximum levels between e20 to d3 in bursacytes and d3 to d7 in thymocytes (P<0.05). GA enzyme activity increased from e20 to d7 in bursacytes (P<0.05) and did not change in thymocytes (P>0.05). CPT enzyme activity did not change over time in either cell population (P>0.05). In experiment three, bursacytes and thymocytes were sampled (n=6) on d1, d7 and d14. Fluorescent D-glucose uptake by bursacytes and thymocytes was greater on d14 compared to d1 and d7 (P<0.05). Results indicate that (a) developing B and T lymphocytes increase glucose metabolism with no change in fatty acid metabolism during the first two weeks after hatch; (b) developing B lymphocytes and not T lymphocytes increase glutamine metabolism after hatch.

Key Words: lymphocyte, glucose, glutamine