

buffer and analyzed by two-dimensional gel electrophoresis. Comparative analysis of Coomassie blue stained protein spots from individual samples from both the groups using a Melanie software, revealed 7 and 3 differentially expressed proteins in tissue and conditioned media respectively. Tryptic digests of the protein spots were analyzed by automated matrix assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF-MS) followed by a MASCOT data base search. Five of the tissue proteins were identified as calumenin, actin, chondroitin sulphate, matrilin, and one predicted protein of *Gallus gallus*. The conditioned media proteins corresponded to collagen II, triose phosphate isomerase alpha chain, and an unidentified protein. All these proteins were down regulated in TD except calumenin. Functional relevance of these proteins indicates that they may be involved in the maintenance of cartilage integrity such as their survival, maturation, hypertrophy, and mineralization. The compromise of these functions is likely to affect endochondral bone formation leading to the development of TD.

Key Words: tibial dyschondroplasia, 2D gel electrophoresis, proteomics

199 Astrovirus infection induces sodium malabsorption and redistributes sodium hydrogen exchanger expression. P. K. Nighot*, A. J. Moeser, R. A. Ali, A. T. Blikslager, and M. D. Koci, *North Carolina State University, Raleigh*.

Turkey astrovirus type 2 (TAstV-2) is recognized as a major cause of enteritis in poult in the US and around the world; however our understanding of how it induces disease is limited. Previous studies have demonstrated TAstV-2 infection induces severe watery diarrhea and growth suppression with no significant change in intestinal morphology. More recently, other groups have reported the capsid virus of human astroviruses can induce changes to intestinal cell barrier function *in vitro*. To determine the patho-physiology of TAstV-2-mediated diarrhea we assayed for changes in barrier function and ion transport of control and infected poult jejunum by mounting either intact or stripped intestinal mucosa from poult at 4 days post-infection (peak of clinical signs) on Ussing chambers. Isotopic flux studies demonstrated impaired Na⁺ absorption with reduced short circuit current and increased conductance

in astrovirus-infected jejunum. Electron microscopic examinations of these tissues revealed the terminal web region displayed frequent dense aggregations in TAstV-2 infected intestine. Rearrangement of apical F-actin in form of thick, dense aggregations, particularly in the areas of viral localization, was seen in TAstV-2-infected intestine. The actin rearrangement was limited to morphological alterations as total actin content and ratio of G: F actin in intestinal mucosa was not altered after infection with TAstV-2. Although the total protein expression for the major apical membrane Sodium Hydrogen Exchanger, NHE3, was not changed in western blot analyses of astrovirus-infected jejunum, a significant shift was observed for expression of NHE3 from detergent insoluble fractions to detergent soluble fractions of TAstV-2 infected jejunum. Alternatively, total expression of NHE2 was up regulated in infected jejunum. Taken together, astrovirus TAstV-2 induces malabsorptive diarrhea associated with actin re-arrangement and redistribution of apical membrane NHE3.

Key Words: astrovirus, diarrhea, NHE3

200 Biosecurity, surveillance and outbreak response management. A. N. Akunzule*, *Animal Production Institute, Accra, Ghana*.

Ghana experienced outbreaks of highly pathogenic H5N1 avian influenza (AI) in local poultry in April 2006, May 2007, and June 2007. Development agencies provided financial and material aid to assist the government of Ghana to expand their capacity and expertise should another outbreak occur. A course teaching Ghanaian officials about appropriate surveillance and the proper use of commodities was provided by the United States Agency for International Development (USAID). A plan to greatly expand the capacity was then developed using the "train the trainer" concept where experts are developed that will then teach others. In Ghana, this was done by developing four master training of trainers workshops at the national level in Accra. The master trainers were then tasked with providing this training at the regional and village level in three agro-ecological zones of Ghana in Aburi, Tamale and Kumasi. There are now 213 people capable of responding safely to the next H5N1 AI outbreak.

Key Words: biosecurity, surveillance, outbreak

Immunology II

201 Synergistic effect of dietary *Curcuma*, *Capsicum*, and *Lentinus* on enhancing local protective immunity against *Eimeria acervulina* infection. H. Lillehoj*¹, S. Jang¹, D. Kim¹, C. Ionescu², D. Bravo², and S-H. Lee¹, ¹*Animal and Natural Resources Institute, Agricultural Research Service-U.S. Department of Agriculture, Beltsville, MD*, ²*Pancosma S.A., Geneva, Switzerland*.

The protective effect of orally administered *Curcuma longa* (turmeric), *Capsicum annum* and *C. frutescens* (hot pepper), and *Lentinus edodes* (shiitake mushroom) on avian coccidiosis was evaluated in young broilers. Broiler chickens were continuously fed with a standard diet or standard diet supplemented with *Curcuma*, *Capsicum/Lentinus* or *Curcuma/Capsicum/Lentinus* from hatch. Body weight gain, fecal oocyst shedding, antibody titers, and pro-inflammatory cytokine gene expression were measured as parameters of protective immunity following challenge with *E. acervulina*. Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet showed significantly improved body weight gain

compared with birds on the standard diet alone or birds given *Capsicum/Lentinus*-supplemented diet following challenge with *E. acervulina*. Chickens fed the *Curcuma/Capsicum/Lentinus*-supplemented diet shed significantly reduced fecal oocysts and produced higher serum antibody titers compared to the groups fed the standard diet alone or fed *Curcuma* or *Capsicum/Lentinus*. Finally, the levels of local cytokine transcripts of IL-1 β , IL-6, IL-15, and IFN- γ were consistently higher in the *Curcuma/Capsicum/Lentinus*-fed group compared to the controls fed only the standard diet, *Curcuma*, or *Capsicum/Lentinus* groups. This study provides first immunological evidence that dietary supplementation of turmeric, pepper, and shiitake work synergistically to enhance local innate immunity and provide higher protective immunity against *E. acervulina* infection.

Key Words: broiler, coccidiosis, *Curcuma*, *Capsicum*, cytokine

202 Chicken CD25⁺: Chicken T regulatory cells. R. Shanmugasundaram and R. Selvaraj*, *The Ohio State University, Wooster.*

T regulatory cells are a subset of T cells that specialize in immune suppression. In mammals FoxP3 is the master gene regulator of Tregs. We were not able to identify a putative FoxP3 analogue in the chickens and zebra finches. This suggests that avian taxa lack FoxP3 (and hence Tregs) and/or avian Tregs are independent of mammalian FoxP3 signals. Chickens CD25⁺ cells were isolated and characterized. The distribution of CD25⁺ cells in chicken lymphoid organs and peripheral blood was studied. On an average, 5 to 8% of lymphocytes in different organs were CD25⁺. CD25⁺ cells were distributed in three major subpopulations, CD4⁺25⁺, CD8⁺25⁺ and CD4⁺8⁺25⁺. The ratio of these three subpopulations differed widely among organs. The thymus had the greatest CD4⁺8⁺25⁺, while the spleen had a greater CD4⁺25⁺ and blood had a greater CD8⁺25⁺ subpopulations. Both naïve and in vitro-grown CD25⁺ cells from thymus, spleen and blood suppressed T responder cell proliferation, while CD25⁻ did not suppress T responder cell population. Cytokine production profile of CD25⁺ cells was also quantified. The data suggest that CD25⁺ are the chicken analogue of T regulatory cells.

Key Words: T regulatory, avian, CD25

203 Elucidation of genes and pathways regulated by the avian miRNA, *miR-10a*. J. A. Hicks*, N. Trakooljul, and H. C. Liu, *North Carolina State University, Raleigh.*

In recent years it has been discovered that small non-coding RNA (ncRNA) can post-transcriptionally regulate gene expression. MicroRNA (miRNA) is a family of small (19-24nt) ncRNA that serves as a key regulator of a variety of biological processes, such as cell differentiation, and has been linked to disease development, particularly cancer. The initial focus on elucidating the role(s) of miRNA in eukaryotic gene regulation has been on expression profiling. This profiling has revealed that the eukaryotic genome encodes hundreds of miRNAs which display a wide range of temporal and spatial expression patterns. The next step in the study of miRNA will be to identify target genes and pathways controlled by miRNAs. We recently reported the miRNA expression profiles of miRNA in the spleen and bursa of the developing chick embryo. We found that the miRNA, *miR-10a*, is highly expressed in the embryonic spleen, which suggests that this miRNA is an important gene regulator during embryonic spleen development. In order to further elucidate the role(s) of *miR-10a* in avian spleen development, the potential genes and pathways targeted by *miR-10a* were identified. The miRNA target prediction algorithm miRanda was employed to identify potential *miR-10a* target genes. These target genes were then validated using a retroviral-based RNA interference (RNAi) strategy. Additionally, to identify *miR-10a* regulated pathways, a microarray strategy was employed into which a *miR-10a* inhibitor was introduced into spleen cells in order to knock-down *miR-10a* expression. Our findings suggest that *miR-10a* regulates hematopoiesis and megakaryocytopoiesis as it potentially targets the genes kruppel-like factor 11 and WD repeat domain 1. Additionally *miR-10a* is also likely an important regulator in the immune system as it can target integrin β 1. Furthermore our microarray data suggests that *miR-10a* is also involved in regulating the complement system. Together these results suggest that *miR-10a* plays a variety of roles in the avian spleen.

Key Words: avian development, immune system, gene regulation, microRNA, microarray

204 Associations of chicken Mx polymorphism with antiviral responses in avian influenza virus infected embryo and broilers. Y. Wang*¹, V. Brahmakshatriya², B. Lupiani², S. Reddy², R. Okimoto³, X. Li¹, H. Chiang¹, and H. Zhou¹, ¹Texas A&M University, Department of Poultry Science, College Station, ²Texas A&M University, Department of Veterinary Pathobiology, College of Veterinary Medicine, College Station, ³Cobb-Vantress, Inc, Siloam Springs, AR.

Avian influenza (AI) is a major respiratory disease of poultry that can cause catastrophic losses to the commercial poultry industry. It is imperative to develop effective control and treatment measures to prevent the spread of AI. The Mx protein has been shown to confer antiviral responses to influenza viruses in mouse. One nonsynonymous substitution (S631N) in the chicken Mx protein has been reported to be associated with resistance to avian influenza virus (AIV) in vitro in chickens. In this study we examined the associations of Mx polymorphism with the antiviral response in chicken embryo and young chicks. The embryo and young chicks were generated from the cross of Mx heterozygous parents with expected segregating ratio of 1:2:1 in the progeny. A PCR-RFLP was developed to genotype the Mx gene (NN, NS and SS) from 119 embryos and 24 chickens. Thirteen day old embryonated chicken eggs were inoculated with 10 EID50 H5N9 AIV. Hemagglutination assay was used to evaluate virus replication in chicken embryos. Hemagglutinating units (HAU) in allantoic fluid were determined at 48 hours post-inoculation for all infected embryos. Mean virus titers for the different phenotypes were 256 HAU for NN, 222.86 HAU for NS and 337.79 HAU for SS. For the in vivo study, 24 one-week old broilers were inoculated with 106 EID50 H5N3 AIV and virus titers in lungs were evaluated at day 4 post inoculation. The mean virus titers for three genotypes were 398.11, 501.19 and 1000 TCID50/ml for NN, NS and SS, respectively. Our results indicate that there is a tendency for birds with NN genotypes to have lower virus titer than ones with SS both in ovo and in vivo, although the association between genotypes and virus titers was not significant ($P > 0.05$). Our results indicate that further studies including additional Mx alleles and more animals are needed. The knowledge generated by this study provides valuable information on the effect of the Mx gene on the genetic resistance to AIV in chickens and its potential application in the poultry breeding industry.

Key Words: chicken, Mx gene, avian influenza, virus

205 High energy electron-beam irradiation: A vaccine development technology for pathogens in commercial poultry. J. L. McReynolds*¹, M. A. Davidson², K. J. Genovese¹, P. R. Jesudhasan², S. E. Duke¹, J. A. Byrd¹, M. A. Cepada², and S. D. Pallai², ¹USDA-ARS-SPARC-FFSRU, College Station, TX, ²Texas A&M University, Department of Poultry Science, College Station.

Our laboratories are investigating the use of High Energy (10 MeV) Electron-Beam (E-beam) Irradiation for its potential use in vaccine development. It is well known that ionizing radiation damages nucleic acids by direct and/or indirect effects thereby inactivating the organism. Though the cells are inactivated, our studies suggest that the surface antigenic properties of *Salmonella enteritidis* (SE) are unaltered. The present investigations (3 replicates) were performed to evaluate the efficacy of the E-beam vaccine on SE colonization during an induced molt. Laying hens were divided into 3 groups (12 hens/group): negative control, positive control, and SE-vaccinated. SE-vaccinated birds received a 1×10^6 CFU/mL/bird injection in the breast muscle; controls were sham injected with saline. One wk prior to the beginning of the study, hens were placed on an 8-h light and 16-h dark photoperiod that

continued for the 12-day induced molt. Positive control and vaccinated birds were challenged with 1×10^6 CFU/mL/bird (15 days post vaccination) via oral gavage. SE colonization in the liver, spleen, ceca and ovaries was significantly ($P < 0.05$) reduced in the E-beam SE vaccinated birds compared to the positive controls. E-beam technology can be customized to meet an integrators needs and can be extremely inexpensive since there is no need for R & D costs to develop the irradiation source or vaccine organisms. The results show that the immunological effects of this E-beam SE provided protection against SE colonization in laying hens during an induced molt.

Key Words: chicken, electron-beam, vaccination, *Salmonella*

206 Evidence of Mx independent genetic resistance to viral infection. M. D. Koci*, R. A. Ali, M. D. Quiles, E. Strain, and C. M. Ashwell, *North Carolina State University, Raleigh.*

The ability of individual cells to detect and respond to the presence of virus is a critical first step in the body's ability to resist viral infection and disease. Much effort has focused on identifying proteins involved in the antiviral pathway. Several gene products have been described as part of the innate antiviral response, but our knowledge of how these proteins function or how polymorphisms in these genes affect the antiviral response is limited. In mammals, the Mx gene has been described

to have antiviral properties against various several virus families. Initial studies which identified the avian Mx homologue demonstrated it lacked antiviral activity. More recent studies have demonstrated that commercial and research lines of chickens have polymorphisms in their Mx gene. One variant in particular (G2032A) has been described to have antiviral activity. Sequencing of 12 unrelated individuals from each of 9 commercial layer lines lead to the identification of additional variants in the Mx gene. To begin to understand if the sequence polymorphisms in the Mx alleles are associated with antiviral activity, we isolated chicken embryo fibroblast (CEF) cells from commercial layers. Four CEF cell lines were produced (3, 6, 7, and 8) and genotyped for Mx and MHC I. These CEF cells were infected in vitro with an interferon sensitive virus (vesicular stomatitis virus) and assayed for resistance to virus infection. The results from these studies demonstrated two CEF lines with increased resistance (3 and 7) and two lines with decreased resistance (6 and 8). To further assess the role of Mx and MHC in viral resistance, genetic lines which produced CEF line 7 and line 8 were crossed, and resulting embryos were genotyped such that additional 11 CEF lines that provide each possible Mx and MHC combination were generated. Subsequent in vitro analysis of the crossed CEF cells demonstrated resistance to infection is not associated with Mx or MHC genotype. These results suggest that while some genotypes of Mx have been described to have antiviral activity, expression of the Ser631Asn is not sufficient to increase the innate resistance of CEF cells to viral infection.

Key Words: antiviral, innate immunity, Mx

Extension and Instruction

207 A survey of the economic, environmental, public policy and production issues facing the poultry industry in Louisiana. T. A. Lavergne*, S. M. Derouen, and G. M. Hay, *Louisiana State University AgCenter, Baton Rouge.*

A survey was conducted to determine the opinions of commercial producers, integrator representatives, allied industry representatives, regulatory personnel, and academic personnel concerning issues facing the poultry industry in Louisiana. Respondents were asked to rate 11 items within four categories of issues: Economic issues, Environmental issues, Public policy issues, and Production issues. The items were rated from 5 (extremely important) to 1 (not important). Of the 74 respondents, 45 were commercial growers, 12 were employed in the allied poultry industry, two were employed in the state's regulatory agency, six were academics, and nine were employed by integrator companies. The ratings of commercial poultry producers were similar to the average of the responses of all other categories of respondents. For economic issues, the ratings of commercial producers and all others ranged from 4.1 to 4.8 and 3.54 to 4.86, respectively. Rising input costs received the highest rating for economic issues. For environmental issues, the ratings of commercial producers and all others ranged from 3.3 to 4.4 and 3.0 to 4.5, respectively. Public perception of the environmental effects of animal agriculture received the highest rating for environmental issues. For public policy issues, the ratings of commercial producers and all others ranged from 3.5 to 4.5 and 3.3 to 4.5, respectively. Consumer confidence in food and animal product safety received the highest rating for public policy issues. For production issues, the ratings of commercial producers and all others ranged from 3.5 to 4.8 and 3.0 to 4.6, respectively. Improving production efficiency received the highest rating for production issues. All respondents rated each item as moderately, substantially, or extremely important. Economic issues received the most substantially important and higher ratings. The responses indicate that economic,

environmental, public policy, and production issues are important to these respondents involved in Louisiana's poultry industry.

Key Words: poultry, economic issues, environmental issues, public policy issues, production issues

208 Multifacet, grower-driven education program. M. Miller¹, J. P. Jacob*², A. J. Pescatore², D. G. Overhults³, and R. S. Gates⁴, ¹*Kentucky Poultry Federation, Winchester, KY*, ²*University of Kentucky, Animal & Food Sciences, Lexington*, ³*University of Kentucky, Biosystems & Agricultural Engineering, Lexington*, ⁴*University of Illinois, Agricultural & Biological Engineering, Urbana.*

The Kentucky Poultry Federation received a grant from the KY Ag Development Board to support the state broiler industry. The project objective is to enhance the productivity and sustainability of KY poultry growers. The project is divided into 2 parts. In the 1st part energy audits are completed for a sample of poultry houses for each of the 6 integrators in the state. The objective of these audits is 2-fold. The 1st goal is to identify common areas where growers can improve energy efficiency by properly operating and maintaining existing equipment. The 2nd goal is to develop recommendations for cost effective upgrades that will help growers reduce their energy use. Information obtained from the audits is then used to develop complex-specific educational workshops for all their growers. The same information is used to develop an educational binder which is designed to serve as a reference book for the producers. The growers are kept up to date on the progress of this part of the project by means of a website and quarterly newsletter.

In the 2nd part of the project, a state-wide producer education conference is held each year and addresses current issues affecting all producers