

once-used litter with 50 birds each. Experiment 1 was conducted February-March; Experiment 2, April-May; and Experiment 3, June-July. Birds received a corn-soybean meal starter (0.68 kg/bird; 22% CP, 3087 kcal/kg ME), grower (1.36 kg/bird; 20% CP, 3131 kcal/kg ME), and finisher (c.a. 2.27 kg/bird; 17.5% CP, 3197 kcal/kg ME) to 42 days of age. Treatments were control (CON); Poultry Litter Treatment (PLT), Poultry Guard (PG) or 50:50 All-Clear:PLT (ACPLT) at 100 lbs/1,000 ft² and liquid alum (A-7) at 30 gals/1000 ft² with six pens per treatment. Treatments were top-applied 24-hrs prior to placement with a 14-day downtime between experiments. Feed and water were provided ad libitum with 23 hr light. Litter samples were obtained initially, at experiment termination, and during litter reconditioning between experiments. Trace element levels are presented on a dry matter basis.

Specific treatment regimens resulted in increased concentrations of litter trace elements. As compared to CON, PLT contributed an increase in Na accumulation (10,546 vs. 6576 ppm); PG increased Al (1018 vs. 623 ppm) and Fe (1433 vs. 1176 ppm); ACPLT increased both Na (8959 vs. 6576 ppm) and Al (1251 vs. 632 ppm); and A-7 increased Al to 2865 ppm as compared to CON (623 ppm). Initial levels of minerals such as P, Ca, and K; Cu, Fe, Zn, and Mn were 1.06, 1.99, and 1.56%; 22.4, 649, 217, and 219 ppm, respectively. At the end of the third experiment, levels were 2.16, 3.83, and 2.89%; 35.7, 1212, 409, and 397 ppm, respectively. Nitrogen composition following each experiment increased from 3.32 to 3.52 to 3.73%, respectively. Reconditioning of litter between experiments yielded an average reduction in nitrogen of 26.2 and 17.9% prior to the start of Experiment 2 and 3, respectively. Trace element loading of soils from prolonged application of poultry litter requires additional investigation to determine its detrimental effects on soil and water quality.

Key Words: trace element, litter treatment, broiler

161 Using mortality compost in vegetable production. C. Dunkley*¹, D. Cunningham², C. Ritz², and S. Rahjeev¹, ¹University of Georgia, Tifton, ²University of Georgia, Athens.

A study was conducted to determine the effectiveness of composting to breakdown the carcasses of poultry mortality and destroy pathogenic microbes that may be present. The study was divided into two phases, the first involving the composting of daily mortality while the second used the composted material to amend vegetable plots planted with cabbage seedlings. Phase 1 was first conducted during the summer and then repeated during the winter to determine whether time of year had an effect on the temperature profile achieved or the length of time needed for the process to be completed. Daily mortalities from a broiler flock that was approximately 24 to 30 days old were collected from a producer and layered in a compost bin each day for four days. Samples were collected from the litter before it was placed in the bin and every other day for a week after the bin was compiled. Samples thereafter collected once per week until the process was completed. Samples were evaluated for microbial content. Temperature was taken and recorded from random points in the bins on a daily basis. For Phase 2 the finished composted material was used as a soil amendment in two vegetable plots. A third plot without compost material was used as the control. Soil samples were collected from each of the plots prior to application of the compost material after which cabbage seedlings were planted in each of the plots. Vegetative and soil samples were collected and evaluated for microbial profile prior to planting and again at 1 wk, 3 wk, 7 wk, and at harvest. The summer compost had the highest temperature of 156°F on d9 during the primary phase while the winter compost had the highest temperature of 156°F on d42 during the secondary phase of the compost. The summer compost samples were salmonella negative from d2 of the trial but mixed bacterial colonies remained for the duration of the study. The vegetative samples showed coliform levels up to 2.6 log₁₀/gm at wk3. The results show that while winter composting can effectively breakdown poultry carcasses summer compost is more efficient.

Key Words: compost, mortality, broiler, microbes, vegetable

Genetics

162 Ascites-resistant and susceptible broiler lines express different genes in their right ventricles. R. L. Taylor, Jr.*¹, M. E. Chapman², R. F. Wideman, Jr.², N. B. Anthony², and C. M. Ashwell³, ¹University of New Hampshire, Durham, ²University of Arkansas, Fayetteville, ³North Carolina State University, Raleigh.

Inadequate pulmonary vascular capacity in fast-growing broilers promotes susceptibility to pulmonary arterial hypertension (PAH) and subsequent pulmonary hypertension syndrome (PHS, ascites). Lower pulmonary vascular capacity requires the right ventricle (RV) to boost pulmonary arterial pressure (PAP) to maintain sufficient circulation to the lungs. Elements that raise cardiac output, lower pulmonary vascular capacity, or elicit pulmonary vasoconstriction comprise possible contributors to PAH. Yet, factors that initiate PAH remain unknown. To profile RV gene expression, we used ascites-resistant (RES) and -susceptible (SUS) broiler lines selected using hyperbaric hypoxia, which have >10x differential ascites incidence. Tenth generation RES and SUS line male progeny were housed in environmental chambers with ad libitum access to feed and water. Beginning at d 45, ten birds of each line were injected i.v. with cellulose microparticles to challenge the RV by

increasing the pulmonary vascular resistance. PAP values were recorded for 60 min post-injection followed by sampling of the RV tissue from each bird. Extracted RNA was reverse transcribed to cDNA, indirectly labeled with either Cy3 or Cy5 and hybridized (including dye swap) to a focused 70-mer oligonucleotide microarray containing 320 genes. Each gene was spotted twelve times increasing sensitivity to detect sample group differences. Thirty-eight genes with differential expression were divided equally between the two lines. Interferon regulatory factor 2 (Irf2), phospholamban, glyceraldehyde-3-phosphate dehydrogenase, and heart and neural crest derivatives expressed protein 2 were among higher expressed genes in the RES Line. Genes in the SUS Line having higher expression included insulin-like growth factor binding protein 1, CD36 molecule (thrombospondin receptor), homeobox A13, and CD3d molecule. Heart development and ventricular cardiac muscle morphogenesis were biological processes related to the genes identified. Network analysis found that genes identified included biomarkers associated with signaling in cardiac hypertrophy.

Key Words: ascites, gene expression, microarray, cardiac hypertrophy

163 Fine mapping of the sex-linked Barring gene within a 680 Kb region on chromosome Z. B. J. Dorshorst* and C. M. Ashwell, *North Carolina State University, Raleigh.*

First described as a sex-linked dominant gene by Spillman in 1908, barring (B) is a very familiar phenotype to most poultry breeders and scientists. The Barred Plymouth Rock breed is the standard example, exhibiting alternating white and black bars on the feathers covering the entire body. In addition to the primary phenotype barring has also been shown to 1) inhibit pigmentation in the dermis of the shank and beak 2) to interact with Dominant White (I) to more completely inhibit black spots in the feathers of I/i birds and 3) to dilute chick down color with a characteristic white spot on the top of the head. The specific mechanism and corresponding genetic mutation eliminating pigmentation from alternating regions of the feather has not been identified. Previous work using tissue grafts has shown that the microenvironment of the feather follicle and corresponding melanocytes are responsible for the barring phenotype as opposed to a systemic or endocrine mediated defect. Two alternative but perhaps complimentary hypotheses for the mechanism behind the barring phenotype are that of the diffusible inhibitor of melanin synthesis (Nickerson, 1944) and the buildup of cytotoxic melanin biosynthesis products resulting in premature cell death (Bowles, 1988). Utilizing an F2 population derived from a single Black Polish male and a single Barred Plymouth Rock hen we have mapped the barring gene to a 680 Kb region on the distal end of the long arm of chromosome Z. The development of the F3 generation of this cross has enabled the fine mapping of the barring gene demonstrating a novel genetic mechanism of melanocyte regulation. The identification of the causal mutation of the barring variant allows for the selection of this potent pigmentation inhibitor which is otherwise undetectable in the Dominant White (I) and/or Recessive White (c) genetic background of today's typical commercial flock.

Key Words: barring, pigmentation, melanocyte

164 High-throughput gene expression analysis of chicken intestinal intraepithelial lymphocytes (IEL) following oral feeding with carvacrol, cinnamaldehyde, and capsicum oleoresin. D. K. Kim*¹, H. S. Lillehoj¹, S. H. Lee¹, S. I. Jang¹, C. L. Keeler Jr.², C. Ionescu³, and D. Bravo³, ¹*Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Beltsville Agricultural Research Center, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD,* ²*University of Delaware, Department of Animal and Food Sciences, Newark,* ³*Pancosma S. A., Research Department/ Nutrition & Technology, Le Grand-Saconnex, Switzerland.*

The present study was conducted to investigate the immunomodulatory effects of three dietary phytonutrients with known health promoting effects using functional genomics approach. Carvacrol, cinnamaldehyde and capsicum oleoresin are dietary phytonutrients with well-known anti-inflammatory or antibiotic effects. Broiler chickens were fed standard diets supplemented with carvacrol, cinnamaldehyde and capsicum oleoresin, and their intestinal lymphocytes were examined for changes in immunity-related gene expression using an avian intestinal intraepithelial lymphocyte microarray (AVIELA). When compared to untreated controls, carvacrol-fed chickens showed the altered expression of 190 (64 up-regulated, 126 down-regulated) mRNAs, and cinnamaldehyde led to changes in gene expression of 235 (84 up, 151 down) mRNAs. In the case of capsicum, altered expression was observed in 657 (272 up, 385 down) transcripts after 7 days intake compared with untreated controls. Among >2.0-fold up- and down-regulated genes in carvacrol, cinnamaldehyde or capsicum treated groups, most changes occurred in

the genes associated with 'protein metabolism and modification'. The genes involved in 'signal transduction' and 'nucleoside, nucleotide and nucleic acid metabolism' also showed significant modulation in all three groups fed phytonutrients. In the genes altered by capsicum, the highest scored molecular network indexed using Ingenuity Pathways Analysis software included genes associated with inflammatory disease, immunological disease and gastrointestinal disease. These results indicate that feeding carvacrol, cinnamaldehyde and capsicum oleoresin induced changes in the local gene expression profiles that may be related to anti-inflammatory and antibiotic effects of these phytonutrients.

Key Words: carvacrol, cinnamaldehyde, capsicum oleoresin, microarray, chicken

165 Gene expression profiling among liver, bone marrow and kidney in broilers. W. K. Chou*, X. Li, C. Bailey, and H. Zhou, *Texas A&M University, Department of Poultry Science, College Station.*

Our main interest is to elucidate molecular mechanism how vitamin D₃ (VD₃) affect variety of biological functions, including innate and cell-mediate immunity, and bone mineralization. Liver, bone marrow and kidney are the major organs to convert the pre-VD₃ to activate hormonal VD₃. Bone marrow is the classic target for VD₃. The objective of this study is to profile gene expression of liver, bone marrow and kidney in chickens using chicken 44K Agilent microarray. A total of 20 different broilers were randomly selected from 10 pens of birds (2 birds per pen). Total RNAs were isolated from liver, kidney and bone marrow collected at day 21. The samples were labeled with Cy3 or Cy5 with dye swap. Signal intensity from each gene was globally normalized using LOWESS by R program. A mixed model including tissue, dye, slide, array was used to identify differentially expressed genes between any two tissues at the 1% significance level. There were 12,887 genes (6359 genes up-regulated, 6060 genes down-regulated, respectively) between bone marrow vs. kidney, 14376 genes (7736 genes up-regulated, 5592 down-regulated, respectively) between bone marrow vs. liver, and 10876 genes (6123 genes up-regulated, 4559 genes down-regulated, respectively) between liver vs. kidney. Gene ontology analysis showed Wnt receptor signaling pathway functional group was significantly enriched by 3.42 fold from differentially expressed genes in the comparison between bone marrow vs. kidney. The complement activation and humoral immune response functional groups were also significantly enriched by 7.05 fold in the comparison between kidney vs. liver. This data lays the foundation for further investigation of the molecular mechanism of vitamin D₃ in broilers.

Key Words: gene ontology, chicken, microarray

166 Inbreeding and population structure of lines of chickens divergently selected for high and low 8-week body weight. G. C. Márquez*, R. M. Lewis, E. N. Wiegand, and P. B. Siegel, *Virginia Polytechnic Institute and State University, Blacksburg.*

A population of chickens has undergone long term selection for high or low 8-week body weight to evaluate effects of selection over many generations. A pedigree of 6003 individuals was assembled to characterize inbreeding and population structure of the lines. Inbreeding quantifies the probability that genes in an individual are identical by descent. Individual inbreeding coefficients (F) were calculated separately for each line from the founding generation (1957 hatch) to generation 48 (2005 hatch). The founders were a panmictic population. Maximum F was 0.59 (generation 41) and 0.53 (generation 36), with means of 0.28

and 0.26, in the high and low lines, respectively. The effective population size (N_e) is the number of individuals accounting for observed inbreeding if the population was randomly mating. The N_e was 44.5 and 49.3 in the high and low lines, respectively. There were 56 founders per line (8 male and 48 female). The effective number of founders (f_e) is the number of equally contributing founders expected to produce the observed inbreeding in the population. The f_e was 15.9 and 17.6 for the high and low line, respectively. Family size statistics were calculated as large variation in these lead to higher inbreeding when some animals are used more widely than others. Family sizes of sires in the high line ranged from 1 to 21 offspring, with mean 5.1 and variance 10.3; for dams they ranged from 1 to 10, with mean 2.2 and variance 2.3. Family sizes of sires in the low line ranged from 1 to 16 offspring, with mean 4.9 and variance 7.0; dams ranged from 1 to 7 offspring, with mean 1.9 and variance 1.2. Accumulated inbreeding was high and N_e low, which was expected in closed lines. The f_e is low compared to the number of actual founders, as some contributed more than others. Family size statistics confirmed fewer males than females were used, which led to observed levels of inbreeding. Given similar maximum and mean F , N_e and f_e within lines, the effort to maintain similar population structures was achieved.

Key Words: chicken, genetic diversity, population structure, selection, inbreeding

167 New microsatellite markers for chickens. J. E. Fulton*, A. M. McCarron, K. N. Pinegar, and A. R. Johnson, *Hy-Line International, Dallas Center, IA*.

Microsatellites (MS) are simple sequence repeats (SSR) in which short nucleotide motifs are repeated multiple times. Their distribution throughout genomes, often in non-coding regions, as well as their high level of polymorphism makes MS very useful as genetic markers. MS were originally identified by cloning and sequencing of repetitive elements and their flanking regions. This laborious technique identified approximately 1200 genetic markers that became the core used to develop early molecular-based chicken genetic maps. This existing MS marker set was limited as it did not cover all chromosomes, particular the microchromosomes, and many large chromosomal regions were poorly covered.

With the public availability of the chicken genome sequence, researchers now have tools to develop markers in targeted regions. The UCSC chicken genome browser (<http://genome.ucsc.edu/cgi-bin/hgGateway>) and Repeatmasker information were used to identify repetitive genomic elements. Primers to amplify these elements were designed using the program Primer3Plus (<http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi>). To enable consistent genotype scoring, development focused on markers having repeat elements between two and five base pairs while avoiding single base pair repeats. Extensive testing and optimization assured production of robust PCR amplicons. More than 700 new markers have been added to all chromosomes, with emphasis on the smaller chromosomes. The markers were tested on DNA from 10 commercially utilized layer populations, representing both brown egg layer and white egg layer genetics. High polymorphism was noted with these markers possessing between 1 and 48 alleles with a mean of 6.4 alleles per marker. Four percent of the markers were monomorphic. In most instances, the observed size range was similar to the size range predicted from the chicken genome sequence. All of these new markers have been submitted to GenBank with the prefix HYL.

Key Words: microsatellite markers, DNA, repetitive sequences, genetic polymorphisms

168 Characterization of Minos and piggyBac transposons as “gene-trap” mutagens in the chicken. R. B. Beckstead*¹, B. Jordan¹, and M. Stark², ¹University of Georgia, Athens, ²Brigham Young University, Provo, UT.

The chicken is a well-established model system for studying vertebrate embryogenesis, but has not been extensively adapted to take advantage of modern genetic tools. In response to the newly published chicken genome, our lab is adapting genetic and molecular tools for use in the chicken to carry out a functional transposable element (TE) mutagenesis screen. Our long-term goal for this project is to identify candidate genes for economically important traits. Transposable elements that have been shown to function in other model systems were analyzed for use in chicken. We tested two different TE constructs based on Minos (Mi) and piggyBac (PB) transposons. The transposable element constructs for each TE system contained a strong splice acceptor followed by an IRES (internal ribosomal entry site) and an EGFP reporter gene flanked by inverted repeats specific to the transposable system used. Both systems use a cut and paste mechanisms where the transposase excises the TE construct from the donor plasmid and inserted it randomly into the genome. In the event that the TE element inserts into an expressed gene the IRES-GFP reporter will be spliced into the subsequent mRNA allowing for GFP protein expression. Constructs were tested by electroporation of the TE donor plasmid and a plasmid encoding the transposase gene into chick fibroblast cell culture and two-day old chick embryo. We observed that both the Minos and piggyBac TE systems efficiently inserted into the chicken genome as visualized by GFP expression in both cultured cells and chick embryos. These results suggest that a transposon base gene-trap mutagenesis screen could be performed in the chicken.

Key Words: gene, transposon, chicken, Minos, piggyBac

169 Diversity of Mx gene variants in commercial chickens. C. M. Ashwell*, C. Rot, and M. D. Koci, *North Carolina State University, Raleigh*.

Previous studies have demonstrated the Mx gene of mammals possesses antiviral properties against various viral pathogens including influenza virus. Subsequent studies demonstrated that chickens did express an Mx gene however this gene did not appear to have antiviral properties. More recent studies have demonstrated commercial and research lines of chickens contain numerous polymorphisms in their Mx and one variant in particular (G2032A) leads to a Ser631Asn amino acid change which is apparently capable of conferring antiviral activity to chicken Mx. Sequencing of 12 unrelated individuals from each of 9 commercial layer lines identified many segregating sequence variants in the Mx gene including the afore mentioned variant resulting in the Ser631Asn amino acid change. Upon completing the sequencing of all 13 exons as well as flanking sequence and the 5'-untranslated region, 5,039 bp of sequence was obtained from each of the 108 DNA samples for a total of 544,212 bp. In total, 23 sequence variant locations within Mx were observed in the 108 DNA samples. These 23 sequence variants included both synonymous and non-synonymous mutations, however there were only 11 distinct haplotypes segregating in the 9 lines. Of these haplotypes 4 have been observed by other groups reporting on Mx sequences of domestic and local breeds. 7 novel haplotypes were identified in the commercial layer lines. The novel haplotypes all include amino acid changes that may affect gene function and will be the subject of phenotypic evaluation for potential Mx effects on virus response in future studies.

Key Words: Mx, viral resistance, sequence diversity, avian influenza