

about medicated feeds. Participants were least knowledgeable about poultry feed composition and consumption in the nutrition section.

An understanding of these topics may be more effectively communicated to exhibitors, and the increasing population of backyard poultry owners, if it is known where poultry extension specialists should begin the edu-

cation process. The information discovered in the course of this survey will be used to develop extension materials and continuing education symposiums that incorporate poultry health and nutrition topics.

Key Words: extension, health, nutrition, exhibition poultry, backyard flock

Poster Session: Genetics

281P Quantitative trait loci for eggshell-related traits in the F₂ families from the Oh-Shamo (Japanese Large Game) and White Leghorn. T. Goto*¹, A. Ishikawa², S. Onitsuka¹, N. Goto¹, Y. Fujikawa¹, T. Umino¹, M. Nishibori¹, and M. Tsudzuki¹, ¹Hiroshima University, Higashi-Hiroshima, Japan, ²Nagoya University, Nagoya, Japan.

Recently, a great number of quantitative trait loci (QTLs) has been detected in experimental animals and livestock. In the chicken also, over 1,200 QTLs have been discovered for growth, meat, egg, behavior, and disease resistance traits. However, for eggshell-related traits, a considerably small number of QTLs have been reported. In the present study, we performed QTL analysis of eggshell-related traits with the Map Manager QTX b20 software in a unique chicken resource population of the 421 F₂ hens from intercrosses of an Oh-Shamo (Japanese Large Game) male and three White Leghorn females. The traits analyzed were eggshell size (the length of long and short axes of the egg: LLA and LSA), eggshell weight (EW), eggshell strength (ES), and eggshell thickness (ET), and they were measured at 300 and 400 days of hen ages. 118 microsatellite markers were genotyped. Interval mapping revealed nine significant QTLs with main effects on 300 d LLA (on chromosomes 8 and 11), 400 d LLA (chr. 8), 300 d LSA (chr. 8), 400 d LSA (chr. 8), 300 d EW (chr. 8), and 300 d ET (chrs. 1 and 9), and 400 d ET (chr. 1). The identified QTLs explained 6-14 % of the total phenotypic variance. The 300 d LLA QTL on chromosome 11 and 300 d ET QTLs on chromosomes 1 and 9 appeared to be new loci based on their map locations.

Key Words: chicken, eggshell, quantitative trait loci, mapping, microsatellite marker

282P The mitochondrial genome sequence and molecular phylogeny of the budgerigar, *Melopsittacus undulatus*. X. Guan*¹, D. Samuels², Z. Tu³, and E. Smith¹, ¹Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg, ²Vanderbilt University, School of Medicine, Nashville, TN, ³Virginia Polytechnic Institute and State University, Department of Biochemistry, Blacksburg.

The budgerigar, more commonly known as budgie, *Melopsittacus undulatus*, is a bird widely used as a pet and as a model for biomedical research into auditory and oxidative stress questions. Despite this significance, there is very little published genetic information about the budgie. Here, we describe the budgie's mitochondrial genome (mtGenome) sequence, a resource that could facilitate not only investigation into the budgie's extraordinary ability to deal with relatively higher levels of oxidative stress, but its relationship to parrots and other birds. The potential utility of the sequence, developed using PCR, was carried out by screening the D-loop and 16S rRNA for single nucleotide polymorphisms (SNPs) and using these to evaluate the phylogenetic relationship between the budgie and other avian species. The estimated total length of the mtGenome sequence was 18,193 bp, which includes a duplicated hyper variable region, a feature unique to only a few birds,

13 protein coding genes, and 24 RNAs (22 tRNA and 2 rRNA). The duplicated non-coding regions showed 86% sequence similarity. The coding region structure implicates gene conversion in the budgie mtGenome. Further, mtGenome-based phylogenetic analysis suggests that the budgerigar is most closely related to kakapo (*Strigops habroptilus*). The mitochondrial sequence of the budgie described here will form a useful resource for both parrot phylogeny and the role of the mtGenome in budgie longevity.

Key Words: budgerigar, mtGenome, rearrangement, control region, phylogenetics

283P Genetic diversity of *Campylobacter* populations in chicken ceca. P. Singh* and Y. M. Kwon, University of Arkansas, Fayetteville.

Campylobacter species is the most common human pathogen causing gastrointestinal infections and poultry is a major source of this pathogen. In this project, we aim to study the genetic diversity of *Campylobacter* strains within individual chickens using cecal samples to understand the nature of intestinal colonization by *Campylobacter* species. Genotyping was conducted based on the DNA sequence of Short Variable Regions (SVR) in the flaA gene. Cecal samples collected from ten market age chickens were used for isolation of *Campylobacter* genomic DNA and SVR was amplified with flaA gene-specific primers, cloned and sequenced. Sequencing results obtained from 85 clones (~10 clones/bird) showed that on an average 24.3 % of clones had mutations within individuals. When translated SVR sequences were analyzed, there was on an average 20.6 % of sequences carrying altered amino acids within individuals. The mutation did not show any consistent pattern, suggesting a random nature of the mutations. Four translated sequences had nonsense mutations to produce truncated proteins. These results suggest that there are multiple genotypes colonizing in a cecum and the occurrence of truncated FlaA protein may represent a novel mechanism for evasion of adaptive immune responses.

Key Words: *Campylobacter*, chicken, short variable region, cecum, genetic diversity

284P Association of single nucleotide polymorphisms in candidate genes with phenotypic traits in fat or lean chicken lines. X. Liu*¹, L. Cogburn², M. Muchow¹, E. Le Bihan-Duval³, J. Simon³, and T. E. Porter¹, ¹University of Maryland, Department of Animal and Avian Science, College Park, ²University of Delaware, Department of Animal and Food Science, Newark, ³Station de Recherches Avicoles-INRA, Nouzilly, France.

As a result of selection for rapid growth, excess fat accumulation in broiler chickens is a problem in the poultry industry. The current study investigated associations between single nucleotide polymorphisms

(SNPs) and multiple traits in experimental fat (FL) or lean (LL) chicken lines. In previous research, cDNA microarrays were used to identify differentially expressed genes in FL and LL chickens. Based on these results, four candidate genes located in chromosomes with known quantitative trait loci (QTL) for abdominal fat were selected: superoxide dismutase (SOD3), aldo-keto reductase (AKR1B10), glypican (GPC3), and syndecan (SDC1). Within the promoter region of these genes, eight SNPs identified through sequencing of genomic DNA were unevenly distributed between the FL and LL birds. The current objective was to genotype a three-generation experimental population produced through a reciprocal intercross of the FL x LL. TaqMan genotyping assays and pyrosequencing were used to determine SNP genotypes for 463 F2, 48 F1, and 30 F0 chickens. A univariate model was used to estimate several production traits, with sire and hatch as random effects and SNP as a fixed effect. Significant associations ($P < 0.05$) were detected between AKR1B10 SNP1 and GPC3 SNP1 and fat yield and fat weight. SDC1 SNP1 was significantly associated with fat weight ($P < 0.05$). SOD3 SNP2 and SNP3, which were completely linked with each other, were associated with breast yield ($P < 0.05$). A factor analysis was performed to take multiple traits into consideration. GPC3 SNP1 and SDC1 SNP1 were associated ($P < 0.05$) with a "muscle" yield factor, which combined thigh weight, breast weight, shank diameter and shank length. A repeated measures analysis of growth rate revealed that GPC3 SNP1 interacts with body weight from 1 to 9 weeks of age ($P < 0.05$). Finally, QTLs on chromosomes 1, 3 and 4 for body fat, initially identified using 127 microsatellite markers, were refined by incorporating these SNPs into the QTL analysis. These genetic markers could be of great value for marker-assisted selection of chickens with lower abdominal fat and improved meat yield.

Key Words: fat, QTL, MAS

285P MicroRNA expression and methylation signatures induced by Marek's disease virus infection in chickens. F. Tian*¹, H. Zhang², and J. Song¹, ¹University of Maryland, College Park, ²USDA, ARS, Avian Disease and Oncology Laboratory, East Lansing, MI.

MicroRNAs are tiny, non-coding RNAs regulating gene expression at post-transcriptional level. Reported experimental evidences show that microRNAs can serve as oncogenes or tumor suppressor genes. Interestingly, methylation of microRNA genes can down regulate microRNA gene expression, similarly as it does to some protein coding genes. Since Marek's disease virus (MDV) induces T cell lymphoma in Marek's disease (MD) susceptible chickens, MDV infected and non-infected chickens, both from a MD resistant and a MD susceptible lines (63 and 72, respectively) of chickens, were utilized to identify microRNAs that might be involved in the host defense to the viral infection. Through microarray analysis, 24 microRNAs were identified with significantly expressional differences between the infected and non-infected line 72 chickens ($P < 0.01$; FDR < 0.01) at 21 days post infection, suggesting an association between MDV infection and microRNA downregulation in susceptible chickens. These microRNAs may influence diverse pathways and biological processes through their targets. We also noticed that while the microRNA expression was down-regulated, the Dicer 1 and Drosha expressions remained steadily in the infected line 72 chickens. The methylation levels of several microRNAs were significantly higher in infected line 72 chickens than that in infected line 63 chickens. Taken together, we concluded that microRNA expression and methylation levels may serve as signatures of resistance or susceptibility for MDV induced chicken lymphoma.

Key Words: MDV, microRNA, methylation

286P Removed

287P Global gene expression associated with feed efficiency in male broilers. W. G. Bottje¹, B. Kong*¹, J. J. Song², T. Wing³, A. Pazcek³, R. Okimoto³, and K. Lassiter¹, ¹University of Arkansas, Department of Poultry Science, Fayetteville, ²University of Arkansas, Department of Mathematical Sciences, Fayetteville, ³Cobb-Vantress, Inc., Siloam Springs, AR.

Since feed remains the highest input cost in animal production, feed efficiency (FE) remains an important genetic trait. This study was conducted to investigate global RNA expression using microarray technology. Breast muscle was obtained from male broilers with high FE ($n = 6$) or low FE ($n = 6$) out of a group of 100 birds as previously described (Poult. Sci. 81:546-555, 2002). RNA isolated from muscle samples were tested for quality by agarose gel electrophoresis. The samples were pooled into high and low FE groups and labeled with either Cy3 or Cy5 fluorescent cRNA probes (Agilent Tech., CA). The Cy3 and Cy5 cRNA probes were hybridized on a 4 x 44 K Agilent chicken oligo microarray and, after washing and incubation, were scanned (Genepix 4000B scanner, Molecular Devices, CA). Background-corrected red and green intensities for each spot were used in the subsequent analysis. Global normalization based on local polynomial regression (loess) was applied to the intensities such that only biological variations remained. A moderated t-statistic and its corresponding p-value for each gene were computed to identify differentially expressed genes and adjusted for multiple testing by false discovery rate. Genes with an adjusted p-value below

0.05 were identified as ones being differentially expressed between the low and high FE groups. Quantitative PCR performed on 12 genes was used to validate microarray results. The results revealed that there were approximately 3800 genes that were differentially expressed in the high and low FE groups. Of these, 423 genes were expressed at least 50% different between the two groups. Out of these 423 genes, 249 were higher in the Low FE group with 35 being expressed at 2 fold greater compared to the High FE group. The high FE group had 174 genes that were expressed at 50% or more and 17 of the 174 were expressed at greater than 2 fold higher compared to the Low FE group. The results of this study indicate that by using a global gene expression approach, it may be possible to identify specific genes or gene pathways that are differentially expressed in broilers selected for FE.

Key Words: broiler, feed efficiency, global gene expression

288P Association of immune-related gene expression with SNPs flanking those genes or the transcription factor, NF-Kappa-B. E. Beach, C. Ciraci, B. Abasht, J. C. M. Dekkers, and S. J. Lamont*, *Iowa State University, Ames.*

Genetic markers (SNPs) for gene expression may be useful additions to marker assisted selection in chicken breeding programs. Signalling by the transcription factor, NF-Kappa-B, is important in initiating and maintaining an effective immune response against pathogens. The objective of this experiment was to determine whether SNPs in the genomic regions flanking NF-Kappa-B (NFKB) and immune-related genes were associated with the level of mRNA expression of the latter group of genes. Gene expression was quantified by RT-PCR for 26 immune-related genes from spleen and cecal tissue of 60 chickens of two lines infected with *Salmonella enteritidis*. The RNA was isolated from tissues harvested at 6 or 7 days after inoculation of day-old chicks. A maximum of 6 segregating SNPs were identified within 5.5 megabases from the target genes (median distance 1.0 megabase). These SNPs were considered cis-acting SNPs by evaluating associations with corresponding gene expression. Six SNPs were also identified within 4 megabases of the NFKB gene (median distance 1.8 megabase), and these were evaluated as trans-acting SNPs relative to expression of each immune-related gene. Associations were identified by fitting a regression model in JMP by line and tissue, with SNP, sex, and necropsy period as fixed effects and q-PCR plate, sire, and room housed as random effects. Both cis- and trans-located SNPs were found to be significantly ($P < 0.05$) associated with gene expression level in both tissues and in both lines. Acknowledgements: EB is a National Needs fellow, USDA-CSREES grant no. 2007-38420-17767.

Key Words: eQTL, immune, NF-Kappa-B, SNP, transcription factor

289P Epigenetic analysis of CD4 gene in SPF chicken lines resistant or susceptible to Marek's disease. J. Luo¹, Y. Yu³, H. Zhang², and J. Song*¹, ¹*University of Maryland, College Park*, ²*USDA-ARI ADOL, East Lansing, MI*, ³*China Agricultural University, Beijing, China.*

Marek's disease (MD) is a lymphoma caused by Marek's disease virus (MDV) in domestic chickens, which results in economic loss of the poultry industry. CD4 is a surface receptor expressed on CD4+ T cells, which recognize invading pathogen during virus infection. In this study, the influence of MDV infection on epigenetic status of the CD4 gene was examined. With the aid of pyrosequencing technology, promoter

methylation levels of the CD4 gene in spleens of chickens from a MD resistant line (63) and a susceptible line (72) were compared at 10 and 21 days post infection (dpi) with MDV. We found that in the promoter region of the CD4 gene the methylation levels of the line 72 chickens were drastically decreased between 10 dpi and 21 dpi ($p < 0.01$), whereas in the line 63 chickens the methylation levels were slightly decreased ($p < 0.05$). Moreover, the methylation levels between the non-infected and infected chickens did not differ both in line 63 and line 72 at 10 dpi ($p > 0.05$), but significantly differ in line 72 ($p < 0.01$) and slightly differ in line 63 ($p < 0.05$) at 21 dpi. These results indicated that the promoter methylation status of CD4 gene took on conspicuous effects in MD susceptible line 72 than in resistant line 63. As CD4+ T cells play an important role in immune-protection, the results of this study demonstrated that relative stableness of promoter methylation status of the CD4 gene may contribute to the host resistance against MDV pathogenicity.

Key Words: Marek's disease, epigenetics, methylation, immunology, genetics

290P Generating novel genomic libraries of the guinea fowl: Hypothalamus. J. Tyus II*, S. N. Nahashon, N. Adefope, and D. Wright, *Tennessee State University, Department of Agricultural Sciences, Nashville.*

Excessive fat accretion in poultry negatively impacts feed efficiency, lean tissue growth and consumer acceptability of poultry products. Annual losses incurred by poultry processors through excess fat extraction and disposal are estimated at 250-300 million USD. Saturated animal fat in the human diet has been linked to obesity, cardiovascular disease and certain cancers. Understanding hypothalamic influence of fat deposition in birds will be useful in designing feeding regimens, breeding programs and management practices for improving carcass quality and minimizing production costs. While much progress has been made in generating genetic sequence information in chickens, turkeys and quail, there is paucity of such pertinent data in the guinea fowl (GF). Such information is essential in understanding the genome of the GF and in comparative mapping of avian species. The primary aim of this study was to construct a comprehensive complementary DNA (cDNA) library of genes expressed in GF hypothalamus (hypo). Messenger RNA was isolated from GF hypo, reverse transcribed into cDNA and cloned into the pBluescript plasmid vector using the Stratagene[®] cDNA Library Construction Kit. Approximately 300 clones were selectively screened, cycle-sequenced by the polymerase chain reaction and analyzed with the ABI PRISM[®] 3100-Avant Genetic Analyzer. Realized nucleotide sequences were subjected to sequence homology searches carried out through the NCBI databases using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>). Nucleotide sequence similarity between GF and other avian species averaged 79.5%. Nucleotide sequences exhibiting high homology (ie., $\geq 80\%$) with other avian species averaged 655.6 bases in length and ranged from 373 to 1,025 bases. Nearly 10% of the nucleotide sequences analyzed showed no significant similarity to any available sequence data. Ultimately, information obtained from this and other genomic libraries will provide an invaluable tool for comparative mapping of the avian genome and further our understanding of the mechanisms underlying appetite, satiety and nutrient utilization in poultry.

Key Words: guinea fowl, hypothalamus, cDNA library, genomics

291P Initial assembly of the turkey whole genome sequence. R. A. Dalloul*¹, O. Folkert¹, E. J. Smith¹, K. M. Reed², O. Crasta¹, A. P. McElroy¹, R. A. Coulombe³, E. A. Wong¹, J. B. Dodgson⁴, and D. W. Burt⁵, ¹Virginia Polytechnic Institute and State University, Blacksburg, ²University of Minnesota, St. Paul, ³Utah State University, Logan, ⁴Michigan State University, East Lansing, ⁵Roslin Institute, Midlothian, United Kingdom.

A community-driven consortium has initiated sequencing the genome of the domesticated turkey, *Meleagris gallopavo*. Turkey meat is currently the fourth protein choice for American consumers with estimated US production of 271 million birds raised in 2008. In addition to the economic impact, the turkey serves as a model organism for a number of metabolic and medical diseases. The Turkey Genome Sequencing Consortium comprised of US and international scientists has used the latest 454 sequencing technology during this initial phase, with other sequencing platforms to be employed soon. The DNA for sequencing

was isolated from a female turkey (NT-WF06-2002-E0010, referred to as "Nici" (Nicholas inbred)). Nici is from an inbred sub-line (sib-mating for nine generations) originally derived from a commercially significant breeding line, with 88% monomorphism based on SNP genotyping. Roche/454 GS-Titanium sequencing at Virginia Tech has already produced more than 5x random and paired-end genome coverage (> 8 billion bases sequenced). The latest sequence assembly contains ~880 million base pairs in 428,910 large contigs, with average size of ~2 kb. The expected outcome of the fully sequenced (99% coverage) turkey genome is a rich genomic resource suitable for future academic and industrial, basic and applied poultry research. It will also provide solid foundation for the development of species-specific SNP panels for genome-based selection and improvement, and comparative genomics in poultry and other avian species.

Key Words: turkey, genome, *Meleagris gallopavo*, 454 sequencing

Poster Session: Immunology

292P WITHDRAWN.

293P Immunobiological effects of three phytonutrients, carvacrol, cinnamaldehyde, and capsicum oleoresin on chicken cells cultured *In vitro*. D. K. Kim*¹, H. S. Lillehoj¹, S. H. Lee¹, S. I. Jang¹, C. Ionescu², and D. Bravo², ¹Animal Parasitic Diseases Laboratory, Animal and Natural Resources Institute, Beltsville Agricultural Research Center, United States Department of Agriculture, Agricultural Research Service, Beltsville, MD, ²Pancosma S. A., Research Department/ Nutrition & Technology, Voie des Traz 6, Le Grand-Saconnex, Switzerland.

The present study was conducted to investigate the effects of three different plant-derived phytonutrients, carvacrol, cinnamaldehyde and capsicum oleoresin, on innate immune responses and tumor cell growth. To evaluate their effects, lymphocyte proliferation and the growth rate of tumor cell were accessed using a non-radioactive CCK-8 assay, and nitric oxide production was also measured using an *in vitro* culture treated with the three phytonutrients (carvacrol, cinnamaldehyde and capsicum oleoresin). Quantitative real-time RT-PCR was performed to measure the transcriptional expression of cytokine genes in macrophages in response to treatment with each phytonutrient. Results showed that each phytonutrient induced significant proliferation of spleen lymphocytes compared with the untreated control, and all stimulated robust nitric oxide production to the levels similar to that induced by recombinant chicken interferon- γ . All phytonutrients inhibited the growth of chicken tumor cells in a dose-dependent manner. The levels of mRNAs encoding IL-15 and IL-18 were enhanced when macrophages were treated with cinnamaldehyde. Capsicum oleoresin provoked the high expression of IL-18 and TNFSF15. The genes of IFN- α , IL-1b, IL-6 and IL-12 were not significantly influenced by the treatment of cinnamaldehyde or cap-

sicum. These results suggest that these three phytonutrients, carvacrol, cinnamaldehyde and capsicum oleoresin, enhance host innate immune system in chicken.

Key Words: carvacrol, cinnamaldehyde, capsicum oleoresin, innate immunity, chicken

294P Ethanol-induced changes in oxidative stress and immunological parameters of the chicken, *Gallus gallus*. H. Deng*¹, X. Guan¹, K. B. Gyenai¹, J. Xu¹, R. Dalloul¹, R. M. Gogal², R. E. Pearson³, and E. J. Smith¹, ¹Virginia Polytechnic Institute and State University, Department of Animal and Poultry Science, Blacksburg, ²Virginia Polytechnic Institute and State University, Department of Biomedical Science and Pathobiology, Blacksburg, ³Virginia Polytechnic Institute and State University, Department of Dairy Science, Blacksburg.

Oxidative stress is believed to be responsible for many diseases and physiological abnormalities in animals. However, the potential effect of oxidative stress on the immune system of chicken has not been investigated. Using ethanol as an inducer of oxidative stress, the main objective of this study was to examine the possible association between oxidative stress and immunosuppression in chickens. To evaluate these relationships, 4-week-old White Leghorn chickens were randomly divided into 4 groups of 24 birds each, and provided ad libitum starter diet and drinking water containing 0, 2, 6, and 10% ethanol for 2 weeks. As oxidative stress increased, plasma IgG but not IgM decreased. Similarly, the weights of the major immune organs, thymus, spleen and bursa, were inversely correlated with oxidative stress. The data presented here suggest that supplementation of drinking water with ethanol at 2% level is optimum for enhancing immunocompetence in chickens, while higher levels of ethanol increased oxidative stress and reduced immunocompetence.

Key Words: chicken, ethanol, oxidative stress, antibody response, organ weight