Breeding and Genetics

12 In vivo estimation of breast muscle depth in the turkey (Meleagris gallopavo) using ultrasound technology and its correlation to breast meat yield. L. A. Case*1,2, B. J. Wood2,1, and S. P. Miller1, 1University of Guelph, Guelph, Ontario, Canada, 2Hybrid Turkeys, Kitchener, Ontario, Canada.

Currently, in vivo estimation of breast meat yield is based on conformation score in the turkey industry. Ultrasound measurements of muscle depth were analyzed to determine if these objective trait measurements could be used for selection in a breeding program. Two measurements of breast depth, one taken horizontally across the chest and one parallel to the keel, were captured using an ultrasound machine. Including the muscle depth traits in a linear regression on breast meat yield increased the proportion of variation explained by 4% and 11% in the male and female lines, respectively. The heritabilities of the muscle depth traits ranged from 0.42 – 0.67. These values were higher than the heritability for conformation score in both the male line (0.37) and female line (0.38). The ultrasound traits also showed high genetic correlations to breast meat yield, ranging from 0.50 to 0.74, indicating that selection on the depth traits would result in a correlated improvement in breast meat yield. This has the potential to improve the rate genetic gain in breast meat yield, which would increase turkey production profitability.

Key Words: ultrasound, breast meat yield, heritability, selection

13 Genetic factors contributing to fat deposition in chicken. M. K. Nassar* and G. A. Brockmann, Humboldt-Universität zu Berlin, Germany.

While excess accumulation of body fat reduces the nutritional and economic value of a chicken considerably, increased intramuscular fat content could be a favorable trait for meat quality. In the present study, a genome scan was performed to detect genomic loci that affect the fat deposition in white adipose tissues and muscle in 278 males at 20 weeks of F2 populations of reciprocal crosses between 2 divergent chicken lines, the partially inbred line New Hampshire (NHI) and the inbred line White Leghorn (WL77). The NHI line had been selected inbreeding for high meat yield and WL77 for low egg weight before inbreeding. The inbreeding coefficients of the NHI and WL77 lines were about 86 and 99%, respectively. Chickens were genotyped for 123 marker loci covering 25 chromosomes. Linkage analysis provided evidence for a highly significant (F = 11.28) quantitative trait locus (QTL) influencing white adipose tissue mass on GGA4 with a peak F-value at 77.29 Mb and a significant (F = 8.96) QTL on GGA2 at 43.70 Mb, which explained 7.6 and 6.7% of the phenotypic F2 variance, respectively. The NHI QTL allele affecting white adipose tissue mass on GGA4 had negative additive effect (~5.3 g), while the QTL on GGA2 had positive additive effect (4.3 g). A highly significant (F = 11.92) QTL for intramuscular fat content was mapped on GGA2 at 33.10 Mb that accounted for 9% of the phenotypic F2 variance. The confidence interval overlapped with the QTL region for white adipose tissue mass. The GGA2-QTL had additive effect (0.3%), where the allele derived from superior line NHI tended to increase intramuscular fat content. Additionally, highly significant loci for intramuscular fat content were identified on GGA15 and 26, which have not been reported previously in other crosses. Our crossbred populations provide valuable basis for the further fine-mapping and subsequent candidate-genes identification for fat deposit traits. The final identification of genes contributes to our understanding of the complex inheritance pattern of the fat deposit traits in chicken.

Key Words: inbred chicken line, quantitative trait loci, white adipose tissue, intramuscular fat content

14 Validation of microsatellites linked to a candidate gene, as markers for ascites and economically important traits in broilers. S. Krishnamoorthy*, R. F. Wideman, D. D. Rhoads, G. F. Erf, and N. B. Anthony, University of Arkansas, Fayetteville.

Ascites syndrome continues to inflict financial losses to the world poultry industry despite years of investigation on its underlying cause. In the United States, ascites is generally controlled by slowing early growth performance which ultimately reduces meat yield and limits realization of the true genetic potential for broilers. A linkage group of 2 microsatellite markers and a candidate gene associated with ascites susceptibility have been identified on chromosome 9 in research lines divergently selected 15 generations for ascites susceptibility. These same microsatellite markers were found to also be descriptive for 3 commercial broiler lines. For one of the 2 microsatellite markers, a BB homozygote was present in 60% of all the birds in the ascites resistant research line, but only 6% in the ascites susceptible line. In 2 commercial lines, that same genotype was 8% and 14% of all genotypes. In a hypobaric model where about 50% of the commercial birds developed ascites, the BB homozygotes showed 0% and 7% ascites susceptibility. Interestingly, the BB genotype was absent in the third commercial line which had a higher incidence of overall ascites mortality compared with the other commercial lines. The second microsatellite marker also had genotypes found to be associated with ascites resistance. These microsatellite markers show utility in several different lines and therefore, could be used for marker assisted selection to improve ascites resistance. However, previous studies have shown the resistant and susceptible research lines to differ in feed efficiency and breast yield. Preliminary data also indicates that these markers are associated with performance traits. Data will be presented to more clearly elucidate the quantitative effects of this locus on production traits.

Key Words: ascites, microsatellites, marker-assisted selection

15 Novel use of an in vivo reagent to transfec Germ Line stem cells in chicken. B. J. Jordan*1, R. B. Beckstead1, and M. Stark2, 1University of Georgia, Athens, 2Brigham Young University.

The chicken is a well-established model system for studying vertebrate embryogenesis, but creating transgenics to study mutants has proven difficult. Viral infections are predominantly used to create transgenic chicks, but the rate of transgenesis is low. Additionally, there are size and sequence restraints when using virus, which make it unsuitable for many applications. To create a more versatile method of chick transgenesis, we are using a transposable element (TE) system paired with an in vivo transfection reagent to generate transgenic chicks. The TE system incorporates a transposase enzyme, which recognizes a specific DNA sequence called a transposon. The enzyme excises the transposon from its original location and inserts it into another genomic location. The transposon contains a GFP gene for tracking of insertion into active genes by fluorescent microscopy. We have proven this system effective with in vitro cell culture and in vivo embryonic injection experiments. The TE system is delivered to cells using an in vivo
transfection reagent, JetPEI. This reagent is a charged polymer that interacts with DNA to form a bundle, which is endocytosed by cells. Once inside, the bundle ruptures and the TE DNA is released into the cell where integration can occur. We tested the efficacy of the in vivo delivery system by injecting a mixture of in vivo reagent with a constitutively expressed GFP gene. This mixture was injected into Stage X embryos and incubated for 5 d. The embryos were removed, imaged for GFP expression and sectioned and stained with germ cell markers. Imaging showed localization of GFP to the germinal ridge, along with other tissues. Staining with germ cell markers confirmed that the reagent transfected DNA into germ cells. With transfection possible, we can now pair the JetPEI reagent with the TE system to utilize a novel and powerful tool for creating transgenic chicks.

Key Words: transgenic, transposable element, transfection reagent, embryogenesis, germ cells

16 Gene expression profiles of ceca in different broiler lines infected with wild-type and mutant Campylobacter jejuni. A. Nazmi1*, 1J. Zhang1, X. Li1, C. L. Swaggerty2, M. H. Kogut2, H. Chiang1, Y. Wang1, K. Genovese2, H. He2, V. J. Drita1, I. Pevzner4, and H. Zhou1, 1Texas A&M University, College Station, 2United States Department of Agriculture, College Station, Texas, 3University of Michigan, Ann Arbor, 4Cobb-Vantress Inc., Siloam Spring, AR.

The gram-negative bacterium Campylobacter jejuni (C. jejuni) is a serious human pathogen associated with several million cases of diarrhea around the world annually. Chicken products contaminated with C. jejuni are one of the major sources for human infection. Two broiler lines, line A and line B, have been previously shown to confer resistance (line A) or susceptibility (line B) to C. jejuni. In the present study, a chicken whole genome microarray was used to profile gene expression in the cecum after C. jejuni inoculation. One-day-old chicks from each line were challenged orally with mutant, wild-type C. jejuni or PBS, and ceca were harvested at 24 and 36 h post-inoculation (PI). After total RNA was isolated, 8 biological replicates were used to study infected and non-infected birds at each time point within each line. The LOWESS method was used to normalize the signal intensity of each gene and data was reported on the log2 scale. A mixed model including line, treatment, time point, array, dye, and all 2-way interactions among treatment, time, and line was used to identify differentially expressed genes (P < 0.01). For line A, there were 382 and 106 genes differently expressed at 24 h PI, and 271 and 347 genes at 36 PI in mutant and wild-type, compared with non-infected birds, respectively. For line B, there were 672 and 1021 genes differently expressed at 24 h PI, and 2393 genes at 36 h PI in mutant and wild-type, compared with non-infected birds, respectively. These results suggest that there was significantly more host immune response in the susceptible line than in the resistant line. Further signal pathway analysis between A line and B line in respond to C. jejuni inoculation is underway.

Key Words: broiler, Campylobacter jejuni, cecum, immune response, microarray

17 Blood leukocyte transcriptomics of broiler chicks infected with avian pathogenic Escherichia coli. E. Sandford1, M. Orr1, X. Li2, H. Zhou2, T. Johnson2, S. Kariyawasam4, P. Liu1, L. K. Nolan1, and S. J. Lamont1*. 1Iowa State University, Ames, 2Texas A&M University, College Station, 3University of Minnesota, St. Paul, 4Pennsylvania State University, University Park.

Colibacillosis caused by avian pathogenic Escherichia coli (APEC) causes significant losses to the poultry industry. Enhanced genetics of host defense mechanisms will reduce reliance on antimicrobials. To develop effective genetic selection programs, greater understanding of the genetics controlling immune response to infection is needed. A chicken 44K microarray was used to measure gene expression in peripheral blood leukocytes (PBL). Commercial broiler chicks (2 wk old) were vaccinated or mock-vaccinated against APEC. Two weeks later, chicks were experimentally infected with APEC or mock-infected. Whole blood was collected 1 or 5 d post-infection and PBL isolated. A pathology category (mild or severe) based on internal lesions was assigned at necropsy to mock-vaccinated, infected chicks. This generated 10 total treatment groups: vaccine status, infection status, and necropsy day as a priori factors; pathology category as a posteriori. Linear mixed model approach was used to test for significant differences in contrasts of interest. Many (1914) genes were differentially expressed (DE) in PBL collected on d 5, between mild and severe pathology categories of mock-vaccinated, infected birds (Q value <0.05). On both days, many DE genes were detected between groups with severe lesions and the mock-vaccinated, mock-infected control groups (1097 and 506 genes on d 1 and 5, respectively). In groups with severe lesions, 107 DE genes were detected between d 1 and 5. Severe pathology resulted in greater induction in gene response than repression. No vaccination effect was detectable in microarray analysis of the PBL, despite significantly lower lesion scores among vaccinated chicks. Examination of biological processes by DAVID revealed many terms related to immune response and metabolic processes. We report expression differences not only between infected and mock-infected chicks, but also between mild and severe pathologies, providing a foundation for candidate gene studies and development of marker assisted selection for effective immune response and resistance to APEC. Funding: USDA 2008–35604–18805 and 2007–38420–17767

Key Words: APEC, microarray, PBL

18 Genetic characterization of Red junglefowl (Gallus gallus) in India. M. Thakur1, 1, M. Fernandes1, R. Kalsi2, R. Kaul3, and S. Sambandham1, 1Wildlife Institute of India, Chandrabani, Dehradun, Uttarakhand, India, 2Mukundlal National College, Yamuna Nagar, Haryana, India, 3Wildlife Trust of India, Sector-Noida, U.P 201 301, India, 4Kurukshetra University, Kurukshetra, Haryana, India.

Genetic diversity of Red Junglefowl (RJF), the sole ancestor of present-day chicken, has been explored throughout its distribution range in India to propose a conservation action plan for the genetically endangered species. In total, 145 RJF samples were collected from 5 different zones i.e., Northern, Eastern, Central, Southeast and Northeast of the country and 358 alleles were distinguished across 22 microsatellite markers. The observed heterozygosity was lowest (Ho = 0.3636) in the central RJF population while it was highest (Ho = 0.6459) in the northeastern RJF population. Number of alleles per locus (Na) and the effective number of alleles per locus (Ne) ranged from 6 to 19 and 2.7317 respectively. These results suggest that there was significantly more host immune response in the susceptible line than in the resistant line. Further signal pathway analysis between A line and B line in respond to C. jejuni inoculation is underway.

Key Words: APEC, microarray, PBL
observed among pairs of loci. Nei’s Genetic distances were calculated for the 5 RJF populations and a dendrogram was constructed using Unweighted Pair Group method with arithmetic mean (UPGMA) which showed RJF populations in India formed 3 clusters: (i) northern and eastern, (ii) central and southeastern, and (iii) northeastern. Correspondence analysis also showed the similar pattern of clustering RJF population in India. Genetic bottleneck hypothesis were also tested on the captive population of Northern India, suggesting that RJF has not experienced a genetic bottleneck in the recent past.

**Key Words:** Red Junglefowl, microsatellite markers, genotyping, genetic diversity, genetic bottleneck.

20 Study of phylogenetic relationship of three Indian chicken populations based on mitochondrial D-loop region. R. Javed*1,2, B. Mishra3, M. S. Tantia1, and R. K. Vijh1, 1National Bureau of Animal Genetic Resources, Karnal, Haryana, India, 2KurukShetra University, KurukShetra, Haryana, India, 3Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India.

We had drawn a phylogenetic relationship among 3 (Aseel, Daothigir and Punjab Brown) indigenous breeds of chicken based on D-loop region. D-loop region was amplified and multiple sequence alignment was done using Clustal W software. A sequence of 652 base pairs was taken for further analysis. In total, 17 different haplotypes were found, of which, 9 in Aseel, 3 in Daothigir and 5 different haplotypes were observed in Punjab Brown. Haplotype-type 1 and haplotype-type 3 were shared among 3 breeds. Relative haplotype frequencies in the 3 populations ranged from 0.05 to 1.00 in Aseel, 0.05 to 0.35 in Daothigir and 0.0435 to 0.478 in Punjab Brown. Significant linkage disequilibrium was observed in 3, 2 and 1 pair of loci in Punjab brown, Daothigir and Aseel, respectively. The haplotype diversity was estimated to be 0.79474, 0.79474 and 0.74308 for Aseel, Daothigir and Punjab Brown. The number of migrants based on haplotype data information was Nm = 2.06 while a value of Nm = 1.27 was obtained using sequence data information. The effective number of migrants Nm based on the Fst estimator has been found to be 0.1890. The Fst values which is a parameter for the population differentiation revealed maximum genetic differentiation between Aseel and Punjab Brown and was 22.27% while the minimum population differentiation was between Daothigir and Punjab Brown (16.60%). The inter haplotype distances were utilized for the preparation of trees using NJ and UPGMA algorithm. The results revealed that the Aseel population was the ancestral population and other population arose from it. The study of indigenous chicken populations is important for biodiversity analysis to identify the genetic structures, migration patterns and genetic relationship among these populations/breeds. The study of these chicken breeds also assumes importance for their increased utility in chicken improvement programs in terms of disease resistance and quality meat production.

**Key Words:** phylogenetic analysis, D-loop region, chicken, Aseel, Daothigir and Punjab Brown.

21 Genome screening of native Egyptian chickens selected for increased body weights using microsatellite markers. E. A. El-Gendy1, E. M. El-Komy*2, A. A. El-Far1, and A. A. El-Gamry2, 1Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt, 2Department of Animal Production, National Research Center, Giza, Egypt.

An experiment was conducted to study the molecular characteristics of a native Egyptian chicken line (CE1) that has been developed as a local broiler line by selection for increased 6-wk body weight for 7 generations. For genetic comparisons, the local genetic control line (CE2), a slow-growing commercial broiler strain (SGB) and a fast-growing commercial broiler strain (FGB) were used and the F1 reciprocal crosses CE1*SGB and SGB*CE1 were obtained. Twenty-seven microsatellite markers were used to screen the genomes of the different genetic stocks. The average numbers of detected allelic bands, over all markers, ranged from 4.93 in line CE2 to 6.07 in line CE1 in the parental generation and from 4.85 in the cross SGB*CE1 to 6.11 in line CE1. Polymorphism ranged from 57.2% of the total allelic bands in line CE2 to 65.2% in line CE1 in the parental generation, and from 52.9% in the cross CE1*SGB to 65.5% in line CE1 in F1 generation. The stock-specific alleles averaged 1.2% of the total number of detected alleles in line CE1 and 7.5% in line CE2 in the parental generation, versus 2.0% in line CE1 and 7.4% in line CE2 in F1 generation. The crosses CE1*SGB and SGB*CE1 had specific alleles of 1.2 and 3.1%, respectively. Similar percentages of monomorphic alleles were obtained for the genetic stocks and averaged 2.11%. Also, more genetic variation was observed for line CE1 compared with the control line CE2 and strains SGB and FGB. The cross CE1*SGB showed high similarity with the parental line CE1, however the cross SGB*CE1 showed high similarity with the parental strain SGB. The results indi-
cated that line CE1 has been genetically differed from its control line (CE2) as a result of the selection scheme.

**Key Words:** local chickens, microsatellite loci, polymorphism, reciprocal crosses

### 22 An approach to marker-assisted selection for increased body weights in local chickens in Egypt. E. A. El-Gendy and M. A. Helal*, Department of Animal Production, Faculty of Agriculture, Cairo University, Giza, Egypt.

A study was conducted to reveal the microsatellite loci that can be used to assist in selection for increased 6-wk BW in 2 local chicken lines in Egypt. The lines have been developed by selection for high 6-wk BW for 8 selected generations as normally feathered local broilers (Line CE1), and naked-neck local broilers (Line CE3). The screening of genomic microsatellite loci has been carried out on selected generations 6, 7 and 8, using 27 microsatellite markers. Six-wk BW were obtained on family basis for each line and generation. The selected high BW families of line CE1 have performed 6-wk BW of 1.14 to 1.96 folds of their genetic controls over the 3 selected generations, and the selected high body weight families of line CE3 have performed 6-wk body weight of 1.16 to 1.79 folds of their genetic controls. There have been several polymorphic and monomorphic loci detected by the microsatellite markers. The study revealed line-specific microsatellite allelic bands, among them several bands were family-specific. Significant associations between the family means and the appearance of several loci were observed. In line CE1, primer ADL0299 detected a band of 180 bp with increased frequency in subsequent generations and was associated with high 6-wk BW. In line CE3, primer ADL0299 detected a band of 200 bp with increased frequency in subsequent generations and was associated with high 6-, and 12-wk BW. In line CE1, primer ADL0022 detected a band of 180 bp with high frequency in generation 8 and was associated with 8-, and 12-wk BW. In line CE3, primer LEI0075 was detected a band of 280 bp with increased frequency in subsequent generations and was associated with high 6-wk BW. It is concluded that specific allelic bands can be combined to the phenotypic and genotypic data in a marker assisted selection (MAS) program for increased juvenile body weights of local chickens.

**Key Words:** body weight, local breeds, MAS, microsatellite loci

### 23 Productive performance and immunocompetence parameters of naked necks and normally feathered chicken genotypes issued from different maternal lines. A. Galal* and M. Mahrous*, Poultry Production Dept., Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Productive performance, humoral, cell-mediated immunities and phagocytic ability were examined in naked neck (Nana) and normally feathered (nana) genotypes issued from different maternal lines (Brown and White Hy-line breeder hens). Cell-mediated immunity was examined by phytohemagglutinin-P (PHA-P) assay at 30 wk of age. At 32 and 34 wk of age, 20 birds from each genotype within dam lines were injected sheep red blood cells (SRBCs), and blood samples were collected at 7 and 14 d post-primary injection (PPI) and post-secondary injection (PSI). Phagocytic ability was measured by carbon clearance assay at 35 d of age. Regarding adaptability, comb and wattle lengths were significantly increased by the naked neck gene allowing for increased heat dissipation. In the absence of interaction, the presence of Na gene significantly increased egg number compared with normally feathered counterparts. Also, the naked neck hens had a larger egg weight. With respect to immunocompetence parameters, the results showed that the naked neck genotype had significantly (P < 0.05) higher total antibody titers to SRBCs than their nana counterparts. In both dam lines, the naked neck genotype had significantly faster carbon clearance ability than the nana sibs.

**Key Words:** naked neck gene, immunity

### 24 Eggshell ultrastructure of naked neck, frizzle and normally feathered genotype chickens. M. Mahrous* and A. Galal, Poultry Production Dept., Faculty of Agric., Ain Shams University, Cairo, Egypt.

An experiment was conducted to evaluate mechanical and ultrastructural properties of eggshell in naked neck (Nana), frizzled (nanaFf) and normally feathered (nanaFf) genotype chickens. To assess eggshell quality, a total of 200 eggs (50 each genotype) were randomly collected at 35 weeks of age. The eggs produced from birds carrying Na gene in a single manner or interact with F gene had owned better thickness and breaking strength of eggshell compared with other produced from nanaff sibs. The presence of Na gene in a single state or combined with F gene significantly increased relative palisade thickness compared with nana genotype. Opposite trend was noticed for relative mammillary thickness. Type B bodies, which are rounded and located among mammillary caps, were more frequent in eggshell of nanaff genotype resulting in poor eggshell quality. However, Nanaff and NanafFf genotypes had good rounded caps and early fusion as compared with nanaff ones. In conclusion, the birds carrying Na gene in a single state or combined with F gene had genetically not only better mechanical eggshell properties but also good ultrastructural formation of eggshell compared with normally feathered genotype.

**Key Words:** naked neck gene, frizzled gene, eggshell, ultrastructure