

211 Effects of dietary protein and energy on broiler live performance to 36 days. F. I. L. Hernandez^{*1}, D. R. Korver², R. A. Renema², and M. J. Zuidhof¹, ¹Alberta Agriculture and Food, Edmonton, AB, Canada, ²Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.

A study was carried out to investigate the effects of energy and protein levels on performance of Cobb Avian 48 broilers to 36 d. Chicks were randomly assigned to a 2 × 2 × 3 × 5 factorial arrangement of treatments, with 2 sexes; 2 levels of early nutrition (0 to 11 d); and after 11 d, 3 metabolizable energy (ME) levels and 5 dietary balanced protein (DBP) levels, balanced for 4 limiting amino acids. The 3 ME levels were 94, 97, and 100% of Cobb-Vantress dietary specifications for maximum growth rate and feed conversion ratio (FCR). The 5 DBP levels were 85, 92.5, 100, 107.5, and 115% of these same specifications. From 0 to 11 d, 2 prestarter nutrient densities were used, based on Cobb's starter recommendations for maximizing growth rate and FCR (HIGH), or for reduced feed cost (LOW). From 12 to 36 d of age, the prestarter treatment was nested within pens, which housed ME × DBP × sex interactions. By 36 d, the HIGH prestarter increased BW (2.14 vs. 2.08 kg; $P < 0.0001$). Male broilers were heavier than females ($P < 0.005$) in all levels of ME and DBP. Females had a higher cumulative FCR than males ($P < 0.0001$). In females, DBP did not affect cumulative FCR at 36 d. Males fed 100% through 115% DBP had a lower cumulative FCR than those fed 85 and 92.5% DBP ($P < 0.05$). Covariate analysis (adjusted for BW) indicated that breast yield was higher at 107.5 and 115% DBP than at 85% through 100% DBP (range: 389.7–381.4 g vs. 372.6–378.5 g, respectively; $P < 0.05$). Fat pad decreased with increasing DPB levels ($P < 0.0001$). Fat pad was higher at 100% ME than at 97% ME and 94% ME. Females had higher fat pad weight and lower eviscerated BW and legs yield. In conclusion, HIGH prestarter improved growth rate, males were more efficient than females, low DBP levels increased fat pad, and breast yield increased by 1 g for every 1.5% increase in DBP.

Key Words: live performance, metabolizable energy, dietary balanced protein

212 Methionine deficiency in chicks reduces hepatic recycling of dehydroascorbic acid and up-regulates renal L-gulonolactone oxidase activity. D. V. Maurice^{*}, S. F. Lightsey, and J. E. Toler, Clemson University, Clemson, SC.

The effects of oxidative stress are well known and antioxidants alleviate adverse responses. Ascorbic acid (AsA), a potent antioxidant (AO) synthesized by poultry, is oxidized to dehydroascorbic acid (DAsA) that can be converted to AsA. Methionine (MET) plays a central role in metabolism and recent findings demonstrate that it functions not only as an antioxidant but also induces the expression of an AO protein. The paucity of information on the relationships between AsA and MET lead us to examine the effect of MET deficiency on tissue AsA, plasma total antioxidant capacity (PTAC), hepatic recycling of DAsA, and L-gulonolactone oxidase activity (GLOX). In each experiment commercial broiler chicks were grown in wire-floor cages to 24–25 days of age. A pen of 6 birds formed an experimental unit with 8 pens per treatment and 1 chick was sampled from each pen at termination. A corn-peanut meal-soybean meal diet with added MET (0.27%) and lysine HCl (0.3%) was the control diet (22.4% CP; 0.58% MET; 0.90% SAA) and the same diet without added MET was the MET deficient diet (22.4% CP; 0.32% MET; 0.63% SAA). The assayed values for the deficient diet were 21.8% CP; 0.31% MET; 0.65% SAA; 0.21% added lysine and 0.01% added MET (courtesy Evonik Degussa, Atlanta). Body weights were comparable in Expt 1 (851 vs. 834 g) with deficiency induced increase in feed intake (42.1 vs. 46.5 g/day) and significantly reduced by deficiency in Expt 2 (819 vs. 748 g). Plasma AsA, bursal AsA and PTAC were significantly reduced by MET deficiency but significant differences were not detected in splenic and testicular AsA. Hepatic recycling of DAsA was reduced by 18% in deficient chicks (17.8 vs. 13.7; $P < 0.01$) but changes in relative weight and hepatic AsA were not detected. In both experiments GLOX was significantly up-regulated by 28–29% in MET deficient chicks (464 vs. 364 units $P < 0.01$ and 566 vs. 438 units $P < 0.01$). The results demonstrated that MET deficiency induced increased GLOX and suggest that the 2 nutrients form a link in the AO defense system and contribute to homeostasis.

Key Words: ascorbic acid, L-gulonolactone oxidase, methionine deficiency

Genetics

213 The paternal effect of *Campylobacter jejuni* colonization in broilers. X. Li¹, C. Swaggerty², M. Kogut², H. Chiang¹, Y. Wang¹, K. Genovese², H. He², N. Stern³, and H. Zhou^{*1}, ¹Texas A&M University, College Station, ²ARS-USDA, College Station, ³ARS-USDA, Athens, GA.

Day-one broilers from 2 pure lines (A and B) and 2 F1 reciprocal crosses C and D were challenged with 105 cfu (colony forming unit) of *Campylobacter jejuni* (*C. jejuni*). Cloacal swab were collected on 3, 6, 10, and 13 dpi, and cecal contents on 7 and 14 dpi for *C. jejuni* counting, respectively. Colony categories in the swab and the number of *C. jejuni* colonies in cecal contents of each bird were recorded and relative bacterial numbers for the swab in each line were calculated. Number of bacteria in cecal content in line B was significantly higher than in line A ($P < 0.05$), and line C (A♀×B♂) significantly higher than line D (A♂×B♀) ($P < 0.05$) at both 7 and 14 dpi, respectively. Similar results were observed in cloacal swab. There was significant correlation between *C. jejuni* count in cloacal swabs and that in cecal content. The

results indicated that paternal effect might be one of important genetic factors influencing the resistance to *C. jejuni* colonization in broilers.

Key Words: *C. jejuni*, paternal effect, challenge

214 Differentially expressed soluble proteins in aortic cells from atherosclerosis-susceptible and resistant pigeons. S. C. Smith, E. C. Smith, M. L. Gilman, J. L. Anderson, and R. L. Taylor Jr.^{*}, University of New Hampshire, Durham.

Atherosclerotic cardiovascular disease is the leading cause of death in economically developed countries. Numerous hypotheses have attempted to explain the initiation of lesions but the underlying cause(s) remains unclear. Complex gene-environment interactions are believed to be involved in the disease. In attempts to understand genetic compo-

nents of this disease, the susceptible-resistant pigeon (*Columba livia*) model has been employed. The White Carneau (WC) pigeon develops spontaneous atherosclerosis without elevated plasma cholesterol levels and in the absence of other known risk factors. These noninduced atherosclerotic lesions have morphological and ultrastructural similarity to those seen in humans. The Show Racer (SR) pigeon is resistant to this lesion formation. Soluble proteins in aortic smooth muscle cells cultured from atherosclerosis-susceptible WC and atherosclerosis-resistant SR pigeons were extracted and separated on 2-dimensional electrophoresis gels. Spots were analyzed with Phoretix™ software and compared between the 2 breeds. Proteins differentially expressed were plotted using molecular weight (MW) against pI. Eight discrete zones were identified, 5 which included only proteins unique to susceptible cells and 3 which included proteins unique to resistant cells. Of the 88 differentially expressed proteins from susceptible cells, 41 were located in unique zones while 29 of 82 differentially expressed proteins from resistant cells were in unique zones. Selected proteins from susceptibility and resistance zones were annotated by peptide mass fragments, MW, pIs, and correspondence with genes differentially expressed between cells from the 2 breeds. Some of the annotated proteins (smooth muscle myosin phosphatase, myosin heavy chain, fatty acid binding protein, ribophorin, heat shock protein, and TNF α -inducing factor), corresponded to current hypotheses to explain atherogenesis. In addition, the unique electrophoretic migration zones of proteins should prove valuable to further distinguish atherosclerosis susceptible or resistant phenotypes.

Key Words: proteomics, atherosclerosis, smooth muscle cells

215 The inheritance of embryonic chick edema and its penetrance in dwarf SCWL chickens. W. A. Given, T. F. Savage*, and J. C. Hermes, *Oregon State University, Corvallis*.

The concept of genetic penetrance, “the frequency of manifestation of a genetic factor” was introduced by Timofeef-Ressovsky (*Naturwissenschaften* 19:493, 1931). Incomplete penetrance has been used to explain the absence of phenotypic expression when otherwise anticipated. Embryonic chick edema, ECE a semilethal heritable condition was initially described in a line of dwarf SCWL chickens in 1998 [Poult. Sci. 77 (Suppl. 1):69]. The condition was characterized by the presence of one or more subcutaneous fluid filled sacs present most commonly on the embryo’s thighs. These sacs varied in diameters from 0.5 to 6 cm. The condition was originally reported as the gene expression of 2 autosomal recessive loci expressing incomplete penetrance ranging for 0 to 100%. With the inclusion of a third hypothesized contributing locus responsible for ECE, the data was reevaluated. Heterogeneity and pooled chi square tests when applied to the data confirm the hypothesis that ECE expression is the result of autosomal dominant genes at 2 loci and one homozygous recessive locus. This study suggests that a more accurate explanation for the phenomenon of incomplete penetrance is the hypothesized number of gene loci responsible for the trait.

Key Words: inheritance, embryo edema, penetrance

216 Genetic variations in immune-related genes in free-run and free-range chicken flocks. F. Izadi*¹, S. J. Lamont², M. G. Kaiser², and K. M. Cheng¹, ¹*Department of Animal Science, University of British Columbia, Vancouver, BC, Canada*, ²*Department of Animal Science, Iowa State University, Ames*.

Chickens raised under free-range conditions have likely been exposed to more environmental variations, including pathogen types, than commercial chicken lines raised in a highly controlled environment. To test the hypothesis that free-run/free-range chickens have more genetic variation related to immune response than highly selected commercial chicken lines, we examined SNP variations of 7 immune-related genes (IFN- γ , CHB6, TGF- β 3, Md2, TLR4, iNOS, CASP1) in 30 samples of 2 free-range stocks (Taiwanese cross, Silkies), a Rhode Island Red \times Barred Plymouth Rock cross, and 2 commercial stocks (Commercial Brown, Commercial White). Six of the 7 genes were polymorphic in 1 or more stocks. Observed heterozygosity within lines ranged from 0.25 to 0.455. Unexpectedly, the free-range stocks did not have higher heterozygosity in these immune-related genes than the commercial stocks. This may be related to their generally small population size or the timing when the crosses were made.

Key Words: single nucleotide polymorphisms, genetic variability, immune-related genes

217 The inheritance of clubbed down in *Coturnix* embryos and its associated genotype-environment interaction. M. A. Charbonneau, T. F. Savage*, and J. C. Hermes, *Oregon State University, Corvallis*.

During a long-term genetic selection study to increase hatchability of *Coturnix* embryos incubated at 102°F, a heritable embryonic lethal condition described as clubbed down was characterized and its mode of gene transmission was studied. This condition was characterized by a bulbous-like or nodular swelling/s in the proximal region of the embryos down plumules. The aberrant plumules were most prominent in the ventral body pterygiae of the sternum and neck though some embryos possessed the clubbed down condition over the majority of embryo’s body. The numbers of clubbed down plumules varied from 1 to 20+ per 5 mm² of body surface. Some nodules were red in appearance due to the observed presence of erythrocytes. Plumules with the nodules were detectable as early as 8 days of incubation and some affected embryos were observed to be alive at 14 days of incubation. A reduction in embryo size was often associated with the presence of clubbed down plumules. Some of the clubbed down embryos observed also exhibited other deformities, amaxilla and or celosomia. Neither of these deformities was associated with clubbed down. Histological examinations of the clubbed down were not informative while scanning electron micrographs revealed the interiors of the nodules to be devoid of cellular matter.

The clubbed down condition was determined to be the expression of an autosomal recessive allele in the homozygous condition. Further studies revealed that the expression of clubbed down occurred when the homozygous embryos were incubated at 102°F.

Key Words: clubbed down, *Coturnix* embryo, inheritance

218 Association of FMO3 gene with egg production and quality traits in brown layers. P. Settar*, J. E. Fulton, J. Arango, J. A. Arthur, and N. P. O'Sullivan, *Hy-Line International, Dallas Center, IA*.

Fishy taint occurs in eggs of some brown egg layer stocks when fed diets high in choline or choline precursors (such as sinapine from rapeseed meal). It has recently been determined that this phenotype is due to a genetic defect in the FMO3 (flavin-containing mono-oxygenase 3) gene such that the enzyme can not efficiently convert TMA (trimethylamine) to the nonodorless form. Identification of the genetic defect as a single gene and the subsequent development of rapid DNA-based tests for detection of the defect means that this defect can be quickly removed from commercial brown egg lines. It is important to know if there are any associations between the FMO3 gene and economically important traits. In this study, the association between FMO3 genotypes and 10 economically important traits was evaluated. A total of 12,600 birds from 2 consecutive generations brown egg layer line with complete phenotypic records were genotyped for the FMO3 gene. Traits evaluated include: age at sexual maturity (SM), egg weight (EW3) and shell color (SC3) of the first 3 eggs laid, egg weight (EW), shell color (SC), shell strength (SS), albumen height (AH) and yolk weight (YW) measured in 5 consecutive eggs at 26 wk of age, along with body weight (BW) at 38 wk of age and production (PD) during the entire laying cycle. Association tests were carried out by using marker information as a fixed effect in the model along with hatch and generation effects. Sire and dam effects were included in the model as random effects. There were no associations between FMO3 and SC3, SS, AH, YW, PD, and BW traits. However, SM, SC, E3, and EW showed significant association with FMO3 genotype. Differences between wild type and mutant FMO3 allele were calculated as 0.6 days for SM, 0.8 units for SC, 0.4 g for E3, and 0.5 g for EW. These results show that elimination of the mutant allele from this particular population should be slow and systematic in order to avoid any negative impact on egg weight and age at sexual maturity.

Key Words: brown egg layer, FMO3, marker association

219 A new cytogenetic model for the order of the nucleolus organizer region (NOR), major histocompatibility complex (MHC) -B, and -Y on microchromosome 16 in chicken. M. E. Delany*¹, C. M. Robinson¹, R. Goto², and M. A. Miller², ¹University of California, Davis, ²City of Hope Beckman Research Institute, Duarte, CA.

The MHC-B complex and the NOR were determined to be linked on chicken chromosome 16 through trisomy mapping (Bloom and Bacon, 1985); a second MHC-like complex, the Rfp-Y (now termed MHC-Y) was also determined to be linked on GGA 16 (Miller et al., 1996). A chromosomal model was developed to explain the apparent lack of genetic linkage between MHC-B and MHC-Y which proposed that the NOR was located in between the 2 MHC complexes. That is, it was postulated that the size of the NOR (6 Mb) and its propensity for recombination could be the underlying basis for inheritance data showing segregation of the MHC-B and MHC-Y as if these complexes were encoded by different chromosomes. Here we present new evidence from high resolution fluorescence in situ hybridization analysis of mitotic prometaphase and pachytene chromosomes using BAC probes specific for MHC-B and MHC-Y, which provides an alternative explanation. The cytogenetic results indicate that the MHC-Y is proximal (adjacent) to the NOR on the q arm with the MHC-B located distal and near the q terminus although on the same side of the NOR as the MHC-Y; it is the

presence of a large GC-rich chromosomal region which separates the MHC-Y from the MHC-B, rather than the NOR. The updated model will be discussed and compared with that described for other avian species. Research was supported by funds from the USDA-CSREES Multistate Research Project NE-1016 (MED: CA-D*-ASC-7281-RR) and USDA-CSREES-NRI (MAM: 2006-35205-16678).

Key Words: cytogenetics, NOR, MHC-Y, MHC-B, fluorescence in situ hybridization

220 DNA based test for the identification of fishy taint in chickens. J.E. Fulton*¹ and D. E. Harry^{2,3}, ¹Hy-Line International, Dallas Center, IA, ²Oregon State University, Corvallis, ³Genetic Foundations, Philomath, OR.

Fishy taint in commercial brown egg layer stocks has been determined to be due to a mutation of the FMO3 (flavin-containing mono-oxygenase 3) gene. This mutation is a single base pair change that results in an amino acid substitution in an active site of the FMO3 enzyme. Females homozygous for this defective enzyme were found to have high levels of the fishy odor compound TMA (trimethylamine) in their eggs after being fed high levels of choline chloride. This study was done to determine if this same genetic defect and subsequent phenotype was present in unrelated brown eggs stocks. A total of 592 females from 3 Hy-Line brown egg layer pure lines were fed high levels of choline chloride and TMA levels were measured in their eggs. The sequence of exon 7 of the FMO3 gene was determined from 24 of these individuals. Those hens that produced TMA in their eggs were found to have the same genetic change in their FMO3 gene as had been reported. An allele specific PCR test was developed to more rapidly detect homozygous affected individuals, heterozygous carriers, and homozygous unaffected individuals as well as to identify males carrying the defective gene. This test provides unambiguous results within 24 hours of sampling, allowing rapid detection of this undesirable gene in breeding stocks. The FMO3 gene is located at one end of chromosome 8, in a region known to contain genes and QTL that influence several traits of commercial interest. Even though rapid DNA based tests can be developed, rapid elimination of genes must be approached with caution so as to not affect the frequency of linked desirable genes.

Key Words: brown egg layer, FMO3, fishy taint

221 A diallele cross to investigate the impact of selection age on growth response in Japanese quail. I. D. Harford*, L. K. Stamps, and N. B. Anthony, *University of Arkansas, Fayetteville*.

To study the effect of selection age on growth response, 4 Japanese quail lines were developed that were selected for increased body weight at 10 (H10), 17 (H17), 28 (H28) or 40 (H40) days of age. These selection ages represent reported key points on the growth curve. In these quail populations, it has been observed that selection for rapid growth at any selection age resulted in increased final body weights when compared to the randombred control. Between the selected lines, however, the various selection ages resulted in clear differences on both growth response and final body weights. Growth response was observed to be stepwise in that maximal asymptotic body weights ranked highest for H40, followed by H28, H17 and H10. In order to further investigate the

effects of selection age on growth and development, a complete diallele cross was made between the 4 selected quail populations. Growth and yield was measured on 4 replicate pens for each cross, with each pen containing a minimum of 20 birds. Growth and yield for all crosses were evaluated at each of the 4 selection ages: days 10, 17, 28, and 40. Of particular interest in this study is the change in both reciprocal and heterotic effects over time. In this study it was observed that reciprocal and heterotic effects did change over time. In both cases nonadditive genetic variation seems to play a much greater role prior to rather than post point of inflection (POI). In general, the utilization of a line selected at or prior to POI is advantageous as it maximizes growth potential due to the effects of nonadditive variation. This advantage was apparent for both growth and yield parameters in this study. It is interesting to note that the crosses that resulted in the most heterosis were those that included a line selected at POI and a second line selected for late body weight.

Key Words: Japanese quail, diallele cross, point of inflection

222 Gene expression profiling of host responses to Marek's disease virus: A comparison of genetically defined resistance and susceptibility. A. J. Sarson*^{1,2}, P. Parvizi¹, D. Lepp², M. Quinton¹, and S. Sharif¹, ¹University of Guelph, Guelph, ON, Canada, ²Guelph Food Research Centre, Agriculture and Agri-Food Canada, Guelph, ON, Canada.

Marek's disease virus (MDV) is a cell-associated oncogenic herpesvirus that induces lymphoid tumours in chickens. Genetic resistance to Marek's disease (MD) has been largely associated with the major histocompatibility complex (MHC). For example, chickens with the B21 and B19 haplotypes have been strongly correlated with resistance and susceptibility to MD, respectively. However, MD resistance is under polygenic control and therefore we sought to compare the gene expression profiles of MDV infected B19 and B21 chickens, to assess the role of non-MHC genes in MDV infection. Birds from both B21 and B19 haplotypes were divided into 2 groups with one group infected (10,000 plaque-forming units of JM-16, MDV at 5 days posthatch). On days 4, 7, 14 and 21 postinfection, gene expression profiles in spleen tissue were obtained using a chicken immune-specific microarray. LOWESS normalized signal intensity was analyzed for differential expression using a mixed model ($P \leq 0.05$). Microarray results indicated significantly differential gene expression across time and treatments (AH221, Bu-1, IgG, IgA, IgM, MHC class II β chain, granzyme A, and STAT2). However, only immunoglobulin genes were differentially expressed when comparing B19 and B21 infected chickens. A subset of genes derived from microarray analysis was validated by quantitative PCR. Overall, we noted gene expression patterns that differed between lines

in infected birds, as well as within lines when comparing infected and uninfected birds. These gene expression data have suggested novel candidate genes for resistance or susceptibility to MD emphasizing the involvement of several gene families, including those encoding immunoglobulins, MHC molecules, signal transduction molecules, and cytolytic proteins. Future directions will be aimed at further dissecting the function of these genes and their products in immunity to MD and genetic regulation of resistance to this disease.

Key Words: Marek's disease, disease resistance, microarray

223 Cholesterol levels in divergently selected Japanese quail lines following 35 generations of relaxed selection. C. B. Stephens*, H. L. Marks, M. P. Lacy, E. H. Foster, and M. M. Compton, *Department of Poultry Science, University of Georgia, Athens.*

The purpose of this experiment was to determine if cholesterol levels in a high cholesterol line (HL) and low cholesterol line (LL) of Japanese quail would drift back toward control line (CL) levels if selection pressure was removed. Japanese quail were divergently selected over 18 generations for high and low plasma cholesterol. After 18 generations, the cholesterol levels of high line quail averaged 28% more than controls and the cholesterol levels of low line quail averaged 31% less. The divergently selected lines were then regenerated without additional selection pressure for 35 generations. At 26 weeks of age, 15 male and 15 female quails from each genetic line were weighed and serum samples were obtained. Samples were analyzed for total cholesterol using an enzymatic cholesterol quantification assay. Mean total cholesterol for the HL (400 mg/dL) differed significantly ($P < 0.05$) from the LL (295 mg/dL) and CL (272 mg/dL). There was no significant difference between the LL and CL. No significant differences between cholesterol levels of males and females were observed in any of the lines. There was no significant correlation between cholesterol levels and body weight. These results suggested that genetic differences are still present in HL quail even after long term relaxed selection; however, cholesterol levels in the LL quail appear to have returned to CL levels. Minimum cholesterol levels are essential to many physiological functions including cell membrane development and maintenance, vitamin metabolism, and reproduction. This may be one explanation as to why LL quail returned to CL levels when selection pressure was removed. In the protocol used to generate the 2 divergent lines, quail with high and low cholesterol levels were selected at 4 weeks of age. In the current study, cholesterol was measured in 26-week-old birds. These results indicated the need for further research including assaying cholesterol at 4 weeks of age.

Key Words: Japanese quail, cholesterol, divergent selection

Metabolism and Nutrition VI: Feed Additives

224 Cardiac morphology, fatty acid status and eicosanoid production in broiler birds: Effect of maternal omega-3 fatty acids. J. Bautista-Ortega, G. Cherian*, and D. E. Goeger, *Oregon State University, Corvallis.*

Metabolic, inflammatory (e.g., joint abnormalities), sudden death, ascites, and cardiovascular irregularities are major causes of morbidity and mortality in broiler chickens. The objective of this research was to

evaluate the contribution of a maternal diet rich in n-3 fatty acids on cardiac morphology, polyunsaturated fatty acid and eicosanoid metabolism in broiler birds. Fertile eggs with diverse n-6 and n-3 fatty acids were obtained by feeding breeder hens diets containing 3.0% sunflower oil (Low n-3), 1.5% sunflower oil and 1.5% fish oil (Medium n-3), or 3.0% fish oil (High n-3). These oils were chosen due to their high content of n-6 or n-3 fatty acids. The hatched chicks were fed a diet devoid of long chain n-6 or n-3 fatty acids for 6 weeks. Chicks hatched from Low n-3