

## Genetics: Genetics I

**79 The effect of genotype on internal egg temperature and embryo growth in turkeys.** M. S. Lilburn\* and J. Anderson, *Ohio State University/OARDC, Wooster.*

Within the broiler industry, there has been considerable interest in the interactions between genotype and incubation environment, particularly the airflow and temperature relationships with embryo temperature, hatch, and immediate post-hatch condition of the chick. These relationships have not been as completely explored in turkeys. The objective of the current experiment was to monitor internal temperatures in eggs differing greatly in size due to genotype. The four genotypes used in the this study were as follows: E line, long term selection for egg production; R1, a randombred control line from which E was selected; RBC2, a randombred control line representative of the commercial turkey of the 1960s<sup>TM</sup>; F line, originated from the RBC2 and selected for 16 wk body weight. On day 8 of incubation, egg weight and internal egg temperature were measured on 15 fertile eggs from each line. Four eggs were removed from the incubator at a time (one per line) and the genotypic order of internal egg temperature measurements was altered for each group. On day 23 of incubation, the same measurements were taken and yolk-free embryo weight was determined together with internal egg temperature. The same procedure as described for day 8 was followed. The following egg wt and internal temperature measurements (F) were recorded at day 8: E line, 65 g, 96 F; R1, 88 g, 96.4 F; RBC2, 85 g, 97.2 F; F line, 96.7 g, 97.5 F. On day 23, average internal egg temperature had increased to 98.4 F in all lines and the following yolk-free embryo wt were recorded by line: E line, 31.4 g; R1, 36.1 g; RBC2, 38.1 g; F line, 43.6 g. The data suggest that between 8 and 23 calendar days of incubation, there is an approximate 1-2 F increase in internal egg temperature in all four lines but the absolute temperature is consistent despite big differences in egg size and embryo yolk-free weight.

**Key Words:** Turkeys, Incubation, Temperature

**80 Evaluation of embryonic turkey myogenic cells in two divergent lines.** M. Malone\*, J. Reddish, M. Wick, and M. Lilburn, *The Ohio State University, Columbus.*

Data from previous studies in the literature suggest that differences exist in the proliferation rates of breast muscle myogenic cells cultured post-hatch from two divergent turkey lines. The two lines are a randombred control line (RBC2) and a line developed from the RBC2 selected for body weight at 16-wk of age (F-line). To date, all the data supporting differences in myogenic cell activity have used tissue from post-hatch poultts originating from the RBC2 and F-lines. The objective of the current study was to evaluate potential genetic differences in myogenic cells harvested from embryos from the two lines. Egg weights (91.3 vs. 79.8 g) and embryo weights (24.9 vs. 21.1 g) were increased in the F-line vs. RBC2 line at ED 20. Myogenic cell counts were evaluated in ED 14 and ED 20 embryos. Primary cultures of myogenic cells were isolated from equal amounts of pectoralis major breast muscle from embryos in both lines. Following a 2-h pre-plating period to minimize the proportion of non-myogenic cells, equal numbers of myogenic cells were plated and cultured at 37°C for up to 7 d. During this time, cell counts were determined in five random fields within each culture plate using a light microscope. The cell counts were made at various time points from d 1 up to d 2. Digital photographs were taken to establish a visual time line of cell proliferation over a period of up to 7 d. Cell counts conducted in ED 14 embryos showed greater numbers of cells for the F-line vs. RBC2 line on d 1 (80 vs. 68 cells; mean of five fields per plate, four plates per line) and d 2 of culture (161 vs. 95 cells). Similar trends were observed in muscle cells from F-line and RBC2 embryos at ED 20 on d 1 (95 vs. 80 cells) and d 2 (102 vs. 74 cells) of culture. These results suggest that genetic differences exist in embryonic breast muscle myogenic cell proliferation. The results, however, may not be a direct effect of selection but could be simply due to selection associated increases in egg weight and embryo weight.

**Key Words:** Embryonic, Turkey, Myogenic cell

**81 Broiler growth and development as influenced by divergent selection for ascites.** H. O. Pavlidis\*, L. K. Stamps, J. M. Balog, and N. B. Anthony, *University of Arkansas, Fayetteville.*

Ascites syndrome is an often fatal condition that affects rapidly growing meat-type poultry and results in tremendous economic losses for the poultry producer through on-farm mortality and processing condemnations. Current broiler management practices address this issue through programs designed to slow early growth. These include, but are not limited to, feed restriction, lighting programs and watering systems. A long term selection study using hypobaric hypoxia has yielded research lines that have very different susceptibilities to ascites. In fact, the Susceptible (SUS) line had a reported incidence of ascites of 98.5% while line Resistant (RES) was only 26% when reared under simulated high altitude conditions. Exploration of these lines has revealed that after only 8 generations of selection significant differences in growth pattern were observed. Significant line differences were observed for hatch weight with line RES being higher than SUS. Interestingly, by Day 3 the line differences in BW are gone and by Day 7 a complete reversal in line ranking is apparent with Line SUS being heavier than RES. It appeared that the selection against ascites induced by hypobaric hypoxia has favored a broiler that self-restricts growth. Selection for increased ascites has emphasized a bird that grows very rapidly immediately post hatch. These observed growth differences continue to Day 42 where SUS remains heavier than RES. These differences in BW and growth at Day 42 have translated into both absolute and relative differences in *Pectoralis Major* with Line SUS being greater than RES. By Day 56, however, there was no absolute difference in *Pectoralis Major*, but Line SUS maintained a higher relative breast weight and a smaller relative WOG than Line RES. Selection for ascites resistance has resulted in an alteration of early growth that appears to carry over to economically important traits such as body weight and breast yield when measured at processing ages. Concurrent selection for ascites and respective production traits may counteract these losses.

**Key Words:** Ascites selection, Growth pattern, Breast yield

**82 Broiler lines differing in a major dominant gene for resistance to the ascites syndrome.** S. Druyan\*<sup>1</sup>, A. Ben-David<sup>2</sup>, S. Yahav<sup>3</sup>, and A. Cahaner<sup>1</sup>, <sup>1</sup>*The Hebrew University, Rehovot, Israel,* <sup>2</sup>*Be'er Tuvia Poultry Disease Laboratory, Be'er Tuvia, Israel,* <sup>3</sup>*Agricultural Research Organization, Bet Dagan, Israel.*

Genetic control of the tendency of broilers to develop ascites syndrome (AS) was suggested, but research is hindered by low incidence of AS in commercial populations. Therefore divergent selection aimed at developing AS-resistant and AS-susceptible lines was initiated. Progeny of 85 sires (So generation) from a commercial dam-line were evaluated under AS-inducing conditions. Incidence of AS (%AS) per family ranged from 0 to 50%; and was highly heritable (about 0.6).

Additional chicks (generation S1) were produced from the So parents of families with lowest %AS and highest %AS, and were tested under experimental AS-inducing condition. Significant genetic divergence was established between AS-susceptible and AS-resistant lines already by a single cycle of selection. Among S1 progeny of high-%AS families, 70% of the chicks exhibited AS, in contrast to only 20% among progeny of the low-%AS families. This divergence further increased in the following generations: among the S2 and S3 progeny, %AS was over 90% in the AS-susceptible line, and lower than 20% in the AS-resistant line.

Pedigree analysis suggested that a major dominant gene is responsible for AS resistance, with the majority of AS-resistant birds being homozygous for dominant allele, while AS-susceptible chicks are homozygous for the recessive allele for susceptibility. Broilers of these two lines facilitate a more reliable and sensitive research on various aspects of AS: 1. Determining the genetic basis of AS, using the divergent lines and crosses between them. 2. Developing more reliable physiological criteria for early phenotypic identification AS-resistant and AS-susceptible individuals. 3. Examining the correlated responses to selection

against AS-susceptibility, by comparing the divergent lines under normal environmental conditions.

**Key Words:** Ascites syndrome, Selection, Heritability

**83 Statistical comparison of nonlinear breast yield curves.** M. Zuidhof\*, Alberta Agriculture, Food and Rural Development, Edmonton, AB, Canada.

Statistical comparison of nonlinear relationships is difficult since estimation procedures are not a standard part of most statistical packages. A methodology described by Motulsky and Ransnas (1987) was used to compare breast yield curves of two commercial broiler strain crosses. Single males (M) and females (F) of Ross x Ross 308 (R308) and Ross x Ross 708 (R708) strains were dissected twice per week from 0 to 56 d of age. Additionally, two and ten birds per group were dissected at 53 and 56 d of age, respectively. Huxley's allometric relationship ( $y=ax^b$ ) was employed to relate breast weight to BW, using the NLIN procedure of SAS. Yield curves for M and F were compared within strain, and within strain comparisons were made for both M and F. The following describes the methodology used to compare M and F within a strain. Optimal parameter estimates were obtained separately for M and F. Sums of squares were determined for each sex. Next, optimal parameter estimates were determined for the combined data, and the sum of squares was determined for the pooled data set. To determine whether the separate estimates were better than the pooled estimate, an F ratio was calculated, and a corresponding P value was determined. For each group, nonlinear breast yield curve comparisons showed significant differences (see table).

Reference:

Motulsky, H. J. and L. A. Ransnas. 1987. Fitting curves to data using nonlinear regression: a practical and nonmathematical review. *FASEB J.* 1:365-374.

**Table. Significance of breast meat yield curve comparisons.**

Group 1	Group 2	F value	P > F
F R308	M R308	4.086	0.0222
F R708	M R708	5.157	0.0088
F R708	F R308	6.409	0.0032
M R708	M R308	8.162	0.0008

**Key Words:** Nonlinear model, Breast meat yield, Statistical methodology

**84 Developmental profiling of telomerase genes by quantitative real-time pcr in the chicken embryo.** T. H. O'Hare\* and M. E. Delany, University of California, Davis.

Telomerase activity is essential for maintaining telomere size which has a major impact on genome stability and thus cellular lifespan. Telomerase consists of an RNA template (telomerase RNA, TR) and a reverse transcriptase (telomerase reverse transcriptase, TERT), which catalyzes the addition of telomeric repeats to the chromosome ends. In the chicken, telomerase activity is high during early differentiation and organogenesis, with activity decreasing in many somatic tissues (e. g., brain, heart, liver) and disappearing before hatch. Whereas, stem cells, highly proliferative tissue layers (e. g., intestinal epithelium), and the gamete lineage maintain strong activity at all ages. TR and TERT expression patterns and their correlation with telomerase activity are unknown in the chicken; therefore, the objective of this study was to evaluate developmental expression of TR and TERT. Quantitative real-time RT-PCR (q-PCR) was used to assay mRNA levels by the comparative Ct method. Here we present data from four organs (brain, heart, liver, and intestine) at two ages, 7 and 14 days of incubation (DI) which correspond to when initial changes in telomerase activity occur. TERT levels did not change between the two ages. However, TR levels increased 18-fold in intestine and 3-fold in liver between 7 and 14 DI; these organs maintain high and moderate telomerase activity, respectively. Brain TR levels decreased 5-fold and heart TR levels stayed the same between the two ages. The

latter two organs demonstrate a decrease in telomerase activity between 7 and 14 DI. These results suggest that changes in TR expression rather than TERT are the basis for ontological shifts in telomerase activity in the chicken.

**Key Words:** Telomerase, Development, q-PCR

**85 Association of polymorphisms in MC3R/4R and UCP genes with ascites resistance.** P. Sharma\*, W. Bottje, and R. Okimoto, University of Arkansas, Fayetteville.

Sequence analysis of MC3R, MC4R and avUCP genes revealed novel missense mutations in each gene segregating in a commercial broiler line. Of the two missense mutations found in MC3R, Gly104Ser heterozygote was associated with higher feed efficiency and higher body weight. UCP Ala118Val allele was associated with higher feed efficiency in a male population and MC4R Ser76Leu heterozygote was found to be associated with higher body weight in a male population. We decided to screen these alleles across three ascites lines: the control (unselected), ascites resistant, and susceptible lines. Allele frequency differences were observed between lines. Two SNPs in MC3R gene Met54Leu and Gly104Ser were found to segregate in the population as three MC3R haplotypes Leu-Gly, Leu-Ser, Met-Gly. The MC3R Leu-Ser haplotype was found to be lost by the resistant line, and it was found to be present at low frequency in the control and susceptible lines. The frequency of avUCP Val118, MC3R Met-Gly haplotype, and MC4R Ser76 was higher in the susceptible line. The resistant and susceptible lines are under selection and maintained using 24 males and 3 females mated to each male. Although allele frequency differences have been observed between lines, the possibility of genetic drift cannot be ruled out. Given the frequencies of these alleles, they may not be the major contributors to ascites susceptibility.

**Key Words:** Ascites, Allele, Polymorphism

**86 Myosin isoform transitions in genetically distinct turkey lines.** F. Cihla\*, S. Graham, J. M. Reddish, M. Wick, and M. S. Lilburn, The Ohio State University, Columbus.

Over the past 20 years, there have been significant increases in body weight and breast muscle proportion in commercial broiler and turkey strains. The genetic increases in breast muscle proportion have been accompanied by an increased incidence of muscle quality anomalies, similar to but not the same as the pale, soft, exudative (PSE) genetic muscle defect in swine. Myosin makes up approximately 40% of total breast muscle protein and there are distinct isoforms of myosin corresponding to specific stages of development (i. e. embryonic, post-hatch or neonatal, adult). The objective of this study was to compare the myosin isoform transition pattern in three genetic lines of turkeys. A randombred control turkey line (RBC2) has been maintained at Ohio State for approximately 35 years and this line corresponds to the commercial turkey of the late 1960s. A line was developed from the RBC2 based solely on selection for body weight at 16 wk (F line) and selection now stands at over 35 generations. This line has significantly larger breast muscles than the RBC2 but proportional breast muscle wt is similar in the two lines. The third line studied was a commercial line which is similar in weight to the F line but with a significant increase in the proportion of breast muscle. The hypothesis to be tested was that concomitant with genetic increases in absolute and proportional muscle wt, the transition to the adult myosin isoform would be accelerated in the two heavy turkey lines compared with the RBC2 line. The adult isoform was detected by 14 d of age in all three lines and this is similar to what was reported by Maruyama and Kanemaki (1991). From 14 to 21 d, there appeared to be a rapid increase in the concentration of the adult isoform in the F line compared with the other two lines. In the F line compared with its randombred control (RBC2), selection for body weight is associated with a rapid transition to the adult myosin isoform but this transition pattern does not appear to be affected by selection for both body weight and relative breast muscle weight, as would be the case in modern commercial turkeys.

**Key Words:** Turkeys, Breast muscle, Myosin

**87 A retrovirus insertion causes abnormal expression of the tyrosinase gene in recessive white chickens.** C. M. Chang<sup>\*1</sup>, J. L. Coville<sup>1</sup>, J. P. Furet<sup>2</sup>, A. Oulmouden<sup>4</sup>, G. Coquerelle<sup>1</sup>, D. Gourichon<sup>3</sup>, and M. Tixier-Boichard<sup>1</sup>, <sup>1</sup>National Institute for Agronomic Research, UMR INRA/INA P-G, Jouy-en-Josas, France, <sup>2</sup>National Institute for Agronomic Research, UEPSD, INRA, Jouy-en-Josas, France, <sup>3</sup>National Institute for Agronomic Research, INRA, Nouzilly, France, <sup>4</sup>INRA/University of Limoges, Limoges, France.

The purpose of this study was to identify the causal mutation in the tyrosinase (*TYR*) gene, which could explain the recessive white phenotype in chickens. A restriction fragment length polymorphism (RFLP) was first observed with 3 different enzymes in all chickens carrying the recessive white mutation at C locus, by using a total chicken *TYR* cDNA probe. Several partial probes were then used to confirm and localize the rearrangement region within intron 4. After amplifying intron 4 by long PCR, a major size difference was observed

between the mutant (14kb) and the normal genotype (around 7kb). The amplified fragment was digested by HindIII, then the intronic fragments obtained for the mutant and for the normal were cloned and sequenced. A complete retrovirus insertion was found in intron 4 of *TYR* gene of recessive white chickens. RT-PCR and quantitative RT-PCR tests were performed in order to compare and measure the gene expression level in both recessive white and wild type genotypes. The RT-PCR study showed that the *TYR* gene was transcribed in skin samples for both recessive white and the wild-type chickens, but with an apparently lower intensity in the recessive white genotype than in the wild type. The quantitative RT-PCR study showed that the expression level of *TYR* gene is at least 1000 times decreased in recessive white chickens. It is strongly suggested that the retrovirus insertion affects the splicing procedure and induces the abnormal expression of *TYR* gene in the recessive white chickens.

**Key Words:** Tyrosinase gene, Retrovirus, Recessive white

## Nutrition: Amino Acids

**88 Digestible sulfur amino acid requirement variation in broilers due to sex, rearing environment, and performance parameters.** B. Lumpkins<sup>\*</sup> and A. Batal, University of Georgia, Athens.

Two experiments were conducted to evaluate variations in the estimated digestible sulfur amino acid requirement (DSAAR) for body weight gain (BWG) and gain to feed ratio (GF) due to sex and rearing environment from 7 to 21 d of age. Chicks were sexed immediately after hatch, and placed in either starter batteries or floor pens. A standard corn-soybean meal diet with 23% CP and 3,200 Kcal ME/kg was fed from 0 to 7d of age, after which the chicks were sorted and allocated to the experimental diets. The experimental diets were formulated to be isocaloric and isonitrogenous, and varied only in the level of digestible sulfur amino acid. The digestible sulfur amino acid levels were 0.54, 0.64, 0.74, 0.84, and 0.94% for Experiment 1, and 0.61, 0.71, 0.81, 0.91, and 1.01% for Experiment 2. The true sulfur amino acid digestibility was determined using the total fecal collection precision-fed rooster assay with adult cecectomized roosters. The DSAAR was estimated using one slope broken-line methodology. In Experiment 1, when chicks were reared in starter batteries, the estimated DSAAR based on BWG was higher for females (0.81%) than males (0.76%). However, the estimated DSAAR was higher for males (0.78%) than females (0.68%) based on GF. There was only a slight difference in the estimate DSAAR for males based on either BWG or GF. In females, the estimated DSAAR was higher based on BWG than GF. In Experiment 2, for chicks reared in starter batteries, the estimated DSAAR for males (0.73%, 0.75%) and females (0.73%, 0.74%) was very similar based on both BWG and GF, respectively. However, when the chicks were reared on the floor, the estimated DSAAR was higher for females (0.76%, 0.87%) than males (0.73%, 0.81%) based on BWG and GF, respectively. The estimated DSAAR, based on GF, was higher for chicks reared on the floor compared to chicks reared in the batteries. The inconsistent results suggest no real difference in the estimated DSAAR due to sex. The estimated DSAAR for maximal efficiency may be higher for both males and females when chicks are reared on the floor.

**Key Words:** Sulfur amino acids, Sex, Rearing environment

**89 Standardized ileal amino acid digestibility of crystalline amino acids is close to 100% regardless of the standardization method.** D. Hoehler<sup>\*1</sup>, A. Lemme<sup>1</sup>, C. O. Brito<sup>2</sup>, and H. S. Rostagno<sup>2</sup>, <sup>1</sup>Degussa Corporation, Kennesaw, Georgia, <sup>2</sup>University of Vicosa, Vicosa, Minas Gerais, Brazil.

The use of methionine, lysine, threonine, and tryptophan supplements is common practice in animal nutrition. In the future, potentially some more amino acids might be commercially available for feed production. Amino acids from supplemental amino acid sources were often found to be 100% digestible based on the determination by total tract or excreta amino acid digestibility. This approach has some methodological drawbacks which can be overcome by determination of ileal amino acid digestibility. In the present experiment, 84 male ROSS 308 birds were distributed to 2 treatments with 6 replicates of 7 birds

each at 17 d of age. Birds were housed in metabolism crates. Experimental diets and water were offered for ad libitum consumption from day 17 to 22. Two experimental diets were formulated: a protein-free diet consisting mainly of starch but containing also oil, fiber, mineral and vitamin sources; and a test diet in which 9.1% of the starch of the protein-free diet (PFD) was replaced by a mixture of crystalline amino acids. At d 22 birds were slaughtered by cervical dislocation and ileal digesta from the terminal ileum was collected for digestibility determination. Ileal digestibility figures were corrected for basal endogenous amino acid losses determined firstly by feeding the PFD and secondly by using figures obtained by feeding enzymatically hydrolyzed casein (EHC, based on literature data). Basal amino acid losses at the terminal ileum were between 80 (Met, Trp) and 500 (Thr) mg/kg dry matter intake for individual amino acids. Standardized ileal amino acid digestibilities of crystalline amino acids were: Lys 100.3%, Met 99.6%, Met+Cys 98.7%, Thr 97.0%, Trp 101.6%, Arg 100.5%, Ile 99.9%, Leu 100.3%, and Val 99.9% when corrected for basal endogenous losses based on feeding the PFD diet. The digestibility figures are very close to 100% regardless of the way of standardization methodology (PFD or EHC), suggesting a complete absorption of crystalline amino acids until the terminal ileum in growing broiler chickens.

**Key Words:** Amino acids, Ileal digestibility, Broiler

**90 Effects of age on amino acid digestibility in turkeys.** O. Aimiwu<sup>\*</sup> and M. Lilburn, The Ohio State University/OARDC, Wooster.

In turkeys, there is not much documented research on the relationship between age and amino acid digestibility. Since the protein and amino acid requirements for poulters are the highest of all commercial poultry species, it is important to ascertain the digestibility coefficients of common feedstuffs at different ages so as to formulate diets as accurately and as economically feasible as possible. Three ileal amino acid digestibility trials were conducted to study the effects of age on the ability of poulters to digest protein from common protein sources. Poulters were fed semi-purified diets containing meat and bone meal (MBM), soybean meal (SBM), fishmeal and a commercial 60% CP blend (blend) as the sole sources of protein. Two complete diets formulated to commercial specifications were also tested. One diet contained corn with a high level of SBM (CS) and the other contained corn, fish meal and a lower level of SBM (CSF). There was an overall diet effect on digestibility ( $P < 0.05$ ). At 5 d of age, poulters fed the diets containing MBM and fishmeal had significantly lower digestibility coefficients ( $P < 0.05$ ) compared with 3 and 6 week-old poulters. There were no significant age effects on essential amino acid digestibility in poulters fed the commercial protein blend, CS and CSF complete diets except for threonine in the protein blend, histidine, methionine and valine for the complete diets, including lysine for the CS diet. It is therefore concluded that the effects of age on feed ingredient digestibility is influenced by the type of feed ingredient.