

Breeding and Genetics: Molecular Genetics

T24 Quantitative genetics and differential performance and gene expression of half-sib families of hybrid striped bass in communal ponds. S. A. Fuller*, B. H. Beck, M. McEntire, and D. Freeman, *USDA ARS Stuttgart National Aquaculture Research Center, Stuttgart, AR.*

The US is one of the world's largest importers of seafood. A major constraint in producing hybrid striped bass is suboptimal production efficiency due to large performance variation of fish from undomesticated brooders. The objectives of this first-year study were to determine the genetic basis of production traits for selective improvement, and RNaseq superior and inferior performing representatives to identify global expression differences and develop predictive SNP markers as part of a multi-year improvement project. Domesticated F8 white bass and F4 striped bass were bred in a partial diallel breeding design and reared in replicate family tanks until large enough to tag with a PIT tag. Thirty-two fish from each of 44 half-sib families were tagged and initial length and weight was recorded before being randomly assigned to one of 4 0.04 ha communal ponds resulting in 5632 individually tagged fingerlings. Fish were allowed to grow for 115 d before harvest. At harvest tags were scanned to reveal family of origin, final length and weight were taken, fish were humanely sacrificed and a liver and muscle sample were flash frozen for qPCR and RNaseq analyses. Following pond production, hybrid striped bass averaged 235.3 ± 17.8 (SD)mm and 192.1 ± 48.7 g across all families and ponds, with a range from 110 to 288mm and 47.3–371.7g. Analyses of covariance demonstrated highly significant differences in length and weight of fish among different paternal and maternal half-sib families with initial weight as the covariate ($P < 0.0001$). Estimates of heritability were high for both traits, with values for weight and length, respective, ranging from 0.74 to 0.97 for dams and 0.52 to 0.99 for sires. Liver RNaseq data are currently being analyzed from high and low performing families and individuals and SNP markers validated to identify markers for future marker assisted selection. Incorporating crossbred offspring performance into a genetic improvement program could be used to successfully produce more rapidly growing hybrid striped bass and improve the profitability of the industry.

Key words: hybrid striped bass, aquaculture, genetic improvement

T25 Effects of transgenic myostatin depression on reproductive parameters and placental superoxide dismutases in mice. S. Yarl-agadda, C. N. Lee*, Y. S. Kim, J. Yang, and W. Y. Ho, *University of Hawaii-Manoa, Honolulu.*

Double muscled cattle carrying non-functional myostatin mutations have high incidences of dystocia, low calf viability and higher heat intolerance. Myostatin-null mice and transgenic mice with depressed myostatin function by its propeptide overexpression appeared normal in reproduction as no dystocia was observed in our colony. To gain insights into the effects of depressing myostatin on reproduction, we compared pelvic width, uterine length, hormonal profiles and the activities of placental superoxide dismutases (SOD) of transgenic mice overexpressing myostatin propeptide to those of their wild-type littermate controls. Ten pregnant transgenic females (TG, B6SJL strain) and 10 wild type females (WT) of greater than 2 mo old were used. Pelvic width of TG mice at 10 and 16 d of pregnancy was not different from wild type mice. Serum estrogen was not affected by genotypes while TG mice had higher concentrations of serum progesterone at 10 d of pregnancy than WT females (213 ± 38.9 pg/ml vs 101 ± 17.5

pg/ml, $P < 0.05$). However, by d16, WT females had higher serum progesterone vs TG females ($447.4 + 37.3$ pg/ml vs $318.4 + 37.1$ pg/ml). Mn-SOD and Cu/Zn-SOD protein levels in placenta, 2 antioxidant defense proteins, decreased significantly from d 10 to 16 of pregnancy. However, their expression levels in placenta or ovarian tissue were not different between TG and WT mice. These results suggest that myostatin suppression has no effects on placental or ovarian tissue antioxidant proteins, serum estrogens and pelvic development.

Key words: myostatin suppression, superoxide dismutases, reproductive parameters

T26 Study of polymorphism at CSD gene in *Apis mellifera meda*. S. Karimi*¹, A. Nejati Javaremi¹, S. R. Miraei Ashtiani¹, and H. Alizadeh², ¹University of Tehran, University College of Agriculture and Natural Resource, Department of Animal Science, Tehran, Karaj, Iran, ²University of Tehran, University College of Agriculture and Natural Resource, Agronomy & Plant Breeding Department, Tehran, Karaj, Iran.

About 20% of animal species have a haplodiploid system of sex determination. Males are haploid from unfertilized and females are diploid from fertilized eggs. It is known that a complementary sex determining (csd) gene is responsible for sex determination in honeybees. In this species, csd acts as the primary sex-determining signal with several alleles segregating in populations. Males are hemizygous and females are heterozygous at this locus; non-reproducing diploid males occur when the locus is homozygous. If inbreeding increases between bees, the possibility of cross between queen with drones with similar alleles at this locus will increase. Other studies indicated that this phenomenon leads to increase in the percentage of diploid drones in population which, in turn, leads to decrease of colony growth and, subsequently, economic losses. Genotypes of 17 queens from several apiaries were determined through genotyping of about 6 of their haploid drone progenies. A total of 108 samples from 16 colonies were made. RNA extraction was done with High pure RNA Isolation kit. Then sscDNA was produced by RevertAid MMuLV RT enzyme. EF-1alpha was used as housekeeping gene and positive control. In the next stage with a pair of specific primers, the region between exons 5 and 9 of csd gene with 300 to 400 bp in length in different alleles was reproduced by using polymerase chain reaction. PCR product was run on 1.5% agarose gel and each of the 2 different alleles of each queen were sequenced. In cases where polymorphism could not be recognized by length differences of the PCR products, they were digested using SspI enzyme. Digested products were then run on the 6% polyacrylamide gel. Two different alleles of each queen were selected for sequencing. About 25 samples were sequenced. Seven functionally different alleles were found. Queens within the same apiary have more similar genotypes compared with the genotypes of queens from other apiary. Genotyping of queens of apiaries involved in production of commercial honeybee queens may help reduce the incidence of diploid drones.

Key words: haplodiploid, honeybee, drone

T27 Growth-related differential gene expression in the longissimus thoracis muscle of Iberian \times Landrace back-crossed pigs. J. Casellas*^{1,2}, J. L. Noguera², R. N. Pena^{2,3}, J. M. Folch¹, M. Muñoz⁴, and N. Ibáñez-Escriche², ¹Departament de Ciència Animal i dels Aliments, Universitat Autònoma de Barcelona, Bellaterra, Spain, ²Genètica i

Millora Animal, IRTA-Lleida, Lleida, Spain, ³Departament de Producció Animal, Universitat de Lleida, Lleida, Spain, ⁴Departamento de Mejora Genética Animal, SGIT-INIA, Madrid, Spain.

The aim of this study was to identify growth-related differential gene expression in the longissimus thoracis muscle of finished pigs. A total of 102 Iberian (25%) × Landrace (75%) back-crossed pigs were reared under standard management conditions, weighted at 90 d (34.4 ± 0.6 kg), 105 d (43.6 ± 0.7 kg), 120 d (54.3 ± 0.8 kg), 135 d (65.0 ± 1.0 kg), 150 d (74.0 ± 1.1 kg), 165 d (86.8 ± 1.2 kg) and 175 d of age (96.1 ± 1.3 kg), and slaughtered at an average age of 179.9 ± 0.3 d. Samples of the longissimus thoracis muscle were collected at slaughter, snap frozen and stored until analysis. For each sample, total RNA was isolated and individually hybridized in the GeneChip Porcine Genome array (Affymetrix, Santa Clara, CA). After normalizing raw data with the RMA algorithm from the Bioconductor package, gene expression scores from 13,547 probes were analyzed with the GEAMM software under a multivariate mixed linear model accounting for the systematic effect of each array as well as 4 sources of variation modeled under normal priors: probe, sex (male or female), fattening batch (3 levels) and pig growth during fattening (continuous effect). Pig growth was calculated as the regression coefficient (i.e., slope) of pig weight against pig age across all weighting events during fattening (0.59 ± 0.1 kg/d). The Bayesian analysis launched a unique Monte Carlo Markov chain with 110,000 iterations, the first 10,000 of them being discarded as burn-in. Focusing on the link between pig growth and gene expression, 14 probes reached the significance threshold after correcting for multiple testing by false discovery rate ($\alpha = 0.05$; $P < 0.000052$), although 3 of them belonged to the same locus (GAPDH) and showed similar estimates. It is important to highlight that most of the significant loci could be grouped on the basis of their biological pathway, i.e., carbohydrate metabolism (ENO3, GAPDH, LDHA and PGM1), muscle contraction (MYL1 and TNNT3) and ribosomal structure (RPL36A).

Key words: gene expression, longissimus thoracis, pig growth

T28 Path analysis of candidate genes for intramuscular fat in pigs. N. V. L. Serão*^{1,3}, J. Braccini Neto², A. M. F. Ribeiro³, P. V. Silva³, S. L. Rodríguez-Zas¹, and S. E. F. Guimarães³, ¹University of Illinois at Urbana-Champaign, Urbana, ²Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, ³Universidade Federal de Viçosa, Viçosa, MG, Brazil.

High levels of intramuscular fat (IMF) have generally a positive influence on the sensory experience associated with eating pork. This study aimed to identify gene expression profiles with direct and indirect association with IMF content in pigs. Longissimus dorsi samples from 72 male and female pigs representing 3 genetic groups slaughtered at different weights were used to quantify the IMF content and to measure the expression of 9 candidate genes: ATN1, EEF1A2, FABP3, LDLR, MGP, OBSCN, PDHB, RYR1 and TRDN. The IMF content was determined by ether extraction and expressed as percentage in fresh meat. Gene expression was measured using real-time PCR. To determine the basic causal model of the path analysis for IMF and associated genes, 10 path models were created. The first model included IMF as the outcome variable and the 9 genes as predictors. The other path models had one gene as outcome variable and the other 8 as predictors, until all 9 genes were used as the outcome variable. Predictor genes were selected in the model following a stepwise method ($P < 0.1$). Using these relations and path analysis, the associations between genes and IMF were inferred using the PATH statement of the TCALIS procedure

(SAS version 9.2). The basic causal model was sequentially analyzed, removing the least significant relation at a time until all remaining genes were significant ($P < 0.05$). This model included 29 direct relations, where only FABP3, EEF1A2 and LDLR had direct effects on IMF. After a progressive selection, the final causal model included 13 direct relations ($P < 0.05$). The same direct predictors of IMF were kept, but ATN1 was dropped from the path model. The higher effect was observed from OBSCN to RYR1 (0.7219) and the smallest from MGP to RYR1 (-0.1785). All genes in the model acted as predictors and outcomes, with the exception of MGP (only as predictor). Genes FABP3 through LDLR and PDHB through FABP3 showed indirect effect of their expression on IMF. The results of this analysis provided an intuitive and comprehensive path diagram with estimates of direct associations among candidate genes, and direct and indirect associations between these genes and IMF.

Key words: gene expression, meat quality

T29 Evaluating statistical models to assess differential gene expression in PRRSV infected pigs using plasmode datasets. M. E. Arceo*¹, C. W. Ernst¹, M. Wysocki², J. K. Lunney³, and J. P. Steibel¹, ¹Department of Animal Science, Michigan State University, East Lansing, ²Lehrstuhl für Tierzucht, Technische Universität München, Munich, Germany, ³Animal Parasitic Diseases Laboratory, ARS, USDA, BARC, Beltsville, MD.

Porcine reproductive and respiratory syndrome virus (PRRSV) causes substantial economic losses for US farms. The variability of pig response to PRRSV infection suggests a host genetic component involved in the pathogenesis of the disease. With data collected from Hampshire-Duroc cross and NE Index line pigs infected with PRRSV, Petry identified low (LR) vs. high (HR) PRRSV burden pigs (with low vs. high viremia, good vs. low/no weight gain, and few vs. many lung lesions). Microarray technology has been applied to identify differentially expressed genes using RNA from lung and bronchial lymph node (BLN) tissues of HR and LR pigs. The objective of this work was to use this data to assess different statistical models for analyzing microarray data using plasmode data sets. To build the plasmode data sets, we permuted array-treatment labels resulting in 34 independent data sets where no differential expression is expected, but the normal biological variation is conserved. Plasmode data were used to compare linear fixed models to linear mixed models. Test statistics that borrow information from data across all genes (moderated tests) to estimate variances and assess significance were also considered. Type I errors were evaluated at nominal 0.05, 0.01, 0.005, 0.001 and 0.0001 levels. To attain control of nominal type-one error rates, moderated tests required the use of permutations to compute p-values. We modified R/maanova software to obtain such permutations more efficiently (3-fold decrease in elapsed computational time) at the expense of re-using estimated variance components from the data. The most powerful results were obtained from a mixed effects model with moderated tests (Fs test) and unmodified permutation scheme (69 significant genes at FDR 10%). Using the modified permutation scheme resulted in less power than a fixed effects model with unmodified permutations (3 versus 21 significant differences at FDR 10%). In summary, powerful analysis of gene expression data remains a computationally challenging task. This work was supported by the PRRS CAP, USDA NIFA Award 2008–55620–19132.

Key words: PRRSV, microarray, linear models

T30 Structural changes at bovine IgE as related to variation at the DNA level. I. Rivera, M. Pagan*, E. Jimenez, and G. Ortiz, *Department of Animal Industry, University of Puerto Rico at Mayaguez, Mayaguez, PR.*

Bovine immunoglobulin E (IgE) was evaluated as a candidate gene to study potential variations in resistance to parasite infestation and anthelmintic efficiency. A DNA pooling and nucleotide sequence strategy was used to identify single nucleotide polymorphisms (SNPs) at the IgE heavy chain constant region gene (GenBank Accession no.: U63640). General (n = 319 bulls) and individual breed pools were constituted using DNA from Angus (n = 39), Senepol (n = 60), Charbray (n = 43), Charolais (n = 62), Bos Indicus (n = 39), and crossbred (n = 76) cattle. Polymerase chain reaction primers were designed to amplify regions of exons 1–3. At exon 1, a cytosine/guanine transversion and a cytosine/thymine transition resulting in silent mutations (threonine and serine, respectively) were identified. At exon 3, an adenine/guanine transition corresponding to an arginine to glycine amino acid change was recognized (only the Angus bulls were completely homozygous GG for such SNP). Meanwhile, exon 2 was highly polymorphic (n = 5 SNPs). Of the SNPs located in that part of the IgE gene, a cytosine/guanine transversion was silent (alanine). However, a cytosine/thymine substitution changed a polar amino acid (proline) by another (leucine). In this case, all the Charbray animals were classified as homozygous TT. The other 3 SNPs corresponded to a proline (non polar) to histidine (basic), proline to glutamine (polar), and an asparagine (polar) to aspartic acid change. In the last 2, homozygosity was observed within the Angus and Charolais breeds (AA and GG for Pro/Glu and Asp/Asn, respectively). Moreover, the SNP responsible of the Pro/His residue substitution was not segregating in Angus. Because polymorphism at IgE has been implicated in resistance to gastrointestinal nematodes infection in ovinines, the structural changes reported herein in bovines needs further evaluation to elucidate potential association with immune response and overall health.

Key words: IgE, polymorphisms, bovine

T31 Association between SNPs in candidate genes and residual feed intake in Angus cattle. A. I. Trujillo*, A. Casal, P. Grignola, J. P. Marchelli, and P. Chilibroste, *Departamento de Produccion Animal y Pasturas, Facultad de Agronomia, Universidad de la Republica, Montevideo, Uruguay.*

Residual feed intake (RFI) is a measure of feed efficiency which is an economically important trait in livestock. Single nucleotide polymorphisms (SNPs) that show associations with RFI may be useful for marker-assisted selection. There is limited research examining the relationship between specific genes mutations and RFI. Neuropeptide Y (NPY), leptin (LEP) and insulin like growth factor-1 (IGF-1) are candidate genes due to their roles in the regulation of feed intake, energy balance, and growth. This study examined the relationship between SNPs previously identified in NPY (A/G, intron 2), LEP (C/T, exon 2) and IGF-1 (C/T, promoter region) genes with feed efficiency and performance in beef Angus calves. Thirty 8 female calves were selected from a total of 1700 genotyped calves born in spring 2009. Half of the calves were carrying 3 “favorable” alleles simultaneously (V, validation group) while the other half was carrying 3 “unfavorable” alleles (C, control group). Calves were allocated to individual pens in a completely randomized design (initial BW = 186.2 ± 32 kg). Individual feed intake (FI) and body weight (BW) were measured during 56 d. Calves were fed twice daily of a mixed diet (60:40 as fed) compound concentrate: chopped alfalfa hay. FI was estimated daily

by difference between feed offered and refused. Phenotypic RFI was calculated as the residuals from a regression model regressing FI on ADG and mid test metabolic weight (MMW). Mean ADG, FI, feed conversion ratio (FCR), and RFI were 1.24 ± 0.19, 7.89 ± 1.19, 6.22 ± 0.73 and 0.00 ± 0.42 kg/d, respectively. Differences among groups in ADG and FCR were not significant ($P = 0.42$, $P = 0.86$, respectively). However, calves of V group tended to have lower FI ($P = 0.065$) and to be more efficient (RFI = -0.115; $P = 0.105$) than animals of C group. RFI was correlated with FI ($r = 0.36$) and FCR ($r = 0.54$), but not with ADG or MMW. Our preliminary results show a suggestive association between the SNPs studied and RFI in Angus cattle despite further research is warranted.

Key words: residual feed intake, beef cattle, SNPs

T32 Identification of a JY-1 gene variant in Nelore cattle. G. M. F. de Camargo*¹, A. C. de Freitas¹, A. C. Andrade¹, F. M. M. Gil¹, D. F. Cardoso¹, P. D. S. Fonseca¹, F. R. P. Souza¹, M. Cervini¹, F. Baldi¹, L. G. de Albuquerque¹, L. C. A. Regitano², and H. Tonhati¹, ¹Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, ²Brazilian Agricultural Research Corporation - Southeast Cattle Center, Sao Carlos, Sao Paulo, Brazil.

The JY-1 protein is found in monoovulatory species and was first described in cattle. It is an oocyte specific protein and it plays a key role in the regulation of the granulose cells functions. It also influences the early embryo development. Other genes with similar functions were described in polyovulatory animal. The aim of this study was to analyze a region of the exon 3 of the JY-1 gene in Nelore cattle to investigate possible polymorphisms and their association with reproduction performance. DNA was extracted from tail hair of 298 Nelore heifers by the phenol-chloroform-isoamyl alcohol protocol. The heifers were divided in 2 groups: 149 heifers that conceived at 16 mo of age and 149 heifers that failed to conceive at 16 mo. The primers 5'ATCAAAGTGAACAGGGCAGA3' and 5'AAGTATGACAAGA-GATACGGTCAGG3' were designed to amplify a partial region of exon 3. The fragment amplified by the PCR has 373 bp. After, the RFLP analyses were done with the restriction enzyme SspI whose restriction site is 5'AATATT3'. It was possible to identify 2 genotypes (TT and TC) and characterize 2 variants: T and C (GenBank accession numbers: JF262042 and JF262043). The genotype TT has 2 bands with 208 bp and 165 bp and the genotype TC has 3 bands of 373 bp, 208 bp and 165 bp. The allelic frequencies were 0.97 and 0.03 for variant T and variant C, respectively. The genotypic frequencies were 0.94 and 0.06 for the genotypes TT and TC, respectively. The genotypic frequencies deviated ($P < 0.05$) from Hardy-Weinberg equilibrium, which could be due to the low frequency of variant C. The sequences available at GenBank for this region (NM_001110098.1, EF642497.1 and EF642496.1) characterize the variant C in *Bos taurus taurus*, which is the variant with the lowest frequency in *Bos taurus indicus*. The correlation between the genotypes and the pregnancy at 16 mo was not done because of the divergence between the frequencies and also because of the equal distribution of the patterns between the heifer groups. So, future studies must be done to identify other polymorphisms in JY-1 gene segregating in Nelore cattle and analyze their possible correlations with heifer pregnancy.

Key words: PCR-RFLP, polymorphism, SNP

T33 Novel associations between a SNP in the bovine DDEF1 gene and production traits in Nelore breed. P. C. Tizoto*¹, S. L.

Meirelles¹, G. B. Veneroni¹, M. M. de Souza¹, F. Siqueira², A. do Nascimento Rosa², L. O. Campos da Silva², R. de Almeida Torres², S. R. Medeiros², R. R. Tullio³, M. M. de Alencar³, G. Feijó², and L. C. de Almeida Regitano³, ¹Federal Universidade of São Carlos, São Carlos, São Paulo, Brazil, ²Embrapa Beef Cattle National Center, Campo Grande, Mato Grosso do Sul, Brazil, ³Embrapa Southeast Cattle Research Center, São Carlos, São Paulo, Brazil.

Concomitant with the traditional selection, which has produced interesting results, marker assisted selection (MAS) can help breeding programs and improve profiles for economically important traits. The identification of markers associated with interest production traits is a fundamental step for implementing MAS in breeding programs. This project aimed to study the association between a SNP (G/A) in intron 13 of bovine DDEF1 (development and differentiation enhancing factor 1) gene and the traits weaning weight adjusted to 240 d (WW), yearling weight adjusted to 450 d (YW), backfat thickness (BFT), ribeye area (RYA) and shear force at 24 h postmortem (SF), in reference families of Nellore breed. We used about 270 steers, descendants of 20 Nellore bulls selected to represent variability within the Nellore breed. SF measures were available from only 140 steers. At approximately 18 mo of age, animals were transferred from grazing systems to feedlots, where they were finished for about 100 d before slaughtering for meat quality data collection. The genotypes were determined by amplification refractory mutation system (ARMS-PCR). A mixed model with fixed effects of contemporary group and genotypes, and age of the animal at the time of measurement (linear effect) as a covariate and the random effect of bull was used to evaluate the marker effect. For SF data the model also included pH as a covariate. Analyses were done by the maximum restricted likelihood (REML) using PROC MIXED of the Statistical Analysis System (SAS). The SNP in DDEF1 gene showed significant association with WW ($P = 0.0021$), YW ($P = 0.0109$), RYA ($P = 0.0109$) and SF ($P = 0.0083$). It represented 2.18%, 4.61%, 4.28% and 1.13% of total additive variance and 6.16%, 4.32%, 4.74% and 17.51% of total phenotypic variance for WW, YW, RYA and SF, respectively. Therefore, this SNP is a good candidate for application in MAS of meat production and quality traits in Nellore breed.

Key words: beef, SNP, DDEF1

T34 CAPN4751 and UOCAST effects on feed efficiency, carcass traits and feedlot performance in Nellore (*Bos indicus*) cattle. R. C. Gomes^{*1}, M. E. Carvalho², M. H. A. Santana¹, S. L. Silva¹, P. R. Leme¹, P. Rossi³, and J. B. S. Ferraz¹, ¹Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo (FZEA/USP), Pirassununga, SP, Brazil, ²Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (ESALQ/USP), Piracicaba, SP, Brazil, ³Departamento de Zootecnia, Universidade Federal do Paraná (UFPR), Curitiba, PR, Brazil.

The calpain system is related to protein turnover and may affect productive traits in livestock. Thus, the aim was to examine associations among SNP polymorphisms in the calpain and calpastatin genes and carcass traits, growth and feed efficiency in beef cattle. Nellore steers and bulls ($n = 290$; 378 ± 42 kg BW, 23-mo \pm 42d old) were feedlot fed in tests conducted at FZEA/USP, Pirassununga, Brazil, from 2007 to 2010. Dry matter intake (DMI) and average daily gain (ADG) were recorded for 50 to 84 d and carcass traits were assessed by ultrasound. Feed conversion ratio (FCR), gross feed efficiency (GFE), partial efficiency of growth (PEG) and residual feed intake (RFI) were computed. Cattle were genotyped for CAPN4751 (C/T, 6545 bp of AF248054) and UOCAST (C/G, 282 bp of AY008267) in the cal-

pain and calpastatin genes, respectively. The genotyping was carried out using TaqMan Real Time PCR assays. Association analyses used a linear mixed model with contemporary group, sex and genotype as fixed effects, age as covariate and sire as a random effect. Frequencies for C and T alleles of CAP4751 and C and G alleles of UOCAST were 0.13, 0.87, 0.55 and 0.45, respectively. Genotypic frequencies were 0.02, 0.22 and 0.76 for CC, CT and TT of CAP4751 and 0.29, 0.51 and 0.20 for CC, CG and GG genotypes of UOCAST, respectively. Because the low CC frequency, CAP4751 effects were tested by CT vs. TT contrasts. No CAP4751 effects were observed for DMI ($P = 0.11$), GFE ($P = 0.25$), FCR ($P = 0.46$), PEG ($P = 0.67$), RFI ($P = 0.75$), backfat thickness (UBFT; $P = 0.74$) and ribeye area (UREA; $P = 0.76$). There were differences between CT and TT genotypes of CAP4751 on final BW (486 vs. 473 kg, $P = 0.03$), ADG (1.53 vs. 1.44 kg/d, $P = 0.04$) and rump fat thickness (URFT, 7.5 vs. 6.70 mm, $P = 0.04$). UOCAST did not affect BW ($P = 0.59$), DMI ($P = 0.48$), GFE ($P = 0.12$), FCR ($P = 0.31$), PEG ($P = 0.40$), RFI ($P = 0.45$), UBFT ($P = 0.76$), URFT ($P = 0.90$) and UREA ($P = 0.61$). An additive effect was observed for UOCAST on ADG (-0.0685 ± 0.026 kg/d, $P = 0.0093$). The UOCAST and CALP4751 polymorphisms can affect growth and carcass traits in Nellore cattle but not feed efficiency.

Key words: *Bos indicus*, DNA marker, residual feed intake

T35 Biallelic expression studies of CAST gene in bovine muscle. M. M. de Souza¹, S. C. M. Niciura², A. M. G. Ibelli¹, S. L. Meirelles¹, M. I. Rocha¹, P. C. Tizoto^{*1}, G. Gasparin³, M. E. Carvalho³, G. B. Veneroni¹, F. A. Bressani², P. S. N. de Oliveira¹, F. Siqueira⁴, L. L. Coutinho³, and L. C. de Almeida Regitano², ¹Federal University of São Carlos, São Carlos, São Paulo, Brazil, ²Embrapa Southeast Cattle Research Center, São Carlos, São Paulo, Brazil, ³University of São Paulo, Piracicaba, São Paulo, Brazil, ⁴Embrapa Beef Cattle National Center, Campo Grande, Mato Grosso do Sul, Brazil.

Bovine meat tenderness is the main feature appreciated by consumers and is influenced by calpastatin protein activity. This protein is codified by CAST gene and is the main modulator of μ -calpain protease, which in turn, degrades the myofibrillar proteins of skeletal muscle in the post-mortem period. In general, models used for gene-phenotype associations consider equal expression of both alleles. Therefore, departures from biallelic expression patterns must be incorporated in models of quantitative genetic analysis because it may result in differences in males and females breeding values. This study aimed to analyze allelic expression pattern of CAST gene in muscle tissue of Nellore steers immediately after slaughter. A group of 270 animals were genotyped for the polymorphism A/G within the 3' UTR of CAST gene by using TaqMan probes in real-time PCR. RNA was extracted from muscle of 14 heterozygotes to produce first strand cDNA. The allelic expression has been analyzed by using the same TaqMan probe used for genotyping. A standard curve was made to normalize the specific probe fluorescences for each allele in the TaqMan assay. This was made with dilutions of genomic DNA from 2 homozygous animals AA and BB; the dilutions were 8:1, 4:1, 2:1, 1:1, 1:2, 1:4 and 1:8. For each dilution the \log_2 (FAM intensity/VIC intensity) was calculated at the last cycle (40) of PCR, thus the allele fluorescence ratio of the 14 steers was extrapolated in the standard curve. Therefore it was possible to measure whether there were differences in allelic expression and whether it was inherited from mother or father. The non-parametric randomization test was used for statistical analysis. Parental origin was not found to affect allele expression ($P > 0.05$). Although the expression of the G allele was 1.4 times that of the A allele, that difference was not significant. Finally, considering that other published papers described

allelic specific gene expression using smaller sample size, it is possible to conclude that the CAST gene shows biallelic expression in skeletal muscle during the post-mortem period in Nelore cattle breed, compatible with general quantitative models for marker-assisted selection.

Key words: CAST, SNP, imprinting

T36 The polymorphism Msp I in intron 3 of the growth hormone gene in Nelore cattle (*Bos taurus indicus*). D. F. Cardoso¹, G. M. F. de Camargo*¹, P. D. S. Fonseca¹, F. M. M. Gil¹, M. G. Chiquitelli¹, F. R. P. de Souza¹, L. G. de Albuquerque¹, M. E. Z. Mercadante², and H. Tonhati¹, ¹Department of Animal Sciences, Sao Paulo State University, Jaboticabal, Brazil, ²Animal Science Experimental Station, Sertãozinho, SP, Brazil.

The growth hormone gene is very studied in animals due to its key role in biological functions. In *Bos taurus taurus*, this gene is correlated to growth, body composition and even milk composition. The growth hormone in cattle (bGH) has a polymorphic structure that characterizes it as a potential molecular marker that may assist selection process. The aim of this work was to verify and analyze the polymorphism described in the intron 3 of *Bos taurus taurus* in Nelore cattle (*Bos taurus indicus*) to validate these associations in this breed. The DNA was extracted from blood samples of 238 Nelore cattle belonging to 3 lines selected for growth from the selection program of the Animal Science Experimental Station in Sertãozinho-SP, Brazil. A fragment of 329 bp from intron 3 was amplified by PCR with the primers 5'CCCACGGGCAAGAATGAGGC3' and 5'TGAGGAACTGCAGGGGCCCA3'. The RFLP was done with the restriction enzyme MspI. It was possible to identify 3 migration patterns, the first one has one fragment of 329 bp and corresponds to the homozygote $-/-$, the second one has 2 fragments of 224 bp and 105 bp and corresponds to the homozygote $+/+$, the third one has these 3 fragments above and corresponds to the heterozygote $+/-$. The allelic frequency was 0.26 to the allele MspI(+) and 0.74 to the allele MspI(-). The genotypic frequencies were 0.06, 0.41 and 0.53 to the genotypes $+/+$, $+/-$ and $-/-$, respectively. Although these animals belongs to a selection program, the frequencies are in Hardy-Weinberg equilibrium at 5%, indicating that this locus of the bGH is not affected by selection. It is important to emphasize that the polymorphism recognized by the endonuclease MspI has different frequencies in Zebu and European cattle. The MspI(+) allele that is correlated to milk production and composition has high frequencies in European breeds and low frequencies in *Bos taurus indicus*. The results indicate that the PCR-RFLP/MspI is efficient to detect the polymorphism in the intron 3 of the bGH in Nelore cattle and it may be associated with important economic traits. Financial Support: FAPESP

Key words: molecular marker, beef cattle, RFLP

T37 Polymorphisms of the IGF1 and MSTN genes in Nelore beef cattle (*Bos indicus*) and in their crosses with *Bos taurus*. R. A. Curi¹, M. R. S. Fortes², D. M. Vankan², J. A. V. Silva*¹, H. N. Oliveira³, M. D. S. Mota¹, and A. C. Silveira¹, ¹Faculdade de Medicina Veterinária e Zootecnia, Unesp, Botucatu, São Paulo, Brasil, ²School of Veterinary Science, University of Queensland, St. Lucia, Queensland, Australia, ³Faculdade de Ciências Agrárias e Veterinárias, Unesp, Jaboticabal, São Paulo, Brasil.

The aim of this study was to estimate the segregation of the single nucleotide polymorphism (SNP) AF_017143.1:g.198C > T of the IGF1 gene and AF_320998.1:g.433C > A of the MSTN gene in Nelore

(*Bos indicus*) and Nelore × *Bos taurus* beef cattle, and to evaluate their effects on carcass and meat traits. A total of 300 animals (114 Nelore and 186 crosses) were genotyped and phenotyped for rib eye area (REA), backfat thickness (BT), intramuscular fat (IF), shear force (SF) and myofibrillar fragmentation index (MFI). The allele substitution effects for each of the polymorphisms on the traits of interest were estimated by regression of the phenotypes analyzed on the number of copies of a particular allele using the General Linear Model procedure. The polymorphism of the IGF1 was non-informative in Nelore animals with allele C was found to be fixed. Although association between allele C and greater REA has been verified in animals from crossing ($P < 0.05$), this is no longer significant after the Bonferroni correction of hypothesis tests for multiple comparisons. The allele A of the SNP of the MSTN was absent in Nelore and it is only found in 2 crossbred animals, impairing association studies. The present results suggest the lack of potential for application in marker-assisted selection of the analyzed SNPs in cattle with breed compositions similar to those described here. Furthermore, the absence of these SNPs in Nelore cattle, a situation that may extend to other *Bos indicus* breeds, indicates the need to use other identified polymorphisms or the search for new polymorphisms in the IGF1 and MSTN genes to carry out futures association studies involving this subspecies.

Key words: fat deposition, meat tenderness, molecular markers

T38 Characterization of polymorphism in the ORL1 gene in Nelore cattle (*Bos taurus indicus*) by PCR-RFLP. P. D. da Silva Fonseca¹, F. R. P. de Souza¹, G. M. F. de Camargo*¹, F. M. Gil¹, D. F. Cardoso¹, M. G. Chiquitelli¹, L. G. Albuquerque¹, M. E. Z. Mercadante², and H. Tonhati¹, ¹São Paulo State University, São Paulo State University, Jaboticabal, Brazil, ²Animal Science Experimental Station, Animal Science Experimental Station, Sertãozinho, Brazil.

The ORL1 gene, under normal conditions, has low expression in adipocyte cells. In obese animals its expression is higher and it causes an increase in the cholesterol content and promotes the capture of fatty acids. Cholesterol and triglycerides concentration are highly correlated with fat deposition, so the ORL1 gene is a candidate gene to subcutaneous and intramuscular fat deposition in cattle. The aim of this study was to investigate the existence of the ORL1 polymorphism in Nelore cattle and characterize the allelic and genotypic frequencies. The DNA was extracted from blood of 240 animals from Animal Science Experimental Station, Sertãozinho, Brazil. The animals were genotyped by PCR-RFLP using the restriction enzyme *Pst*I. Three genotypes were obtained. The genotypes AA, AC and CC have the frequencies 0.22, 0.25 and 0.53, respectively. The allelic frequencies of A and C were 0.35 and 0.65, respectively. These results indicate that there is a good distribution of the alleles among the animals, therefore permitting verification of the association of the SNP with growth and carcass traits in Nelore cattle. Financial support: CNPq and Fapesp

Key words: PCR-RFLP, adiposity, SNP

T39 Analysis of MUC1 alleles in Nelore cattle using single-allele and multi-allele models. F. R. P. Souza¹, S. Sartore², S. Maione², D. Sogli², V. Spalenza², G. M. F. de Camargo*¹, P. Sacchi², R. Rasero², and M. E. Z. Mercadante³, ¹Sao Paulo State University, Jaboticabal, SP, Brazil, ²University of Torino, Grugliasco, TO, Italy, ³Instituto de Zootecnia, Sertãozinho, SP, Brazil.

The aim of the present study was to analyze the association of the highly polymorphic mucin MUC1 with economic traits in Nelore

cattle. A total of 295 Nelore heifers, born between 2003 and 2005, from a selection experiment running at Instituto de Zootecnia, Sertãozinho - São Paulo/Brazil, were used. The animals were genotyped by PCR. The traits analyzed were birth weight (BW), weaning weight (W210), yearling weight (W550), yearling height (YH550), longissimus muscle area (LMA), subcutaneous backfat thickness (BF), and rump fat thickness (RF). Data were analyzed by a mixed model including absence or presence of the allele (coded as 0 and 1, respectively), contemporary group (selection line and year of birth, 1, ..., 9) and month of birth (September, October, November) as fixed effects, age of dam and age at recording (only for YH550 and the carcass traits LMA, BF and RF) as linear covariates, and the random effect of sire (1, ..., 41). Initially, "single-allele" models were used for each trait, subsequently "multi-allele" models, including all alleles, were applied. Five alleles were identified (1–5). Single-allele model results showed that the allele 3 was associated with W550 ($P = 0.03$). Considering the multi-allele model, significant effects of the alleles 1 and 4 were found on BW ($P = 0.02$ and 0.04 , respectively), however, allele 3 did not affect W550 significantly. Despite these findings, application of this marker in marker-assisted selection will require more studies with a larger number of animals genotyped to increase the accuracy of the statistical analyses.

Key words: VNTR, molecular markers, QTL

T40 Association between a SNP in intron 1 of the ghrelin gene with milk production traits in Murrah buffaloes (*Bubalus bubalis*). F. M. M. Gil, F. R. P. Souza, G. M. F. de Camargo*, P. D. S. Fonseca, D. F. Cardoso, R. R. Aspilcueta-Borquis, G. Stefani, and H. Tonhati, *São Paulo State University, Jaboticabal, São Paulo, Brazil*.

Ghrelin is a gastrointestinal hormone and a potent release stimulator of growth hormone (GH) in the somatotrophic cells of the hypophysis and hypothalamus. It also influences the general metabolism of the body. Studies demonstrated that ghrelin and GH have high plasmatic concentration in dairy cows with high breeding value. Other studies consider GH the most important hormone related to milk yield in lactating cows. The characterization of the ghrelin gene (GHRL) in buffaloes is important because it is a candidate gene to identify molecular markers related to growth, carcass and milk production traits. The aim of this study was to associate the SNP A/G in intron 1 (GenBank accession number: GU071074 and GU071075) of the GHRL gene in Murrah buffaloes with milk production traits. The DNA was extracted from hair of 212 dairy buffaloes from one farm in the São Paulo state, Brazil. The animals were genotyped by PCR-RFLP using the restriction enzyme BstUI. Three genotypes were obtained. The genotypes AA, AG and GG have the frequencies 0.37, 0.47 and 0.16, respectively. The allelic frequencies of G and A were 0.4 and 0.6, respectively. In analyses, the GLM procedure of SAS was used, the model included as fixed effects birth season, birth year and genotype, and as a covariable the age of the buffalo. The possible association of the polymorphism with the phenotypic values of milk yield, protein yield, fat yield, protein percentage and fat percentage at a statistical significance of 5% was tested. The results indicate that there is no association of the SNP described with milk yield ($P = 0.3965$), fat yield ($P = 0.2320$), protein yield ($P = 0.5334$), fat percentage ($P = 0.6224$) and protein percentage ($P = 0.1305$) in dairy Murrah buffaloes.

Key words: restriction enzyme, PCR-RFLP, milk yield

T41 Identification of polymorphism in leptin gene in *Bubalus bubalis*. V. A. Ferreira Junior¹, G. M. F. de Camargo*¹, A. L. F. Lima², F. M. M. Gil¹, and H. Tonhati¹, ¹Sao Paulo State University, Jaboticabal, SP, Brazil, ²Santa Catarina Federal University, Florianopolis, SC, Brazil.

The leptin is a hormone synthesized by the adipocyte tissue and regulates the feed intake in many species as well as in ruminants. It is highly correlated with body weight and adiposity. The gene of the leptin is a potential molecular marker because it is related to feed intake, a trait that is difficult to be measured especially in systems based on pastures. The trait also has high economic value and is correlated with production traits. The aim of this study was to investigate polymorphisms in partial region of intron 2 of the leptin gene in Murrah buffaloes. The DNA was extracted from hair of 150 dairy buffaloes from one farm in the Sao Paulo state, Brazil. The animals were genotyped by PCR-RFLP. In the PCR reaction the primers used were 5'GTCTGGAG-GCAAAGGGCAGAGT3' and 5'CCACCACCTCTGTGGAGTAG3' and in the RFLP reaction, the restriction enzyme used was Bsa AI. Three genotypes were obtained. The first one has one fragment of 522 bp and corresponds to the homozygote AA, the second one has 2 fragments of 441 bp and 81 bp and corresponds to the homozygote GG, the third one has 3 fragments (522 bp, 441 bp and 81 bp) and corresponds to the heterozygote AG. The genotypes AA, AG and GG have the frequencies 0.3, 0.52 and 0.18, respectively. The allelic frequencies of A and G were 0.56 and 0.44, respectively. The technique was efficient to detect the SNP A/G in intron 2 in buffaloes. The same SNP is also present in cattle. The genotypic frequencies are in Hardy-Weinberg equilibrium at 5%. This SNP seems to be interesting to be studied because it is a SNP conserved between species and also because the frequencies are well distributed among the animals.

Key words: SNP, buffaloes, PCR-RFLP

T42 Relationship between kappa-casein genotype in inseminated bulls and the milk composition of their daughters. J. Bezdicsek*¹ and J. Riha², ¹Agriresearch Rapotin, Ltd., Rapotin, Czech Republic, ²Research Institute for Cattle Breeding, Ltd., Rapotin, Czech Republic.

Using genotypes that positively influence cattle milk production is an important breeding aim. The goal of this study was to evaluate the relationship between one milk protein kappa-casein genotype (CSN3) in inseminated Czech Fleckvieh and Holstein bulls, used between the years 1997–2007 and the milk composition of their daughters. We included only cows with a milk yield C100 (5000–7500 L) and H100 (7000–10 000 L). The statistical analysis was done using SPSS 15.0 program for Windows and PowerMarker (Liu, K., Muse, S. V., 2005). The average genotype frequency in the case of Czech Fleckvieh bulls ($n = 136$) was AA = 0.39; AB = 0.48; AE = 0.01; BB = 0.09; BE = 0.02; and EE = 0.01 and allele frequencies were A = 0.64; B = 0.34 and E = 0.02. Genotype frequencies found in Holstein bulls ($n = 60$) were AA = 0.55; AB = 0.28; AE = 0.12; BB = 0; BE = 0.03 and EE = 0.02 and allele frequencies were A = 0.75; B = 0.16 and E = 0.09. In the Holstein breed we found a higher frequency of the E allele and a higher frequency of genotypes with this allele. The genetic diversity and heterozygosity in Czech Fleckvieh Bulls were the following 0.47; 0.52. In Holstein Bulls 0.40; 0.43. These results show the higher genetic variability of bulls of the Czech Fleckvieh breed. At the same time we carried out an assessment of the milk fat and protein percentage of the daughters of the observed bulls. The measurement was made on cows in the 1st lactation separately for the Czech Fleckvieh ($n =$

607 cows) and Holstein (n = 702 cows). Significance of differences is marked as $^*(P \leq 0.05)$, $^{**}(P \leq 0.01)$. A found higher protein content in Czech Fleckvieh was associated with particular genotypes: BB (3.56%) > AB (3.55%) > *AA (3.48%) > AE (3.43%) > EE (3.41%) > * BE (3.37%). Protein in Holstein: AB (3.32%) > AA (3.31%) > AE (3.30%) > BE (3.28%) > * EE (3.26%). Fat in Czech Fleckvieh: AB (4.2%) > AA (4.14%) > BB (4.11%) > BE (4.09%) > EE (4.05%) > **AE (3.93%). Fat in Holstein: AB (3.86%) > AA (3.85%) > EE (3.83%) > AE (3.82%) > ** BE (3.72%). From the above it is clear that differences between CSN3 genotypes were not considerable and only in some cases significant.

Key words: kappa-casein, milk composition

T43 Effect of DGAT1, TG and leptin gene polymorphisms on milk production traits in Holstein-Friesian cows in Hungary. I. Anton^{*1}, K. Kovács¹, G. Holló², V. Farkas³, F. Szabó³, and A. Zsolnai¹, ¹Research Institute for Animal Breeding and Nutrition, Herceghalom, Hungary, ²University of Kaposvár, Faculty of Animal Science, Kaposvár, Hungary, ³University of Pannonia, Georgikon Faculty of Agriculture, Keszthely, Hungary.

The objective of this study was to estimate the effect of the acylCoA-diacylglycerol-acyltransferase 1 (DGAT1), thyroglobulin (TG) and leptin locus on milk fat, milk protein and milk yield in Hungarian Holstein-Friesian cows. A lysine/alanine (K232A) polymorphism in DGAT1 -a microsomal enzyme that catalyzes the final step of triglyceride synthesis- has been proved to affect milk yield, as well as milk fat and protein content in different dairy cattle breeds. The effect of a 5'-polymorphism of TG gene- which product is the precursor of hormones that influence lipid metabolism- has been shown to affect milk fat content in cattle. Polymorphisms in the leptin gene have been associated with milk protein yield and milk yield. A total of 417 blood samples have been collected from different Holstein-Friesian herds and genotypes were determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) assay. Milk production data were recorded throughout 3 consecutive lactations and statistical analyses have been carried out to find association between individual genotypes and milk production traits. The data set was analyzed with SPSS 15.0 for Windows software. Multivariate ANOVA (general linear model, GLM) was applied to determine differences in milk production traits, where DGAT1, TG and leptin genotypes, birth year, number of lactations and calving season were included as fixed effects and 305-d-milk yield, 305-d-milk fat percentage and yield and 305-d-milk protein percentage and yield were considered as dependent variables. In case of DGAT1 locus, AA homozygous animals produced the highest values of fat yield and protein yield ($P < 0.05$). Milk yield decreased consistently ($P < 0.05$) from genotype AA/AA through to GC/GC. Among TG genotypes, TT animals had the highest ($P < 0.05$) 305-d-milk fat percentage and yield values. Referring to leptin polymorphism, CC cows produced higher ($P < 0.05$) 305-d-milk protein values than TC animals. The project was supported by the Hungarian Scientific Research Fund (project 78174).

Key words: DGAT1, TG, leptin

T44 Correlation analysis of hepatic transcript abundance and lactational performance in postpubertal Holstein dairy heifers. J. Doelman, J. M. Kim^{*}, H. Cao, N. G. Purdie, and J. P. Cant, *University of Guelph, Ontario, Canada.*

Dairy genomic research has recently grown in popularity, though investigation into the use of transcript abundance as a marker of future performance remains limited. The objectives of this study were to employ a statistical method to reduce variability within a microarray data set and subsequently identify correlations between gene expression signal intensity and performance measures during first lactation of 81 Holstein dairy heifers. Pearson correlation can be used to determine the underlying structure of a large data set through identification of a data subset that is well correlated to a particular variable. Partial Least Squares regression seeks to model dependent variables by means of a set of predictor variables but has yet to be applied in the field of dairy genomics. These 2 types of analysis were performed on microarray data from previous research that quantified gene expression signal from yearling Holstein heifers. To reduce the total number of genes used in the data set for regression analysis, the linear dependence of all genes in the entire normalized data set was measured against 18 DHI variables using Pearson correlation analysis. Results were pooled to generate 4 lists based on coefficient values and significance of $P < 0.05$. List 4 featured 1541 genes ($r^2 > 0.04$), list 3 contained 453 genes ($r^2 > 0.09$), list 2 was comprised of 140 genes ($r^2 > 0.12$) and list 1 consisted of 31 genes ($r^2 > 0.16$). Test set validation was used to fit the partial least squares model by creating a test and training set using the normalized expression data sets. The strongest correlation coefficients, $r^2 = 0.62$ (protein percentage) and $r^2 = 0.54$ (fat percentage) were obtained using list 1. Strong correlations were also found for 305 d protein yield ($r^2 = 0.40$, list 3) and protein percentage ($r^2 = 0.33$, list 4). Moderate correlation coefficients were also identified for breed class average milk ($r^2 = 0.21$, list 1) and protein ($r^2 = 0.24$, list 1). Identification of gene expression patterns in a predictive nature such as this offers a potential selection tool to be employed by producers.

Key words: heifer, correlation, gene expression

T45 Identification of a SNP in the gene IL2 and its association with resistance against gastrointestinal infection by nematodes in goat. F. A. Bressani^{1,5}, P. C. Tizioto^{*2}, S. L. Meirelles², W. Malagó Junior^{1,2}, R. Giglioti³, A. M. G. Ibelli², J. G. G. Gromboni⁴, E. Carrilho⁵, L. G. Zarus⁶, L. da Silva Vieira⁷, and L. Correia de Almeida Regitano¹, ¹Embrapa Southeast Embrapa Southeast Cattle Research Center, São Carlos, São Paulo, Brazil, ²Federal University of São Carlos - UFSCar, São Paulo, São Paulo, Brazil, ³State University of São Paulo - UNESP, Jaboticabal, São Paulo, Brazil, ⁴UNICEP, São Carlos, São Paulo, Brazil, ⁵University of Sao Paulo, São Carlos, São Paulo, Brazil, ⁶Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil, ⁷Embrapa Goats and Sheep, Sobral, Ceará, Brazil.

The gene IL2 encodes an interleukin which plays a role in inducing the maturation of T and B cells, important factors in the immune response to parasites in several species. Two hundred twenty-nine individuals of a F2 goat population were studied aiming at finding genetic markers for resistance to gastrointestinal infection. To accomplish this, a SNP in the IL2 gene was identified and its association with resistance to gastrointestinal infection was tested. The population investigated was an F2 generated from F1 Saanen (susceptible to gastrointestinal endoparasites), Anglo-nubian (resistant) crosses. Phenotypes consisted of eggs per gram (EPG) and were obtained by parasitological examination of feces samples. The data were transformed as $\log_{10}(\text{EPG}+1)$ and analyzed using the mixed procedure of SAS. Fixed effects included in the model were sex, sampling order, and age at sampling; while animal was fitted as random effect. Based on this analysis, 44 individuals with extremes EPG were selected. The gDNA of these animals

was obtained from isolated leukocytes by the salting-out method. Specific oligonucleotides were designed to obtain PCR products from IL2 gDNA which were sequenced in the ABI Prism 3100 Avant Sequencer (Applied Biosystems). The sequences were further analyzed using the Phred, Phrap, and Consed programs. A SNP (G/A) identified within the intron 2 of IL2 gene was analyzed by Fisher test and showed association with resistance against gastrointestinal infection by nematodes ($P = 0.0489$). Further studies with the whole F2 population are in progress to confirm this association.

Key words: IL2, SNP, goat

T46 Effect of the DGAT1 gene polymorphism on the backfat thickness and fat-tailed weight in Iranian Lori-Bakhtiari sheep.

H. Mohammadi*, M. Moradi Shahrehabak, and M. Sadeghi, *Department of Animal Science, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Tehran, Iran.*

Backfat thickness refers to the amount of fat over the animals back and strongly associated with percentage of retail product, represents a valuable sheep quality trait, and fat-tail demands sheep industry attention for many reasons. To name a few, lean to fat-tail ratio improvement means better feed conversion efficiency. The DGAT1 catalyzes the final step of triglyceride synthesis and the gene is located on the centromic end of bovine chromosome 14. The DGAT1 gene has been mapped to ovine chromosome 9. Polymorphism in the DGAT1 gene has been associated with milk fat percentage and body fatness. The objective of this study was to evaluate the effect of the DGAT1 gene locus on ovine quality traits in the Lori-Bakhtiari sheep breed in Iran. A total of 152 blood samples were obtained from different sex Lori-Bakhtiari sheep. PCR primers were assumed from previously reported studies (Xu et al., 2008). A 309 bp fragment from exon 17 was amplified and digested by AluI with PCR-RFLP method. The association between genotypes and fat-tailed weight and backfat thickness was analyzed. DGAT1 CC animals showed the highest fat percentage values for fat-tail and backfat thickness, the difference between CC and TT genotypes was significant ($P < 0.05$). The results of this study identified novel associations; The C allele had a positive effect on fat-tail weight and backfat thickness in fat-tailed sheep. The results of this study suggest that the TT genotype of DGAT1 could be regarded as a molecular marker for breeding programs to improve carcass traits in fat-tailed sheep breed.

Key words: DGAT1, single nucleotide polymorphisms (SNP), fat deposition and carcass traits

T47 Identification and evaluation of an IGF-I gene polymorphism in a Zel sheep population using RFLP/HaeII.

S. M. Kazemi*¹, C. Amirinia², S. Gharaveysi¹, H. Emrani², and A. Yilmaz³, ¹*Department of animal Science, Islamic Azad University, Qaemshahr Branch, Qaemshahr, Mazandaran, Iran,* ²*Department of Animal Biotechnology, Animal Science Research Institute of Iran, Karaj, Alborz, Iran,* ³*Department of Animal Sciences, The Ohio State University, Columbus.*

The IGFs play an important role in regulating somatic growth, and they are affected by nutritional and other conditions during growth. Polymorphisms of IGF genes are reported to be significantly associated with many traits including growth and reproductive traits. In this study, 142 DNA samples from Zel sheep were used to detect a promoter region polymorphism in the insulin-like growth factor-1 (IGF-1) gene. To extract DNA from blood we used a Salting-out procedure.

Primers were obtained for amplification of the specific segment. Polymerase Chain Reaction (PCR) was accomplished after finding the best reaction conditions and the specific segment amplified well. RFLP fragments were used to detect the polymorphism in the segment. RFLP analysis was performed by incubating the PCR products with HaeII restriction enzyme at 37°C for 4 h. Gels (3.5% agarose) were visualized by using ethidium bromide. The polymorphism was observed and the evaluation of the results relieved 2 alleles and 3 genotypes. The alleles were A and B with frequencies of 0.71 and 0.29, respectively. The genotypes AA, BB and AB had frequencies of 0.47, 0.06 and 0.47, respectively. The data were analyzed for genetic variation statistics using PopGene software (version32) and no deviation from Hardy-Weinberg equilibrium was observed in this study.

Key words: Zel sheep, IGF-1, polymorphisms

T48 Haplotype structure of telomerase reverse transcriptase (*turTERT*) gene in the turkey, *Meleagris gallopavo*.

A. M. J. B. Adikari*, J. Xu, X. Guan, and E. Smith, *Virginia Polytechnic Institute and State University, Blacksburg.*

The recently released turkey genome sequence offers an opportunity to characterize and define the role of some genes that affect turkey performance and productivity. Our objective for this study was to screen the telomerase reverse transcriptase (*turTERT*) gene for structural variation based on single nucleotide polymorphisms (SNPs) and haplotypes using a diversity panel consisting of birds from heritage, commercial, and wild varieties. The rationale is that TERT influences some metabolic diseases including heart diseases, metabolic syndrome, and hypertension. Further, the levels of functional telomerase are critical for telomere maintenance whose shortening is associated with organismal aging and concomitant metabolic diseases. Primers used for long-range polymerase chain reaction (LR-PCR) were designed using the Primer 3 software. Each amplicon was gel purified, sequenced, and the SNPs detected using standard methods. Linkage disequilibrium (D') among SNPs was estimated using Visual Haplotype software. From 34 kb of *turTERT* gene screened, a total of 4 SNPs were detected in the introns. Allelic diversity ranged from 0.14 to 0.68. A total of 3 haplotypes were derived from the SNPs with frequencies that ranged from 0.09 to 0.59. While the diversity panel maximized detection of variation, both the SNPs and haplotypes appear to show that the Royal Palm's *TERT* alleles appear to be distinct. Visual haplotype analysis revealed that the first 3 SNPs, which were about 300 bp apart, were strongly associated ($r^2 = 0.87-1.00$) while the fourth, about 9 kb from the nearest SNP, was not strongly linked ($r^2 < 0.1$) to the others. The distribution of the SNPs and haplotypes, as well as the D' , provide a foundation that will facilitate future association and linkage studies between *turTERT* and metabolic diseases in the turkey.

Key words: Turkey, single nucleotide polymorphisms, linkage disequilibrium

T49 Changes in the proteome and metabolic profiles of broiler chickens during adipose tissue accretion.

G. Kelley*, X. Wang, F. Chen, and S. Nahashon, *Tennessee State University, Nashville.*

Fat accretion in poultry directly influences the efficiency of feed utilization and consumer acceptability of poultry and poultry products. Losses estimated at about US\$250–300 million are incurred by consumers and processors annually in pollution control, extraction and disposal of excess carcass fat. Understanding underlying mechanisms of excessive fat deposition in poultry will aid in improving carcass

quality and minimize production cost. We hypothesized that chicken adiposity is highly influenced by factors beyond the genome. Therefore, the aim of this study was to employ a proteomics approach to identify proteins that may be associated with fat accretion in broiler chickens. Metabolic profiles of the experimental birds were also evaluated. One hundred and 20 1-d-old broiler chickens were randomly assigned to floor pens and fed standard broiler diet for 8 weeks. At 8 WOA, experimental birds were bled, sacrificed and adipose tissue from the abdominal and visceral areas was collected, weighed and frozen in liquid nitrogen before storage at -80°C until used. Adipose proteome from the birds with the highest and lowest abdominal fat percentage (8 birds each) was assayed using 2-dimensional differential gel electrophoresis (2D-DIGE) followed by in-gel digestion and Matrix Assisted Laser Desorption/ionization Time-of-Flight (MALDI-TOF) mass spectrometry. A total of 132 spots were found to be differentially expressed between the extreme birds ($P < 0.05$). Several of the proteins are unique and some are involved in metabolic pathways that are associated with fat accretion including vimentin, apolipoprotein, and annexin. Obese birds exhibited high levels of potassium and serum glutamic oxaloacetic transaminase than their lean counterparts. The lean birds on the other hand exhibited higher levels of alkaline phosphatase than obese birds.

Key words: broiler chickens, adipose tissue proteome, metabolic profiles

T50 PCR-RFLP analysis of promoter region of Interferon gamma gene in high and low immunocompetent Aseel native chicken. S. Choudhary^{*1}, S. Kumar², and B. Nautiyal¹, ¹MJP Rohilkhand University, Bareilly, U.P. India, ²Central Avian Research Institute, Bareilly, U.P. India.

Resistance to diseases is under the control of certain immune response genes. Interferon gamma (INFG), a cytokine is one such candidate gene that plays a critical role in immune system function. In this study, DNA polymorphism of INFG gene at promoter region was studied using polymerase chain reaction-restriction fragments length polymorphism (PCR-RFLP) technique in 48 random bred Aseel native chicken, 24 in high and 24 in low immunocompetence index group. Two sets of PCR primer, set I for full length promoter and set II for partial length promoter, amplified product with 670 bp and 495 bp in size, respectively. Agarose gel electrophoresis and DNA sequencing of PCR amplified product confirmed amplification of INFG gene promoter region. PCR-RFLP analysis of full length promoter with enzymes, *EcoRI* and *TaqI* and partial length promoter with enzymes *Alu I*, *Hinf I*, *Dde I* and *TaqI* was monomorphic, whereas, full length promoter with enzyme *Tsp509 I* was polymorphic. Gene frequencies of 2 alleles, allele A (168, 123, 99, 88, 64 and 54 bp fragments) and allele B (123, 104, 99, 88, 64 and 54 bp fragments) were 0.64 and 0.36. The genotypic frequencies of genotypes AA, AB and BB were 0.17, 0.30 and 0.53, respectively. Heterozygote (AB) demonstrated higher magnitude of all immunocompetence traits. *Tsp509 I* PCR-RFLP of INFG gene at promoter region was suggestive of development of genetic markers for high humoral immune response in chicken.

Key words: Aseel, PCR-RFLP, interferon gamma

T51 Association of BMPR-IB gene polymorphism with breeding value of growth and reproductive traits in Mazandaran native chicken. Sh. Niknafs*, A. Nejati Javaremi, and M. Sadeghi, *Agricultural Faculty, University of Tehran, Karaj, Alborz, Iran.*

The aim of the current study was to investigate A287G SNP of the chicken BMPR-IB gene and its association with the breeding value of growth and reproductive traits. Hence, a sample of 206 individuals including 10 males and 196 females of Mazandaran native chicken were genotyped using PCR-RFLP technique. On the other hand, for estimating breeding value of the traits, phenotypic information of 18 successive generations of selection in breeding station of Mazandaran native chicken (north of Iran) was analyzed using a univariate animal model in ASREML procedure. Investigated traits included body weight at hatch (35287 records), at 8 (43067), at 12 weeks of age (38297), at sexual maturity (31147), egg number (31349), age at first egg (31349), egg weight of first (27249), of 28 (17225), of 30 (19031), of 32 (18955) weeks of age, average egg weight of first 12 weeks of production (18847), egg mass (28725) and egg production intensity (31349). Finally, marker-trait association analyses were performed using estimated breeding value of the traits, as dependent variable, in GLM procedure of SAS 9.1. The significant differences of least squares means were tested with Tukey-Kramer multiple range tests, and a P-value of < 0.05 was considered statistically significant. Two alleles and 3 genotypes were identified. Genotypic frequency of AA, AG and GG were 0.349, 0.544 and 0.107 respectively. Results showed, for all investigated traits, no significant differences among breeding value LSmeans of the genotypes existed ($P < 0.05$). In conclusion, we found no significant association between BMPR-IB gene and breeding value of the growth and egg production traits in Mazandaran native chicken.

Key words: BMPR-IB gene, SNP, growth and reproductive traits

T52 Association of a single nucleotide polymorphism in NPY gene with growth and reproductive traits in Mazandaran native chicken. S. Niknafs*, A. Fatemi, H. Mehrabani Yeganeh, and A. Nejati Javaremi, *Agricultural Faculty, University of Tehran, Karaj, Alborz, Iran.*

The objective of the current study was to investigate a SNP (with accession number of M87298) for the chicken NPY gene and its association with breeding value of growth and reproductive traits in chicken. A breeding station of Mazandaran native chicken was established in 1988 with 2 main objectives: extension and genetic improvement of the local breed. From 1988 to 2009, 18 generations of selection was done for traits of 8-wk BW (BW8), egg number, age at first egg and average egg weight as selection criteria. Recorded traits consisting of body weight at hatch (35287 records), at 8 (43067), at 12 weeks of age (38297), at sexual maturity (31147), egg number (31349), age at first egg (31349), egg weight of first (27249), of 28 (17225), of 30 (19031), of 32 (18955) weeks of age, average egg weight of first 12 weeks of production (18847), egg mass (28725) and egg production intensity (31349). A total of 206 individuals, from generation 17, were selected at random and genotyped for the SNP using PCR-RFLP technique. Phenotypic information of the 18 generations was analyzed genetically to estimate breeding value of the traits for genotyped individuals. Genetic analysis was performed by univariate animal model in ASREML software. Fixed effects of sex, generation and hatch were considered in the model where would have significant effects. Marker-trait association analysis was done using breeding values (as dependent variable) and SNP genotypes (as independent variables) in GLM procedure of SAS 9.1. Three genotypes of BB, Bb and bb with the frequencies of 0.885, 0.100 and 0.015 were respectively identified. Results suggested that there were significant differences among breeding value LSmeans of genotypes for body weight at sexual maturity. In conclusion, chicken

NPY gene may be associated with body weight and may be considered in MAS program to improve growth performance.

Key words: growth and reproductive traits, NPY gene, SNP

T53 Association of a single nucleotide polymorphism from GnRHR gene with growth and egg production traits in Mazandaran native chicken. S. Niknafs*, A. Fatemi, H. Mehrabani Yeganeh, and A. Nejati Javaremi, *Agricultural Faculty, University of Tehran, Karaj, Alborz, Iran.*

Gonadotropin releasing hormone receptor (GnRHR) is mainly associated with the development and function of the reproductive axis in avian species. To study the association of this gene with growth and egg production traits in Mazandaran birds, a total of 206 individuals were selected at random and PCR-RFLP technique was used to genotype chickens for one SNP (accession number AJ506779) of this gene. Three genotypes of AA, Aa and aa with the frequencies of 0.379, 0.469 and 0.152, respectively, were identified. Association of these genotypes with both breeding values and phenotypic records of growth and egg production traits were examined. Breeding values were estimated by a univariate animal model in ASREML methodology using information that pertained to 18 generations of selection in breeding station of Mazandaran native chicken. A total of 206 genotyped chickens for this study belonged to generation 17. Investigated traits included body weight at hatch (35287 records), at 8 (43067), at 12 weeks of age (38297), at sexual maturity (31147), egg number (31349), age at first egg (31349), egg weight of first (27249), of 28 (17225), of 30 (19031), of 32 (18955) weeks of age, average egg weight of first 12 weeks of production (18847), egg mass (28725) and egg production intensity (31349). Marker-trait association analysis showed no significant differences among phenotypic LSmeans of the genotypes. However, least squares means of breeding value for traits of egg number and egg mass demonstrated significant differences among genotypes. More egg number and egg mass were observed for the genotype AA. In conclusion, GnRHR gene may be associated with egg production traits genetically and additively in Mazandaran native chicken.

Key words: GnRHR gene, SNP, chicken

T54 Investigation of three single nucleotide polymorphisms of STAT5B gene and their association with growth and reproductive traits in Mazandaran native chicken. S. Niknafs*, A. Nejati Javaremi, M. Sadeghi, and A. Fatemi, *Agricultural Faculty, University of Tehran, Karaj, Alborz, Iran.*

The objective of the current study was to investigate the association of 3 SNPs of the STAT5B gene with breeding value of growth and reproductive traits in Mazandaran native chicken (north of Iran) breed. A total of 205 individuals were selected randomly and genotyped for 3 SNPs of STAT5B gene (C4535156T, G4533675C and G4533815A) using PCR-RFLP technique. Phenotypic information of 18 generations was employed in estimating breeding value for different traits using univariate animal models in ASREML. Investigated traits included body weight at hatch (35287 records), at 8 (43067), at 12 weeks of age (38297), body weight at sexual maturity (31147), egg number (31349), age at first egg (31349), egg weight of first (27249), at 28 (17225), at 30 (19031), at 32 (18955) weeks of age, average egg weight of the primary 12 weeks of production (18847), egg mass (28725) and egg production intensity (31349). Marker-trait associations analysis were performed with the GLM procedure of SAS 9.1. Significant differences of least squares means were tested with Tukey-Kramer multiple tests correction, and corrected P-values <0.05 were considered statistically significant. Two alleles and 3 genotypes were observed for each SNP. Alleles of A (0.663) and a (0.337) for C4535156T SNP, B (0.646) and b (0.354) for G4533675C SNP, C (0.804) and c (0.196) for G4533815A SNP were identified. Four significant associations of C4535156T SNP with body weight at hatch, G4533675C SNP with egg weight of first and G4533815A SNP with body weight at 8 and 12 weeks of ages were found ($P < 0.05$).

Key words: STAT5B gene, growth and reproductive traits, chicken