M86 Heat storage and HSP expression of Holstein females – an *in vivo* study. Ana C. A. P. M. Geraldo[1,2], Thays M. C. Leme[1], Reíssa A. Vilela[1], Cristiane G. Titto[1], Evaldo A. L. Titto*[1], Paulo Infante[3], Fernando J. Moreira da Silva[4], and Alfredo M. F. Pereira[2], *[1]Animal Science and Food Engineering Faculty, University of São Paulo, Pirassununga, São Paulo, Brazil, [2]Institute of Mediterranean Agricultural and Environmental Sciences,University of Évora, Évora, Portugal, [3]Mathematics Department, University of Évora, Évora, Portugal, [4]Department of Agrarian Sciences, University of Azores, Angra do Heroísmo, Azores, Portugal.*

One of the main factors that affect animals' performance is high temperature, causing several changes, including at the cellular level. These changes lead to an increased expression of heat shock proteins. This experiment aimed to study the *HSPA1A* and *HSP90AA1* gene expressions of Holstein cows after exposure to direct solar radiation. The heat tolerance test was performed and rectal temperature and respiratory rate measured, and blood samples collected. After the erythrocytes lysis to obtain the buffy-coat, the RNA was isolated by the TRIzol method and RT-PCR performed with SuperScript III after digestion with DNase I. The qPCR apparatus took place in 7500 Real Fast Time, using TaqMan Gene Expression Assays for HSPA1A and HSP90AA1 target genes, ACTB and PPIA as endogenous genes. The ΔCt ($Ct_{target} - Ct_{endogenous}$) were calculated as well as gene expression through the $2^{-\Delta\Delta Ct}$ method. The treatments considered for statistical analysis were $t < 44°C$ and $T \geq 44°C$ (with T being the black globe temperature). We used linear mixed models in the program R Project Software (version 3.0.1). There weren't significant differences between treatments for any of the variables. This way we can say that animals were in a moderate stress condition, which did not allow the identification of differences in *HSPA1A* and *HSP90AA1* gene expression.

Key Words: dairy cattle, *HSPA1A*, *HSP90AA1*

M87 Gene and pathway analysis of metabolic traits in dairy cows. Ngoc-Thuy Ha[1,2], Josef J. Gross*[1], Jens Tetens[3], Martin Schlather[4], Rupert M. Bruckmaier[1], and Henner Simianer[2], *[1]Veterinary Physiology, Vetsuisse Faculty University of Bern, Bern, Switzerland, [2]Animal Breeding and Genetics Group, Department of Animal Sciences, Georg-August-University Goettingen, Goettingen, Germany, [3] Institute of Animal Breeding and Husbandry, Christian-Albrechts-University Kiel, Kiel, Germany, [4]Chair of Mathematical Statistics, University of Mannheim, Mannheim, Germany.*

During excessive body fat mobilization in early lactation, dairy cows experience a severe metabolic load. Here, a failure in metabolic adaptation often results in an increased susceptibility to health problems. In this study, we analyzed the genetic basis of the metabolic adaptability during the transition period. To this end, blood samples were taken from 178 cows (Holstein, Red Holstein, Fleckvieh and Braunvieh) at 3 critical stages: T1 = wk 3 before expected calving (not lactating and no metabolic load); T2 = wk 4 postpartum (lactating and high metabolic load), and T3 = wk 13 after parturition (lactating and no noteworthy metabolic load). Plasma concentrations of nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHBA) and glucose, 3 metabolites characterizing the metabolic status and adaptability, were measured at T1, T2, and T3. All cows were genotyped with the Illumina next-generation High-Density Bovine BeadChip resulting in a data set of 777,692 SNPs. After quality control and filtering, the SNPs were annotated to known genes (Ensembl Genes Database) and pathways (KEGG Database). For each gene G with g SNPs, we performed a score test based on the linear regression model $y = \gamma_1 C_1 + \ldots + \gamma_m C_m + \beta_1 S_1 + \ldots + \beta_g S_g + \varepsilon$, where C_i are environmental or breed effects and y are either one of the 3 metabolites measured at T1, T2 or T3 or the ratio of their concentrations measured at the different points of time, to assess the association of the gene G to the phenotype y. The results were then used to identify pathways enriched with significant genes using a weighted Kolmogorov-Smirnov test. As a result, we found 99 significant genes associated with at least one of the 3 metabolites. For each metabolite, we found genes that are significant at T2 but not at T1 and T3 or vice versa. This strongly suggests the genes to be potential candidates for the adaptive regulation. We further find 3 pathways (steroid hormone biosynthesis, ether lipid metabolism and glycerophospholipid metabolism) to jointly affect the 3 metabolites. In conclusion, this may be regarded as evidence for the genetic basis for the adaptation performance of dairy cows and, at the same time, reveals its complexity.

Key Words: genome-wide association study, gene-based score test, dairy cow

M88 A novel intronic SNP marker candidate which associated with fatty acids profile in Korean cattle (Hanwoo). Yoonseok Lee*[1], Dongyep Oh[3], Jaejung Ha[3], Jinduk Bok[1], Sangkee Kang[1,2], YunJaie Choi[1,2], and Myunggi Baik[1,2], *[1]Institute of Green Bio Science & Technology, Seoul National University, Pyeongchang-gun, Gangwon-do, South Korea, [2]Department of Agricultural Biotechnology, Seoul National University, Gwanak-gu, Seoul, South Korea, [3]Gyeongsangbuk-do Livestock Research Institute, Yeongju, Gyeonsangbuk-do, South Korea.*

Recently, consumers have become increasingly health-conscious and demand not only palatable but also health-beneficial meat in the market. In Korea, degree of the intramuscular fat deposit (marbling) is one of the crucial indicators to judge beef quality. Thus, the fatty acids profile in beef is important because it affects both flavor and health-benefit. Various trials have been conducted to change fatty acids profile into health-beneficial composition, such as poly-unsaturated fatty acids-abundant meat, in Korean beef. The fatty acid binding protein 4 (*FABP4*) gene is a cytosolic protein abundantly expressed in adipocyte and plays an important role on lipid synthesis and intracellular fatty acid trafficking. An SNP located on the splice site variation between the third exon and the third intron of bovine FABP4 showed a statistically significant effect on intramuscular fatty acid deposition. The aim of this study was to investigate the relationship between SNP within the third intron region of FABP4 and fatty acids profile of longissimus dorsi area in Korean cattle. We sequenced the third intron region of bovine FABP4. Among this SNPs, the g.3996 C>T SNP show statistically significant effect on oleic acid and polyunsaturated fatty acids ($P < 0.001$). Especially, the animals with CC genotype have a lower ratio of omega-6/-3 fatty acid (5.4:1) than the ratio of animals with TT genotypes (9.9:1). Therefore, our result suggest that g.3996 C>T intronic SNP will be used as a useful marker-assisted selection for breeding on fatty acids profile of Korean cattle.

Key Words: Korean cattle, intronic SNP, fatty acid binding protein 4 (*FABP4*)

M89 A QTL on BTA16 is associated with *Mycobacterium avium* ssp. *paratuberculosis* (*Map*) tissue infection. Jennifer N. Kiser* and Holly L. Neibergs, *Washington State University, Pullman, WA.*

Johne's disease is a contagious bacterial infection in cattle caused by *Mycobacterium avium* ssp. *paratuberculosis* (*Map*) infection. A previous genome wide association study (GWAS) in Holstein cattle identified QTL on bovine chromosome 3 (BTA3) and BTA9 that were highly associated ($P < 5 \times 10^{-7}$) and on BTA1, BTA16, and BTA21 that were moderately associated ($P < 5 \times 10^{-5}$) with *Map* tissue infection. The objective of this study was to validate these GWAS results in Holsteins from the Pacific Northwest (PNW, n = 191) and a combined population from the PNW and the Northeast (PNW+NE, n = 432). DNA was genotyped using the Illumina BovineSNP50 BeadChip. Cases were ileo-cecal node positive for *Map* by PCR, and controls were *Map* tissue negative. Individuals were removed if the SNP call rate was < 90%, and SNPs were removed if the genotype call rate was < 95% or they had a MAF of < 0.01. After filtering, 162 cases, and 247 controls and 44,445 SNPs remained for analysis. A GWAS for the PNW and PNW+NE was conducted using an efficient mixed-model association eXpedited (EMMAX) method using 3 gene effect models. No loci were associated with the recessive model in PNW or PNW+NE. For the PNW cows, 4 dominant QTL were identified; 3 on BTA21 ($P = 1.18 \times 10^{-6}$, 8.15×10^{-6}, 2.13×10^{-5}) and 1 on BTA3 (9.88×10^{-6}). In the additive model, a new QTL on BTA21 (5.14×10^{-7}) and on BTA1 ($P = 5.82 \times 10^{-5}$) were identified. In the PNW+NE population, 2 QTL were identified with the dominant model: one on BTA14 ($P < 3.17 \times 10^{-5}$) and one on BTA16 ($P < 3.17 \times 10^{-5}$). The BTA16 QTL was also identified by the additive model ($P < 4.59 \times 10^{-5}$) and identified previously by our group using the NE cows alone ($P < 2.57 \times 10^{-5}$). SNPs associated with *Map* tissue infection on BTA16 lie within introns of *CDC42BPA*, a Serine/Threonine-protein kinase that has downstream effects on *CDC42*. Through the regulation of *CDC42, CDC42BPA* has been linked to several immunological pathways in humans including B-cell receptor and chemokine signaling pathways. Although none of the previous QTL in the NE GWAS were validated in the PNW population, a QTL on BTA16 was associated with susceptibility to *Map* tissue in both the NE and PNW+NE populations.

Key Words: bovine paratuberculosis, genomics

M90 Effect of the STAT5A *Bst*EII polymorphism on reproductive parameters of Holstein dairy cows. Pedro A. S. Silveira[1], Walter R. Butler[2], Carlos C. Barros[1], Marcio N. Corrêa[1], and Augusto Schneider*[1], [1]*Federal University of Pelotas, Pelotas, RS, Brazil,* [2]*Cornell University, Ithaca, NY.*

The signal transducer and activator of transcription 5A (STAT5A) is a transcription factor that mediates the function of various hormones and cytokines, including growth hormone (GH). Mutations in the STAT5A gene, such as the substitution of a G by a C in exon 8, have been associated with differences in production and reproductive performance of dairy cows. The aim of this study was to evaluate the effect of STAT5A *Bst*EII polymorphism on the days from calving to first ovulation and calving-conception interval (CCI). For identification of the polymorphisms DNA was extracted from blood and a fragment of the STAT5A gene was amplified by PCR. The presence of the G and C alleles was determined after digestion of the PCR products with the *Bst*EII enzyme and gel electrophoresis. For this study 73 Holstein cows were followed from 21 d prepartum to 210 d in milk (DIM). At 55 DIM the cows were submitted to an OvSynch-TAI protocol, which was repeated in cows diagnosed as not pregnant. From calving, milk production was recorded, milk samples for progesterone measurement were collected twice a week to determine ovulation day until 60 DIM, and the CCI was evaluated until 210 DIM. Serum samples for insulin-like growth factor I (IGF-I) measurement were collected at −21, 0, 7, 21 and 60 DIM. Data were analyzed using the GLM procedure of SAS. In total, 19 cows (26%) were of the CC genotype, 32 cows (43.8%) of the GC genotype and 22 cows (30.2%) of the GG genotype. The calving to ovulation interval was not different between genotypes ($P > 0.05$): 28.4 ± 3.1, 29.5 ± 2.5 and 29.4 ± 2.9 DIM for the CC, CG and GG genotypes. The CCI was 101.3 ± 9.4, 102.7 ± 7.6 and 93.9 ± 9.4 DIM for the CC, CG and GG genotypes ($P > 0.05$). Milk production was similar between the 3 genotypes ($P > 0.05$). Serum IGF-I was also not different between genotypes, being 66.3 ± 7.2, 61.8 ± 5.8, 65.6 ± 6.7 ng/mL for CC, CG and GG genotypes ($P > 0.05$). Therefore, the STAT5A *Bst*EII polymorphism did not affect the calving to ovulation interval, CCI, milk production or IGF-I concentrations in Holstein dairy cows. It should be noticed that this study used a small number of cows and larger studies are necessary to confirm current results.

Key Words: SNP, growth hormone (GH), insulin-like growth factor I (IGF-I)

M91 Differentially expressed genes for beef fatty acid profile in Nellore cattle. Mariana P. Berton*[1], Marcos V. A. Lemos[1], Hermenegildo L. J. Chiaia[1], Fabieli L. B. Feitosa[1], Carolyn Aboujaoude[1], Larissa F. S. Fonseca[1], Bianca F. Olivieri[1], Daniela F. R. J. Gimenez[1], Bruno L. Utembergue[2], Lucia G. de Albuquerque[1], Aline S. M. Cesar[2], Angélica S. C. Pereira[2], and Fernando Baldi[1], [1]*State University of Sao Paulo, Jaboticabal, Sao Paulo, Brazil,* [2]*University of Sao Paulo, Pirassununga, Sao Paulo, Brazil.*

The aim of this study was to use the RNaseq technique to identify differentially expressed (DE) genes in the *Longissimus thoracis* muscle of Nellore cattle finished in feedlot with extreme phenotypes for beef fatty acid profile. After slaughter, a muscle tissue sample was collected of each animal for the extraction of RNA and in the deboning a sample was taken for determining the fatty acid profile. The fatty acids were quantified by gas chromatography (CG-2010 Plus; Shimadzu), using capillary column SP-2560. The following fatty acids were quantified: myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1 *cis*-9), linoleic (C18:2 *cis*-*cis*-9–12), CLA (C18:2 *cis*-9 *trans*-11) and linolenic (C18:3), total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), the ratio of polyunsaturated fatty acids on saturated (PUFA/SFA), n-3 (omega-3) fatty acids and n-6 and ratio of n-6 fatty acids on n-3 (n-6/n-3). Two groups of animals with extreme phenotypes for the composition of meat fatty acid were formed, being considered 10 animals with the highest (H) and 10 animals with the lowest (L) concentrations for each fatty acid. The RNA sequencing data were generated on the Illumina HiSeq System platform. The differential expression of RNA was determined using the iPlant Collaborative platform, containing the FastQC package (version 0.10.1), TopHat2 (version 2.0.9) and Cuffdiff (2.1.1). The analysis of the metabolic pathways of differentially expressed genes was performed with the DAVID tool. The ACSS1 was upregulated ($q < 0.05$) for saturated fatty acids (palmitic, stearic, oleic, total saturated), and downregulated ($q < 0.05$) for unsaturated acids, (omega-3). This gene assists in the conversion of acetate, into acetyl-CoA to incorporate it into the fatty acids by the action of acetyl CoA synthetase. Other DE genes involved in metabolic pathways of fatty acids synthesis were found, such as: BDH1_BOVIN, ACSM3, CBPE_BOVIN, F1N650_BOVIN. Through the RNaseq technique was identified possible genes acting

in the synthesis of beef fatty acids, which can be beneficial for human health.

Key Words: *Bos indicus*, transcriptomic, fatty composition

M92 Polymorphisms in the promoter of interleukin-12β2 and interleukin-23 receptor genes influence milk production traits in Chinese Holstein cows. Yongjiang Mao*, Xiaorui Zhu, Shiyu Xin, Huiming Zhang, and Zhangping Yang, *College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiansu, China.*

Interleukin-12 (IL-12) and interleukin-23 (IL-23) are proinflammatory cytokines produced by macrophages and dendritic cells in response to infection with intracellular pathogens. Given the importance of IL-12 and IL-23 for modulating inflammation and the host immune response, the IL-12 and IL-23 receptor genes may be suitable candidate genes for studying disease resistance in dairy cattle. Twenty Chinese Holstein cows were selected randomly for PCR amplification and sequencing, and used for SNP discovery in the bovine IL-12Rβ2 and IL-23R promoter region. One SNP (c.-246G>T) in IL-12Rβ2 gene and 2 SNPs (c.-856A>G and c.-207T>C) in IL-23R gene were identified. Chinese Holstein cows (n = 866) were then genotyped using Sequenom MassARRAY (Sequenom Inc., San Diego, CA) based on the 3 identified SNPs, and the associations between SNPs or haplotype of the genes and milk production traits, SCS were analyzed by the least squares method in the GLM procedure of SAS. The IL-23R c.-856A>G and IL-23R c.-207T>C showed close linkage disequilibrium ($r^2 = 0.89$). No association was found with SCS, but associations were found between 3 of these SNP with milk protein content and lactose content. The software MatInspector revealed that these SNPs were located within several potential transcription factor binding sites, and may alter gene expression, but further investigation will be required to elucidate the biological and practical relevance of these SNP.

Key Words: interleukin-12 receptor β2 (IL-12Rβ2), interleukin-23 receptor (IL-23R), SNP

M93 Evolution of mutational variance associated with age and sex of the parent for weaning weight in C57BL/6J mice. Mayela Castillo[1], Juan F. Medrano[2], and Joaquim Casellas*[1], *[1]Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]University of California, Davis, CA.*

New mutations are a very relevant component of polygenic variability and they must be viewed as the raw material for the maintenance of genetic diversity. Nevertheless, the biological phenomena that originate new mutational variants are poorly understood, particularly in regard to the effects that factors such as a parent's age may have. This research focuses on the analysis of weaning weight (10.48 ± 0.02 g) in 12,644 C57BL/6J mice from 46 non-overlapping generations. Data (y) were analyzed under the following model: $y = Xb + Z_1p + Z_2a + Z_2m_p + Z_2m_m + e$, where systematic (b), permanent environmental (p), and additive genetic effects (a, m_p and m_m) were linked by appropriate incidence matrices (X, Z_1 ande Z_2). Note that mutational effects where modeled depending on their origin (i.e., paternal, m_p; maternal, m_m), and an independent set of parent-specific mutational effects were estimated on the basis of new mutations arising to each individual in the pedigree file (i.e., $m_p = m_{p,1} + m_{p,2} + \ldots + m_{p,12644}$). Within this context, $m_{p,i}$ was assumed multivariate normal (MVN) distributed, $p(m_{p,i}|M_i,\sigma_{mp}^2,\lambda_p) = MVN(0,0.5M_i\sigma_{mp}^2\,[1+\varepsilon_{p,i}\lambda_p])$ M_i being the matrix of mutational relationships specific to new mutations arising in the *i*th individual, σ_{mp}^2 (σ_{mm}^2) being the paternal (maternal) mutational variance, $\varepsilon_{p,i}$ being sire's age when conceiving *i*th individual, and λ_p being a linear regression coefficient linking mutational variance and sire's age. This model was solved under a standard Bayesian approach. Mode (and credibility interval) for σ_{mp}^2 and σ_{mm}^2 were 0.113 (0.069 to 0.163) and 0.052 (0.020 to 0.097), respectively. Whereas linear regression coefficient for dam's age collapsed to 0 and discarded additional changes in σ_{mm}^2, λ_p reached a modal estimate of 0.001 (10^{-5} to 0.051); e.g., this linearly increased σ_{mp}^2 from ~0.11 g^2 (puberal sires) to 0.154 g^2 (one-yr-old sires). This parameterization allowed us to clearly characterize different mutational patterns associated with the sex of the parent, as well as the effect of the accumulation of new mutations along breeding stock's lifetime.

Key Words: age-related mutation, C57WL/6J, weaning weight

M94 A molecular evaluation of bovine respiratory disease and carcass traits in feedlot steers. Samantha Miller[1], Ryon Walker[3], Timothy Page[1,2], and Matthew Garcia*[1,2], *[1]Louisiana State University, Baton Rouge, LA, [2]Louisiana State AgCenter, Baton Rouge, LA, [3]Louisiana State AgCenter Hill Farm, Homer, LA.*

Bovine respiratory disease (BRD) is the most common disease affecting feedlot cattle and economic losses incurred by affected animals is estimated to be $640 million annually. The objective of the current study was to evaluate single nucleotide polymorphisms (SNP) and their potential associations with bovine respiratory disease susceptibility and carcass traits in feedlot steers. A population of 314 crossbred steers born from 2010 to 2013 and raised at LSU AG Center Central Research Station (CRS) in Baton Rouge, LA and LSU Ag Center Hill Farm Research Station (HFRS) in Homer, Louisiana, were utilized in the current study. Prior to shipping to a commercial feedlot, the measurements of birth weight, weaning weight and hip height were collected from each steer. After weaning, and the completion of a 45-d preconditioning period, steers were shipped to a commercial feedlot. A total of 16 steers over the entire 4-year evaluation period were affected by BRD. A total of 309 steers were harvested at a commercial packing plant and the traits of marbling score, rib eye area, back fat thickness and yield grade were collected. A total of 74 SNP were selected from a previously described BRD QTL region spanning between 40 and 80 MB on BTA 6. This same region has also been identified to have QTL associated with kidney pelvic heart fat percentage. A total of 33 SNP were selected from a previously described BRD QTL region spanning 0–30 MB on BTA 20. This region has been identified to have QTL's associated with kidney pelvic heart fat percentage, marbling score, intramuscular fat, sheer force and carcass weight. A mixed model design was fit with individual carcass traits, BRD status, and individual SNP genotype as dependent variables and sire breed and year fit as independent variables in the model to identify SNP that were significantly associated with the traits of interest. Although multiple SNP were identified as being significantly associated with carcass traits and BRD susceptibility, these SNP must be validated in larger, and more diverse populations before implementation into selection strategies.

Key Words: bovine respiratory disease, feedlot, single nucleotide polymorphism

M95 Association of a polymorphism in the paraoxonase 1 (*PON1*) gene with reproductive performance, health and production of Holstein cows. Pedro A. S. Silveira[1], Walter R. Butler[2], Carlos C. Barros[1], Marcio N. Corrêa[1], and Augusto Schneider*[1], *[1]Federal University of Pelotas, Pelotas, RS, Brazil, [2]Cornell University, Ithaca, NY.*

Paraoxonase 1 (PON1) is a negative acute phase protein associated with uterine health conditions in postpartum dairy cows and may affect reproductive performance. Recently, a single nucleotide polymorphism (SNP) was found in the promoter of the *PON1* gene in dairy cows associated with serum PON1 activity. The aim of this study was to evaluate the association of the *PON1*(A/G)-221 SNP with reproductive performance, disease incidence and milk production in Holstein cows. For the study, 85 Holstein cows were followed from 21 d prepartum to 210 d in milk (DIM). For SNP identification DNA was extracted from blood and the tetra primer ARMS-PCR technique was used. The primers produced a control 700 bp product, and smaller specific products of 500 bp (allele A) or 200 bp (allele G). After gel electrophoresis it was possible to genotype all cows and some were confirmed by sequencing of the products. At 55 DIM the cows were submitted to an OvSynch-TAI protocol, which was repeated in cows diagnosed as not pregnant. From calving, milk production was recorded, milk samples for progesterone measurement were collected twice a week to determine ovulation day until 60 DIM, and disease incidence, the number of inseminations/pregnancy (AI/P) and the calving- conception interval (CCI) was evaluated. Data were analyzed using the GLM procedure of SAS and by survival analysis and Chi-squared on GraphPad Prism. After genotyping, we detected 57 cows (67.0%) of the AA genotype, 20 cows (23.6%) of the AG genotype and 8 cows (9.4%) of the GG genotype. Cows of the GG and AG genotype ovulated earlier than AA cows (27.6 ± 2.9 and 32.1 ± 2.2 DIM, respectively; $P = 0.02$). There was no difference between genotypes for milk production, number of AI/P or CCI ($P > 0.05$). Also, there was no difference for the occurrence of disease (metritis and mastitis) between the 3 genetic groups ($P > 0.05$). Therefore, the presence of at least one G allele at the position −221 of the *PON1* gene is associated with an earlier postpartum ovulation, although more studies on the mechanism for this effect are needed.

Key Words: *PON1*, SNP, dairy cow

M96 Effect of *POU1F1* gene polymorphism and dairy traits in Holstein cattle from Antioquia, Colombia. Jose V. Isaza*, Albeiro Lopez-Herrera, and Jose J. Ecehverri, *Universidad Nacional de Colombia Sede Medellin, Medellin, Antioquia, Colombia.*

Genetic improvement has allowed great advances in selecting individuals with interest for determine characteristics. Knowledge about bovine genome and genetic markers along each chromosome allow the search for genetic variants that affect important productive and reproductive traits, making possible the genetic improvement. The goal of this study was to determinate the association between the *POU1F1* gene with productive and reproductive traits in a population of Holstein cows from Antioquia, Colombia. The *POU1F1* gene belongs to transcription factors POU, which regulates growth and development in animals. This gene participates on pituitary development and hormonal expression in mammals, regulating the production of growth hormone, thyroid stimulating hormone and prolactin. DNA was extracted from 523 samples, and 2 alleles were identified using the PCR-RFLP technique. The frequency for the alleles A and B was 21.26 and 78.74%, respectively, and the 3 genotypes AA, AB and BB showed frequencies of 2.87, 36.78, and 60.34%, respectively. The association between the genotypes and the studied traits was analyzed with a linear mixed model including the fixed effect of *POU1F1* genotype, parity, year and month of birth, and the random effect of the animal. The analyzed SNP had no significant effect on the evaluated traits milk yield, protein percentage, fat percentage, calving interval and services per conception. The absence of association between the SNP and evaluated traits could be due to 2 reasons; the interaction of this gene with other genes involved in the POU1F1 pathway which may hide the individual effect of this SNP on the evaluated traits, and the genotype-environment interaction, the latter has a large effect on gene expression. This way, the *POU1F1* gene is not a good candidate to be used in breeding programs assisted by molecular markers (MAS) for the evaluated population.

Key Words: dairy herd, molecular marker, polymorphism

M97 Identification of genes and networks for the response to thermal stress. Hoyoung Chung*, *National Institute of Animal Science, Suwon, KY, Korea.*

This study has been aimed to investigate genetic responses for the genes related to heat stress when exposing Holstein calves that were selected based on no significant relationship (inbreeding coefficient <0.01). A total of 10 animals aged from 4 to 6 mo were selected, and heat stress was imposed on animals directly in an environmentally controlled house that was managed for 33°C and 90% of humidity based on THI values. After exposing heat stress for 8 h a day, animals were placed in a normal condition at least 12 h to recover from the heat stress. The blood samples, which were collected at the starting point (09:00 a.m.) and at the end of heat stress (21:00 p.m.), were immediately placed in liquid nitrogen. The RNA was extracted from blood samples between control and treatment, and cDNA library was constructed for each individual. After sequencing analysis that produced a minimum 3 G byte, expression analysis confirmed that 53 genes were differentially expressed according to the severe heat stress. The analysis also verified 4 major pathways (MAPK signaling; T-cell receptor signaling; B-cell receptor signaling, and Chemokine signaling) that were related to heat stress. As expected that heat-shock protein (*HSP*) is related to thermal stress and response, the analysis verified that *HSP70* presented 656.4 fold changes between control and treatments. To verify fold changes, 100 primer pairs from 51 genes with accession numbers were tested using real-time PCR, and the results confirmed 17 genes in statistical significances. The identified genes should be served as reference genes for the selection of superior animals against thermal stress.

Key Words: thermal stress, differentially expressed gene

M98 Transcriptome analysis of muscular tissue in Nellore cattle divergently ranked for meat tenderness. Larissa Fernanda Simielli Fonseca*[1], Daniele Fernanda Jovino Gimenez[1], Fernando Baldi[1], Jesus Aparecido Ferro[2], Rafael Espigolan[1], and Lucia Galvão Albuquerque[1], *[1]Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, SP, Brazil, [2]Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias, UNESP, Jaboticabal, SP, Brazil.*

The objective of the present study was to identify differential gene expression related to meat tenderness in Nellore cattle. Meat samples from 132 animals belonging to the same contemporary group were used. Meat tenderness was measured by shear force through Warner Bratzler method and 20 divergently ranked animals for this trait (10 with tough meat and 10 with tender meat) were selected. Means and the respective

standard deviations for tough and tender meat groups were 7.4 ± 0.78 kg and 4.41 ± 0.40 kg, respectively. Total RNA was extracted from the selected samples and sequenced (RNA-Seq) using the HiSeq 2500 System (Illumina). The results were analyzed in the iPlant Collaborative platform. The workflow included: FastQC; TopHat2 and Cuffdiff. A total of 17 differentially expressed genes (q- value <0.05) were identified, and among them, 4 are highlighted. The genes *Q3ZCJ1* (transmembrane protein 37), *C1QTNF7* (C1q and tumor necrosis factor related protein 7) and *BDH1* (3-hydroxybutyrate dehydrogenase, type 1), were more expressed in tough meat samples, whereas the gene *ATP1A1* (ATPase, Na^+/ K^+ transporting, α 1 polypeptide) was more expressed in tender meat samples. The Q3ZCJ1 protein is involved in the same metabolic pathway of actin and myosin proteins that constitute the myofibrils, organelle which acts on muscle contraction. C1QTNF7 protein acts in complement and coagulation cascade metabolic pathway. This pathway is activated after an injury and the coagulation system is activated by exposed collagen after an injury. There is a direct link between collagen content and meat tenderness. The BDH1 enzyme is involved in the synthesis and degradation of ketone bodies that, if present, causes a decrease in muscle pH. The ATP1A1 enzyme is related to protons synthesis, which causes a decrease in intracellular pH during ATP degradation to ADP. Muscle pH decrease is related to meat tenderness during the post-mortem process. These genes seem to be involved in the meat tenderness process. This study was supported by São Paulo Research Foundation FAPESP (grants 2009/16118-5 and 2013/09190-7)

Key Words: RNAseq, differentially expressed gene, quality meat

M99 Environmental risk assessment by genetically engineered mice as transgenic animal model. Dailu Guan[1], Qian Yu[1], Erhu Zhao[1], Yong Wang[2], and Yongju Zhao*[1], *[1]College of Animal Science and Technology, Southwest University; Chongqing Key Laboratory of Forage & Herbivore; Chongqing Engineering Research Center for Herbivores Resource Protection and Utilization, Beibei, Chongqing, China, [2]Department of Laboratory Animal Science, College of Basic Medicine, Third Military Medical University, Sapingba, Chongqing, China.*

Environmental safety on transgenic animals is often a controversial issue within the researchers and the public. Here, a total of 12 full-sib transgenic mice expressing enhanced green fluorescent protein (eGFP) were used for assessing foreign gene expression by single-line passage method and evaluating effects on GE gene with the environment with a total of 12 full-sib mice (positive: control = 6:6, with the same size of male and female mice). PCR method and fluorescent protein observation system (FPOs) were used for detecting the inserted exogenous genes in these mice. The results showed that the expression level of eGFP and WRPE gene (vector sequence) were not significantly different, but it significantly reduced by F1 to F4 generation (F1, F2, F3 and F4) ($P < 0.05$) by Real-time quantitative PCR (RT-qPCR). The effect of GE mice on the environment with 4- or 8-week-old full-sib transgenic mice offspring was evaluated. The exogenous genes were not detected by PCR in the mice manure. Microbial flora of transgenic mice were not significantly different with those of the control group ($P > 0.05$), neither male or female, at 4 or at 8 week-old. In addition, there was no significant difference of the microbial communities in mice gut as assessed by PCR-DGGE or by 16S rDNA sequencing between positive and control transgenic mice offspring. Furthermore, the phylogenetic analyses showed that the manure bacteria sampled during each of the 2 stages belonged primarily to 3 groups, *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. No unknown microbial flora were found in the mice manure. [Supported by the National Natural Science Foundation of China (No. 31172195), the Fundamental Research Funds for the Central Universities(No: XDJK2014A010) and the 2013 Innovation Team Building Program in Chongqing universities (KJTD201334).]

Key Words: transgenic mouse, environmental risk, gene drift

M100 Associations between HEL5, AFZ1, ILSTS002, BMS3004, IDVGA-51, LHR, and FSHR alleles on reproductive evaluation of bulls. Gabriel R. Pereira*[1], Silvio R. O. Menegassi[1], Paulo R. Aguiar[2], Katiana S. Pereira[2], Celso Koetz[3], Flavio G. Lopes[3], Vanerlei M. Roso[4], Vanessa Peripolli[1], Fernanda G. Moojen[1], and Julio O. J. Barcellos[1], *[1]Federal University of Rio Grande do Sul - UFRGS, Porto Alegre, RS, Brazil, [2]Lutheran University of Brazil - ULBRA, Canoas, RS, Brazil, [3]University of Northern Paraná - UNOPAR, Arapongas, PR, Brazil, [4]GenSys Association, Porto Alegre, RS, Brazil.*

This study emphasized the importance to develop new molecular tools to accurately identify candidate genes in predicting semen quality in bulls that can be used in livestock production. We assessed the frequency distribution of molecular markers linked to insulin growth factor I, follicle stimulating hormone (FSH), luteinizing hormone (LH) and leptin genes in Braford and Hereford population. All bulls (Braford and Hereford; n = 188) showed good body condition scores throughout the experiment. Blood sampling and measurements of scrotal circumference were performed on weaned bulls at 7 and 24 mo of age during the bull breeding soundness evaluations. Semen collection was performed using an electroejaculator on bulls at 24, 28, 32 and 36 mo. Blood samples for DNA extraction were collected by puncture of the coccygeal vein. Five microsatellites or short tandem repeats (HEL5, AFZ1, ILSTS002, BMS3004, and IDVGA-51) and 2 SNP markers (LHR and FSHR) were evaluated by the amplification of DNA products, which then underwent electrophoresis in 10.5% polyacrylamide gel. Molecular markers were analyzed with PROC GLM ANOVA using SAS and significant were considered when < P < 0.05. Hereford bulls that expressed the variation in the IDVGA51 allele was associated ($P < 0.05$) with sperm motility and vigor traits in Hereford bulls. Hereford animals showed polymorphic information content (PIC) of 0.36 to 0.75% along with expected heterozygosity (H) of 0.49 to 0.78%. However, Braford bulls that expressed the ILSTS002 and AFZ1 alleles were associated ($P <$ 0.05) with major and minor defects, respectively. Braford PIC ranged from 0.28 to 0.78% with an expected H of 0.35 to 0.81%. We found no relation between HEL5, BMS3004, LHR and FSHR to predict semen quality in bulls ($P > 0.05$). Therefore the present study revealed that 3 microsatellite alleles are important male reproductive biomarkers for improving semen parameters in bulls. We concluded that the ILSTS002 and AFZ1 alleles in the Braford and the IDVGA51 allele in the Hereford may be used for improving the reproductive traits in bulls.

Key Words: molecular marker, microsatellite, genetic variability