

## Breeding and Genetics: Molecular Biology and Genomics

**W54 Protection and stabilization of whole blood at room temperature does not influence DNA yield, purity, and integrity.** R. Flores\*<sup>1</sup>, M. Udtha<sup>1</sup>, J. E. Sanner<sup>1</sup>, E. A. Backes<sup>2</sup>, L. S. Wilbers<sup>2</sup>, and J. D. Caldwell<sup>2</sup>, <sup>1</sup>The University of Texas Health Science Center at Houston, Houston, <sup>2</sup>Lincoln University, Jefferson City, MO.

Preservation of bio-specimens for molecular biological applications traditionally involves freezing which increases laboratory and research project costs. Protection and stabilization of DNA at room temperature (RT) may eliminate the costs associated with freezer storage. However, there is a paucity of information describing the yield, purity, and integrity of DNA stored at RT. Objectives were to evaluate the yield, purity, and integrity of DNA extracted from whole blood samples stored at RT, low (−20°C), and ultra-low (−80°C) temperatures. Sheep (n = 4 [7 mL]) and human (n = 6 [4 mL]) whole blood samples were collected and aliquots stored at RT, −20°C, and −80°C. Blood samples at RT were stored utilizing bio-stabilization technology (DNAstable Blood and DNAgard Blood; Biomatrix, San Diego, CA) designed to protect genomic DNA in whole blood from degradation resulting from unprotected RT storage. Negative control samples were stored unprotected at RT. Genomic DNA was extracted by using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA). Quantification and purity of the extracted DNA was determined by spectrophotometry and the integrity was assessed following electrophoresis. The DNA yield was not influenced by a species × storage temperature interaction ( $P = 0.84$ ). Similarly, storage temperature did not influence DNA yield ( $P = 0.52$ ) and averaged  $13.6 \pm 1.2$  ng/μL across all storage temperatures. Among samples stored protected at RT, species, type of technology utilized, and the interaction did not ( $P > 0.13$ ) influence DNA yield with yield averaging  $12.5 \pm 1.2$  and  $12.7 \pm 1.2$  ng/μL for DNAgard and DNAstable, respectively. The 260/280 ratio was influenced by a species × storage temperature interaction ( $P = 0.01$ ). In general, the 260/280 ratios were higher ( $P < 0.05$ ) for human samples stored at low and ultra-low temperatures compared with sheep samples stored at similar low temperatures. In conclusion, ambient temperature-based bio-stabilization technologies may offer an alternative to traditional low temperature-based bio-specimen preservation and storage.

**Key Words:** blood, DNA, temperature

**W55 Maximum differences analysis: An empirical method for genome-wide association studies.** M. Cellesi, N. P. P. Macciotta,\* G. Pulina, G. Gaspa, and C. Dimauro, *Dipartimento di Agraria, Università di Sassari, Italy.*

Genome-wide association studies have rapidly become the most popular method to discover associations between SNPs and quantitative traits. Marker significance is traditionally assessed by using the Bayesian or the frequentist approach. Both methods have positive and negative aspects. In the present study we suggest an empirical method which combines the simplicity of the frequentist approach with the reliability of Bayesian inference. We called this method maximum difference analysis (MDA). A total of 2,093 Italian Holstein bulls were genotyped with the Illumina BovineSNP50 BeadChip. Polygenic EBV for milk yield, fat and protein content were available. MDA starts by randomly choosing 1,500 animals and retaining the best 100 (B) and the worst 100 (W) animals for a particular trait. Relative genotypes frequencies

for each marker were calculated for B and W group, respectively. Then the difference between genotype having the highest frequency in B and the corresponding frequency in W were calculated. In every single chromosome, the above mentioned differences were considered as a random variable and, after standardization, SNPs with difference higher than 1.96 standard deviations were retained as possible candidate for association. This procedure was repeated 5,000 times and the frequency (fi) of the previous detected association for each SNP annotated. A marker was considered significantly associated with the specific trait if  $\pi_i = f_i/5000 > 0.99$ . For milk yield, fat and protein, 93, 40 and 92 markers were selected, respectively. Several SNP were found to be significantly associated to more traits: 19 for milk and protein, 19 for milk and fat, 12 for fat and protein and 10 among all traits. Moreover, 19, 7 and 17 SNPs, for milk, fat and protein, respectively, were selected with  $\pi_i = 1$ . The reliability of results obtained by MDA was confirmed by the detection of several SNPs located near genes known to affect the specific trait, as the DGAT1 and the casein cluster.

**Key Words:** gene association

**W56 Adjustment of selection index coefficients and polygenic variance to improve regressions and reliability of genomic evaluations.** P. M. VanRaden, J. R. Wright,\* and T. A. Cooper, *Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD.*

In multi-step genomic evaluations, direct genomic values (DGV) are computed using either marker effects or genomic relationships among the genotyped animals, and information from non-genotyped ancestors is included later by selection index. The DGV, the traditional evaluation (EBV), and a subset breeding value (SBV) estimated using pedigree relationships among the genotyped animals are combined according to theoretical weights based on reliabilities of the 3 terms. In official yield trait evaluations of young Holstein bulls, the weights average 0.99 for DGV, 0.12 for EBV, and −0.11 for SBV. Alternative weights have been proposed by other countries to increase reliabilities and regressions of predicting future data from past. Most US regressions were close to expected values and increased when some weight was removed from the DGV and added to either the EBV or SBV or both. Reliabilities decreased slightly for Holsteins with weight added to either EBV or SBV, but reliability increased for some traits of Jerseys if weight was added to SBV. Maximum weights on DGV of 1.0, 0.9, and 0.8 were compared. For each 0.1 decrease, regressions increased by about 0.02. Regressions for a few traits were lower than expected, and limiting the DGV weight to 0.9 or 0.8 instead of the theoretical value of 1.0 helped bring the regressions into compliance with validation tests. Adjustments to polygenic variance also increased the regressions by about 0.02 for each 0.1 increase, but reliabilities were slightly reduced when compared with adjusting the selection index weights. Finding optimum percentages of polygenic variance required more computation and was less flexible than finding optimum weights, because recombining 3 terms in a final step is easier than re-estimating all marker effects. Conclusions are that index adjustments can help to pass genomic validation tests for some traits by removing small biases in regressions, but that the theoretical selection index weights currently in use are close to ideal.

**Key Words:** multi-step evaluation, direct genomic value, validation

**W57 Use of canonical discriminant analysis to distinguish among three bovine breeds by using a low number of selected SNP markers.** C. Dimauro,\* M. Cellesi, R. Steri, S. Sorbolini, and NPP Macciotta, *Dipartimento di Agraria, Università di Sassari, Sassari, Italy.*

Multivariate statistical techniques are commonly used to synthesize genomic information in animal breeding studies. In the present work, the multivariate canonical discriminant analysis (CDA) was used to discriminate between 3 bovine breeds. Data consisted of 2,090 Holstein, 750 Brown, and 480 Simmental bulls genotyped by using the Illumina BovineSNP50 BeadChip. After data editing, SNP in common to the 3 breeds were 30,055. A sample of 30 randomly selected bulls (10 for each breed) were used as new observations in the cross-validation data set. When 3 groups are involved, CDA derives 2 canonical functions (CAN1 and CAN2) which are linear combination of the original variables (SNP genotypes coded as 0, 1 and 2). Coefficients of the linear combination are the canonical coefficients (CC) and represent the correlation among SNP and canonical functions. CDA was first applied separately on the 29 bovine autosomes. For each chromosome, SNP with  $CC > 0.90$  in CAN1 and  $CC > 0.50$  in CAN2 were selected. Results indicated that CDA was able to efficiently separate the 3 breeds also at chromosome level. At the end of the procedure, 295 SNP out of 30,055 were retained. These markers were then used to develop a CDA on the entire genome. The Mahalanobis distance test was highly significant ( $P < 0.001$ ) for all comparisons (distances: Holstein vs. Brown 2,941, Holstein vs. Simmental 3,127, Simmental vs. Brown 410). In particular, CAN1, which accounted for 95% of the total variability, separated Holstein from Brown and Simmental bulls. This result could be due to the high selection pressure exerted on Holstein population and the 129 SNP with  $CC > 0.90$  could be the related signatures. CAN2 was able to significantly separate Brown and Simmental groups. In this case 7 SNP with  $CC > 0.60$  were found. Finally, individuals in the cross-validation data set were 100% correctly classified. The above described procedure could be therefore tested for developing a test for the traceability of breed products. Low-density SNP panels (295 markers in this work), with moderate price, could be specifically developed for routine analysis.

**Key Words:** genomic association

**W58 Reliability of genomic breeding values at different reference population's designs when some or all animals are genotyped.** M. Pszczola<sup>1,3</sup>, T. Strabel<sup>\*3</sup>, J. A. M. van Arendonk<sup>2</sup>, and M. P. L. Calus<sup>1</sup>, <sup>1</sup>*Animal Breeding and Genomics Centre, Wageningen UR Livestock Research, Lelystad, the Netherlands*, <sup>2</sup>*Animal Breeding and Genomics Centre, Wageningen University, Wageningen, the Netherlands*, <sup>3</sup>*Department of Genetics and Animal Breeding, Poznan University of Life Sciences, Poznan, Poland.*

Reliability of genomic breeding values with different structure of a small reference population including ungenotyped animals was investigated. Data reflecting a dairy cattle population structure was simulated for a trait with heritability of 0.3. Reference populations were small and consisted of cows only. Reference populations consisted of highly, moderately, lowly, and randomly related animals by choosing appropriate family structure. Evaluated animals were one generation younger than the reference populations. Four scenarios in which reference population and evaluated animals were not genotyped (AA), reference population was genotyped and the evaluated animals were not genotyped (GA), reference population was not genotyped and the evaluated animals were genotyped (AG) and both groups were genotyped (GG). Reliabilities of direct genomic values were predicted deterministically using selection index theory. For GG, reliabilities were considerably higher than in the

other cases. In AG, reliabilities were somewhat higher, than in GA. AG achieved substantially higher reliabilities than AA. Reliabilities increased with decreasing average relationship within the reference population. The main source of the gain in reliability is genotyping the evaluated animals, however, the benefit from genomic selection is substantially higher when all animals are genotyped.

**Key Words:** genomic selection, reliability, dairy cattle

**W59 Dealing with uncertainty of dependent variables in genome wide association studies.** S. Smith,\* E. H. Hay, and R. Rekaya, *University of Georgia, Athens.*

In genome-wide association studies (GWAS) using multiple-step procedures, the dependent variable (DV) is often a pseudo-observation such as estimated breeding values (EBVs) or de-regressed proofs. Thus, these "estimated" DVs include a certain level of uncertainty. If the sampling errors (SE) attached to these variables are constant across all records, regression analyses will accommodate this situation without major difficulty. However, when SE are heterogeneous across observations, the case with pseudo-records used in GWAS in livestock applications, the situation is more complex. The residual terms of the regression models include 2 components, the SE due to estimation of the DV, i.e., EBVs, and white noise that will exist if it was possible to observe (measure) the DV. Unfortunately, this type of data are often analyzed using an ordinary least square (OLS) assuming homogeneous residuals or weighted least square (WLS) where both components of the residual are assumed to be heterogeneous when only the first component is. In this study we present a method for analyzing uncertain DVs when only one component (SE) of the residual term is heterogeneous with applications in GWAS. Further, we assumed SE to be either completely known or only known up to a proportionality constant to mimic scenarios of using EBVs as DVs. A real data set consisting of 1989 animals genotyped for around 56k SNPs was used. EBVs for growth and associated prediction error variance (PEV) and reliability were computed using phenotypic and pedigree information. After SNP selection using single marker analyses and stepwise regression, 209 SNPs were retained for association with growth EBVs. Four analyses were conducted: 1) OLS (M1); 2) WLS (M2); 3) WLS assuming only heterogeneous SE using the estimated PEV (M3) and 4) WLS assuming only heterogeneous SE using a transformation of the estimated reliabilities (M4). Using a 5-fold cross validation, the results (correlation between EBVs and the prediction) showed a superiority of M3. This superiority ranged from 3% between M3 and M4 to 8% between M3 and M1.

**Key Words:** uncertainty, genome-wide association, prediction error variance

**W60 Increased use of young bulls in dairy cattle breeding programs.** H. D. Norman, J. L. Hutchison,\* and J. B. Cole, *Animal Improvement Programs Laboratory, ARS, USDA, Beltsville, MD.*

Availability of genomic evaluations since 2008 has resulted in many changes in breeding programs. The contribution of young bulls (0.8 to 3.9 yr old) to those programs was investigated. The number of Holstein sires of young bulls doubled from 2008 (126 sires) to 2011 (276 sires); 14% of sons were sired by a young bull in 2008 compared with 40% in 2011. Corresponding values for Jerseys were 34 and 54 sires of young bulls in 2008 and 2011, respectively, with 19 and 40% sired by young bulls. From US breeding records from 2007 through 2011, 19,359,730 Holstein and 1,133,090 Jersey breedings were examined. Young bulls were used for 28% of Holstein breedings in 2007, 29% in 2008, 39%

in 2009, 43% in 2010, and 48% in 2011; annual percentages for young genotyped Holstein bulls were 0, 8, 36, 42, and 48%. Genotyped bulls accounted for 0, 26, 92, 98, and 99% of breedings to young Holstein bulls annually from 2007 through 2011. Young bulls were used for 25, 27, 31, 33, and 40% of Jersey breedings annually, with 0, 0, 22, 32, and 39% of breedings to young genotyped bulls; genotyped bulls accounted for 0, <1, 72, 98, and 98% of breedings to young Jersey bulls. Percentage of female progeny sired by young bulls was calculated by progeny birth year for 5,035,103 Holstein and 496,062 Jersey heifers. Young bulls annually sired 24, 23, 23, 33, and 40% of Holstein heifers born from 2007 through 2011 and 29, 27, 30, 33, and 35% of Jersey heifers. Mean sire age for Holstein progeny born in 2011 was 23 mo younger than in 2006 for bulls and 12 mo younger for heifers; corresponding values for Jerseys were 15 and 4 mo. Mean net merit from December 2011 weighted by number of breedings was \$290 for 2008 breedings, \$339 for 2009 breedings, and \$361 for 2010 breedings of active Holstein bulls and \$230, \$483, and \$532 for genotyped Holstein bulls (a difference of \$60, \$144, and \$171, respectively). Corresponding values for Jerseys were \$270, \$286, and \$324 for active bulls and \$396, \$448, and \$510 for genotyped bulls (differences of \$126, \$162, and \$186). Use of young bulls has greatly reduced the generation interval and improved the rate of genetic gain since implementation of genomic evaluations.

**Key Words:** breeding, service sire, genomics

**W61 Accuracy and bias for final score in US Holsteins from adding genomic information on bulls and cows.** S. Tsuruta<sup>\*1</sup>, I. Misztal<sup>1</sup>, and T. J. Lawlor<sup>2</sup>, <sup>1</sup>University of Georgia, Athens, <sup>2</sup>Holstein Association USA Inc., Brattleboro, VT.

Obtaining genomic information on animals should lead to a greater opportunity for earlier and more accurate selection. Selective genotyping of validation bulls will lead to a regression coefficient (b) for later daughter deviations on early genomic parent averages to be less than 1.0. A recent study by Olson, et al. (2011) indicates that the current expected b-value for US final score for bulls is 0.781. The objective of this study was to compare the change in accuracy and potential bias from adding genomic information on bulls and cows for final score of US Holsteins. A total of 39,741 animals (5,235 cows and 34,506 bulls) had either a final score themselves or progeny with a final score. A SNP chip containing 42,503 usable markers was available for all genotyped animals. The full data set included 10,944,571 records in 2011. The reduced data contained 9,602,030 records in 2007. Validation animals were 760 young bulls born after 2004 and with at least 50 daughters in 2011 and 2,098 young cows born after 2006. Genomic breeding values were calculated with a single-step procedure. Including genotypes on bulls only, raised the R<sup>2</sup> from 23.4 to 33.3 along with a slight increase in the b-value. Including genotypes on both bulls and cows improved the R<sup>2</sup> to 34.2 and slightly lowered the b-value for the bulls. Including genotypes on bulls and cows improved the cow's accuracy (R<sup>2</sup>) from 16.1 to 24.2 with a b-value that went from slightly overestimating the genomic breeding values to slightly underestimating it, i.e., 0.978 and 1.042, respectively. Inclusion of bull and cow genotypes in the single-step procedure improves the accuracy of selection with a small change in the indication of bias from the parent averages.

**Key Words:** genomic evaluation, final score, US Holstein

**W62 SNPs that affect microRNA binding sites in the bovine ACACA gene are associated with polyunsaturated fatty acid (PUFA) content of Canadian Holstein cows.** E. M. Ibeagha-Awemu<sup>\*1</sup>, K. A. Akwanji<sup>2</sup>, Z. Wang<sup>3</sup>, and X. Zhao<sup>2</sup>, <sup>1</sup>Dairy and

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Bovine milk has beneficial lipids that contribute positively to human health. These lipids include some isomers of CLA, oleic and  $\alpha$ -linolenic acids and represent a very small portion of the total fatty acids (FAs) in milk. FAs in milk arise from dietary sources or are synthesized de novo in the mammary epithelial cells through the action of enzymes including acetyl-CoA carboxylase- $\alpha$  (ACACA). Fat synthesis is a complex trait that is regulated by genetic, environmental and epigenetic (e.g., microRNA) factors. microRNAs are functional non-coding small RNA molecules that have emerged as important regulators of gene expression. microRNA dis-regulation has been shown to be associated with disease conditions in humans and production traits in farm animals. In this study, we identified 6 SNPs in the 3'UTR of the ACACA gene by sequencing 50 samples and genotyping of 150 samples from Canadian Holstein cows by Sequenom iPLEX Gold technology. The FA profiles of milk from the same cows were analyzed by GC and HPLC. In silico analysis showed that SNPs ACACA-80 A>G affected a binding site for bta-miR-126 while ACACA-83 C>T and ACACA-84 G>A disrupted the binding site for bta-mir-3432. Relationship between FAs and SNPs were analyzed using PROC MIXED of SAS (version 9.3) with genotype as fixed factor. Multiple comparisons of means was done with Turkey adjustment. Significant associations were found between SNPs, total PUFA and other FA isomers in the milks of Canadian Holstein cows. Cows ACACA-80 AA had higher concentrations ( $P < 0.05$ ) of PUFA ( $2.902 \pm 0.122$ ) as compared with cows GG ( $2.333 \pm 0.159$ ) but similar to cows AG ( $2.576 \pm 0.090$ ). ACACA-80 AA also had significantly higher concentrations of C16:1T, C18:1n9c and lower content of total saturated FAs as compared with cows AG and GG. Similarly, cows ACACA-83 CT ( $3.126 \pm 0.185$ ) had significantly higher PUFA content as compared with ACACA-83 CC ( $2.608 \pm 0.067$ ) and ACACA-83 TT ( $2.256 \pm 0.586$ ). PUFA of cows ACACA-84 GG ( $2.839 \pm 0.107$ ) was also higher ( $P < 0.05$ ) as compared with ACACA-84 AA ( $2.334 \pm 0.158$ ) and ACACA-84 AG ( $2.488 \pm 0.129$ ). SNPs ACACA-83 and 84 also affected the content of C16:1, C16:1T, C18:2n6cc, C20:3n6, C6:0, C8:0, C10:0 and C12:0. These results demonstrate that SNPs in the 3'UTR of the ACACA gene may be involved in the regulation of PUFA synthesis and as such may act as potential candidate genes for breeding for improved PUFA content of bovine milk. Our results further suggest that microRNA may be involved in the regulation of mammary milk FA synthesis. We are in the process of validating our results with a larger data set.

**Key Words:** ACACA gene, Canadian Holsteins, SNP

**W63 Genomic-polygenic evaluation of postweaning weight and ultrasound carcass traits in an Angus-Brahman multibreed population.** M. A. Elzo<sup>\*1</sup>, C. A. Martinez<sup>1</sup>, G. C. Lamb<sup>1</sup>, D. D. Johnson<sup>1</sup>, M. G. Thomas<sup>2</sup>, I. Misztal<sup>3</sup>, D. O. Rae<sup>1</sup>, J. G. Wasdin<sup>1</sup>, and J. D. Driver<sup>1</sup>, <sup>1</sup>University of Florida, Gainesville, <sup>2</sup>Colorado State University, Fort Collins, <sup>3</sup>University of Georgia, Athens.

The objectives of this study were to estimate the fractions of additive genetic variances for postweaning weight (PW) and 3 ultrasound traits explained by SNP included in the Illumina3k chip, to compare EBV rankings predicted using genomic-polygenic, genomic, and polygenic models, and to assess EBV trends as Brahman fraction of calves increased from 0 to 1 in an Angus-Brahman multibreed population. Ultrasound traits were ribeye area (UREA), percent of intramuscular

fat (UPIMF), and backfat thickness (UBF). Trait measurements on 623 calves born between 2006 and 2010 were collected at the Feed Efficiency Facility of the University of Florida. Single-trait genomic-polygenic (GP) models were used to estimate variance components using Markov Chain Monte Carlo procedures (option VCE from program GS3). Fixed effects were contemporary group (year-pen), age of dam, sex of calf, age of calf, Brahman fraction of calf, and heterozygosity of calf. Random effects were additive SNP, additive polygenic, and residual. Subsequently, EBV were computed with option BLUP of program GS3 using GP models and models that included only genomic (G) or polygenic (P) effects. The fractions of the additive genetic variance explained by SNP in the Illumina3k chip were 0.09 for UREA, 0.38 for UBF, 0.06 for UPIMF, and 0.08 for UW. Rank correlations between EBV from GP and P models were high (0.89 to 0.99), and moderate (0.51 to 0.65) between EBV from G and P models. Regression coefficients for all models and traits showed that calf EBV tended to decrease as Brahman fraction of calves increased suggesting that calves with higher Brahman fraction were leaner, had smaller ribeye areas, and grew more slowly than calves with higher Angus fractions. The low fraction of additive genetic variances accounted for by the markers in the Illumina3k chip indicated that higher density chips would be needed to more completely account for additive genetic variation in multibreed populations.

**Key Words:** cattle, genomic, multibreed

**W64 Genomic-polygenic evaluation of Angus-Brahman cattle for carcass traits with the Illumina 3K chip.** M. A. Elzo<sup>\*1</sup>, G. Hu<sup>1</sup>, C. A. Martinez<sup>1</sup>, G. C. Lamb<sup>1</sup>, D. D. Johnson<sup>1</sup>, M. G. Thomas<sup>2</sup>, I. Misztal<sup>3</sup>, D. O. Rae<sup>1</sup>, J. G. Wasdin<sup>1</sup>, and J. D. Driver<sup>1</sup>, <sup>1</sup>University of Florida, Gainesville, <sup>2</sup>Colorado State University, Fort Collins, <sup>3</sup>University of Georgia, Athens.

The objective of this research was to assess the effect of including genotypic information from the Illumina 3k chip on the genetic evaluation of animals for 5 carcass traits in an Angus-Brahman multibreed population using genomic-polygenic (GP), genomic (G), and polygenic (P) models. Fractions of additive genetic variances associated with markers in the Illumina3k chip were computed, and animal EBV rankings and EBV trends as calf Brahman percent increased from 0 to 100% were compared across models. Traits were hot carcass weight (HCW), dressing percent (DP), ribeye area (REA), fat over the ribeye (FOE), and marbling (MAB). Phenotypic and genotypic data were from 202 steers born from 2006 to 2010. Data were analyzed with single-trait models. All models had contemporary group (year-pen), Brahman fraction of calf, heterozygosity of calf, and slaughter age as fixed effects. Random effects were additive SNP (GP and G models), additive polygenic (GP and P models), and residual. Program GS3 was used to compute variance components and heritabilities with option VCE (Markov Chain Monte Carlo), and EBV using option BLUP. Heritabilities were 0.72 for HCW, 0.25 for DP, 0.53 for REA, 0.44 for FOE, and 0.23 for MAB. Fractions of additive genetic variance explained by Illumina 3k SNP were 0.08 for HCW, 0.47 for DP, 0.19 for REA, 0.27 for FOE, and 0.23 for MAB. Higher rank correlations existed between EBV from GP and P models (0.94 to 0.99;  $P < 0.0001$ ) than between EBV from G and P models (0.78 to 0.84;  $P < 0.0001$ ). Regressions of calf EBV on Brahman fractions were non-significant for all traits indicating that calves of comparable EBV for carcass traits existed across all breed compositions. The low fractions of additive genetic variances accounted for by the Illumina3k chip indicated that GP models would need to be used to compute EBV if this chip were used to help predict animal EBV, and that higher density

chips would be needed to better account for additive genetic variation in multibreed populations.

**Key Words:** carcass, cattle, genomic

**W65 Using low-density commercial DNA-marker panels on prediction accuracy for expected progeny differences of selection criteria: An application in a marker-assisted breeding program for Nelore cattle in Brazil.** J. B. S. Ferraz<sup>\*1</sup>, F. M. Rezende<sup>1</sup>, R. C. G. Silva<sup>1,2</sup>, X. Wu<sup>3</sup>, S. Bauck<sup>2</sup>, J. P. Eler<sup>1</sup>, and E. C. Mattos<sup>1</sup>, <sup>1</sup>University of Sao Paulo/FZEA/ZAB/GMAB, Pirassununga, SP, Brazil, <sup>2</sup>Igenity Livestock Production Business Unit, Merial Ltd., Duluth, GA, <sup>3</sup>Department of Animal Science, Univ. of Wisconsin, Madison.

The objective of this study was to evaluate the effect of inclusion of molecular information on estimated accuracy for expected progeny differences of the following selection criteria of a breeding Nelore project in Brazil: weaning weight (WW), post weaning gain up to 18 mo (PWGAIN), scrotum circumference (SC) and muscle score (MUSCLE), when predicted molecular breeding values (MBV) of these traits, based on low-density SNP panels developed by Igenity in Brazil (released in June 2011), were included in the analysis. Low-density SNP panels were selected using the parallel Bayes CpiC package, in which a BayesC  $\pi$  model was used for feature selection and a BayesC model was used for post-selection statistical inference and cross-validation. (Co)variance components and genetic parameters were estimated using a large data set with 97,580 animals included in the  $A^{-1}$  matrix. Heritability were estimated to 0.19 for WW, 0.82 for MBV of WW, and genetic correlation between them was estimated to be  $r_g = 0.36$ . Heritability and genetic correlation for other characters were: 0.21 (PWGAIN), 0.86 (MBV of PWGAIN), with  $r_g = 0.50$ ; 0.52 (SC), 0.67 for (MBV of SC), with  $r_g = 0.33$ ; 0.20 for (MUSCLE), 0.70 (MBV of MUSCLE) with  $r_g = 0.32$ . Further, those genetic parameters were applied to genetic evaluation analyses with single traits (considering phenotypes only in appropriate models) and 2 traits (phenotypes as trait 1 and MBV for the same trait as trait 2), using REML approach, in a large data set ( $A^{-1} = 520,169$ ) and from that analysis new predictions of EPD were obtained. From the large data set, close to 3,500 animals were genotyped. BIF accuracies were calculated for all animals in single-trait analysis. The effect of including MBV on accuracy of prediction of marker-EPD varied with subgroups of animals as well as different traits: prediction accuracy obtained for young replacement bulls with genotypes showed an increase of up to 22%; similar increment of predictive accuracy was observed for bulls 13 progenies or more. It is hence concluded that selected low-density SNP marker panels was useful in marker-assisted selection programs for Nelore population analyzed.

**Key Words:** MAS, beef breeding program, impact on accuracy

**W66 SNP AY428575.1:g.346G>A of the bovine TCAP gene: Genotyping with PCR-RFLP and occurrence in Nelore animals (*Bos indicus*) and Angus (*B. taurus*) × Nelore.** B. Borges<sup>\*1</sup>, R. Curi<sup>2</sup>, A. Tamanaha<sup>2</sup>, and L. A. Chardulo<sup>3</sup>, <sup>1</sup>College of Agrarian and Veterinary Sciences, UNESP, Jaboticabal, SP, Brazil, <sup>2</sup>College of Animal Production and Veterinary Medicine, Animal Breeding and Nutrition Department, UNESP, Botucatu, SP, Brazil, <sup>3</sup>Bioscience Institute, Chemistry and Biochemistry Department, UNESP, Botucatu, SP, Brazil.

Genetic mapping studies and physiological analysis have resulted in identification of candidate genes related to meat quality on beef cattle, including the titin-cap gene (*TCAP*). It encodes teletonin, a protein found

at skeletal and cardiac muscles linked to the Z1-Z2 domains of titin. This protein is degraded into the *post mortem* period and influences the reduction of tenderness between 24 and 72 h after slaughter. The major objective of this work was genotyping the SNP AY428575.1:g.346G>A of the bovine *TCAP* gene by PCR-RFLP technique, and report its use for the first time. Using the forward primer 5' GGGAGTGAGCAGTCATCATGGC 3' and reverse primer 5' AGAGGCAGCACCCGCTGGT 3', amplification products of 517 bp were acquired. They were submitted to digestion with *BtsCI* enzyme, which resulted in the genotypes AA (approx. 177, 154, 128, and 58 bp), AG (approx. 305, 177, 154, 128, and 58 bp) and GG (approx. 305, 154 and 58 bp). A total of 118 Nelore (*B. indicus*) and 8 Angus × Nelore (*B. taurus* × *B. indicus*) animals were genotyped. The use of the PCR-RFLP for the genotyping of SNP of the bovine *TCAP* gene was inexpensive and robust, which will greatly facilitate analysis of this polymorphism by basic laboratory equipment and reagents when compared with the single-base extension method. There were slight variation among Nelore animals with just 1 genotype AG and 117 genotypes GG. In the same way, there were found 6 Angus × Nelore with genotype AG and 2 GG. These preliminary results suggest the worthlessness of SNP AY428575.1:g.346G>A of the bovine *TCAP* gene to association studies with traits of interest to Nelore breed and, probably, to the subspecies *B. indicus*. On the other hand, they show the viability of these studies with the crossbreed Angus × Nelore, with Angus and, possibly, to all subspecies *B. taurus*. Acknowledgments to FAPESP for financial support.

**Key Words:** beef cattle, candidate gene, meat quality

**W67 Association study of heat shock protein 70 gene with serum biochemical indices in Sanhe cattle.** Y. Wang<sup>\*1</sup>, L. Liu<sup>1</sup>, Q. Xu<sup>2</sup>, Q. Chu<sup>3</sup>, Y. Yu<sup>1</sup>, H. Wu<sup>4</sup>, D. Wang<sup>4</sup>, P. Yuan<sup>4</sup>, and A. Liu<sup>5</sup>, <sup>1</sup>College of Animal Science and Technology, China Agricultural University, Beijing, China, <sup>2</sup>College of Biology, Beijing Jiaotong University, Beijing, China, <sup>3</sup>Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China, <sup>4</sup>Xiertala Breeding Farm, Hailaer Farm Buro, Hailaer, Inner Mongolia, China, <sup>5</sup>Hailaer Farm Buro, Hailaer, Inner Mongolia, China.

Resistance to extreme cold is one of the distinct characteristics of Sanhe cattle, a dual purpose breed developed in Northern China. To quantify the ability of cold resistance and explore the genetic mechanism of cold resistance, some physiological and biochemical indicators in the serum were chosen and polymorphism of Heat Shock Protein 70 gene (HSP70) were screened, and the association between those indicators and polymorphism of HSP70 was analyzed. Blood samples from 170 Sanhe cows were collected and 6 biochemical indicators-corticosteroid (CORT), adrenocorticotrophic hormone (ACTH), thyroxin (T4), triiodothyronine (T3), Glutathione peroxidase (GSH-Px) and erythrocyte potassium in the serum were tested. Pooled DNA sequencing method (36 unrelated Sanhe cows) was used for identifying the polymorphisms of promoter and exon in HSP70. Individual genotypes were detected using the MALDI-TOF-MS technique. For the association study, a fixed effect model considering parity, sire and genotype of each single SNP was employed. Descriptive statistics of CORT, ACTH, T4, T3, GSH-Px and erythrocyte potassium in Sanhe cattle were 277.79 ± 30.47 ng/mL, 23.81 ± 9.29 pg/mL, 66.43 ± 13.91 ng/mL, 1.18 ± 0.36 ng/mL, 777.73 ± 126.40 U/mL and 22.74 ± 2.48 mmol/L, respectively. Research showed that serum biochemical indices changes when animal are exposed to hot or cold environment, and variations were found in Sanhe cattle, so serum biochemical indices can be used as auxiliary indicators for assessing the degree of cold stress. Totally 13 SNPs (6 SNPs in promoter region and 7 SNPs in exons) were detected using pooled DNA sequencing method, and all were found polymorphic in the sampled population. Minimum

allele frequency of these SNPs was 0.027–0.423. The association study showed that parity significantly ( $P < 0.05$ ) effected CORT level and sire significantly ( $P < 0.05$ ) effected ACTH level in all situations. Three novel SNPs were found significantly ( $P < 0.05$ ) associated with some biochemical indices, namely A1679G with ACTH level, C620G with GSH-Px activity and C1784T with erythrocyte potassium concentration. Current results provide evidence that HSP70 gene is an important gene associated with serum biochemical indices, therefore, assumed to have influence on cold resistance ability.

**Key Words:** HSP70 gene, Sanhe cattle, serum biochemical index

**W68 Molecular characterization of constitutive androstane receptor (CAR) and its association with feed efficiency of Nelore (*Bos indicus*) cattle.** P. Alexandre, M. H. A. Santana, R. C. Gomes, J. B. S. Ferraz,\* and H. Fukumasu, College of Animal Science and Food Engineering - Animal Breeding and Biotechnology Group (USP/FZEA/ZAB/GMAB), Pirassununga, SP, Brazil.

The constitutive androstane receptor (CAR) was initially characterized as a key regulator of xenobiotic metabolism. CAR has also been implicated in various physiological pathways such as energy metabolism and homeostasis of lipids, triglycerides, cholesterol and other endogenous hydrophobic molecules. Here, our focus was to detect genetic polymorphisms (SNP) of CAR of Nelore beef cattle, predict their functional role and associate them to residual feed intake (RFI) and residual body weight gain (RIG), a recently proposed measure of feed efficiency. Genomic DNA was extracted from blood of 50 Nelore bulls and the entire CAR gene was amplified and sequenced with 8 pairs of primers. Molecular characterization was performed for polymorphism identification, phylogenetic analysis and prediction of functional consequences of SNPs. Also, statistical association with RIG was performed with PROC MIXED (SAS). We found 24 SNPs in CAR gene, being one in the promoter region, 8 exonic, 13 intronic and 2 SNPs after the 3' UTR. We did not find any SNPs in the DNA binding domain of the receptor (DBD); however, 2 SNPs were found between DBD and the ligand binding domain (LBD), both being synonymous: SNP10 (Leu→Leu) and SNP11 (Ser→Ser). The LBD was the most genetically variant region presenting 5 SNPs, being 4 synonymous: SNP14 (Asp→Asp), SNP17 (Ala→Ala), SNP18 (His→His) and SNP19 (Ala→Ala); and 1 non-synonymous: SNP13 (Ala→Treo). No SNPs were found in exons 8 and 9, which codes for 3'UTR. Phylogenetic analysis demonstrated that exons from CAR gene are highly conserved between *Bos indicus* and *Bos taurus* and are closer to primates than to dog or chicken. The single SNP found upstream of CAR gene (SNP01 c.-81–176G>A) changed the transcription factor binding sites due the variation of G to A. Interestingly, this SNP was the only one associated with RIG ( $P = 0.0221$ ). These results lead us to consider that CAR expression might be associated with the complex physiology of feed efficiency in Nelore. Efforts to comprehend the role of CAR on molecular pathways related to feed efficiency and validate this marker for RIG in Nelore are under investigation by our group.

**Key Words:** Nelore, RFI, SNP

**W69 Assessment of 16 candidate genes for growth and maternal ability traits in Mexican Charolais cattle.** L. A. Meza-García, V. I. Pacheco-Contreras,\* G. M. Parra-Bracamonte, and A. M. Sifuentes-Rincón, Laboratorio de Biotecnología Animal, Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Reynosa, Tamaulipas, México.

Maternal ability, birth and weaning weights, are some of the most important traits considered as selection criterion in Mexican cattle. They are

complex and quantitative traits, and have shown significant association with some gene polymorphisms in different cattle breeds. Here, we evaluate the gene-trait association of a selected panel polymorphisms located at 16 candidate genes in a Mexican Charolais cattle herd to estimated their utility in gene assisted selection. Based on literature reports, we selected 24 polymorphisms located at POU1F1, FGF2, PRL, STAT5A, LEP, ABCG2, CCR2, BLG, CYP11B1, GHRL, GHR, GHRH, Prop1, NPY, UCP3, UCP2 genes, 24 RFLP-based procedures were designed to genotype 57 samples of Charolais cows with records for total maternal (TM), yield milk (YM), birth weight (BW) and weaning (WW) weight. Polymorphism was confirmed for 22 SNPs in the analyzed population. The effect of genotypes on the maternal ability (MA) (measured as TM and YM) and weight traits were analyzed using the least square analysis of the GLM procedure including genotype as fixed effect. No marker showed association with BW and 3 markers (GHR555, UCP2-380 and LEP3100) were significantly ( $P \leq 0.05$ ) associated with MA and WW traits and 5 markers shows trends for these traits. Regression analyses were achieved to determine the allele substitution effect of associated markers on each trait (Table 1). Before these makers can be used to assist the Charolais selection, more studies with larger data sets are necessary to confirm the found associations and observed trends.

**Table 1.** Estimated allele substitution effect  $\pm$  SE of 3 SNP on weaning weight (kg) and maternal ability traits (EBV kg)

SNP ID	Allele substitution	WW	YM	MT
GHR555	G>A	12.16 $\pm$ 5.53		
LEP3100	T>C		0.25 $\pm$ 0.50	0.23 $\pm$ 0.22
UCP2-380	G>C		-0.90 $\pm$ 0.48	

**Key Words:** Charolais cattle, polymorphisms, maternal ability

**W70 Distribution of molecular markers and determination of molecular breeding values associated with feed efficiency, beef tenderness, and marbling in Senepol cattle.** B. Velez,\* B. Diaz, and M. Pagan, *University of Puerto Rico, Mayaguez, Puerto Rico.*

Molecular breeding values (MBV) for feed efficiency (FE; net feed intake), marbling (MAR), and tenderness (TEN) were obtained using a commercially available 56 genetic marker panel to compare the genetics of a group of Senepol bulls ( $n = 153$ ) used as sires in Brazil, Colombia, Puerto Rico, St. Croix (US Virgin Islands) and in the continental US with the currently available MVP database for such economically relevant traits. In addition, the distribution of individual alleles associated with FE, TEN, and quality grade (QG) and its corresponding potential phenotypic differences (traditional GeneSTAR candidate gene panel; Pfizer Animal Genetics) were determined in another group of animals 75% to purebred Senepol ( $n = 47$ ). The multinational sire sample ( $n = 153$ ) showed MAR, FE, and TEN MBVs of -0.31, -0.45, and 0.44, respectively. The current Senepol breed reference MBV average for MAR, FE, and TEN are -0.02, -0.27, and -0.04, respectively, and the across breed average MBVs are 0.03 MAR, -0.15 FE, -0.02 TEN (for FE and TEN, lower MBVs are more desirable). For bulls ( $n = 47$ ) genotyped for individual alleles, from 8 favorable alleles/stars (2 corresponding to thyroglobulin 5, TG5) for QG, 5 alleles were present and arranged in 10 different combinations corresponding to a 4.75% probability of their carcasses been classified as USDA Choice or better. For FE (8 favorable alleles/stars), 13 different allele combinations were present, which indicated a predisposition within these animals to consumed 1.62 Kg (out a maximum of 1.8 Kg for animals with all desirable alleles) less dry matter per day and have a similar or better weight gain than an animal without any desirable allele. For TEN, in average, it would be expected

to have 0.53 kg less of Warner Bratzler shear force from these animals as compared with animals without a tender allele (6 favorable alleles/stars; test candidate genes = calpain-316, calpain-4751, and calpastatin). These results suggest that the Senepol breed have a genetic predisposition to gain weight efficiently and produce lean beef.

**Key Words:** Senepol, molecular breeding value, feed efficiency

**W71 Function analysis of liver X receptor  $\alpha$  regulating fatty acid synthesis in mammary epithelial cells of dairy goats.** W. Wang, J. Luo,\* Y. Zhong, X. Lin, and H. Shi, *Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

Liver X receptor  $\alpha$  (LXR $\alpha$ ) is a nuclear receptor of the transcription factor and known to play a crucial role in lipid metabolism such as bile acid and fatty acid synthesis in humans and rodents. However, very little information is available on the role of LXR $\alpha$  in the regulation of fatty acid synthesis in goat mammary glands. In this study, a novel cDNA was isolated from the mammary gland of a Xinong Saanen dairy goat and designated as goat LXR $\alpha$ . RT-PCR and RACE were used to obtain the full-length cDNA of LXR $\alpha$ , which is comprised 1654 bp with an ORF (open reading frame) of 1344 bp, the 5'- and 3'-UTR regions of 150 bp and 160 bp, respectively. The deduced amino acid sequence encodes 477 amino acids with a predicted MW of 50.4 kDa and a theoretical pI of 6.3. Additionally, homology search and sequence multi-alignment indicated that the putative goat LXR $\alpha$  amino acid sequence shares a high similarity with bovine, mouse, rat, pig and human counterparts. Bioinformatics predictions demonstrated that the LXR $\alpha$  protein, which is located in the nucleus containing characteristic signatures of nuclear receptor with DBD (DNA binding domain) and LBD (ligand-binding domain). Real-time quantitative PCR presented that LXR $\alpha$  was predominantly expressed in goat small intestine, liver, spleen and mammary gland. Treatment of goat mammary epithelial cells with different concentrations (0.01, 0.1, 1  $\mu$ M) of T0901317, a synthetic agonist of LXR $\alpha$ , resulted in elevating SREBP1 (sterol regulatory binding protein 1) and FASN (fatty acid synthase) mRNA levels in response to the LXR $\alpha$  activation. The association between the different T0901317 concentrations and fatty acid compositions in goat mammary epithelial cells also was examined using gas chromatograph. The results showed that activation of LXR $\alpha$  significantly increased goat mammary epithelial cells C18:1 and C18:2 content, but did not affect SFA (saturated fatty acids). These discoveries are consistent with the notion that LXR $\alpha$  plays a key role in controlling lipogenesis and regulating UFA (unsaturated fatty acids) synthesis of goat mammary glands, which may prove useful in regulating milk fatty acid composition in lactating dairy goats.

**Key Words:** dairy goats, LXR $\alpha$ , fatty acid synthesis

**W72 Structural and functional analysis of fatty acid synthase gene promoter of Xinong Saanen dairy goat.** J. Li, J. Luo,\* and Y. Sun, *Shaanxi Key Laboratory of Molecular Biology for Agriculture, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China.*

The objective of the study was to analyze the structure and function of fatty acid synthase (FASN) gene promoter in the mammary gland epithelial cells of Xinong Saanen dairy goat, and further explore the transcriptional regulatory mechanism of FASN gene. The biopsy mammary gland tissue samples were collected from 3 does during lactation for epithelial cell culture; all measurements on epithelial cells were replicated 3 time for statistical analysis by SPSS. The 5' flanking region

of goat FASN gene cloned from genomic DNA included a 591 bp region upstream of the transcription initiation site (+1), exon1, intron1 and partial exon2. TATA box and CAAT box, which are typical eukaryotic promoter elements, were located at -41 and -74 respectively. G and C accounted for 76% of the whole upstream region sequence, and 4 GC boxes (GGCGG and CCGCC) were found upstream of the TATA box. Bioinformatic analysis showed that there were potential transcription factor binding sites of LXR(-400), ER(-390), SREBP-1c(-150), NF-Y(-90) and E-box(-65) in goat FASN gene promoter. To define the core region of the FASN promoter, chimeric constructs containing serial 5' and 3' deletion sequences and the luciferase reporter gene were transfected into goat mammary epithelial cells. The luciferase activity was determined using dual-luciferase reporter assay system. The results indicated that the core region of FASN gene promoter was from -293 to -14 conferring basal transcriptional activity. Moreover, transcription factor binding sites of LXR, ER, SREBP-1c, NF-Y and E-box were mutated using overlap extension PCR. Site-directed mutagenesis analysis showed that binding sites of SREBP-1c, NF-Y and E-box could significantly downregulate the FASN gene promoter activity in goat mammary epithelial cells ( $P < 0.05$ ), while binding sites of LXR and ER had no effect on the gene promoter activity. It is concluded that transcription factors including SREBP-1c, NF-Y and E-box were involved in regulating FASN gene at the transcriptional level.

**Key Words:** dairy goat, FASN gene promoter, site-directed mutagenesis

**W73 Use of different statistical approaches to study genetic variability of OAR6 in sheep breeds farmed in Italy.** R. Steri<sup>1</sup>, A. Criscione<sup>2</sup>, E. Ciani<sup>3</sup>, B. Moiola<sup>4</sup>, P. Crepaldi<sup>5</sup>, L. Nicoloso<sup>5</sup>, D. Marletta<sup>2</sup>, E. L. Nicolazzi<sup>6</sup>, A. Passero<sup>3</sup>, G. Catillo<sup>4</sup>, F. Pilla<sup>7</sup>, and N. P. P. Macciotta\*<sup>1</sup>, <sup>1</sup>Università di Sassari, Sassari, Italy, <sup>2</sup>Università di Catania, Catania, Italy, <sup>3</sup>Università di Bari, Bari, Italia, <sup>4</sup>CRA, Rome, Italy, <sup>5</sup>Università di Milano, Milan, Italy, <sup>6</sup>Università Cattolica, Piacenza, Italy, <sup>7</sup>Università del Molise, Campobasso, Italy.

Dense marker maps allow for the investigation of genomic regions that differentiate between breeds. In this work, 496 sheep belonging to 20 Italian sheep breeds were genotyped with the Illumina OvineSNP50 BeadChip. After data editing, 2,180 SNP located on chromosome 6 were analyzed with 4 different approaches. I) Fst Outlier Detection (FOD), implemented in the LOSITAN software, based on the comparison between Fst calculated on actual data and expected heterozygosity (He) and Fst under an island model. II) Composite Log-likelihood (CLL), based on calculation of CLL of the observed allelic frequencies across overlapping windows of 9 markers. III) Correspondence analysis (CA). VI) Canonical Discriminant Analysis (CDA). The different approaches were able to identify regions at OAR6 that expressed variation between breeds. Highest values for all statistics were found for a region spanning between 35 and 41 Mb known to harbour BMPR1b and ABCG2 loci. SNPs with a relevant discriminating power between breeds were also found at 76, 96 and 107 Mb, near to KIT, IL8 and SCD5 genes respectively. FOD detected 227 not neutral markers (17 under positive and 210 under balanced selection) using a confidence interval of 0.95. A total of 62 windows out of 242 were significant for CLL ( $P < 0.01$ ). Several 85 and 135 SNPs exceeded empirical threshold for CA and CDA, respectively. The discriminating power was high for all methods and in general, they revealed a geographical pattern of variation between breeds. Moreover, each method provided specific information. FOD supplied a relatively low number of markers in divergent selection but it was able to identify loci under balanced selection. CA and CDA allowed a decomposition of total variability in different and uncorrelated variables that could be useful for the identification of genes influencing

complex traits. The use of different statistical methods to study genetic variability between ethnic groups could provide indications about the adaptation to local conditions as well as the effect of selection.

**Key Words:** sheep breeds, SNP, statistical approaches

**W74 Genotyping of five Chinese local pig breeds focused on meat quality by using PCR-RFLP based on halothane and Mx1.** Z. M. Feng, G. G. Lian, X. F. Kong, X. Zhou, and Y. L. Yin,\* *Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China.*

In recent years, due to the increasing demand for pork with high quality, a more convenient and fast way to test pigs is required for breeding, which enhances investigations on genetic variations associated with meat quality. So far, many genetic variations associated with meat quality have been investigated, such as myxovirus resistance protein 1 (Mx1), which was first found to be related to meat quality using samples from Chinese local pig breeds, and Halothane (Hal). Effects of Mx1 on meat quality of Chinese local pig breeds were demonstrated by Li in her Master's thesis in 2005 (Huazhong Agric. Univ. Wuhan, China), which was published in Chinese. The current study focuses on the use of RCR-RFLP of Hal and Mx1 for evaluating meat quality traits of several Chinese local pig breeds (Bama mini-pig, Huanjiang mini-pig, Lantang pig, Ningxiang pig and Xiangxi black pig including Daheping black pig, Pushi black pig and Taoyuan black pig) and Landrace pig (a foreign pig breed). The results indicated that the genotypes of Chinese local pig breeds were mainly shown as HalNN, while rarely in HalNn, rather than Halnn. The recessive allele (n) frequency in Chinese local pig breeds was significantly lower than that in Landrace pig. The recessive allele (B) frequency in the sixth intron of Mx1 in Chinese local pig breeds was generally higher than that in Landrace pig. The B frequency in the ninth intron of Mx1 was commonly higher in both Chinese local pig breeds and Landrace pig. In general, these findings have manifested that the Chinese local pig breeds, particularly Xiangxi black pig and Ningxiang pig, have a better meat quality.

**Key Words:** pig, nutrition, digestion

**W75 Which housekeeping gene can be used in gene expression analysis in Chinese local pig breeds?** Z. M. Feng, J. P. Guo, X. F. Kong, and Y. L. Yin,\* *Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, Hunan, China.*

Relative quantification real-time PCR (RT-PCR) is widely used for the quantification of mRNA expression level. The expression level should be normalized by using one or more reference genes. In this study, 3 frequently-used endogenous reference genes, including glyceraldehyde-3-phosphate dehydrogenase (GAPDH),  $\beta$ -actin (ACTNB), and 18S rRNA (18S), were evaluated by comparing their expression levels in 864 samples from 5 Chinese local pig breeds (Huangjiang mini-pig, Bama mini-pig, Taoyuan black pig, Lantang pig, and Ningxiang pig) and Landrace pigs (a foreign pig breed). The results, which were analyzed by Fisher's Least Significant Difference test after ANOVA using SPASS, showed that ACTNB and GAPDH had no significant different expression in overwhelming majority of the tissues investigated in the present study. We conclude that ACTNB and GAPDH had higher stable expression levels in various tissues, which indicated that both of them could be used as reference genes for the quantification of mRNA expression in some Chinese local pig breeds. For Chinese local pig breeds, ACTNB might be more suitable as a reference gene for target genes with high expression levels, while GAPDH might be better for

target genes with low expression levels. Our findings also suggested that evaluation of reference genes is necessary for research among different local pig breeds. Otherwise, absolute quantification RT-PCR should be recommended.

**Key Words:** local pig breeds, mRNA expression, nutrition

**W76 Genotype imputation accuracy in an F<sub>2</sub> pig cross using high-density and low-density SNP panels.** J. L. Gualdrón Duarte\*<sup>1,3</sup>, R. O. Bates<sup>1</sup>, C. W. Ernst<sup>1</sup>, N. E. Raney<sup>1</sup>, R. J. C. Cantet<sup>3</sup>, and J. P. Steibel<sup>1,2</sup>, <sup>1</sup>*Department of Animal Science, Michigan State University, East Lansing,* <sup>2</sup>*Department of Fisheries and Wildlife, Michigan State University, East Lansing,* <sup>3</sup>*Departamento de Producción Animal, Facultad de Agronomía, UBA - CONICET, Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.*

High resolution SNP genotyping can be used for QTL mapping to facilitate meta-analysis across multiple populations. Typical swine F<sub>2</sub> populations have only a few founders (F<sub>0</sub>), and a relatively small number of recombinations are expected in each generation. For that reason, genotyping the F<sub>0</sub> and F<sub>1</sub> generations with a high-density (HD) panel and typing the F<sub>2</sub> with a reduced number of tagSNP would enable high imputation accuracy of HD genotypes in the F<sub>2</sub> generation at a fraction of the cost. The goal of this study was to estimate imputation accuracy of F<sub>2</sub> genotypes when HD (44,752 SNP after data cleaning) genotypes are available for F<sub>0</sub> and F<sub>1</sub> generations. Starting with genotypes for F<sub>0</sub> and F<sub>1</sub> animals from the MSU Duroc × Pietrain resource population obtained using the Illumina Porcine SNP60 BeadChip, we simulated F<sub>2</sub> genotypes (n = 932) conditional on the real pedigree using a gene-dropping model. Subsequently, we applied 2 different methods to select tagSNP for F<sub>2</sub> animals: 1) statistical criteria based on pair-wise linkage disequilibrium on F<sub>1</sub> haplotypes, and 2) evenly-spaced markers. Imputation was performed assuming that F<sub>0</sub> and F<sub>1</sub> were typed at HD. Average imputation accuracy was calculated as the mean difference between observed and expected allelic dosage. We found that using panels of similar size, from evenly spaced selection or statistical selection resulted in similar imputation accuracy. When both F<sub>0</sub> and F<sub>1</sub> were typed at HD, imputation accuracy was approximately 0.97 if 2% of the HD markers, approximately evenly spaced every 2.1 Mb were used as tagSNP. Based on these results, we recommend using HD SNP genotyping in F<sub>0</sub> and F<sub>1</sub> and evenly spaced tagSNP panels consisting of at least 1200 tagSNP to obtain average inter marker distances of 2.1 Mb and to guarantee imputation accuracy above 0.97 in the F<sub>2</sub>. Furthermore, forward imputation of HD-SNP in

F<sub>2</sub> is appealing, since the resulting association tests could be combined across multiple populations using meta-analysis.

**Key Words:** imputation accuracy, F<sub>2</sub> cross, swine

**W77 The proteome and mRNA expression of vimentin in the adipose tissue of broiler chickens.** G. Kelley,\* A. Stewart-Bohanon, F. Chen, X. Wang, and S. Nahashon, *Tennessee State University, Nashville.*

Increased fat deposition in broiler chickens is a major concern to poultry producers and consumers alike. Genomic approaches have been employed to discern the mechanisms of adiposity in food animals. These approaches have provided key transcriptional information depicting the possible role of gene sequences and their expression on fat deposition. However, they do not provide insight on the translational events that may contribute to adiposity although it is understood that proteins are the executants of most of most biological functions. We hypothesize 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 and to evaluate the mRNA expression of genes that encode these proteins. One hundred twenty 1-d-old broiler chickens were randomly assigned to floor pens and fed standard broiler diet until 8 weeks of age (WOA). At 8 WOA, experimental birds were sacrificed and adipose tissue from the abdominal and visceral areas was collected, weighed and frozen in liquid nitrogen before storage at -80 oC until used. Adipose proteome from 16 birds with the highest and lowest abdominal fat percentage 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 fat and lean birds ( $P < 0.05$ ). Among them was vimentin, which was highly expressed in obese birds and was confirmed by Western blot. The mRNA abundance of vimentin was assayed at 8 WOA using Real Time-Polymerase Chain Reaction. A student *t*-test revealed no significant differences ( $P > 0.05$ ) in the threshold cycles (Ct values) of vimentin between the lean and obese birds. The Ct values were 14.67 and 14.34 for the lean and obese birds, respectively. These results indicate that there are factors beyond the genome that influence vimentin expression in broiler chickens.

**Key Words:** adipose tissue proteome, broiler chickens, vimentin