BREEDING AND GENETICS: APPLICATIONS AND METHODS IN ANIMAL BREEDING—LIVESTOCK I

0957 (W061) Whole genome association analysis for detecting QTLs related to fat and protein production in buffaloes. H. Tonhati*[1], D. F. Cardoso[1], R. R. Aspilcueta Borquis[1], N. A. Hurtado Lugo[2], G. M. de Camargo[1], L. G. Albuquerque[1], D. J. A. Santos[3], D. C. Scalez[1], and M. C. Nakagawa[4], *[1]State University of São Paulo, Faculty of Agriculture and Veterinary Sciences, Jaboticabal, Brazil, [2]Universidade Estadual Paulista "Júlio de Mesquista Filho", Jaboticabal, Brazil, [3]Universidade Estadual Paulista, Jaboticabal, Brazil, [4]State University of São Paulo, Faculty of Agriculture and Veterinary Sciences, Jaboticabal, Brazil.*

Whole genome association studies are important for the livestock industry because they allow incorporation of the QTL detected in genetic evaluations, thus enabling greater selection accuracy and faster genetic progress. Therefore, this study aims at identifying loci associated with fat and protein production in river buffaloes. A total of 452 animals (57 males and 395 females) were genotyped using the 90K panel Axiom Buffalo Genotyping (Affymetrix). For sample quality control, we established the threshold values for: call rate 0.95 and heterozygosity ± 3 standard deviations. For the marker, we adopted call rate > 0.98, MAF > 0.05, HWE up to 10^{-6}, correlation between markers up to 0.998, plus the elimination of coinciding SNPs and with possible errors of physical positioning in relation to the reference map. The number of SNPs left after quality control was 56,716. Statistical analyses were performed using R scripts and the GenABEL software (Aulchenko et al., 2007). The information used in this studied were the de-regressed breeding values to traits production of fat (FY) and protein (PY), according to Garrick et al. (2009). These data were corrected for population substructure using the principal components obtained by multidimensional scaling of genomic similarity matrix, with residuals weighted by $c+(1-r^2)/r^2$. The significance level of 0.05 was corrected for Bonferroni. The five SNPs with the highest P-value (candidate SNPs) were selected for each trait, and through their genomic coordinates (BTAU_4.0 assembly), the annotation of the closest genes was taken out using the NCBI database (http://www.ncbi.nlm.nih.gov). After population structure corrections, the inflation factors (lambda) were estimated as 1.0014 and 1.0078 for FY and protein PY; within the acceptable range. At the significance level corrected by Bonferroni, only two SNPs were deemed significant for FY and PY. The significant SNPs for both traits were present on chromosome 10. Deiodinase type 2 (DIO2) was the closest gene (~150 Kb). This is the main enzyme that converts Thyroxine (T4) to the active Triiodothyronine (T3) (active form). From the prior knowledge that thyroid hormones directly influence lactation, this gene may explain the greater buffalo hardiness during this phase, adapted to feeding conditions poor in protein.

Key Words: milk quality, buffalo, markers

0958 (W062) Evaluation of single nucleotide polymorphism markers on four pig chromosomes for potential associations with halothane sensitivity phenotypes in a population of Yorkshire-Landrace pigs. K. R. Perry*, C. W. Ernst, J. P. Steibel, and R. O. Bates*, Michigan State University, East Lansing.*

We have previously reported that a proportion of pigs homozygous normal for the RYR1 g. 1843 C > T polymorphism were halothane sensitive and had lower post-mortem *Longissimus dorsi* pH. Pigs from this project (n = 363), which were progeny of Landrace sires and Yorkshire-Landrace F1 dams, were subsequently genotyped for 67 SNPs across four chromosomes (SSC6, SSC10, SSC12, and SSC14). These SNPs were located in or near genes responsible for malignant hyperthermia or myopathies in humans (CACNA1S, CPT2, SCN4 and RYR2) that may influence the stress response in pigs, including multiple SNPs within RYR1. The objective of this study was to determine the association of these SNPs with the halothane sensitivity phenotypes recorded in this population. Pigs were evaluated for limb rigidity (RIGID), limb tremors (TREM), and abdominal discoloration (AD) observed after halothane challenge. Halothane sensitivity was assessed after 60 sec exposure to 5% halothane gas in a closed system delivered at 2 L/min. Pigs were considered to be either sensitive (1) to halothane or not sensitive (0) for each trait. Assays were multiplexed and SNP genotypes collected using Sequenom MassArray. Twelve SNPs were discarded from analysis due to low genotyping call rate (< 90%). Twenty-nine of the remaining 55 SNPs were not in Hardy-Weinberg equilibrium ($P < 0.05$). The three halothane response variables were fit to a generalized linear model that included the fixed effects of sex and SNP, and the random effects of replication and litter. False discovery rate (FDR) was used to determine significance. For the 55 SNPs tested for each halothane sensitivity variable, generally there were few SNPs with P-values less than 0.05. Three SNPs for TREMOR, 1 for RIGID and 1 for AD had P-values less than 0.05. The FDR for all SNPs was greater than 0.25 and therefore it was determined that no SNP significantly associated with any of the three halothane response variables. These results indicate that halothane sensitivity in this population is not controlled by variation in these regions of the swine genome, and other genomic

regions should be investigated to determine the genetic control of halothane sensitivity.

Key Words: pig, halothane sensitivity, SNP

0959 (W063) Growth rate of purebred Berkshire pigs housed in hoop buildings in North Carolina.

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This study was designed to estimate growth curves of antibiotic free Berkshire purebred breeding stock reared in hoop buildings at the North Carolina Agricultural and Technical State University Farm. The location features a humid subtropical climate with subtropical summer temperatures and mild winters, and an average annual precipitation of approximately 110 cm. Litters were weaned at 4 wk old, and reared within deep-bedded outdoor hoop houses. Six boars and 21 sows were included in the population. Body weights of a total 124 pigs were collected every 4 wk from birth to 20 wk of age, resulting in 1206 total records. Gompertz growth curves were used to estimate parameters, resulting in 3.681 ± 0.369 as W_0, 0.029 ± 0.002 as m, and 0.006 ± 0.001 as D. Overall average daily gain at 20 wk of age was 0.39 ± 0.11 kg and ranged from 0.16 to 0.64 kg. Average daily gains were 0.38 ± 0.11 kg in boars and 0.40 ± 0.12 kg in gilts. These results were lower than the results reported by others, which may be due to different climates in the test populations and/or due to the closed population used in the present study.

Key Words: Berkshire, growth rate, hoop, outdoor

0960 (W064) Use of the canonical discriminant analysis for selecting a panel of informative markers in 21 Italian sheep breeds.

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SNP markers can be useful to assign individuals to their breed. In the present research, a high density SNP chip was exploited to select a panel of markers useful to verify the origin of 462 individuals belonging to 21 Italian sheep breeds. Animals were genotyped using the Illumina's OvineSNP50 BeadChip and were divided into two groups: the training population (TP) of 420 sheep, 20 for each breed, and the validation population (VP) of 42 animals, two for each breed. SNPs with MAF < 0.20 were discarded and, after data editing, 40,856 markers were used, including 4937 monomorphic SNPs for at least one breed. The canonical discriminant analysis (CDA) was exploited to discriminate among breeds. CDA was first applied at chromosome level and markers with canonical coefficients higher than a fixed threshold were retained. The threshold was obtained through a recursive procedure: the CDA was repeatedly applied by increasing, at each run, the value at which a canonical coefficient is discarded. The procedure stopped when the remaining markers were a pool of linearly independent variables. A genome-wide CDA was then developed with only the selected SNPs, and the effective distance among groups was tested by using the Mahalanobis' distance and the corresponding Hotelling's T-square test. The discriminant analysis (DA) technique was then used to assign the VP to the breed of origin. Finally, the minimum number of significantly discriminant markers was obtained. With a canonical coefficient threshold value of 0.31, 155 linearly independent highly discriminant SNPs were retained. These selected markers provided at Hotelling's T-square test significant separation among all breeds ($P < 0.0000$) and the DA correctly assigned 40 out of 42 VP animals. Among the 155 markers, 46 were monomorphic for at least one breed. The selected markers could be used to develop an assay to routinely track monobreed products. Finally the minimum number of markers able to significantly discriminate all breeds was 48. However, by using this small panel of SNPs, the DA was able to correctly assign only 30 out of 42 VP animals.

Key Words: assignment test, SNP selection

0961 (W065) Genomic differences between Rambouillet sheep selected for high and low reproductive rate.

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The Rambouillet sheep selection program at Montana State University began in 1968 with the establishment of the high (HL) and low (LL) reproductive rate lines. Sheep within these lines were selected based on the index of "I" = total number of lambs born in a lifetime/(age of ewe– 1). The lines have significantly differentiated phenotypically for, number of lambs born, number of lambs born per ewe exposed and per ewe lambing, and total kg of lamb weaned ($P < 0.01$) Furthermore, systemic progesterone concentrations during the luteal phase of the estrous cycle differ for HL and LL ewes ($P < 0.05$). Previous research in these flocks has shown differences between the lines for lambing rate, litter size and ovulation rate. Objectives of the present study were to: 1) evaluate if there are genomic differences between lines, and, 2) identify quantitative trait loci associated with each line and candidate genes within these loci. A sample set of 50 and 46 HL and LL sheep, respectively, were genotyped using the Ovine 60K SNP chip. The data for the genotypes were analyzed using the Golden Helix commercial software package. Principal component analysis indicated distinct clusters for the two lines of sheep when the first two eigenvectors were plotted, demonstrating that these lines

are, in fact, genetically different. Using an additive correlation association model and a Bonferroni correction, there were 14 markers that differed ($P < 0.01$). These markers are on chromosomes 1, 3, 9, and 24. The candidate genes that appear to differ include CHP2, ACOT11, NOS1AP, and EGFR. Further analyses and additional samples from each line are needed to better map the differences between the lines. In conclusion it appears that long-term selection for reproductive, a trait known to have low heritability, can be successful in generating animals, at least in sheep that are phenotypically and genetically different.

Key Words: genetic selection, genomics, physiology, reproduction