BREEDING AND GENETICS: APPLICATIONS AND METHODS IN ANIMAL BREEDING—DAIRY II

0943 (T041) Genome-wide association study on dairy cow mortality in three U.S. regions. S. Tsuruta[*1], I. Misztal[1], and T. J. Lawlor[2], *[1]University of Georgia, Athens, [2]Holstein Association USA Inc., Brattleboro, VT.*

Cow mortality, from DHI reports, is farmer provided information. The termination code = "dead" is the primary reason given for a cow leaving a herd, particularly late in lactation. This trait can be difficult to interpret because its definition and recording method may differ across farms. Our objective was to do a genome-wide association study (GWAS) on cow mortality and 305-d milk yield for three lactations and determine if there were differences in the genetic architecture associated with these traits in three different regions of the United States. Genomic EBV of cow mortality and 305-d milk yield were estimated with a single-step GBLUP using a threshold-linear model. The SNP file contained 42,503 usable SNP markers for 34,506 bulls obtained from USDA-AIPL. Data consisted of the entire U.S. DHI data for three lactations (10,748,430 animals; 6233,306 records) for cows calving from 1999 to 2008. Three U.S. regions— SE: Southeast (648,991 animals; 293,494 records), SW: Southwest (541,777 animals; 272,934 records), and NE: Northeast (1690,481 animals; 883,887 records)— were selected for regional comparison. Heritability estimate for 305-d milk first lactation yield was 29%. Heritability for mortality within the first lactation was 0.04, 0.06, 0.06, and 0.04 for SE, SW, NE, and U.S., respectively. Genetic correlations between first lactation mortality and 305-d milk yield were 0.14, -0.01, 0.02, and 0.25 for SE, SW, NE, and U.S., respectively. The genome was divided into equal segments of 20 sequential SNPs. As expected, a segment on chromosome 14 was significantly associated with milk production in all regions. The proportion of the total genetic variance for 305-d milk yield, explained by this segment, was 1%, 1%, 3% and 4% for SE, SW, NE region, and total U.S., respectively. Chromosome 14 showed a strong association with first parity mortality for the entire U.S., with the NE showing a strong association for all three parities. Milk components (higher or lower %fat) could be a possible explanation. Within the SE and SW regions, chromosome 14 did not show a significant association for any of the three parities. These results suggest that this farmer-recorded trait on mortality is being interpreted differently and/or there are different traits (genomic segments) responsible for cow mortality in different regions of the country.

Key Words: GWAS, cow mortality, region

0944 (T042) Multiple-breed genomic evaluations by using a reduced pool of SNP-markers. M. Cellesi[*1], N. P. P. Macciotta[1], P. Ajmone-Marsan[2], A. Rossoni[3], G. Marras[1], G. Gaspa[1], and C. Dimauro[1], *[1]Università di Sassari, Italy, [2]Università Cattolica del Sacro Cuore, Piacenza, Italy, [3]Associazione Nazionale Allevatori Razza Bruna, Bussolengo, Italy.*

Large reference populations (RP) of genotyped and phenotyped individuals are required to obtain reliable predictions in genomic selection programs. For small breeds, however, assembling such RPs could result particularly challenging. In this study, a multibreed approach was used to enhance the size of the RP. Data were genotypes of 2054 Italian Holstein and 634 Brown Swiss bulls, respectively, genotyped with the Illumina's 50K BeadChip. Phenotypes were deregressed proofs (DRP) for milk, fat and protein yield. An empirical technique, named Maximum Difference Analysis (MDA), was used to select a restricted pool of SNP-markers significantly associated with the considered trait (T). In each breed, animals were ranked according to a T. The best (B) 10% and the worst (W) 10% individuals were selected and the genotypic frequencies were evaluated. For each SNP, the maximum genotypic frequency in B and, in correspondence, the frequency for the same genotype in W were recorded and the difference between the two frequencies was calculated. A bootstrap procedure was then implemented to derive a posterior probability distribution that was used to declare a SNP positively associated with T. Markers negatively associated with T were detected through the same procedure with the only difference that, for each SNP, the maximum genotypic frequency was recorded in W. A BLUP model was used to estimate marker effects that were then used to calculate genomic breeding values of Brown Swiss younger bulls (50animals). Three datasets were used: all original SNPs, only the MDA selected markers (MDA_SNP) and the MDA_SNP for Brown Swiss plus MDA_SNP obtained for Holstein. MDA selected, for both breeds, around 1500 markers for each trait. Accuracies of genomic predictions (Table 0944) evaluated by using MDA_SNPs in the multi-breed scenario were greater than values obtained with all markers and in the single-breed scenario. Results suggested that the MDA applied to a small genotyped bovine population increases accuracies of genomic predictions of about 10%. A further improvement can be obtained in a multibreed scenario.

Key Words: SNP reduction, multiple breeds, genomic selection

Table 0944. Accuracies of genomic predictions for Brown Swiss

Scenarios	Milk	Fat	Protein
Brown Swiss all SNP	0.21	0.35	0.21
Brown Swiss MDA_SNP	0.31	0.43	0.30
Holstein + Brown Swiss MDA_SNP	0.36	0.43	0.34

0945 (T043) Determination of single nucleotide polymorphisms associated with subclinical ketosis in Jersey cattle. R. T. Fugate*[1], L. H. Dauten[2], G. R. Wiggans[3], and H. M. White[4], *[1]University of WI, Madison, [2]University of Connecticut, Storrs, [3]Animal Improvement Programs Laboratory, Agricultural Research Service, USDA, Beltsville, MD, [4]Dep. of Dairy Science University of Wisconsin, Madison.*

Subclinical ketosis is a fresh cow disorder that is costly in terms of lost milk production and treatment cost. Although treatment and prevention strategies are available, prevention requires targeting animals that are likely to develop the disease. Whole-herd genotyping is becoming more common with commercial dairies, and identification of markers for ketosis predisposition would provide a valuable tool to producers. The objective of this study was to identify single nucleotide polymorphisms (SNP) that are associated with subclinical ketosis in Jersey cattle. Ketotic cows were identified by cowside test using the Precision Xtra meter. Blood and hair samples were collected from 54 Jerseys (ketotic and healthy herdmates on the same day) with < 30 d in milk on New England dairy farms. Mean parity of cows was 2.8, with no difference ($P >$ 0.05) between healthy and ketotic cows; no difference ($P >$ 0.05) also was found for milk yield, 305-d mature-equivalent milk yield (ME_{305}), or ME_{305} from the previous parity. Blood serum was analyzed for concentration of nonesterified fatty acid (NEFA) and β-hydroxybutyrate (BHBA). Hair samples were submitted to the American Jersey Cattle Association for genotyping with the BovineSNP50 BeadChip. Concentrations of NEFA and BHBA were analyzed using the SAS 9.2 PROC MIXED; differences in SNP frequency by ketosis status (healthy or ketotic) was analyzed using the $\chi 2$ test from the SAS 9.2 FREQ procedure. As expected, BHBA concentrations were greater ($P \leq 0.05$) for ketotic cows compared with healthy herdmates (1.63 vs. 0.91 ± 0.17 mmol/L). For NEFA, concentrations tended to be greater ($P \leq 0.01$) in ketotic cows compared with healthy cows (0.45 vs. 0.33 ± 0.05 mmol/L). Of the 54,609 SNP analyzed for each genotype, 1685 were different ($P \leq 0.05$) and 1862 tended to differ ($0.05 < P \leq 0.1$) between ketotic and healthy cows. These data suggest that genotypes from the BovineSNP50 BeadChip could be useful in predicting predisposition for ketosis in Jerseys, but examination of a larger data set is necessary to validate the predictive ability of the identified SNP.

Key Words: ketosis, Jersey, SNP

0946 (T044) Multi-trait, multi-breed conception rate evaluations. P. M. VanRaden[1], J. R. Wright*[1], C. Sun[2], J. L. Hutchison[1], and M. E. Tooker[1], *[1]Animal Improvement Programs Laboratory, USDA-ARS, Beltsville, MD, [2]National Association of Animal Breeders, Columbia, MO.*

Heifer and cow conception rates (HCR and CCR) were evaluated with multi-trait, multi-breed models including crossbred cows instead of the previous single-trait, single-breed models. Fertility traits benefit from multi-trait processing because of high genetic correlations and many missing observations, with 4 million HCR and 14 million CCR lactation records stored since 2003 vs. 66 million daughter pregnancy rate (DPR) records since 1960. Conception rates were previously modeled using multiple binary success records per parity (such as no, no, yes) that are now pre-adjusted for environmental effects and combined into lactation records for simpler multi-trait analysis with the continuous trait DPR. Genetic correlation estimates were 0.45 for HCR with CCR, 0.86 for CCR with DPR, and 0.36 for HCR with DPR. Inbreeding depression per 1% inbreeding was -0.21 for HCR, -0.10 for CCR, and -0.13 for DPR. Heterosis was 1.3 for HCR, 3.2 for CCR, and 1.4 for DPR. Crossbred cows get the combined effects of heterosis and no inbreeding compared to purebreds that may average 6%. Genetic differences among breeds were fairly consistent with phenotypic differences. Holsteins had the highest phenotypic and genetic averages for HCR, while Jerseys were highest for CCR. Evaluations from the new and previous models were correlated by > 0.95 for both HCR and CCR for recent Holstein bulls with > 50% reliability, but were less correlated in other breeds because of additional crossbred daughters and contemporaries. For Holstein sires with > 90% reliability, correlations between single-breed and multi-breed evaluations were 0.986 for HCR and 0.992 for CCR, indicating little change in rank when adding the other breeds. Genetic trend for CCR was more negative with multi-trait processing because of the correlation with DPR. Genetic trends were validated using Interbull tests 1 and 3. The genetic correlations with other countries estimated by Interbull changed little for Holsteins, averaging 0.02 higher for HCR and 0.02 lower for CCR, but were more variable for other breeds. The new model implemented in December 2013 combines data from all breeds and uses DPR as a correlated trait to improve HCR and CCR evaluations.

Key Words: conception, evaluation, multi-trait

0947 (T045) Genome-wide genotyping-by-sequencing (GBS) and association analysis of saturated and monounsaturated fatty acids in bovine milk identifies novel markers in Canadian Holstein cows. E. M. Ibeagha-Awemu[*1], S. O. Peters[2], I. G. Imumorin[3], and X. Zhao[4], *[1]Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada, [2]Berry College, Mount Berry, GA, [3]Cornell University, Ithaca, NY, [4]McGill University, Ste. Ann De Bell, PQ, Canada.*

The effect of bovine milk fatty acids (FA) on human development and heath has fueled concerted efforts towards exploitation of existing heritable variation for genetic improvement. Consequently, genomic regions as well as single markers associated with milk fat traits have been identified in many cattle breeds around the world. Since information on population specific markers of milk FA traits in Canadian Holstein cows is limited, we used the high throughput genotyping-by-sequencing (GBS) SNP mining method and association analysis to determine population specific markers associated with milk saturated FAs (SFAs) and monounsaturated FAs (MUFAs). Fatty acid profiles of 1200 cows from seven herds in Quebec were determined by gas chromatography and SNPs were genotyped by GBS method. Genome wide association analysis (GWAS) with 99,814 SNPs (> 70% call rates, accuracy of imputation score > 50% and MAF > 0.01) out of 515,820 SNPS was accomplished with the single-locus mixed linear model procedure (EMMA) implemented in Golden Helix SNP and Variation Suite v8.0 software (www.goldenhelix.com). Genome wide significance (BH *P*-value < 0.05, range 3.86E-05 to 0.049) was detected between 7 SFAs (C4:0, C6:0, C8:0, C13:0, C14:0, C23:0, and C24:0) and several SNPs located in intergenic, coding, splicing and 3' untranslated (UTR) regions. In particular, 15 significant associations for C13:0 are coding variants located in 15 genes, and 10 for C24:0 are also coding variants with many non-synonymous SNPs. One 3'UTR variant within CHFR (E3 ubiquitin-protein ligase) gene significantly associated with C6:0 and C8:0. Furthermore, genome wide significant (BH *P*-value < 0.05) associations were recorded between several SNPs and two individual MUFAs (oleic acid [C18:1n9c] and trans vaccenic acid [C18:1n11t]), total MUFAs and total SFAs. In particular, a synonymous variant (S25_37873086) within ACHE (acetylcholinesterase) gene significantly influenced both total MUFAs and total SFAs. Minor allele frequencies for all reported significant associations are $\geq$ 0.02. Since most of these associations are being reported for FAs for the first time with only six of the genes known to play a role in lipogenesis, our study has uncovered potential novel SNPs and genes that can be used in improvement of milk SFA and MUFA contents through breeding to ensure quality products for human consumption. Moreover, our results also further confirm the use of the GBS technique for identification of population-based unique SNPs for GWAS and for improvement breeding in dairy cattle.

Key Words: genome wide genotyping-by-sequencing, genome wide association study, saturated and monounsaturated fatty acids

0948 (T046) Peroxisome proliferator-activated receptor γ isoforms alter lipogenic gene networks in goat mammary epithelial cells. H. Shi[1], J. Luo[*2], D. Yao[1], and J. Zhu[1], *[1]Northwest A&F University, Yangling, China, [2]Northwest A & F University, Yangling, China.*

Lactation is a highly demanding lipid synthesis and transport process that is crucial for the development of newborn mammals. Peroxisome proliferator-activated receptor-γ (*P*PARG) was reported to promote adipogenesis and lipogenesis in adipose tissue, its role in the lactating mammary gland is less clear. PPARG is present in two isoforms generated by alternative splicing, PPARG1 and PPARG2. Their roles in ruminant lactation mammary gland have been poorly distinguished. To determine which of these isoforms is more closely associated with the regulation of the lipogenic pathways, their distributions were analyzed and key genes in the mammary lipid network were detected by quantitative PCR (qPCR) after overexpression of the two isoforms in goat mammary epithelial cells (GMEC). Various tissues of goats were collected to assay mRNA expression of PPARG isoforms. The adenovirus pAd-PPARG1 and pAd-PPARG2 were generated. The adenovirus (Ad-GFP) was used as a positive control. GMEC at about 80% conñuence were transfected with adenovirus at the same MOI and cultured in the DMEM/F-12, at 37°C in 5% CO_2. Transfected GMEC were cultured with ROSI or DMSO at 50 μM after 24 h of the initial culture and then harvested at 48 h (24 h later) for RNA extraction. Distribution analysis indicated that expression of PPARG2 was markedly greater in adipose than mammary gland and PPARG1 is the mainly isoform in goat mammary tissue. Both PPARG isoforms could significantly upregulate the mRNA expression of NR1H3, INSIG1, PLIN2, CD36, SCD, AGPAT6, DGAT1 under the treatment with rosiglitazone (ROSI). They had no significant effect on SCAP, PNPLA2 and PLIN3 in absence of ROSI. PPARG1 increased the SREBF1, FASN and ACACA while PPARG2 downregulated these genes expression. In conclusion, the data suggest that both PPARG1 and PPARG2 could largely affect fatty acid metabolism when stimulated. However, de novo lipogenesis in mammary cells appear more closely related to PPARG1 activation, with this nuclear receptor acting through its control of SREBF1 and to some extent NR1H3.

Key Words: PPARG, lipogenesis, mammary gland epithelial cells, goat

0949 (T047) Association between polymorphisms in the IGF-I, GHR and STAT5A genes and the interval from calving to conception and milk production in Holstein cows. L. Hax*, A. Schneider, C. Bespalhok Jacometo, P. Mattei, T. da Silva, G. Farina, and M. Nunes Corrêa, *Federal University of Pelotas, Pelotas, Brazil.*

The aim of this study was to investigate the association of polymorphisms in the insulin-like growth factor 1 (IGF-I), growth hormone receptor (GHR) and signal transducer and activator of transcription 5A (STAT5A) genes with the calving to conception interval (CCI) and milk production of Holstein cows. In this study 308 Holstein cows from a commercial herd in southern Brazil were used. The study evaluated cows between the first and sixth lactation with a CCI no longer than 250 d. The animals were reared in a semi-extensive system being milked twice a day. The CCI and milk production data were obtained from the farm management software. Blood samples were collected for DNA extraction. Genotypes were verified by polymerase chain reaction (*P*CR) using the following primers: IGF-I (TTAAATAATTGGGTTGGAAGACTGC and ACCTTACCCGTATGAAAGGAATATACGT); GHR (TGCGTGCACAGCAGCTCAACC and AGCAACCCCACTGCTGGGCAT); STAT5A (GAGAAGTTGGCGGAGATTATC and CCGTGTGTCCTCATCACCTG). The amplified fragments of IGF-I, GHR and STAT5A were digested with 3U of *Sna*BI, *Alu*I and *Bst*EII at 37°C for 3 h, respectively. The resulting fragments were subjected to agarose gel electrophoresis for subsequent UV visualization. Statistical analyzes were performed using the GLM procedure. A value of $P < 0.05$ was considered significant. The average milk production adjusted to 305 d of lactation and CCI were 5652 ± 1170L and 117 ± 57.8 d, respectively. There was no association between milk production and CCI with the genotypes identified for each gene. The presence of none, one or two alleles had no linear or quadratic association with the evaluated traits for IGF-I, GHR and STAT5A. There was no interaction between the genotypes of each studied gene for milk production and CCI. The low level of milk production suggests that these animals were not subjected to a major metabolic challenge. The somatotropic axis acts regulating metabolism and the reproductive system, and its balance is a result of the metabolic condition of the animal. Thus, it is likely that the function of the studied genes was not impaired in this low challenge condition, therefore without any major changes in the GH/IGF-I axis or folliculogenesis. Under such conditions, the effect of the polymorphisms studied were not observed, as previously shown in studies using high-producing dairy cows. In conclusion, the GHR *Alu*I, IGF-I *Sna*BI and *Bst*EII STAT5A polymorphisms are not good molecular markers for selection of dairy cows for milk production and CCI in semi-extensive production systems.

Key Words: genetic selection, molecular markers, reproduction

0950 (T048) A polymorphism within the prolactin gene is associated with milk production in Holstein dairy cows managed under summer heat stress conditions in northwest México. P. Luna*, *Instituto Tecnologico de Sonora, Ciudad Obregon, México.*

Holstein dairy cows managed in northwest México are exposed during summer to extreme ambient temperature and humidity that lead to heat stress. The physiological response from cows exposed to such weather conditions results from the perturbation of a gene network related to heat stress homeostasis. The prolactin signaling pathway has been proposed as an important mediator of this response in cattle. The objective of this study was to assess the association between a SNP polymorphism (rs110494133-A/G within intron 1) in the prolactin gene (PRL) and performance traits such as average and total milk production in Holstein cattle. The SNP allele frequency was 70% for A and 30% for G. No deviations from Hardy-Weinberg equilibrium ($X^2 = 1.00$, $P > 0.38$) were observed in the cow population. DNA was extracted from blood spotted on FTA cards from 118 cows, and genotyped using the sequenom mass array platform. Genotype to phenotype association analyses were conducted using a mixed effects model that included phenotype as the response variable, genotype as a fixed term, sire as a random term, and days in milk as a covariate. Mean values for milk production, serum prolactin, and rectal temperature were 22.1 ± 0.5 kg/d, 32.2 ± 1.2 ng/mL, and 38.3 ± 0.1°C, respectively. In a previous study, we reported that reduced serum levels of prolactin and increased rectal temperature were associated with lower milk performance, which was remedied by spray cooling the cows. In this study, the genotype term was as a significant ($P < 0.05$) source of variation in predicting milk production. Least square means among genotypes AA, AG, and GG were 24.6 ± 1.3, 21.2 ± 1.5, and 21.2 ± 1.7 kg/d for average milk production, and 7403 ± 13.8, 7397 ± 10.5, and 6241 ± 61.8 kg for total milk production, respectively. The A allele from the SNP in the PRL gene was the most favorable ($P < 0.05$) and increased average milk production (1.2 ± 0.3 kg/d) and total milk production (325 ± 63.5 kg). When the mixed model included serum prolactin or rectal temperature as the response variable instead of milk traits, the genotype term also resulted as a significant predictor ($P < 0.05$). We conclude that a SNP within the prolactin gene appears to be a predictor of lactation performance in Holstein dairy cows managed under summer heat stress conditions common to northwest México.

Key Words: heat-stress, prolactin, polymorphism.