

ABSTRACTS

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Late-Breaking Original Research: Late-Breaking Original Research

LB1 Putative *in utero* epigenetic impacts of dam lactation yield and tissue energy stores on daughter first lactation milk production in dairy cattle. R. A. Erdman*¹, J. A. Arias², E. Quinn-Walsh², P. Fisher³, K. Stelwagen⁴, and K. Singh⁴, ¹University of Maryland, College Park, ²Livestock Improvement Corporation, Hamilton, New Zealand, ³AgResearch Ltd, Invermay Agricultural Centre, Mosgiel, New Zealand, ⁴AgResearch Ltd, Ruakura Research Centre, Hamilton, New Zealand.

Environmental factors such as nutrition and physiological state have been shown to induce epigenetic modifications of gene expression. We speculated that dam maternal environment during gestation influenced subsequent daughter phenotype, presumably by epigenetic modification *in utero*. To test this hypothesis, lactation records from 548 dam-daughter pairs from a previously reported Friesian:Jersey crossbreeding experiment (Spelman et al., 2004, Proc. NZ Soc. Anim. Prod. 64:92-95) were used. Dams were second generation (F2) crossbred cows that were jointly managed in a pasture-based system. Dam milk production (3056 ± 956 L) and total body tissue energy (TE) stores (945 ± 134 Mcal) calculated from daily BW and biweekly BCS measurements, were used as indicators of dam environmental, health and nutritional status during gestation. Daughter (F3) first lactation 305 d milk records (2107 ± 823 L) obtained from the New Zealand national milk recording system were analyzed in a statistical model that included the fixed effects of the dam sire, daughter sire, parity, and year, and random effects of dam breeding value, lactation milk production, and dam tissue energy stores at 4 physiological stages: preconception, and the 1st, 2nd, and 3rd trimesters of gestation. The model accounted for 36% of variance in daughter milk yield where regression coefficients for dam milk volume (0.47 ± 0.05 L), preconception (1.96 ± 0.62), 1st (-2.42 ± 0.75), and 3rd trimester (1.09 ± 0.41) TE (Mcal) were significant (P < 0.0001) predictors of daughter 305 d milk production. This suggested that a dam's milk production and TE stores could be used as general indicators of nutritional, health, and physical environmental effects which strongly influence *in utero* epigenetic modifications that alter daughter phenotype. This implies that dam environment not only affects current performance but also her daughter's subsequent performance in a manner that is independent of

her direct genetic effects. We believe that this could be the first example of cross-generational epigenetic regulation in the dairy cow.

Key Words: epigenetics, dairy cow

LB2 Paternity validation and estimation of genotyping error rate for the BovineSNP50 BeadChip. J. I. Weller*¹, G. Glick¹, E. Ezra², Y. Zeron³, E. Seroussi¹, and M. Ron¹, ¹ARO, The Volcani Center, Bet Dagan, Israel, ²Israel Cattle Breeders Association, Ceasarai, Israel, ³Sion, Shikmim, Israel.

Although the BovineSNP50 BeadChip (Illumina, Inc.) was developed for genomic selection, the results can also be used to validate paternity, to determine the genotyping error rate, the minimum number of SNPs required for paternity validation, and to delete defective SNPs from further analysis. Of 576 Israeli Holstein bulls genotyped, the sire was also genotyped for 204 bulls born between 1982 and 2003. Thirteen % of the genotypes were deleted due to call rates < 0.7. SNPs were deleted from this analysis if < half the bulls had valid genotypes, minor allele frequency < 0.05, the SNP was not assigned to an autosome, or the absolute value of the deviation of the observed frequency of heterozygotes from the expected Hardy-Weinberg frequency > 0.2. Of the 54001 SNPs included on the BeadChip, 14,960 (28%) were deleted. For diallelic markers with only a single parent genotyped, a discrepancy with Mendelian inheritance is obtained only if the two individuals are homozygous for different alleles. In addition to analysis with all SNPs, paternity was verified based on each hundredth SNP (388 SNPs) and each four-hundredth SNP (97 SNPs). Paternity was incorrectly recorded for 7 bulls (3.5%). In the analysis of 388 SNPs, the number of discrepancies for these 7 bulls with their putative sire was > 15 with a minimum frequency of > 0.04, while for all other bulls the frequency of discrepancies was < 3 for a maximum frequency of < 0.016. Of the 108 bulls born before 1997, paternity was excluded in 6 cases (5.5%), while for the 96 bulls born since 1997 there was only a single exclusion.

Ninety-seven SNPs were also sufficient to reject paternity for all 7 cases, based on the criterion of >1 discrepancy. In the all-SNP analysis for 197 bulls with validated sires there were 98 SNPs with overall frequency of discrepancies $> 2.5\%$. With these markers deleted, the overall frequency of discrepancies was 0.018%. Based on the estimate that only 21.7% of genotyping errors will result in discrepancies, the overall frequency of genotyping errors was 0.083%.

Key Words: paternity validation, genotyping error rate, BovineSNP50 BeadChip

LB3 Alternative methods for selecting tagSNP panels from the bovine 50K chip to predict marbling in Angus cattle. J. D. Nkrumah*¹, D. J. Garrick², R. L. Fernando², S. L. Northcutt³, B. Bowman³, B. W. Woodward¹, S. W. Bauck¹, D. Vasco⁴, S. D. McKay⁴, T. M. Taxis⁴, M. M. Rolf⁴, J. E. Decker⁴, J. W. Kim⁴, M. C. McClure⁴, R. D. Schnabel⁴, J. F. Taylor⁴, ¹Merial Limited, Duluth, GA, ²Iowa State University, Ames, ³American Angus Association, St Joseph, MO, ⁴University of Missouri, Columbia.

The development of a high density SNP chip by the iBMAC Consortium has made genome-wide association studies feasible in cattle, but the 50K chip may be too expensive for widespread use compared to reduced panels. Similar chips in humans have been used with several dimension reduction methods to select subsets of tagSNP to create prediction equations with small impacts on accuracy. Efficient reduction algorithms ensure that all independent variants contributing to the genetic variation in a trait are selected. We evaluated methods to select subsets of useful SNP from the bovine 50K chip for a reduced panel, including Bayesian model averaging, fixed least squares, and semi-parametric and non-parametric regression methods, to train and predict marbling score in Angus cattle. We used post-filter genotypes for 41,028 SNP across 1,710 bulls born between 1955 and 2003 along with marbling EPD from the Angus Association. Predictability was assessed as the correlation between genomic predictions and marbling EPD in training and cross-validation sets of animals, and by validating on a new set of 275 younger Angus bulls, and on marbling phenotypes on 698 Angus steers. A correlation of 0.70 was achieved in cross-validation using all 41,028 SNP. Prediction equations from each feature selection method were generally repeatable in cross-validation and independent validation. Equations developed from a two-stage approach such as chromosome-wide and genome-wide Bayesian and semi-parametric models produced the most accurate and repeatable effects. Correlations between genomic breeding values and marbling EPD in cross-validation were 0.57, 0.65, 0.69, and 0.73 for the best 50, 100, 150, and 200 selected SNP, respectively. Corresponding correlations in the 275 bulls were 0.51, 0.55, 0.59, and 0.60, and genetic correlations in the 698 steers were 0.28, 0.29, 0.39, and 0.43. Reduced panels of SNP can be selected from the 50K chip to predict marbling with limited impact on accuracy depending upon the size of the reduced panel.

Key Words: high density SNP chip, dimension reduction and feature selection, prediction accuracy

LB4 Genome-wide expression QTL (eQTL) analysis of loin muscle tissue to identify candidate genes in pigs. C. W. Ernst*¹, J. P. Steibel¹, G. J. M. Rosa², R. J. Tempelman¹, R. O. Bates¹, V. D. Rilmington¹, A. Ragavendran¹, N. E. Raney¹, A. M. Ramos¹, F. F. Cardoso^{1,3}, and D. B. Edwards¹, ¹Michigan State University, East Lansing, ²University of Wisconsin, Madison, ³Embrapa Pecuária Sul, Bagé, RS, Brazil.

Integration of transcriptional profiling with genome scans using DNA markers to identify chromosomal regions controlling variation in important phenotypes is referred to as expression quantitative trait loci (eQTL) analysis and enhances candidate gene discovery for complex traits. Here we report the first comprehensive genome-wide eQTL study in livestock. Our specific objective was to conduct an eQTL analysis of loin muscle tissue from F2 pigs in our Duroc \times Pietrain resource population. A genome scan was conducted with 124 microsatellite markers and transcriptional profiling was performed on 176 pigs using the Pigoligoarray (www.pigoligoarray.org) microarray containing 20,400 70-mer oligonucleotides (oligos), most with comparative human gene annotation. The QTL models used for crosses between outbred populations included fixed effects of sex, dye and QTL, and random effects of array and litter. Nominal p-values were corrected for multiple tests using false discovery rate (q-value). To rapidly screen over 20,000 QTL profiles in the eQTL scan, peaks of LOD-score functions for each expression trait in each chromosome were selected using q-values < 0.1 , leaving 975 tests of which 62 were unique peaks (highest peak in a LOD-score function for a transcript within a chromosome). Physical localization to the preliminary pig genome sequence assembly (pre.ensembl.org/Sus_scrofa/, accessed 5/22/09) was determined for 33 oligos. Comparing the positions of putative eQTL with 173 putative QTL previously identified for growth and carcass traits (tQTL) revealed 11 common linkage regions between tQTL and eQTL. Of these, 7 regions involved putative cis-acting eQTL for 8 oligos that provide strong positional candidates for tQTL. For example, a tQTL for LMA on SSC1 is located in the same marker interval as an eQTL for the gene dynein light chain. This study demonstrates the power of integrating genetic markers, phenotypes, transcript profiles, comparative human gene annotation and the preliminary pig genome sequence assembly for identifying candidate genes controlling economically important complex traits.

Key Words: eQTL, genetical genomics, Longissimus dorsi muscle

LB5 Melamine ingested by sheep is deposited in meat. C. W. Cruywagen*, W. F. J. van de Vyver, and M. Stander, Stellenbosch University, Stellenbosch, South Africa.

Eight Dohne Merino rams were used to determine absorption and excretion rates of dietary melamine in sheep and to determine whether melamine would be deposited in meat. Two batches of concentrate pellets were made, one (CON) contained corn gluten meal with no detected melamine and the other (MEL) contained corn gluten meal of Chinese origin with a high melamine content (15117 mg/kg). The MEL pellets contained 1149 mg/kg of melamine. During a 10 day adaptation period, all the animals received a forage based diet and 600 g/d of the CON pellets. In the following 8 day collection period six of the animals received MEL pellets and two received CON pellets. Melamine intake of sheep receiving MEL pellets was 0.69 g/d. Blood samples were taken before first ingestion of MEL pellets on day 1 and again on days 3, 6 and 8 of the collection period for melamine and BUN analyses. Faeces and urine were collected quantitatively over the 8 days for proximate and melamine analyses. All the animals were slaughtered at the end of the trial and samples of the M. Longissimus dorsi, liver, kidneys and abdominal fat were taken for melamine analysis. For the six sheep that received MEL pellets, SE values were determined for melamine concentrations in the collected samples and for apparent digestibility values. Repeated measurement analyses were done on serum data collected over time. Data of the two sheep that received CON pellets for the duration of the trial, confirmed that no melamine was detected in any of the samples and no statistical analyses were performed on their

data. The apparent absorption rate of ingested melamine was 76.7%. Melamine was detected in the urine, blood, muscle and fat tissue of all the animals that received MEL pellets. Serum melamine concentrations reached 5.4 ± 0.76 mg/kg on day 8 of the collection period and the meat contained 9.6 ± 1.2 mg/kg. Calculations on the partitioning of ingested melamine suggested that urine is the major excretion route at 53.2%, followed by faeces at 23.3%. Approximately 3.5% of the ingested melamine was deposited in muscle. It was concluded that ingested melamine is absorbed at a high rate and that a pathway exists for the deposition of dietary melamine in meat.

Key Words: melamine, meat, sheep

LB6 Implementation of genomic selection in egg layer chickens.

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Genomic selection (GS) using breeding values (GEBV) estimated from dense marker data offers promise for genetic improvement. Conventional schemes for egg layer chickens delay final selection until females have produced eggs for about 6 months. A GS program for layer chickens was developed to increase response per year by halving generation intervals through early selection on GEBV. Using a replicate of a commercial layer chicken breeding line, this GS program is being implemented for a multi-trait breeding goal and directly compared to conventional selection using BLUP in the original sub-line over multiple generations. Here we report on initial results from this GS program. Genotypes were obtained for 1,600 birds using a custom Illumina 42k single nucleotide polymorphism (SNP) panel developed to span the entire genome. Training of the GS prediction model was from individuals representing the first 4 of the most recent 5 generations. Validation involved 295 full sib families in the most recent generation since training and was quantified by the correlation between family GEBV and the mean phenotype of on average 7.2 daughters per full sib family. A novel Bayesian mixed linear model procedure (Bayes-CPi) was used to combine individual and progeny mean data, while jointly estimating the proportion of SNPs that have non-zero effects, and the corresponding proportion of variation explained by the markers. Use of this approach for initial training on over 8,400 records for early egg weight on 913 genotyped females and offspring of 293 genotyped males resulted in a correlation of around 0.6 in the validation population. Greater correlations are expected when the validation families are also included in the training data. The posterior mean of the proportion of SNPs with non-zero effects was estimated to be less than 0.02, i.e. fewer than 500 segregating SNPs. Although these results are for one of the more heritable traits in layer breeding programs, the observed correlation is sufficient for the designed GS program to obtain substantially greater responses per year than the traditional program, with limited impact on inbreeding per year.

Key Words: genomic selection, marker-assisted selection, poultry

LB7 *Staphylococcus aureus*: One step closer to a successful vaccine.

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Staphylococcus aureus is a common cause of mastitis, a disease estimated to cost the dairy industry \$2 billion/yr, with no effective vaccine strategy. Our goal is to define the role of immune activating dendritic cells (DC) in the development of protective *S. aureus*-specific T cell memory for use in vaccine development. The objective of this study was to investigate the recognition of *S. aureus* by bovine monocyte-derived DC (MO-DC) and subsequent induction of memory T lymphocyte proliferation. Peripheral blood monocytes (CD14⁺) were isolated from cows previously diagnosed with *S. aureus* mastitis (infected; n= 5). Control cows (n= 5) were chosen based on lack of previous record of *S. aureus* mastitis. Monocytes were cultured (7 d) in RPMI based medium supplemented with recombinant bovine granulocyte-monocyte colony stimulating factor and recombinant bovine interleukin-4. MO-DC were loaded with live (LSA) or irradiated *S. aureus* (ISA) for 24 and 48hr. Real-time PCR analysis indicated a significant induction of *S. aureus* specific pattern recognition receptors (TLR). Specifically, LSA loading induced upregulation of NOD2 (P < 0.05), TLR4 (P < 0.01), and TLR2 (P < 0.01) over time. Loading with ISA induced upregulation of NOD2 (P < 0.03) and TLR2 (P < 0.01) over time. Stimulated MO-DC were incubated with bovine lymphocytes to determine ability to induce cellular immune response. Stimulation of autologous lymphocytes resulted in significant proliferation of cells from infected cows compared to control animals (P < 0.01). Upon further flow cytometric analysis, the proliferation of memory CD4⁺, CD8⁺, and gamma delta WC1⁺ T cells was defined by loss of carboxyfluorescein succinimidyl ester (CFSE) label and using memory cell surface markers CD45RO and CD62L. Taken together, our data indicate the successful response of MO-DC to LSA and ISA and the presence of *S. aureus*-specific memory T cells. The results of these experiments should pave the way for design of a successful cellular based vaccine against *S. aureus* mastitis.

Key Words: mastitis, *Staphylococcus aureus*, vaccine

LB8 Role of behavior in non-invasive disease surveillance of swine influenza virus.

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Labor and expense associated with using serological assays for swine disease diagnostics and surveillance decrease the likelihood of their use on farm. As an alternative method, oral fluid samples are collected from pigs by suspending a rope within a pen. Pigs are naturally inquisitive, and deposit oral fluids during interactions with ropes, but behavioral changes during disease may reduce interactions and saliva deposition. The objectives of this study were to determine 1) the proportion of pigs interacting with ropes, and 2) effects of acute infection on these interactions. Four pens of 10 pigs (n=40) were exposed daily to a single cotton rope per pen, placed at the same location and time each day. On 8 consecutive days, behavior was video-taped for the time that ropes were in place (15-20 mins). Latency of contact with the rope was recorded for each pig. On Day 4, all pigs were inoculated with swine influenza virus (SIV). Logistic regression analysis showed that odds of contacting the rope decreased significantly after inoculation (p<0.0001). On pre-inoculation days, 95-97% of pigs visited the ropes. This fell to 53% on Day 5, but returned to an average of 93% for Days 6, 7 and 8. Latency data was log-transformed to improve normality and analyzed using a failure time regression model. Latency differed significantly across observation days (p<0.05), with a mean of 46s prior to inoculation and 57s post-inoculation. Mean latency was highest on Day 5 (77s). Approximately 88% of all rope contact occurred within one minute of rope placement, and 99% occurred within 10 minutes. In conclusion, ropes sampling provided good representation from group-housed pigs.

Acute disease had an immediate but transient effect on swine motivation to interact with ropes in this experiment. However, a sufficient number of pigs interacted with the rope for successful detection of SIV at the pen level. Despite behavioral changes, cotton ropes remain a viable option for collecting oral fluids, and it is feasible to lower exposure time to 10 minutes to increase the quality of samples.

Key Words: behavior, influenza, surveillance