

and pregnancy diagnosis (G3), and first clinical mastitis after diagnosed pregnant (G4). Within each dairy, every cow in the mastitis groups was matched with a control cow that was in the same lactation, calved in the same month and had a similar 305-d milk yield in the previous lactation. Data were collected for the first 320 days in milk (DIM). Mastitis diagnosis was performed at every milking by the herd personnel. A fore sample of milk was collected from every clinical case for microbiological culture. Reproductive management consisted of estrus synchronization with PGF2a prior to 70 DIM and timed AI afterwards for the first postpartum AI. Cows diagnosed as open at rectal palpation were re-inseminated following a timed AI protocol. Pregnancy was diagnosed 35 d after AI and reconfirmed either at 160 d pregnant or at 300 DIM. Continuous and binomially distributed data were analyzed by the GLM and the LOGISTIC procedures of SAS, respectively. The Kaplan-Meier survival analysis procedure was used to assess the effect of treatment on days open. Results are presented according to the following sequence: G1, G2, and G3. Conception rate at first postpartum AI was decreased by mastitis (28.7 vs 22.1 vs 10.2%,  $P < 0.001$ ). Pregnancy rate at 320 DIM also decreased for cows with mastitis (85.4 vs 72.3 vs 58.5%;  $P < 0.001$ ). Days open were extended for cows with mastitis ( $P < 0.001$ ). Incidence of abortions was 5.8, 11.8, 11.6, and 9.7% for G1, G2, G3, and G4, respectively ( $P < 0.04$ ). Mastitis prior to first AI decreased yields of milk (35.9 vs 34.4 vs 35.3 kg/d;  $P < 0.001$ ) and 3.5% fat-corrected milk (36.5 vs 35.1 vs 36.0 kg/d;  $P < 0.01$ ). Mastitis also increased linear SCC score (1.78 vs 2.75 vs 2.80;  $P < 0.001$ ). Mastitis either prior to or after first postpartum AI decreases conception, increases incidence of abortions and decreases milk production in lactating dairy cows.

**Key Words:** Mastitis, Reproduction, Milk production

**1505 Bacterial counts in bedding and on teat ends of cows housed on sand and sawdust.** M. Zdanowicz\*, J. A. Shelford, C. B. Tucker, and D. M. Weary, *University of British Columbia, Vancouver, Canada.*

The objectives of the study were to compare bacteria counts of mastitis causing organisms in sand and sawdust bedding, and determine the relationship between bacteria counts in bedding with those on the cows' teats. Sixteen cows were housed on either sand or sawdust bedded free-stalls using a cross over design with 3 weeks per bedding type. Fresh bedding was added every 7 days. Visible fecal matter was removed daily as needed to keep stalls clean and dry. Bedding samples were collected on day 0 (prior to cows lying on the bedding), day 1, 2 and 6. Teat ends were sampled prior to the morning milking on day 1, 2 and 6. All samples were analyzed for the growth of coliforms, *Streptococci spp.*, and *Klebsiella spp.*. Bacteria counts in bedding and on the teat ends were analysed using analysis of variance. Treatment differences on each day were tested by Bonferroni multiple comparison test. Correlations among bacterial counts in bedding and on teat ends were determined by Pearson's correlation coefficients. For both bedding and teat end samples, there were significantly more coliform and *Klebsiella* bacteria

associated with sawdust than with sand ( $P < 0.01$ ) but there were more *Streptococcus* associated with sand than with sawdust ( $P < 0.01$ ). In both sawdust and sand bedding, coliform and *Klebsiella* and *Streptococcus* counts increased over the week, although patterns varied with the bedding and the bacteria type. Bacteria counts in bedding were highly correlated to bacteria counts on teat ends. In conclusion, coliform and *Klebsiella* bacteria are more numerous when using sawdust bedding, but *Streptococcus* bacteria are more numerous in sand.

**Key Words:** Environmental bacteria, Stall management, Udder cleanliness

**1506 Sensitivity and specificity of MAS-D-TEC to detect subclinical mastitis in dairy cattle.** H. Ghasemzadeh-Nava\*<sup>1</sup>, M. R. Hosseini<sup>2</sup>, and F. Gharagozloo<sup>1</sup>, <sup>1</sup>Dept. of Large Animal Clinical Sciences, Faculty of Vet. Med; University of Tehran, <sup>2</sup>Private Practitioner, Garmsar, Iran.

Early diagnosis of subclinical mastitis in dairy cows may be important in reducing production losses and enhancing prospects of recovery. In recent years, most effort has gone into the system that uses changes in the electrical conductivity of milk. The purpose of this study was to evaluate the Sensitivity (Se) and Specificity (Sp) of MAS-D-TEC (a manually cow-side detector of mastitis test) for detection of subclinical mastitis by changes in electrical conductivity of foremilk. Fore milk samples of each cow (n=236 quarters) were first examined by MAS-D-TEC device (Westcor Inc; Logan, USA). MAS-D-TEC graded from 0 to 9. On the basis of the factory recommendation, sample grades  $\geq 5$  are highly indicative of presence of infection. Quarter milk samples were then collected aseptically and sent to the diagnostic lab for bacteriological cultures on blood agar and MacConkey agar media. The results of this study revealed that Se of MAS-D-TEC for detection of subclinical mastitis was 100% (137/137, that means all of 137 specimens with  $\geq 5$  grades in MAS-D-TEC procedure were positive in bacteriological cultures), but the Sp of this device was 43.3% (26/60, that means only 26 out of total 60 negative culture specimens in MAS-D-TEC procedure had  $\leq 4$  grades, so 34 of negative culture specimens had  $\geq 5$  grades in MAS-D-TEC procedure). The false positive results of this device to detect subclinical mastitis may be attributed to the stage of lactation, parity, presence of the bacteria in the milk cells (more commonly about the presence of *Staphylococcus aureus* in the macrophage cells), presence of other microorganisms other than bacteria in the milk samples and so on. In conclusion, the present study revealed that milk specimens with degrees  $\leq 4$  in MAS-D-TEC procedure are highly indicative of absence of pathogens in the udder, so it is not necessary to take samples from them for bacteriological cultures, but specimens with degrees  $\geq 5$  may be indicative of subclinical mastitis which bacteriological cultures must be done to ascertain the presence of bacteria and kind of the pathogens involved, necessary for control and preventive mastitis programs in dairy herds.

**Key Words:** Mastitis, Electrical Conductivity, Cattle

## Animal Breeding and Genetics Molecular Genetics

**1507 The bovine gastrointestinal tract: A gene expression profile.** C. Hansen<sup>1</sup>, A. Fu<sup>1</sup>, Y. Meng<sup>1</sup>, C. Li<sup>1</sup>, E. Okine<sup>1</sup>, C. W. Sensen<sup>2</sup>, P. Gordon<sup>2</sup>, and S. S. Moore\*<sup>1</sup>, <sup>1</sup>Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada, <sup>2</sup>Dept. of Biochemistry and Molecular Biology, University of Calgary, Calgary, AB, Canada.

The bovine gastrointestinal (GI) tract is a complex system of chambers that function to break down and absorb what is often low quality feed. Although genes expressed along the GI tract have been intensely studied, for the most part experiments have only reported on one or a few genes at a time. We have used gene expression profiling to catalogue the genes and their level of expression in various tissues of the bovine GI tract and thus gain insight into the functionality of these tissues. Directionally cloned cDNA libraries of each of the segments of the tract were constructed using the Stratagene ZAP cDNA synthesis kit and approximately 2000 expressed sequence tags (ESTs) were generated for each region. Sequences were submitted to the MAGPIE program, a system for the automated analysis of biological sequences, and functional assignments were made for the various ESTs. Gene ontology assign-

ments were made using the GO classification system. Gene expression differences were analyzed statistically using the chi-square test. Tissue specific transcripts were found highly expressed in some regions. For example, the lysozymes were very abundant in the abomasum, but they are virtually absent from other regions. Ribosomal proteins too showed differences in level of expression between regions, indicating differences in the level of protein synthesis. Interestingly enough, a number of the GI regions did not appear to express a particular type of sequence preferentially. Rather, there was a steady, low level of expression of all genes observed. A comprehensive overview of the differences found will be presented.

**Key Words:** Gene Expression, Gastrointestinal Tract, Bovine

**1508 Construction and characterization of ORESTES cDNA libraries generated from bovine mammary gland tissues.** A. F. da Mota\*<sup>1</sup>, T. S. Sonstegard<sup>1</sup>, C. P. Van Tassell<sup>1</sup>, E. E. Connor<sup>1</sup>, A. V. Capuco<sup>1</sup>, M. A. P. Brito<sup>2</sup>, M. A. Machado<sup>2</sup>, M. L. Martinez<sup>2</sup>, and L. L. Coutinho<sup>3</sup>, <sup>1</sup>*Gene Evaluation & Mapping Laboratory*, <sup>2</sup>*EMBRAPA, Gado de Leite*, <sup>3</sup>*University of Sao Paulo-ESALQ*.

Currently, there are approximately 200,000 bovine expressed sequence tags (EST) available in public databases. This is ten-fold less sequence information than that available for human and mouse. As such, additional bovine EST need to be generated to more thoroughly identify, annotate and classify expressed genes. This information will be essential to the interpretation of results generated from functional genomic studies. The objective of the present study was to generate 5,000 EST from the mammary gland, while maintaining a maximum discovery rate of novel EST. Based on this criteria, we chose to construct cDNA libraries using the open reading frame EST (ORESTES) method. This method uses arbitrarily primed cDNA synthesis to generate a partial profile of gene expression that can be PCR amplified, cloned, and arrayed into mini-libraries. The clones are usually sequences representing the central portion of expressed genes. In a preliminary study, primers that amplify estrogen receptor gene family members and mRNA from a pre-pubertal Holstein heifer were used to create six mini-libraries. Clones from three of these libraries were processed for sequence analysis. After processing to assign quality score and trim vector sequence, 455 sequences met minimum GenBank submission criteria. These sequences were assembled using GAP4 (Staden-Package) to assess rate of clonal redundancy and to provide consensus sequences for BLAST analysis. A total of 64 tentative consensus (TC) sequences were assembled leaving 178 singletons (ST) to generate a rate of redundancy of 47%. However, BLAST analysis of the 242 unique sequence elements (TC and ST sequence) against GenBank nt and the *Bos taurus* Gene Index (BtGI) databases revealed that most of these sequences were new relative to other cattle EST. Eighty-one sequences (17%) had no match to the nt database, and 147 (32%) did not match BtGI. Because ORESTES produced a high rate of novel cattle sequences, this method will be exploited to generate EST from mammary gland mRNA isolated from Brazilian dairy cattle (Gir). The presence of novel sequences within these libraries will be a valuable resource for studying gene expression differences in the mammary gland of Holstein and Gir cattle.

**Key Words:** Expressed sequence tags, Functional genomics, Mammary gland

**1509 Influence of a differential allelic expression of bovine kappa-casein gene on micelle properties and renneting parameters.** G. Lapointe\*<sup>3</sup>, G. Robitaille<sup>1</sup>, M. Britten<sup>3</sup>, J. Morisset<sup>1</sup>, Y. Pouliot<sup>3</sup>, and D. Petitclerc<sup>2</sup>, <sup>1</sup>*Sherbrooke University*, <sup>2</sup>*DSRDC, Agriculture & Agri-Food Canada (AAC), Lennoxville (Qc), Canada*, <sup>3</sup>*FRDC, AAC, St-Hyacinthe (Qc), Canada*.

A differential allelic expression of the  $\kappa$ -casein ( $\kappa$ -CN) gene has been recently reported in Holstein cows. Two groups were distinguished within a population of  $\kappa$ -CN AB cows: group HH showed similar amounts of alleles A- and B-specific  $\kappa$ -CN mRNA within mammaryocytes, whereas the allele B-specific gene of group HL was transcribed into mRNA more efficiently than the allele A-specific one. The objectives of the study were to evaluate the impact of differential expression combined with heat treatment on milk rennetability, micelle size, and hydration. At each test-day, milk samples from individual cows (HH n=5, HL n=6) were collected, skimmed, and analysed for protein composition. They were dialyzed overnight at 4°C against bulk milk and standardized for protein content. Micelle size and hydration were determined on non-treated and heated milk (75°C, 64 min). Kinetics of curd formation were monitored using a microplate reader (650 nm) after addition of rennet to intact or heated milk (75°C for 2 to 64 min); coagulation parameters were obtained by fitting optical density data to an appropriate mathematical model. Solubility of whey proteins and micelle hydration decreased significantly with heat treatment ( $P < 0.05$ ), while micelle size tended to increase when heated. Group had no effect ( $P > 0.1$ ) on these parameters. Heat treatment and the group of cows significantly affected ( $P < 0.05$ ) the kinetic of coagulation of milk, but there was no interaction between them. Gel formation rate decreased and clotting time (CT) increased as the heat treatment duration increased, and group HL had a faster gel formation rate and a shorter CT than group

HH. These results suggested that milk from cows of group HL has a better rennetability than milk from cows HH. However, statistical analysis revealed that impact of differential allele-specific expression of  $\kappa$ -CN on the rennetability of heated milk stayed low compared to other genetic effects. Funded by FCAR-NOVALAIT-MAPAQ.

**1510 A differential allelic expression of bovine-kappa casein gene is maintained throughout lactation.** D. Vachon\*<sup>1</sup>, G. Robitaille<sup>1</sup>, M. Britten<sup>3</sup>, J. Morisset<sup>1</sup>, and D. Petitclerc<sup>2</sup>, <sup>1</sup>*University of Sherbrooke, Sherbrooke, (Qc) Canada*, <sup>2</sup>*DSRDC, Agriculture & Agri-Food Canada, Lennoxville (Qc) Canada*, <sup>3</sup>*FRDC, Agriculture & Agri-Food Canada, St-Hyacinthe (Qc) Canada*.

A differential allele-specific expression of kappa -casein (k-CN) gene has been found in Holstein cows (Robitaille & Petitclerc, J. Dairy Sci. 81; 377). Indeed, we identified cows for which allele B-specific gene was transcribed into mRNA with greater efficiency than allele-A specific gene within a population of cows k-CN AB. The objective of this study was to test the effect of stage of lactation on this expression polymorphism. Holstein cows k-CN AB (n=16) were analyzed for the relative proportion of k-CN A and B-specific transcripts in mammaryocytes throughout lactation. Milk samples were collected from individual cows at different stages of lactation (4 to 7 test days/cow) and somatic cells were harvested by centrifugation at 2,500 RPM for 5 min. Cells were lysed with Trizol<sup>®</sup> reagent and total RNA was prepared following standard procedures. The relative proportion of each allele specific mRNA was determined by densitometry following a combination of reverse transcription polymerase chain reaction and single-strand conformation polymorphism. Change in the relative proportion of allele-B specific mRNA according to stage of lactation was analyzed for significance using SAS procedures. A differential allele-specific expression of k-CN gene was observed and mean values for the relative proportion of allele-B specific mRNA during lactation was  $53.8 \pm 0.2\%$ . The relative proportion of allele-B specific mRNA was not significantly affected ( $P > 0.25$ ) by stage of lactation; LSMean values averaged 53.7%, 54.1%, 53.9% and 53.7% for  $< 90$ , 91-180, 181-270 and  $> 271$  days in milk, respectively. In conclusion, the allele-specific k-CN gene expression within mammaryocytes is not affected by stage of lactation. A better understanding of the mechanism involved in the differential allelic expression of k-CN would support the use of k-CN expression polymorphism as a genetic selection marker. Funded by FCAR-NOVALAIT-MAPAC.

**Key Words:** Kappa-casein, Expression polymorphism

**1511 Polymorphism within the bovine kappa-casein gene.** G Robitaille\*<sup>1</sup>, M Britten<sup>3</sup>, J Morisset<sup>1</sup>, and D Petitclerc<sup>2</sup>, <sup>1</sup>*Sherbrooke University*, <sup>2</sup>*DSRDC, Agriculture & AgriFood Canada*, <sup>3</sup>*FRDC, Agriculture & AgriFood Canada*.

Kappa-casein ( $\kappa$ -CN) A and B are the two main genetic variants in Holstein cows. We described a differential allele-specific expression of  $\kappa$ -CN gene that could account, at least in part, for the established association between  $\kappa$ -CN allele B and high  $\kappa$ -CN content in milk (Robitaille et al, 2000, JDR 67:107). Indeed, within cows genotyped AB, we observed a group of cows (HH) with similar level of expression of each allele and another group of cows (HL) over-expressing the allele B-specific  $\kappa$ -CN gene compared to allele A. In this study, we compared DNA sequences of allele A and B gene variants to evaluate single nucleotide polymorphisms (SNPs) associated with A and B genetic variants and with the differential expression of  $\kappa$ -CN gene. Genomic DNA was sequenced from 3 cows HL, 3 cows HH and from homozygous AA and BB cows for  $\kappa$ -CN genetic variants. The  $\kappa$ -CN gene (12 995 base pairs (bp)) contains five exons (64, 61, 207, 612 and 118 bp) and four introns (2426, 5915, 1837 and 1755 bp). We first determined the unpublished sequence of the 2402 bp region within intron II. Then, we compared the gene sequence of genetic and expression variants of  $\kappa$ -CN gene. Exons I, II and III are well conserved and we did not detect any polymorphism within these exons. A total of 35 SNPs were found within exons IV and V and within introns I to IV after comparison between allele-A and allele-B specific genes. SNPs located within the  $\kappa$ -CN gene coming from HL cows were identical to those of HH cows; these SNPs were strictly related to allele A and B-specific sequences. This observation was confirmed using DNA sequence of  $\kappa$ -CN gene from AA and BB cows. In conclusion, the differential allele A and allele B-specific expression pattern cannot be related to specific mutations within the  $\kappa$ -CN gene. Further extensive analysis of the 5' upstream region  $\kappa$ -CN gene is being carried out to find

additional SNPs potentially related to the differential allelic expression of  $\kappa$ -CN gene. Funded by FCAR-NOVALAIT-MAPAC

**Key Words:**  $\kappa$ -casein, Gene expression, DNA sequence

**1512 SNP discovery in candidate genes for a bovine ovulation rate QTL.** K.J. Tessanne\*<sup>1</sup>, K.E. Gregory<sup>2</sup>, and B.W. Kirkpatrick<sup>1</sup>, <sup>1</sup>University of Wisconsin-Madison, <sup>2</sup>USDA Meat Animal Research Center.

The primary aim of this study was to identify candidate gene polymorphisms for an ovulation rate QTL on bovine chromosome 19 (BTA19). This QTL was identified in an elite sire using a three-generation family within the USDA Meat Animal Research Center (MARC) twinning herd, and it was this sire that was screened for polymorphisms. Growth hormone (GH), insulin-like growth factor binding protein-4 (IGFBP-4), and 17 $\beta$ -estradiol dehydrogenase type 1 (17 $\beta$ -HSD) were selected as potential candidate genes and searched for single nucleotide polymorphisms (SNPs) using PCR amplification and DNA sequencing. Two SNPs were found in 5695 bp of sequence, and both were located in GH at positions 253 (C253T) and 1692 (C1692T) of the published bovine sequence. To determine association of these SNPs with ovulation rate, a one-way ANOVA was implemented using deviation from midparent estimated breeding value for ovulation rate as the phenotype of interest. This was calculated for a sample of 177 individuals from the MARC twinning herd born in 1992. No significant association with ovulation rate was detected. In the course of this study four SNP genotyping methodologies were evaluated for accuracy, these being allele-specific PCR, primer extension, forced restriction fragment length polymorphism (RFLP) analysis, and the invader technology. Of these, both the allele-specific PCR and primer extension gave inaccurate genotyping results when compared to the RFLP assay. Primer extension gave an error rate of 41% (n = 41) and allele-specific PCR gave error rates of 74% (n = 23) and 18% (n = 17) for two different primer sets when compared with the forced RFLP results. No errors were observed with the invader technology when compared with forced RFLP results. Efforts to identify polymorphisms in linkage disequilibrium with the QTL were hampered in this case by the broad confidence interval for QTL location. The QTL needs to be refined further before a candidate gene search is performed.

**Key Words:** Genetic marker, Polymorphism, Cattle

**1513 Genetic diversity among the Angus, the American Brahman, the Senepol, and the Romosinuano cattle breeds.** R. A. Brennehan<sup>1</sup>, C. C. Chase, Jr.\*<sup>1</sup>, D. G. Riley<sup>1</sup>, T. A. Olson<sup>2</sup>, and S. W. Coleman<sup>1</sup>, <sup>1</sup>USDA, ARS, SubTropical Agricultural Research Station, Brooksville, FL, <sup>2</sup>University of Florida, Gainesville, FL.

The objective of this study was to quantify the genetic diversity among the breeds under evaluation at the USDA, ARS, SubTropical Agricultural Research Station (STARS). Twenty-six microsatellite loci were used to estimate parameters of genetic diversity among a *Bos indicus* breed, Brahman (B), and three *Bos taurus* breeds, Angus (A), Senepol (S), and Romosinuano (comprised of two distinct bloodlines, R<sub>1</sub> from Colombia and R<sub>2</sub> from Costa Rica). Forty-seven animals from each of the respective STARS herds were selected by pedigree and sampled as breed or bloodline representatives. Analysis was performed using GENEPOP v3.2a. The genetic differentiation detected between the populations was highly significant (P < 0.001). Pairwise measures related to genetic differentiation are shown in Table 1. The R<sub>1</sub> and R<sub>2</sub> populations were most similar, having the smallest genetic distance (D<sub>S</sub>), population subdivision (F<sub>ST</sub>), and proportion of private alleles (Pvt alleles), and the largest gene flow (N<sub>M</sub>). The A and R<sub>1</sub> populations appeared to be most diverse, having the largest F<sub>ST</sub> and proportion of Pvt alleles, and the smallest N<sub>M</sub>. Results indicate that the use of molecular genetics techniques combined with population genetics analyses may be useful to substantiate historical development of these breeds. In conclusion, inferences based on genetic distance were shown to be useful in purebred studies, and may be used to determine relationships between breed diversity and observed heterosis in future crossbreeding studies.

Pairs of breeds	D <sub>s</sub>	F <sub>ST</sub>	P <sub>vt</sub> alleles	N <sub>M</sub>
R2, S	1.106	0.240	0.137	0.299
A, B	1.104	0.204	0.114	0.406
B, R1	0.932	0.262	0.140	0.314
B, R2	0.910	0.240	0.131	0.333
A, R1	0.870	0.287	0.177	0.194
B, S	0.759	0.216	0.122	0.368
R1, S	0.729	0.216	0.137	0.313
A, R1	0.679	0.309	0.184	0.190
A, S	0.615	0.234	0.135	0.296
R1, R2	0.349	0.143	0.106	0.485

**Key Words:** Beef breeds, Genetic distance, Genetic markers

**1514 Identical by descent haplotype sharing analysis: application in fine mapping of QTLs for birth weight in commercial lines of *Bos taurus*.** C. Li<sup>1</sup>, J. Basarab<sup>2</sup>, W. M. Snelling<sup>3</sup>, B. Benkel<sup>4</sup>, B. Murdoch<sup>1</sup>, J. Kneeland<sup>1</sup>, C. Hansen<sup>1</sup>, and S. S. Moore\*<sup>1</sup>, <sup>1</sup>Department of AFNS, University of Alberta, Edmonton, AB, Canada, <sup>2</sup>Lacombe Research Centre, AAFRD, 6000 C&E Trail, Lacombe, AB, Canada, <sup>3</sup>USDA, ARS, US MARC, Clay Center, Nebraska, USA, <sup>4</sup>AAFC, Lethbridge Research Center, AB, Canada.

Fine mapping QTLs will greatly facilitate the identification and cloning of the causative genes. To this end we have utilized historical recombinations in commercial beef cattle lines to better define QTL regions. It is expected that individuals within a semi-closed population, such as a commercial line of cattle, may be derived from one or a limited number of founders. Some common haplotypes originating from the common ancestors should carry on and segregate among the individuals of the breeding line, particularly when selection is applied. These common haplotypes may harbor QTLs of interest and make it possible to locate QTLs segregating in the line. We demonstrated an application of an identical by descent haplotype (IBD) sharing analysis in fine mapping QTLs for birth weight EBV (estimated breeding values) on bovine chromosome 5 in two commercial lines of *Bos taurus* from Beefbooster Inc. Canada. The M1 line was developed from an Angus base and the M3 line from small cows of various breeds. Both lines have been selected for maternal traits for over 30 years. Common haplotypes within each commercial line were identified along BTA5 using 16 microsatellite markers and the association between a common haplotype and the birth weight EBV was tested by regression analysis. Two chromosomal regions at 10 cM to 20 cM and 65 cM to 78 cM were identified having significant associations with the birth weight EBV in both commercial lines. The two chromosomal regions were also confirmed by interval mapping. The QTLs identified using the IBD haplotype sharing analysis were better defined, indicating the effectiveness and accuracy of the IBD haplotype sharing analysis in fine mapping of QTLs in commercial lines of *Bos taurus*.

**Key Words:** QTL, IBD haplotype sharing analysis, Bovine

**1515 Investigation of the association between the estrogen receptor beta gene and reproductive components in swine.** B.J. Isler\*, K.M. Irvin, S.M. Neal, and S.J. Moeller, *The Ohio State University, Columbus, OH.*

The association between a polymorphism in the estrogen receptor beta (ER $\beta$ ) gene and reproductive tract components in swine has been evaluated. The ER $\beta$  genotype of 75 Yorkshire (Y  $\times$  Y), 80 Large White (LW  $\times$  LW), and 99 crossbred (33 LW  $\times$  Y, 66 Y  $\times$  LW) females was determined to be AA, AB, or BB using a PCR-RFLP procedure. All animals were mated to Hampshire or Duroc boars and slaughtered at approximately 75 days of gestation. Data collected from gravid uterine tracts included ovulation rate, uterine weight, uterine horn length, number of fetuses, total fetal weight, average fetal weight, number of mummies, fetal space, and fetal survival. Data were analyzed using a model that included the effects of ER $\beta$  genotype, breed, parity, and significant two-way interactions. Uterine horn was also included in some analyses to determine the presence of between horn effects. ER $\beta$  genotype was found to be associated (P < 0.1) with fetal weight per uterus, average fetal weight per uterus, number of fetuses per horn, fetal weight per horn, and average fetal weight per horn. Animals with the BB genotype had a significantly (P = 0.003) greater number of fetuses per horn

(5.63 ± 0.51) than animals with the AA genotype (4.83 ± 0.15). BB animals also had a significantly ( $P < 0.05$ ) smaller total (1687 ± 75 g) and average (335.4 ± 14.2 g) fetal weight per horn than AA animals (1821 ± 29 g, 368.7 ± 5.7 g). The ER $\beta$  gene appears to act in a dominant manner, with the B allele being dominant over the A allele. Animals with the dominant phenotype gestate larger, lighter litters of piglets than animals with the recessive phenotype. Other traits displayed statistically non-significant trends with respect to the BB genotype: increased fetal survival and total number of fetuses per uterus and decreased uterine weight and fetal space per horn. The ER $\beta$  gene is positively associated with several reproductive tract traits.

**Key Words:** Swine, Genetic Marker, Reproductive Traits

### 1516 Polymorphisms at the mink prolactin locus. T.L. Vardy and A. Farid, *Nova Scotia Agricultural College*.

The objective of this study was to find polymorphisms at the mink prolactin (PRL) locus. This gene plays important roles in mammary gland development, initiation and subsequent maintenance of lactation, termination of embryonic diapause as well as fur growth and coat molt

cycles. Sequence of the cat PRL gene was used to design primers for the amplification of the mink PRL exon 1 and part of exon 2, which were not previously known, by the polymerase chain reaction (PCR). The entire exons 2, 3, 4 and 5 and the intervening introns were also PCR amplified using overlapping primers. PCR products were bidirectionally sequenced in four to seven mink of different colour types (black, pastel, brown, wild). Four nucleotide substitutions were detected in introns, which were in linkage disequilibrium. Genotypes of 86 mink (25 black, 20 pastel, 20 brown, 21 wild) were determined at a NlaIV site (C to G substitution) in intron 3. One allele which was not detected in black mink, had low frequencies in brown (0.05) and pastel (0.025), while it had a moderate frequency (0.20) in wild mink. The result may suggest that the region of DNA containing the PRL locus has been under selection pressure in ranched mink. Three polymorphic tandem repeats; a (GT)<sub>15</sub> and a (TTC)<sub>5</sub>(T)<sub>47</sub> in intron 2, and a (CA)<sub>7</sub>(GA)<sub>14</sub> in intron 4, were also detected. These microsatellites facilitate genetic screening of mink at the PRL locus.

**Key Words:** Mink, Prolactin, Polymorphism

## Dairy Foods Processing

### 1517 Implementation of HACCP system to large scale processing line of plain set yogurt. A. Rabi<sup>1</sup>, R.R. Shaker<sup>2</sup>, A. Banat<sup>1</sup>, and S.A. Ibrahim\*<sup>3</sup>, <sup>1</sup>*Jordan University of Science and Technology*, <sup>2</sup>*Washington State University, Pullman, WA*, <sup>3</sup>*North Carolina Agriculture and Technical State University, Greensboro, NC*.

Limited data on the microbiological quality of traditional dairy products in Jordan are available. Recent studies have shown that yeast is the major contaminant in many of these products. The problem of such contamination could be attributed to many factors. Therefore, it is important to develop a hazard analysis and critical control points (HACCP) system for traditional products. The implementation of such system to yogurt is of great importance in order to produce microbiologically safe dairy product. The system was implemented for yogurt processing line as produced by large dairy company in Jordan. Six critical control points were identified in the flow chart of yogurt production; corrective actions and effective preventive measure were suggested. The microbial results have demonstrated how the hazards at the four critical control points of the process are easily and effectively controlled through implementation of the HACCP system. The microbial results demonstrate how the hazards at the critical control points (CCPs) of the process are easily and effectively controlled through the implementation of the HACCP system to popular dairy products

**Key Words:** yogurt, HACCP, safety

### 1518 Influence of lactic cultures, added linoleic acid, and fructo-oligosaccharides on conjugated linoleic acid concentration in nonfat set yogurt. Tung Lin\*, *Chinese Culture University, Taipei, Taiwan*.

Skim milk mixed with 5% fructo-oligosaccharides and/or 0.1% linoleic acid (LA) was fermented with one of three lactic cultures: *Lactobacillus acidophilus* (CCRC14079), yogurt bacteria (*L. delbrueckii*ssp. *bulgaricus* and *Streptococcus salivarius* ssp. *thermophilus*), and mixed cultures of *L. acidophilus* (CCRC14079), and yogurt bacteria at 37°C for 8-24 h to reach a 0.9% acidity, and the levels of c9,t11-conjugated linoleic acid (c9,t11-CLA) were determined by HPLC. Sensory attributes and Hunter L, a, b values of the products were also evaluated. A significant increase in c9,t11-CLA level was observed in the LA added yogurt inoculated with mixed cultures, and the CLA content was 2.95 µg/g yogurt. The total acceptability ratings ranged from 6.0 to 6.7 were not significant difference among 8 yogurt treatments. Hunter L, a, and b

values showed only slight differences among those yogurts too. Inoculations of mixed cultures with LA addition, therefore, are suggested for CLA-rich nonfat set yogurt production.

**Key Words:** Lactic culture, Conjugated linoleic acid, Fructo-oligosaccharides

### 1519 Viability of bifidobacteria in yogurt products found in North Carolina. J.P. Carr\*, S.A. Ibrahim, G. Shahbazi, M. Worku, and C.W. Seo, *North Carolina Agricultural and Technical State University, Greensboro, NC*.

The use of bifidobacteria as dietary adjuncts is a subject of intense and growing interest. Several probiotic benefits such as improvement of gastrointestinal motility, lactose intolerance systems, and anticholesterol effects have been associated with bifidobacteria. Because of these benefits, there has been an increasing interest in incorporating viable cells of this microbial group into dairy products. However, during processing and storage, the number of viable cells tends to decline. There are few scientific studies reporting the viability of bifidobacteria in commercial yogurt products in the U. S. Therefore, the purpose of this work was to screen the yogurt products for viable yogurt cultures specifically bifidobacteria, and to test these isolates for probiotic properties. Fifty-eight commercial yogurt products (containing bifidobacteria in addition to the traditional yogurt culture) were obtained from local stores. Experiments were performed within 24h of purchase. MRS and G-M17 were used for the enumeration of *Lactobacillus burglariorius* and *Streptococcus thermophilus*, respectively. Modified BIM-25 was used for the enumeration of bifidobacteria. All plates were incubated for 72h at 37C. Isolates of bifidobacteria were examined for the phenotypic and genotypic characteristics. Our results showed that the bacterial counts ranged from 6.00 to 9.89 Log<sub>10</sub>CFU/ml, 6.60 to 9.48 Log<sub>10</sub> CFU/ml, for Streptococcus, and Lactobacillus, respectively. The counts for bifidobacteria among the tested samples ranged from 0.00 to 5.00 Log<sub>10</sub>CFU/ml. Of the 58 products claiming the inclusion of Bifidobacteria in their products, only 44 (75.9 %) contained viable cultures. The β-galactosidase activity for bifidobacteria isolates ranged between 200 and 500 Miller units. One strain showed antimicrobial activity against *E. coli* 0157:H7. The PCR fingerprinting procedure indicated that bifidobacteria isolates were closely related. Regulation on viable probiotic bacterial counts should be more restricted to ensure that products deliver sufficient amount of viable bifidobacteria.

**Key Words:** Bifidobacteria, Yogurt, Viability