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SYMPOSIA AND ORAL SESSIONS

Late-Breaking Original Research

LB1 Identifying new biomarkers in liver for monitoring physiological imbalance and the response to feed restriction for cows during early lactation. K. L. Ingvartsen, E. Bendixen, M. C. Codrea, and K. M. Moyes*, *Aarhus University*, *Tjele, Denmark*.

The aim of this study was to identify new biomarkers in liver for monitoring physiological imbalance and to examine the different coping strategies used during feed restriction (FR) between cows in physiological imbalance and cows metabolically "normal" during early lactation. Twenty-two cows (22–55 DIM) were fed a standard TMR for ad libitum intake. After 5-d, all cows were FR to provide ~40% of NE_L requirements based on body weight, milk production and composition by supplementing the standard TMR with 60% wheat straw. After 4-d of FR, cows returned to full feed. Liver biopsies were collected −1 and 3 d relative to FR. Prior to FR, an index of degree of imbalance was calculated for all cows based on plasma fatty acid, ketone, and glucose concentration. A subset of 6 cows classified as having either the greatest (n = 3) or least (n = 3) degree of imbalance were used for iTRAQ-based quantitative profiling in liver using LC/MS/MS. Prior to FR, 8 proteins were differentially expressed ($P \leq 0.05$) due to physiological imbalance. Proteins upregulated (6) were involved in amino acid metabolism, gluconeogenesis, and β-oxidation of fatty acids. Feed restriction resulted in 20 proteins differentially expressed between cows in physiological imbalance and cows metabolically ′normal′. Upregulated proteins (8) were involved in β-oxidation of fatty acids and gluconeogenesis whereas downregulated proteins (12) were involved in antioxidant defense and glycine metabolism. This study is the first to identify new biomarkers in liver based on a physiological imbalance index and provide a better understanding of the differences in coping strategies used during FR for cows in physiological imbalance. Screening for relevant markers in more accessible samples (i.e., blood and milk) will help farmers identify cows at risk. Results provide new avenues for future management strategies relating to maintaining animal health and productivity during early lactation.

Key Words: physiological imbalance, cow, proteomics

LB2 A novel nonsense mutation of the *DMP1* gene is responsible for inherited rickets in Corriedale sheep. X. Zhao[1], K. E. Dittmer[2], S. Onteru[1], H. T. Blair[2], K. G. Thompson[2], M. F. Rothschild[1], and D. J. Garrick*[1,2], [1]*Iowa State University*, *Ames,* [2]*Massey University*, *Palmerston North, New Zealand*.

Inherited rickets of Corriedale sheep is a recently discovered skeletal disease of unknown prevalence characterized by decreased growth rate, thoracic lordosis and angular limb deformities. Previous outcross and backcross studies suggest it is a simple autosomal recessively inherited disorder. A genome-wide association study was conducted using the Illumina OvineSNP50 BeadChip on 17 sheep diagnosed as affected and an additional 3 known carriers descended from 1 carrier ram. Genomic regions showing association were scrutinized for possible candidate genes that were sequenced for causal polymorphisms concordant with disease status. A homozygous region of 199 consecutive single-nucleotide polymorphism (SNP) loci was identified in all the affected sheep, covering a region of 10 Mbp on ovine chromosome 6. Among 91 candidate genes in this region, exon 6 of the dentin matrix protein 1 (*DMP1*) gene was sequenced using DNA from the 3 carriers to reveal 10 novel SNPs including a nonsense mutation 253T/C. This T/C transition introduced a stop codon (R145X) that could truncate C-terminal amino acids. Genotyping by PCR-RFLP (restriction fragment length polymorphism) for this mutation showed that, all 17 affected sheep were "TT" genotypes and the 27 phenotypically normal sheep were either "CT" or "CC." This locus is not in complete linkage disequilibrium with the other 9 SNPs that can all be ruled out as candidates. Previous research has shown that mutations in *DMP1*gene are responsible for autosomal recessive hypophosphatemic rickets in humans. *Dmp1*_knockout mice also exhibit rickets phenotypes. We believe the *DMP1*_exon6_235T/C mutation to be responsible for the inherited rickets found in Corriedale sheep. A simple diagnostic test can be designed to identify carriers with the defective "T" alleles. Affected sheep could be used as animal models for this form of human rickets, and for further investigation of the role of DMP1 in phosphate homeostasis.

Key Words: rickets, DMP1, genome wide association

LB3 Association of polymorphisms in GPAT4 and SLC27A6 genes with bovine milk fat percentage and fatty acid composition. R. A. Nafikov*[1], J. P. Schoonmaker[2], K. T. Korn[2], K. Noack[1], D. J. Garrick[1], K. J. Koehler[1], J. Minick-Bormann[3], J. M. Reecy[1], D. E. Spurlock[1], and D. C. Beitz[1], [1]*Iowa State University*, *Ames,* [2]*Purdue University*, *West Lafayette, IN,* [3]*Kansas State University*, *Manhattan*.

The purpose of our study was to discover genetic polymorphisms to select animals producing milk with healthier fatty acid composition. The glycerol-3-phosphate acyltransferase-4 (GPAT4) and solute carrier family 27, isoform A6 (SLC27A6) involved in milk triacylglycerol biosynthesis and fatty acid transport into the mammary epithelial cells were candidate genes. We hypothesized that polymorphisms in GPAT4 and SLC27A6 will affect selectivity of fatty acid acylation onto glycerol-3-phosphate and of fatty acid uptake into the mammary epithelial cells, leading to variations in milk fatty acid composition. To test this hypothesis, milk samples were collected monthly over a 305-d lactation from 500 cows and analyzed for fatty acid composition by gas chromatography. After discovering single nucleotide polymorphisms (SNPs) in the genes of interest and genotyping animals for those SNPs, intragenic haplotypes were reconstructed and tested for associations with milk fat percentage and fatty acid composition by linear mixed models. Results showed that the haplotype effect of GPAT4 was associated significantly with concentrations of capric (10:0), lauric (12:0), palmitic (16:0), and oleic (18:1c9) acids, saturated fatty acids (SFA), unsaturated fatty acids (UFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and SFA/UFA. The haplotype effect of SLC27A6 was associated significantly with percentage of milk fat, concentrations of capric, lauric, myristic (14:0), and palmitic acids, SFA, UFA, MUFA, and SFA/UFA. The size of effects for some of the traits was numerically the same or greater than allelic effects of well known diacylglycerol

acyltransferase-1 (DGAT1) A232K mutation. Results of our study provide opportunity for selection for cows with healthier milk.

Key Words: milk fat percentage, milk fatty acid composition, SNP

LB4 Renneting properties of milk containing high molecular weight oat β-glucan. N. Sharafbafi*[1], S. M. Tosh[2], M. Alexander[1], and M. Corredig[1], [1]*Department of Food Science, University of Guelph, Guelph, Ontario, Canada*, [2]*Agriculture and Agri-Food Canada, Guelph Food Research Center, Guelph, Ontario, Canada*.

The effect of concentration and molecular structure of high molecular weight oat β–Glucan (BG) on renneting properties of concentrated skim milk gels were investigated. Incorporation of BG (0.15, 0.3, 0.6, and 0.9% w/w in permeate) into twice-concentrated skim milk resulted in bulk phase separation of gelling systems as shown by reduction in turbidity parameter (1/l*) and diffusion coefficient using diffusing wave spectroscopy (DWS). However, by controlling the kinetics of gelation and phase separation (using shear in renneted milk before BG addition), it was possible to create casein networks with entrapped BG. $CaCl_2$ was added to reduce time of gelation (TG), and increase textural properties, where high concentrations of BG (≥0.6%) resulted in weakening of protein gel network. The effects of shear and the presence of $CaCl_2$ on microstructure and rheological behavior of the renneted gels containing BG were monitored using DWS combined with small deformation rheology. In addition, BG distribution in the rennet gel network was observed by differential staining technique (Calcofluor and Rhodamine B, for BG and protein, respectively) using confocal laser scanning microscopy. The results showed that the onset of protein interactions, illustrated by the turbidity parameter, and the aggregation point, measured by the diffusion coefficient, were strongly affected by increase in BG concentration. BG-containing gels exhibited a significantly lower elastic modulus (G′) compared with their control counterparts ($P \leq 0.05$). Increase in BG concentration delayed the TG, and reduced gel firmness due to the non-interacting nature of the BG polymer. In contrast, the addition of $CaCl_2$ reduced the TG and produced a firmer gel in both control and BG added samples. BG addition could improve the texture and nutritional value of calorie-reduced cheeses, whose hard texture has traditionally been a barrier to their production.

Key Words: β-glucan, concentrated milk, renneting

LB5 Genome-wide association of a novel porcine stress-syndrome and isoflurane sensitivity to dystrophin. D. J. Nonneman*, T. M. Brown-Brandl, S. A. Jones, and G. A. Rohrer, *USDA, ARS, US Meat Animal Research Center, Clay Center, NE*.

Losses of slaughter-weight pigs due to transport stress are an economic concern to pork producers. Historically, the HAL-1843 mutation in the ryanodine receptor 1 gene was considered responsible for most of the losses; however, DNA testing has effectively eliminated this mutation from commercial herds. We identified 2 sibling barrows in the USMARC swine herd that died after transport to a research location at 8 weeks of age. The original mating was repeated along with sire-daughter matings to produce additional offspring. Pigs were challenged with isoflurane anesthesia (3% for 3 min) at 8 weeks. Heart rate and ECG were monitored during anesthesia and blood was collected one week before challenge and immediately after isoflurane administration. Four males from the original sire-dam mating and 2 males from a sire-daughter mating died after one minute of anesthesia. Animals from 6 other litters were identified as having a stress response, sometimes resulting in death, during regular processing and weighing. Their littermates were also challenged with isoflurane. Affected animals tended to have elevated plasma creatine phosphokinase (CPK) levels and cardiac arrhythmias, as determined by ECG. Pedigrees containing 58 pigs including 14 affected animals were genotyped with the Illumina Porcine 60K SNP Beadchip and 2 chromosomal regions were significantly associated with the syndrome at the genome-wide level. One is on SSC14 at position 82.7 to 88.9 Mbp, and the other is on SSCX at 25.1 to 27.7 Mbp over the dystrophin gene. In addition to muscular dystrophies, mutations in human dystrophin can cause dilated cardiomyopathy, rhabdomyolysis, and a malignant hyperthermia-like reaction in response to inhaled anesthesia, which supports this locus as a cause for the observed phenotypes in pigs. The identification of the causative mutation in these families will allow investigation of the prevalence of this disease in commercial populations.

Key Words: swine, stress, genotyping

LB6 Duration of maternal undernutrition differentially alters fetal development and metabolism in twin sheep pregnancies. M. Field*, R. Anthony, T. Engle, S. Archibeque, and H. Han, *Colorado State University, Fort Collins*.

Maternal undernutrition is known to impact fetal development and predispose the offspring to the metabolic syndrome later in life. We examined the impact of maternal undernutrition during early- to mid-gestation or from early gestation until near term on twin sheep pregnancies. Multiparous whiteface ewes (n = 19) were randomly assigned to one of 3 treatments beginning on 28 d of gestational age (dGA). Ewes were either fed 100% (Control; n = 7), or 50% of nutrient requirements from 28 to 78 dGA and readjusted to 100% beginning at 79 dGA (50-100; n = 5) or 50% of requirements from 28 to 110 dGA, followed by a 5% increase at 5 d intervals until 135 dGA (50-50; n = 7). Body weight of the Control (4.3 kg) fetuses were intermediate between the 50-50 (4.1 kg) and 50-100 (4.6 kg; $P < 0.05$). Organ weights differed between 50-50 and 50-100 fetuses ($P < 0.05$). Uterine artery glucose concentrations did not differ ($P = 0.35$), but 50-100 dams (98.8 mg/dL) were numerically higher than Control (76.6 mg/dL) and 50-50 (81.2 mg/dL) dams. The uterine artery:umbilical vein glucose gradient was numerically higher in 50-100 (50-100 = 65.5 mg/dL; Control = 52.3mg/dL; 50-50 = 53.2 mg/dL). The same trend was noted in umbilical vein glucose (50-100 = 34.4mg/dL; Control = 27.7 mg/dL; 50-50 = 25.7 mg/dL; $P = 0.23$). Interestingly, the umbilical vein:artery glucose gradient was less ($P < 0.05$) in 50-50 (4.68 mg/dL) fetuses compared with Control (6.84 mg/dL) and 50-100 (7.02mg/dL) fetuses. A similar trend was seen with umbilical vein:artery differences in O_2 content (50-100 = 4.43 m*M*; Control = 5.02 m*M*; 50-50 = 3.41 m*M*; $P = 0.10$). In conclusion, lower glucose and O_2 content gradients in 50-50 fetuses suggest lower metabolic rates resulting from long-term maternal diet restriction, while mid gestation realimentation may program maternal and placental glucose metabolism, providing for accelerated fetal growth.
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Key Words: undernutrition, pregnancy, sheep

LB7 Bovine mammary stem cells: Transcriptome profiling and the stem cell niche. R. K. Choudhary*[1], R. W. Li[2], C. M. Evock-Clover[2], and A. V. Capuco[2,1], [1]*Department of Animal and Avian Sciences, University of Maryland, College Park,* [2]*Bovine Functional Genomics Laboratory, USDA-ARS, Beltsville, MD*.

Identification and transcriptome analysis of mammary stem cells (MaSC) are important steps toward understanding the molecular basis of mammary epithelial growth, homeostasis and tissue repair. Our objective was to evaluate the molecular profiles of 4 categories of cells within

the bovine mammary epithelium, 2 subpopulations of putative stem cells and 2 subpopulations of control cells, with the goal of localizing and characterizing MaSC in situ. Putative MaSC were identified based upon their ability to retain the thymidine analog, bromodeoxyuridine (BrdU), for an extended period. Five Holstein calves were injected with BrdU, tissue was harvested 45 d later and label retaining epithelial cells (LREC) were identified in mammary cryosections by immunostaining. Using laser microdissection, LREC from basal (LRECb) and embedded (LRECe) layers of mammary epithelium were isolated along with adjacent control epithelial cells (EC). Cells (6–13) in each category per heifer were lysed, cDNA synthesized, amplified and labeled for microarray hybridization. Data analysis revealed 592 differentially expressed genes ($P \leq 0.05$; ≥2-fold change) between LRECb and basal EC, and 110 genes between LRECe and their embedded EC. Of these, 387 genes with enriched expression in LRECb were involved in cell growth and proliferation, cell cycle, and post-translational modifications. Low expression of estrogen receptor-β and high expression of aldehyde dehydrogenase 3B1 in LRECb were consistent with stem cell character. We found high expression of *NR5A2* (pluripotency transcription factor) and no expression of *XIST* (X-chromosome inactivation factor) in LRECb. Comparison between LRECb and LRECe showed downregulation of cell survival and proliferation factors (*IGF2, HSPB6, LAMC1*), nestin (stem cell marker), epigenetic modifiers (*JRID2, METTL33, SMARCC2*), and upregulation of apoptotic genes (*SFRS5, THAP3*) and *XIST* in LRECe. We conclude that BrdU label retention identifies stem and progenitor cells, wherein MaSC (LRECb) are located in the basal region of the mammary epithelium and committed progenitor cells (LRECe) are localized in more apical layers.

Key Words: mammary stem cell, progenitor, microarray

LB8 First metagenomic analysis of the rumen microbiome of dairy cows with subacute ruminal acidosis and identification of specific *Escherichia coli* virulence factors. E. Khafipour*[1], A. C. Little[1], N. C. Berard[1], P. C. Aikman[2], S. Li[1], S. E. Dowd[3], J. C. Plaizier[1], and D. O. Krause[1], [1]*University of Manitoba*, *Winnipeg, MB, Canada*, [2]*University of Reading*, *Earley Gate, Reading, United Kingdom*, [3]*Medical Biofilm Research Institute*, *Lubbock, TX*.

Subacute ruminal acidosis (SARA) is a metabolic disease in high-producing dairy cattle characterized by low rumen pH, reduced feed intake, lower milk fat, and systemic inflammation. We reproduced similar low rumen pH conditions of SARA with an alfalfa-pellet or a high-grain diet, but only the high-grain fed animals showed symptoms of SARA other than rumen pH depression. To further investigate rumen dynamics rumen DNA samples were subjected to next generation high-throughput pyrosequencing of 16S rRNA. A total of 81,416 sequences were generated of which 39,599 were unique. Sequences were converted to categorical data using a standard taxonomy by comparing the sequences to 16S rRNA databases. A negative binomial distribution was fitted to the data and analyzed using generalized linear mixed-model methodology (GLIMMIX) of SAS for categorical data. A total of 16 phyla were represented in the data set, with *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* forming the most dominant phyla. Loss of *Bacteroidetes* was associated ($P < 0.05$) with grain-induced SARA suggesting that members of this phyla (*Prevotella* and *Bacteroides*) have a protective role in the rumen. Gram-negative *Proteobacteria* were associated ($P < 0.05$) with grain-induced SARA. To further evaluate the role of *Proteobacteria* we isolated *Escherichia coli* from rumen samples and performed PCR analysis on 25 *E. coli* virulence factors. *E. coli* were at least 3-logs ($P < 0.05$) higher in grain-induced SARA and curli fibers were highly associated ($P < 0.01$) with *E. coli* from grain induced, but not alfalfa pellet induced SARA. Curli fibers were first identified in *E. coli* isolated from bovine mastitis and attack tight junctions between cells potentially increasing permeability of the rumen epithelium to antigens. This is the most comprehensive metagenomic study of the rumen and the first in which specific virulence factors have been identified that potentially explain the etiology of SARA.

Key Words: subacute ruminal acidosis, rumen microbiome, *Escherichia coli*