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The effect of calving in the summer on the hepatic transcriptome of Holstein cows during the peripartal period

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ABSTRACT

The liver is the main metabolic organ coordinating the adaptations that take place during the peripartal period of dairy cows. A successful transition into lactation, rather than management practices alone, depends on environmental factors such as temperature, season of parturition, and photoperiod. Therefore, we analyzed the effect of calving season on the hepatic transcriptome of dairy cows during the transition period. A total of 12 Holstein dairy cows were assigned into 2 groups based on calving season (6 cows March–April, spring; 6 cows June–July, summer, SU). The RNA was extracted from liver samples obtained at −30, 3, and 35 DIM via percutaneous biopsy and hybridized to the Agilent 44K Bovine (V2) Gene Expression Microarray (Agilent Technologies Inc., Santa Clara, CA). A quantitative PCR on 22 target genes was performed to verify and expand the analyses. A total of 4,307 differentially expressed genes were detected (false discovery rate $\leq$0.05) in SU compared with spring. Furthermore, 73 unique differentially expressed genes were detected in SU compared with spring cows after applying a fold-change threshold $\geq$3 and $\leq$−3. For Kyoto Encyclopedia of Genes and Genomes pathways analysis of differentially expressed genes, we used the dynamic impact approach. Ingenuity Pathway Analysis software was used to analyze upstream transcription regulators and perform gene network analysis. Among metabolic pathways, energy metabolism from lipids, carbohydrates, and amino acids was strongly affected by calving in SU, with a reduced level of fatty acid synthesis, oxidation, re-esterification, and synthesis of lipoproteins, leading to hepatic lipidosis. Glycan-synthesis was downregulated in SU cows probably as a mechanism to counteract the progression of this lipidosis. In contrast, calving in the SU resulted in upregulation of gluconeogenesis but also greater use of glucose as an energy source. Among nonmetabolic pathways, the heat-shock response was obviously activated in SU cows but was also associated with inflammatory and intracellular stress response. Furthermore, data support a recent finding that cows experience endoplasmic reticulum stress around parturition. Transcription regulator analysis revealed how metabolic changes are related to important regulatory mechanisms, including epigenetic modification. The holistic analyses of the liver transcriptome response to calving in the summer at high environmental temperatures underscore how transition cows should be carefully managed during this period, as they experience alterations in liver energy metabolism and inflammatory state increasing susceptibility to health disorders in early postpartum.

Key words: heat stress, lactation, parturition, bioinformatics

INTRODUCTION

During the transition period, several environmental factors including heat stress, photoperiod, and nutritional management affect the health and production efficiency of dairy cows (Dahl et al., 2000; do Amaral et al., 2009). The intensity of heat stress during dry period and lactation can reduce voluntary DMI and negatively affect milk production (do Amaral et al., 2011). Despite the reduced DMI, body fat mobilization does not increase during heat stress, partly due to a reduction in lipolysis and alterations in insulin sensitivity (Baumgard and Rhoads, 2013). The immune system of heat-stressed cows also is compromised (Lacetera et al., 2006) due in part to alterations in gene expression including upregulation of proinflammatory cytokines (Tao et al., 2013). Another well-studied component of cellular responses to heat stress are the heat-shock proteins, which appear to play a major role in eliciting

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immune responses under increased environmental stress in rodents (Campisi et al., 2003) and cows (Catalani et al., 2010).

Although there is some evidence from target-gene analysis that heat stress alters metabolic mRNA expression in liver (do Amaral et al., 2011), it is unknown whether and to what extent other signaling pathways might be affected. For instance, it is well established that the plane of energy nutrition during the dry period can influence the hepatic transcriptome and alter tissue function (Loor et al., 2013; Shahzad et al., 2014). Because of the well-established decrease in DMI induced by heat stress, it is likely that cows calving in the summer (**SU**) compared with spring (**SP**) would experience more pronounced decreases in DMI leading to consequent changes in the hepatic transcriptome. Thus, the main objective of this study was to evaluate the effect of calving season on hepatic molecular adaptations in peripartal cows. Furthermore, we sought to use bioinformatics approaches to uncover novel signaling pathways and gene networks that are affected.

MATERIALS AND METHODS

Experimental Design and Liver Biopsies

Complete details of the experimental design are available in the Supplemental Materials and Supplemental Table S1 (http://dx.doi.org/10.3168/jds.2015-9409). Briefly, 12 Holstein dairy cows (6 cows March–April, spring, SP; 6 cows June–July, summer, SU) were used for transcriptomics. Those are a subset of 24 Holstein dairy cows, of which 12 calved in spring and 12 calved in the summer. Mean temperature–humidity indices for SP (day: 66.4 ± 3.8, night: 56.3 ± 3.0) and SU (day: 79.5 ± 2.9, night: 70.1 ± 4.7) were recorded. At the time of calving, no clinical health problems were observed in cows, and no cow received any treatment for metabolic problems (Basiricò et al., 2011). Liver tissue was harvested via percutaneous biopsy at −30 (±2), 3, and 35 d relative to parturition. The biopsies were performed under local anesthesia, and tissue samples were first frozen in liquid nitrogen and then stored at −80°C until real-time PCR and microarray analysis. Blood samples were harvested at 0800 h from the jugular vein at −30, −18, and −4 d prepartum (with a final ±2 d window variation) and 3, 17, and 35 d postpartum. Milk production was recorded weekly by automatic recorders.

RNA Extraction

The RNA was extracted from frozen liver tissues using QIAzol Lysis reagent (Qiagen, Chatsworth, CA) and following the manufacturer's protocol. During the procedure, the homogenate was separated into aqueous and organic phases by centrifugation. The RNA was precipitated from the aqueous phase by addition of isopropanol. The isolated RNA was resuspended in DNase-free water and stored at −80°C until quantitative PCR (**qPCR**) and microarrays. The RNA quality evaluated via RNA integrity number in the Agilent Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA) was greater than 6.5 for all samples.

Microarrays

cRNA Synthesis, Labeling, and Purification. The microarray experiment was conducted using the 44K-Agilent bovine (V2) gene expression microarray chips (Agilent Technologies Inc.; cat# G2519F-023647). The methods used for labeling and hybridization were those outlined by Agilent Technologies. Briefly, a total of 200 ng of RNA per sample was used to generate first-strand cDNA, which was subsequently reverse transcribed to cRNA using a low-input quick amp labeling kit (Agilent Technologies Inc.; cat# 5190–2306). The resulting cRNA was labeled with either Cy3 or Cy5 fluorescent dye according to the manufacturer's instructions. Purification of the labeled cRNA product was performed with RNeasy mini spin columns (Qiagen, cat# 74104), and it was subsequently eluted in 30 μL of DNase-RNase-free water. The eluted labeled cRNA was quantified in a NanoDrop ND-1000 (Thermo Scientific, Thermo Fisher Scientific Inc., Waltham, MA) to confirm the manufacturer's recommended criteria for yield and specific activity of at least 0.825 μg and ≥6.

Fragmentation and Slide Scanning. The labeled cRNA was fragmented and then hybridized to the microarray slide following the manufacturer's protocol. Briefly, 825 ng of Cy3 and Cy5 labeled cRNA sample were combined; mixed with 11 μL of 10X Blocking Agent (Agilent Technologies Inc.; cat# 5188–5281), 2.2 μL of 25× Fragmentation Buffer (Agilent Technologies Inc.; cat# 5185–5974), and nuclease-free water (to a final volume of 55 μL); and fragmented at 60°C for 30 s. The reaction was then stopped by adding 55 μL of 2× GEx Hybridization Buffer (Agilent Technologies Inc.; cat# 5190–0403), and the samples were loaded onto the slide. These were hybridized in a rotating hybridization oven at 65°C for 17 h. The slides were washed according to the procedures recommended by the manufacturer and scanned using a GenePix 4000B scanner (Axon Instruments Inc., Sunnyvale, CA) and GenePix Pro v.6.1 software. Resulting spots where features were substandard were flagged as bad and excluded from subsequent analysis.