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Body condition score at calving affects systemic and hepatic transcriptome indicators of inflammation and nutrient metabolism in grazing dairy cows

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ABSTRACT

Calving body condition score (BCS) is an important determinant of early-lactation dry matter intake, milk yield, and disease incidence. The current study investigated the metabolic and molecular changes induced by the change in BCS. A group of cows of mixed age and breed were managed from the second half of the previous lactation to achieve mean group BCS (10-point scale) that were high (HBCS, 5.5; n = 20), medium (MBCS, 4.5; n = 18), or low (LBCS, 3.5; n = 19). Blood was sampled at wk −4, −3, −2, 1, 3, 5, and 6 relative to parturition to measure biomarkers of energy balance, inflammation, and liver function. Liver was biopsied on wk 1, 3, and 5 relative to parturition, and 10 cows per BCS group were used for transcript profiling via quantitative PCR. Cows in HBCS and MBCS produced more milk and had greater concentrations of nonesterified fatty acids and β-hydroxybutyrate postpartum than LBCS. Peak concentrations of nonesterified fatty acids and β-hydroxybutyrate and greater hepatic triacylglycerol concentrations were recorded in HBCS at wk 3. Consistent with blood biomarkers, HBCS and MBCS had greater expression of genes associated with fatty acid oxidation (*CPT1A, ACOX1*), ketogenesis (*HMGCS2*), and hepatokines (*FGF21, ANGPTL4*), whereas HBCS had the lowest expression of *APOB* (lipoprotein transport). Greater expression during early lactation of *BBOX1* in MBCS and LBCS suggested greater de novo carnitine synthesis. The greater BCS was associated with lower expression of growth hormone/insulin-like growth factor-1 signaling axis genes (*GHR1A*, *IGF1*, and *IGFALS*) and greater expression of gluconeogenic genes. These likely contributed to the higher milk production and greater gluconeogenesis. Despite greater serum haptoglobin around calving, cows in HBCS and MBCS had greater blood albumin. Cows in MBCS, however, had a higher albumin:globulin ratio, probably indicating a less pronounced inflammatory status and better liver function. The marked decrease in expression of *NFKB1*, *STAT3*, *HP*, and *SAA3* coupled with the increase in *ALB* on wk 3 in MBCS cows were consistent with blood measures. Overall, results suggest that the greater milk production of cows with higher calving BCS is associated with a proinflammatory response without negatively affecting expression of genes related to metabolism and the growth hormone/insulin-like growth factor-1 axis. Results highlight the sensitivity of indicators of metabolic health and inflammatory state to subtle changes in calving BCS and, collectively, indicate a suboptimal health status in cows calving at either BCS 3.5 or 5.5 relative to BCS 4.5.

Key words: body condition score, liver, grazing dairy cow, gene expression

INTRODUCTION

In dairy management systems, BCS is used as an indicator of body fat content and cow nutritional status. Cows should be managed to achieve appropriate BCS both pre- and postpartum to reduce threats to welfare, because BCS at calving may affect early lactation DMI, postcalving BCS loss, milk yield, cow immunity, and fertility (Roche et al., 2009). At calving, DMI and BCS are negatively correlated (Hayirli et al., 2002; Matthews et al., 2012), so that "fat" cows undergo a more pronounced and prolonged depression in DMI, leading to a deeper negative energy balance (**NEB**; Agenäs et al., 2003; Hayirli et al., 2002), an increase in lipomobilization, and a greater and persistent increase in blood NEFA (Dann et al., 2006). When hepatic uptake of NEFA exceeds the normal oxidative or export [as very low density lipoproteins (**VLDL**)] capacity, a greater production of ketone bodies, including BHBA (Drackley, 1999), will occur, leading to ketosis (Gillund

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et al. 2001). Furthermore, if the rate of esterification exceeds the rate of triacylglycerol (**TAG**) export via lipoproteins, fatty liver may develop (Drackley, 1999), thereby increasing susceptibility to other pathologies. Loss of BCS around parturition also is associated with the incidence of milk fever (Roche and Berry, 2006) and displaced abomasum (Bewley, 2008), 2 important metabolic disorders.

Although a recent review of the literature reported that not only overconditioned but also underconditioned dairy cows have a greater incidence of diseases than animals with a normal BCS (Roche et al., 2009), there are no reports on how calving BCS affects molecular signaling patterns in hepatic tissue during early lactation or their association with hepatic indicators of energy balance, liver function, and inflammation. It is likely that calving BCS affects the inflammatory response and liver function partly through alterations in gene expression. Thus, the objective of the present study was to profile the mRNA expression of key metabolic genes associated with fatty acid oxidation, ketogenesis, lipoprotein export, carnitine metabolism, gluconeogenesis, inflammation, and oxidative stress in liver biopsies harvested from cows with high (**HBCS**), medium (**MBCS**), and low (**LBCS**) BCS at calving (Roche et al., 2013). To better appreciate the links between BCS and systemic biomarkers, these data were complemented with liver TAG concentration and concentrations of blood biomarkers of energy balance, liver function, and inflammation.

MATERIALS AND METHODS

Animals, Management, and Sampling

Details of the project management were reported recently (Roche et al., 2013). Briefly, a group of 60 cows was allocated to 1 of 3 groups that underwent nutritional management through late lactation to achieve different BCS targets by the end of lactation [BCS targets were 5.0, 4.0, and 3.0, for HBCS, MBCS, and LBCS, respectively: 10-point system, where 1 is emaciated and 10 obese; Roche et al., 2004]. During the dry period, these cows were fed a ration that allowed for fetal growth and for the cows to gain 0.5 BCS unit before calving. The final targets for mean calving BCS were 5.5, 4.5, and 3.5 for the HBCS, MBCS, and LBCS groups, respectively.

Blood samples were obtained on −4, −3, and −2 wk precalving and 1, 3, 5, and 6 wk postcalving by coccygeal venipuncture into evacuated tubes that contained heparin, EDTA, or no anticoagulant (Vacutainer, Becton Dickinson, Franklin Lakes, NJ). Samples containing heparin anticoagulant were placed immediately into iced water and then centrifuged within 60 min of collection to obtain plasma. Samples without anticoagulant were held at ambient temperature following collection for at least 4 h in order for serum to separate. Details of analyses are included in the supplemental materials (http://dx.doi.org/10.3168/jds.2014-8584). Briefly, serum was used to measure albumin (**ALB**), globulin (**GLO**, as the difference between total protein and albumin), creatinine, aspartate aminotransferase (**AST**), glutamate dehydrogenase (**GDH**), γ-glutamyl transferase (**GGT**), haptoglobin (**HP**), and serum amyloid A (**SAA**). Plasma was used to measure NEFA, BHBA, and glucose.

Liver Biopsy

Liver biopsy was attempted from all cows during the week before calving (−1 wk), and subsequently in 1, 3, and 5 wk. Briefly, the skin was shaved and disinfected, and the area through the skin and body wall was anesthetized with 7 mL of 2% lignocaine (Lopaine 2%, lignocaine hydrochloride 20 mg/mL, Ethical Agents, South Auckland, New Zealand). A stab incision was made through the skin in the right 11th intercostal space at the level of the greater trochanter through which a 12-gauge × 20-cm biopsy needle was inserted into the liver and approximately 1 g (wet weight) of liver tissue was collected. Samples were snap-frozen in liquid nitrogen and subsequently stored at −80°C.

Liver TAG Analysis

Liver TAG content was analyzed in biopsy samples (−1, 1, 3, and 5 wk) from the same 10 cows used for gene expression and blood biomarker analyses by modifying the procedure described in the Wako LabAssay Triglyceride Kit (Wako Chemicals USA Inc., Richmond, VA). Briefly, approximately 100 mg of liver tissue was finely chopped using a sterile scalpel blade, and homogenized in 1 mL of 5% Triton-X100 in water. The homogenized tissue was slowly heated in a water bath (up to 80–100°C) for 2 to 5 min or until the Triton X-100 appeared cloudy. After cooling the sample at room temperature, the heating process was repeated once to solubilize all TAG into solution. The samples were vortexed and centrifuged at 2,000 × g for 5 min at 4°C to remove any insoluble material. The supernatant was collected in sterile 1.5-mL microfuge tubes and stored at −80°C. Approximately 50 μL of sample was diluted 10-fold with distilled water before the TAG assay. The TAG analysis was performed using the standard procedure provided in the Wako LabAssay Triglyceride Kit.