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Abundance of ruminal bacteria, epithelial gene expression, and systemic biomarkers of metabolism and inflammation are altered during the peripartal period in dairy cows

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ABSTRACT

Seven multiparous Holstein cows with a ruminal fistula were used to investigate the changes in rumen microbiota, gene expression of the ruminal epithelium, and blood biomarkers of metabolism and inflammation during the transition period. Samples of ruminal digesta, biopsies of ruminal epithelium, and blood were obtained during −14 through 28 d in milk (DIM). A total of 35 genes associated with metabolism, transport, inflammation, and signaling were evaluated by quantitative reverse transcription-PCR. Among metabolic-related genes, expression of *HMGCS2* increased gradually from −14 to a peak at 28 DIM, underscoring its central role in epithelial ketogenesis. The decrease of glucose and the increase of nonesterified fatty acids and β-hydroxybutyrate in the blood after calving confirmed the state of negative energy balance. Similarly, increases in bilirubin and decreases in albumin concentrations after calving were indicative of alterations in liver function and inflammation. Despite those systemic signs, lower postpartal expression of *TLR2*, *TLR4*, *CD45*, and *NFKB1* indicated the absence of inflammation within the epithelium. Alternatively, these could reflect an adaptation to react against inducers of the immune system arising in the rumen (e.g., bacterial endotoxins). The downregulation of *RXRA*, *INSR*, and *RPS6KB1* between −14 and 10 DIM indicated a possible increase in insulin resistance. However, the upregulation of *IRS1* during the same time frame could serve to restore sensitivity to insulin of the epithelium as a way to preserve its proliferative capacity. The upregulation of *TGFB1* from −14 and 10 DIM coupled with upregulation of both *EGFR* and *EREG* from 10 to 28 DIM indicated the existence of 2 waves of epithelial proliferation. However, the downregulation of *TGFBR1* from −14 through 28 DIM indicated some degree of cell proliferation arrest. The downregulation of *OCLN* and *TJP1* from −14 to 10 DIM indicated a loss of tight-junction integrity. The gradual upregulation of membrane transporters *MCT1* and *UTB* to peak levels at 28 DIM reflected the higher intake and fermentability of the lactation diet. In addition, those changes in the diet after calving resulted in an increase of butyrate and a decrease of ruminal pH and acetate, which partly explain the increase of *Anaerovibrio lipolytica*, *Prevotella bryantii*, and *Megasphaera elsdenii* and the decrease of fibrolytic bacteria (*Fibrobacter succinogenes*, *Butyrivibrio proteoclasticus*). Overall, these multitier changes revealed important features associated with the transition into lactation. Alterations in ruminal epithelium gene expression could be driven by nutrient intake–induced changes in microbes; microbial metabolism; and the systemic metabolic, hormonal, and immune changes. Understanding causes and mechanisms driving the interaction among ruminal bacteria and host immunometabolic responses merits further study.

Key words: gene expression, microbiome, transition cow

INTRODUCTION

The rumen microbial ecosystem is complex and essential for rapid degradation of feedstuffs and production of VFA and microbial protein for use by the cow. Several factors, including nutritional management, can induce modifications of the microbial population (Hernandez-Sanabria et al., 2012). Previous experiments detected changes in microbial populations around parturition, presumably a response to the change in diet from pre- to postpartum (Wang et al., 2012; Pitta et al., 2014; Lima et al., 2015). However, some evidence shows that the core ruminal microbiome is very stable even when cows are switched from a high-forage to a high-concentrate diet (Petri et al., 2013).

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Changes in microbial populations and animal metabolism may affect ruminal epithelial adaptations and nutrient absorption during the transition from late pregnancy to lactation. For instance, inducing SARA by feeding high-concentrate diets or alfalfa pellets resulted in similar decreases in pH and LPS endotoxin concentrations, but treatments induced different inflammatory responses, being more pronounced for grain-induced SARA (Khafipour et al., 2009). Bannink et al. (2012) concluded that to maintain normal function, the ruminal epithelia in the peripartal cow respond in a coordinated manner to rapid dietary changes. However, the mechanisms underlying these adaptations are not fully understood.

The enlargement of ruminal absorptive area as a function of dietary increment of concentrate is well established (Bannink et al., 2012). However, evidence also points at mRNA expression and transporter and enzyme activity in the initial response driving epithelial cell function (Penner et al., 2011). Few studies have investigated the molecular adaptations of ruminal epithelium during the peripartum period (Dionissopoulos et al., 2014; Steele et al., 2015). These studies revealed the existence of interactions among genes of the immune system and those involved in the preparation for the onset of lactation (Dionissopoulos et al., 2014), as well as the presence of growth factors that seem to be regulated after parturition (Steele et al., 2015).

The main objective of this study was to evaluate the adaptations in major microbial species and ruminal epithelium expression of key genes associated with metabolism and tissue development, permeability, transport, inflammation, and immunity. Furthermore, ruminal VFA profiles and systemic biomarkers of energy metabolism, inflammation, and oxidative stress in blood were evaluated to enhance the systems understanding that the cow undergoes around parturition.

MATERIALS AND METHODS

Animal and Experimental Design

All procedures were performed under protocols approved by the University of Illinois Institutional Animal Care and Use Committee (protocol #12094). Briefly, 7 rumen fistulated Holstein cows in their second or greater lactation were selected for this study. Cows were managed during the dry period using the 2-stage approach with a high-straw, lower-energy diet fed from dry-off through −21 d from parturition followed by a lower-straw, higher-energy diet until parturition. The details of diet composition are reported in Supplementary Table S1 (http://dx.doi.org/10.3168/jds.2015-9722). At calving, cows were fed a common lactation diet until d 30 postpartum. Diets were fed as a TMR once daily (0600 h) using an individual gate feeding system (American Calan, Northwood, NH) during the dry period or in open individual mangers during lactation. Collection of BW, BCS, and milk production data and sampling for milk composition analysis were as described previously (Graugnard et al., 2012, 2013). Briefly, cow BW and BCS were obtained weekly throughout the study. Cows were milked 3 times daily after parturition, and milk was sampled thrice weekly for analysis of chemical composition.

Details of sampling methodologies and analytical procedures can be found in the Supplementary Materials (http://dx.doi.org/10.3168/jds.2015-9722). The sampling schedule during the experimental period is summarized in Figure 1. Briefly, blood was sampled from the coccygeal vein on d −14, −7, 10, 20, and 28 relative to parturition and before the morning feeding using a 20-gauge BD Vacutainer needle (Becton Dickinson, Franklin Lakes, NJ) into Vacutainers (10 mL, BD Vacutainer, Becton Dickinson) containing lithium heparin. The plasma obtained after centrifugation was used to asses (1) inflammatory response markers: positive acute-phase proteins (haptoglobin, ceruloplasmin) and negative acute-phase proteins (albumin, paraoxonase); (2) liver function biomarkers: total bilirubin, aspartate amino-transferase, γ-glutamyl transferase; (3) energy and protein metabolism: glucose, cholesterol, NEFA, BHBA, urea, and creatinine; (4) minerals: Ca, Mg, and Zn; (5) other parameters: total proteins, globulins, myeloperoxidase, ferric reducing antioxidant power, and alkaline phosphatase. Methods used for the determination of these parameters are described in the Supplementary Materials (http://dx.doi.org/10.3168/jds.2015-9722).

After blood collection, grab samples of ruminal contents were harvested via the ruminal cannula from the ventral sac of the rumen. Ruminal fluid pH was determined immediately with a glass electrode, then the samples were stored at −20°C and used for DNA extraction and VFA composition. Details of storage protocols and analytical procedures are presented in the Supplementary Materials (http://dx.doi.org/10.3168/jds.2015-9722). At −14, 10, and 28 DIM ruminal papillae were biopsied from the ventral sac of the rumen. The excised ruminal papillae (approximately 500 mg) were immediately washed with sterile 0.01 *M* PBS (pH 6.8). The papillae were scraped to remove attached feed particles and rinsed 3 times to remove the nonadherent bacteria. The cleaned tissues were then frozen and stored in liquid nitrogen until RNA extraction. Details about tissue gene expression analyses are described in the Supplementary Materials (http://dx.doi.org/10.3168/jds.2015-9722).