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The periparturient period is associated with structural and transcriptomic adaptations of rumen papillae in dairy cattle

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ABSTRACT

The structural and functional adaption of the rumen epithelium during the transition period is largely undescribed. To characterize the adaptation of the rumen epithelium during transition, multiparous dairy cattle (n = 12) fitted with rumen fistulas and fed a low-energy dry cow diet (1.37 Mcal/kg, net energy for lactation) were transitioned abruptly to a high-energy lactating cow diet (1.68 Mcal/kg, net energy for lactation) immediately after parturition. Rumen papillae were biopsied at −3, +1, and +6 wk relative to calving. The histology of morphology of the rumen papillae was evaluated under the light microscope and electron microscope, and mRNA profiling was performed using an Affymetrix GeneChip Bovine Gene 1.0 ST Array (Affymetrix, Santa Clara, CA). Data preprocessing was conducted using the robust multi-array average method, and detection of significant genes was conducted using ANOVA. Also, the Benjamini-Hochberg false discovery rate of 0.1 was applied. Microscopic examination of rumen papillae revealed an increase in epithelial desquamation during early lactation as sloughing scores increased from 1.7 ± 0.2 at −3 wk to 4.1 ± 0.3 and 3.4 ± 0.2 at +1 and + 6 wk, respectively. A total of 1,011 (−3 vs. +1 wk) and 729 (−3 vs. +6 wk) differentially expressed genes were identified (false discovery rate of 0.10, $P < 10^{-3}$, fold-change ± 1.2 cut-off). A group of differentially expressed genes involved in desmosome assembly (*DSG1*, *CDSN*), epidermal growth factor signaling (*EGFR*, *EREG*), transforming growth factor β signaling (*TGFB1*), and the insulin-like growth factor-axis (*GHR*, *IGFBP2*, *IGFBP3*, *CTGF*) was also validated using PCR. Gene network analysis found that *EGFR*, *GHR*, and *TGFB1* were focal points of the top pathways, thereby supporting the importance of these regulatory genes to the adaptive response of rumen papillae in early lactation. The microscopic and transcriptomic changes in this study provide insight into the mechanisms responsible for the rapid rate of cellular and molecular adaptations of rumen papillae tissue during the transition period in dairy cattle. In conclusion, the experimental data support the hypothesis that rumen papillae adapt in early lactation by altering their gene expression patterns and, thus, their epithelial structure.

Key words: rumen epithelium, structure, gene expression, transition period

INTRODUCTION

It has become common practice in dairy production to abruptly transition cows from a low-energy to high-energy diet at parturition to compensate for the increased energy demand of lactation. The rumen is the focal point of energy digestion and absorption of the ruminant gastrointestinal tract as it is the site of microbial fermentation of carbohydrates to short-chain fatty acids (**SCFA**), which account for over 70% of the total ME available for the cow (Rémond et al., 1995). Short-chain fatty acids are absorbed by the rumen epithelium (**RE**). The RE overlays rumen papillae, the size of which can proliferate resulting in increased RE surface area and potential for nutrient absorption (Gäbel et al., 2002). For example, the RE responds to the increase in microbial fermentation end products by accelerating cellular proliferation to increase the surface area for SCFA absorption (Sakata and Tamate, 1978); as such, the rumen mass increases by 55% between d 14 and 120 of lactation (Baldwin et al., 2004).

Concomitant with its increased mass and nutrient transport capacity, the RE must maintain the integrity of its stratified squamous epithelium to protect the host against the harsh environment of the rumen (Steele et al., 2011a). The mechanisms responsible for these adaptive changes are poorly understood. However, it is known that RE differentiation and proliferation is associated with growth factors of the IGF-axis (Shen et al., 2004; Steele et al., 2011a, 2012), epidermal growth factor family (**EGF**; Penner et al., 2011) and recently

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the transforming growth factor-β family (**TGFB**; Connor et al., 2014). These studies evaluated gene expression of the RE in the developing ruminant or during a high grain challenge model. It would be extremely valuable to understand how the RE responds to the onset of parturition in concert with the abrupt change in dietary energy.

When dairy cows are fed rapidly fermentable dietary carbohydrate in early lactation they are more prone to develop the common digestive tract disorder ruminal acidosis (Plaizier et al., 2008). During the onset of ruminal acidosis, the structural integrity of the RE is compromised (Steele et al., 2011a), which is linked to the differential expression of various structural and SCFA metabolism genes (Penner et al., 2011; Steele et al., 2011b). In particular, genes associated with desmosomes (Steele et al., 2011a) and claudins (Liu et al., 2013) that are responsible for cellular adhesion have been differentially expressed in ruminants during high grain feeding. During ruminal acidosis, the structure of the RE can be compromised causing a pathological state; however, it is not known if similar changes occur due to the concert of dietary, physiologic, and metabolic changes in early lactation.

Despite recent progress in our understanding of how the RE adapts during various transition periods (weaning, high grain diets), the cellular and molecular events coordinating RE adaptations during the onset of lactation remain largely undescribed. Therefore, the primary aim of the study was to characterize the structural and functional genomic adaptations of whole rumen papillae during transition from a pre-partum low-energy diet to a postpartum, early lactation, high-energy diet transition period using microscopy and transcriptomic approaches. The overall hypothesis was that cell types of rumen papillae adapt in early lactation by altering their gene expression patterns and, thus, their structure and morphology. More specifically, it was hypothesized that the structure of the RE would be compromised during early lactation. With regard to gene expression, it was hypothesized that expression of growth factors (IGF-axis, EGF, and TGF) and cell adhesion genes (desmosomal cadherins, claudins) would be modulated during the transition period from relatively low intake of a low-energy prepartum diet to that of high intake of a high-energy diet after parturition and during early lactation.

MATERIALS AND METHODS

Animals, Experimental Treatments, and Feeding

All procedures before the start of the trial were conducted in accordance with the Canadian Council on Animal Care (1993) and approved by the University of Guelph Animal Care Committee. The experiment was previously described by Dionissopoulos et al. (2014), which focused on describing the changes of immune genes in rumen tissue during the transition period. In brief, 12 Holstein dairy cows consisting of one-half primiparous (644 ± 13 kg of BW, mean ± SD) and the other half multiparous (760 ± 10 kg of BW, mean ± SD) were used in this study. Each cow was fitted with a rumen fistula during the dry period before their first or second lactation as described by Duffield (1999), and housed in a tie-stall facility at the Elora Dairy Research Station for the duration of the experiment (University of Guelph, Guelph, Ontario, Canada). Throughout their lactation, the cattle had unlimited access to water, were milked twice daily in their stalls at 0600 and 1600 h, and allowed to exercise for 2 h starting on 0800 h.

The prepartum diet consisted of a TMR fed once daily (0700 h) and the lactating cattle received a lactation TMR twice daily (0700 and 1500 h), both balanced based to meet nutrient guidelines (NRC, 2001). The prepartum diet consisted of 45% corn silage, 11% alfalfa silage, 27% straw, and 18% protein supplement on a DM basis (TMR composition; 45% DM, 13% CP, 31% ADF, 46% NDF, 34% NFC, 18% starch, 1.37 Mcal/kg of NE_L). For the lactating cow diet, the same corn silage (26% DM), alfalfa silage (26% DM), and straw (6% DM) were used with a lactating cow supplement (21% DM) along with additional high-moisture corn to increase energy density of the diet (TMR composition; 45% DM, 16% CP, 23% ADF, 34% NDF, 43% NFC, 22% starch, 1.68 Mcal/kg of NE_L). Immediately after parturition all cows were abruptly transitioned from the dry cow diet to the lactating cow diet. To achieve ad libitum intake for both diets, the amount of offered feed was adjusted daily with the target of having a minimum of 5 kg of feed refusals/day per cow (as-is basis).

Rumen Papillae Biopsies

Rumen papillae were biopsied from each of the 12 cows on 3 different occasions during the periparturient period: at 3 wk prepartum, 1 wk postpartum, and 6 wk postpartum. The papillae were collected from the ventral sac of the rumen as it has been determined to be the site with the highest capillary blood flow per unit weight mucosa of any location within the rumen (Von Engelhardt and Hales, 1977). In brief, the ventral sac was partially evacuated to facilitate the retraction of the ventral sac to the fistula. Sterile surgical scissors were used to clip approximately 200 mg of rumen papillae that were quickly washed 20 times in ice-cold PBS. After washing, papillae were placed in room