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Changes in gene expression in the rumen and colon epithelia during the dry period through lactation of dairy cows and effects of live yeast supplementation

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ABSTRACT

The objectives of this study were (1) to use endoscopy to collect biopsies from the rumen and colon epithelia to describe changes in gene expression in these 2 tissues as cows move from a dry to a lactation ration and (2) to evaluate the potential influence that supplementation of live yeast could exert on these 2 epithelia. Twenty-one Holstein cows were split into 2 treatments and received either 300 g/d of corn containing 1×10^{10} cfu/d of live yeast (LY; n = 10) or 300 g/d of corn with no supplementation (control; n = 11) starting 21 ± 2.6 d (average ± SD) before until 21 d after calving. At 14 ± 2.6 d before the expected calving date, and exactly at 7 and 21 d after calving, rumen and colon biopsies were obtained from each cow using an endoscope. Total RNA was extracted from rumen and colon tissues, and the expression of *IL10*, *TNFA*, *TLR4*, *IL1B*, *PCNA*, *MKI67*, *SGLT1*, *BAX*, *CASP3*, *OCLN*, *CLDN4*, *HSPA1A*, *HSPB1*, *DEFB1*, *and MCT1* (the latter only in rumen samples) was quantified by quantitative PCR. Overall, fluctuations in expression of the selected genes in the colon between the 2 stages of production and the 2 treatments were smaller than those found in the rumen. In the rumen epithelium, expression of *TLR4* and *DEFB1* was greatest before calving, with LY cows having a greater expression of *TLR4* than control cows. Similarly, expression of *IL10* was greatest in LY cows before calving. Expression of *TNFA* in the rumen epithelium of control cows was lowest at 21 DIM but in LY cows was kept steady among production stages. The expression of *PCNA* and *MKI67* in the rumen epithelium was greatest at 7 DIM, indicating a high proliferation rate of this epithelium after calving. In the colon mucosa, expression of *TLR4* and *DEFB1* was greater than in the rumen, and *DEFB1* expression was greater in LY cows than in control cows. The use of an endoscope allowed us to study the dynamics of rumen epithelium adaptation to increased supply of concentrate after calving, consisting of increased epithelia remodeling, reduction of the *TLR4*, and increased *IL10* expression. Furthermore, the rumen epithelium of dry cows responded rapidly to live yeast, with changes in the expression of genes involved in the immune response becoming evident after 7 d of exposure to yeast. The expression of genes related to the immune response (mainly *TLR4* and *DEFB1*) in the colon mucosa was greater than in the rumen, and the expression of *DEFB1* was further stimulated by live yeast. It is concluded that the use of an endoscope allows the study of gene expression patterns in the rumen and hindgut epithelia. We report marked changes in the rumen wall and more modest changes in the colon when transitioning from a dry to a lactation ration. Furthermore, supplementation of live yeast fostered and increased expression of genes regulating inflammation and epithelial barrier in the rumen, and in the colon it increased the expression of *DFEB1* coding for an antimicrobial peptide.
Key words: colon, endoscope, epithelium, rumen

INTRODUCTION

Both the type and amount of feed consumed by lactating and dry cows differ drastically. Dry cows generally receive diets with high inclusion levels of forage and low contents of concentrate, which typically results in slow fermentation rates in the rumen (Dieho et al., 2016). Furthermore, feed intake of dry cows is commonly <13 kg/d (NRC, 2001), and thus digesta passage rate is also slow. On the other hand, right after calving,

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cows are fed rations with low inclusion levels of forage and high proportions of concentrate (NRC, 2001), which commonly have fast fermentation rates (Offner et al., 2003; Martens et al., 2012). In addition, feed intake increases rapidly and progressively after calving, resulting in increased digesta passage rate (NRC, 2001). Furthermore, the type and amount of forage and concentrate that cows consume influence the rumen microbial composition (Nagaraja and Titgemeyer, 2007; Fernando et al., 2010), rumen microbial activity (Bach et al., 1999), and overall rumen environment, especially pH and fermentation profile (Bevans et al., 2005; Brown et al., 2006; Penner et al., 2007), which all in turn can exert an influence on the epithelium of the gastrointestinal tract. In fact, several studies have reported that rumen epithelia in calves (Górka et al., 2011; Castells et al., 2013; Malmuthuge et al., 2013), dry cows (Reynolds et al., 2004), and even in cows during the transition period (Bannink et al., 2012) were affected by the type of diet offered. Most of these studies have focused on microscopic changes (Beharka et al., 1998; Steele et al., 2011b), although some have looked at changes in gene expression (Zhao and Sun, 2010; Steele et al., 2011a,b). However, little information is available about the possible interaction between the type of diet and changes in gene expression in the epithelium of the rumen (Bannink et al., 2012), and, to our knowledge, no information is available about potential changes in the large intestine of dairy cows during the transition period.

Live yeast supplementation in ruminants has been shown to induce changes in rumen pH, microbial population, and fiber fermentation (Chaucheyras-Durand et al., 2008; Terré et al., 2015), and supplementing live yeast during the transition period could represent an effective strategy to prevent rumen dysfunction after calving (Jouany, 2006). Therefore, it is likely that live yeast supplementation, either directly acting on the epithelial cells or indirectly by altering the rumen microbial population or the ruminal environment (i.e., modulating pH), may exert some effects on the integrity and activity of the rumen epithelium. To our knowledge, no study has evaluated the potential effects of live yeast on expression of selected genes in the rumen epithelium and the colon mucosa.

We hypothesized (1) that the epithelia of both rumen and colon experience changes in their barrier structure, nutrient transport, cell division and integrity, and immunological functions as a consequence of the drastic differences in both the amount and the type of rations as cows move from the dry to the lactation period and (2) that these changes may be influenced by live yeast supplementation due to its effects on both rumen pH and rumen microbial composition. Here we used endoscopy to collect biopsies from the rumen and colon epithelia with the objective of describing changes in the expression of genes involved in nutrient transport, epithelium barrier integrity, immune function, cell division and tissue remodeling, and cell integrity in these 2 tissues of dairy cows as they moved from a dry to a lactation ration and evaluate the potential influence of supplementation of live yeast on these 2 epithelia.

MATERIALS AND METHODS

All procedures used herein were conducted under the approval and supervision of the Ethical Committee of Institut de Recerca i Tecnologia Agroalimentàries (Caldes de Montbui, Spain). Twenty-one Holstein cows (706 ± 85 kg of BW) were split into 2 treatment groups and received either 300 g/d of corn containing 3.3 g/kg (equivalent to 1×10^{10} cfu/d) of live yeast (**LY**, n = 10; *Saccharomyces cerevisiae* CNCM I-1077; Lallemand SAS, Blagnac, France) or 300 g/d of corn with no supplementation (control, n = 11) starting 21 d before calving (259 d pregnant) until 21 d of lactation. The dose of 1×10^{10} cfu/d was chosen because this is the amount commonly fed under commercial conditions, the dose registered in the European Union, and the dose shown to have positive effects on animal performance in a previous meta-analysis (de Ondarza et al., 2010). At each production stage (dry or lactating), the 2 groups of cows (control or LY) consumed the same diet with the only difference being the presence or absence of live yeast. Diets were sampled fortnightly and analyzed for DM (method 934.01), ash (method 942.05), N (method 984.13), and ether extract (method 920.39) content following AOAC (1990). Neutral detergent fiber analyses were performed according to Van Soest et al. (1991). The ingredient and nutrient composition of the dry and lactation rations is depicted in Table 1. Dry cows were housed in group pens with ad libitum access to water and feed and bedded with straw. Lactating cows were housed in freestalls, and the 2 treatment groups were kept in the same pen. Individual feed intake of lactating cows was monitored on a daily basis using electronic scales with controlled individual access for each cow to specific feed bins based on the radio frequency identification system described elsewhere (Bach et al., 2004). Treatment was applied at the cow level. Dry cows received the feed as top dressing while headlocked for 30 min during the morning feeding to ensure that the treatment delivered was entirely consumed, and lactating cows received the treatments mixed in the TMR after obtaining permission to access specific feed bins containing the designated treatment.

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