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Evaluation of the systemic innate immune response and metabolic alterations of nonlactating cows with diet-induced subacute ruminal acidosis

J. C. Rodríguez-Lecompte,*†[1] A. D. Kroeker,* A. Ceballos-Márquez,*‡ S. Li,* J. C. Plaizier,* and D. E. Gomez§
*Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada R3T 2N2
†Department of Pathology and Microbiology, University of Prince Edward Island, Charlottetown, PEI, Canada C1A 4P3
‡Departamento de Producción Agropecuaria, Universidad de Caldas, Manizales, Caldas, Colombia
§Department of Health Management, University of Prince Edward Island, Charlottetown, PEI, Canada C1A 4P3

ABSTRACT

Subacute ruminal acidosis (SARA) increases lipopolysaccharide endotoxin in the rumen, which might translocate into the systemic circulation, triggering a cascade of clinical and immunological alterations. The objective of this study was to characterize the clinical immune and metabolic responses to ruminal-derived lipopolysaccharide in nonlactating cows induced with SARA using 2 challenges, a grain-based SARA challenge (GBSC) or an alfalfa-pellet SARA challenge (APSC). Six dry, nonlactating Holstein cows were used in a 3 × 3 Latin square arrangement of treatments with 4-wk experimental cycles. All cows received the control diet containing 70% forage and 30% mixed concentrates (dry matter basis) for 3 wk. In wk 4, cows received a control diet, GBSC (38% wheat-barley pellets, 32% other mixed concentrate, and 30% forages), or APSC (45% mixed concentrate, 32% alfalfa pellets, and 23% other forages). Total plasma proteins and immunology-related proteins, acute phase proteins, blood cells, serum chemistry, mRNA gene expression of peripheral blood cell surface markers, and selected proinflammatory cytokines were evaluated. Ruminal pH was lower in both groups with induced SARA compared with a control group. Ruminal endotoxins were higher in GBSC; however, plasma endotoxin was not detected in any study group. No significant differences in feed intake, rectal temperature, white blood cell counts, or differentials were found between control and SARA challenge groups; changes in glucose, urea, Ca, and Mg were observed in SARA groups. Total plasma proteins were lower in both SARA groups, and acute phase proteins were higher in GBSC. The expression of *CD14*, *MD2*, and *TLR4* mRNA in peripheral blood leukocytes was not affected by SARA induction. The induction of SARA as a result of GBSC or APSC challenge was successful; however, LPS was not detected in plasma. Changes in clinical, metabolic, and inflammatory responses were not observed in the SARA-challenged cows, suggesting that, in this study, SARA was not associated with a systemic response to inflammation.
Key words: rumen, subacute ruminal acidosis, immune response, inflammation, metabolite

INTRODUCTION

Energy demands associated with milk production have increased the addition of grain into diet composition of dairy cows, which has now become a common practice in milk producers in North America. Similar to lactic acidosis, SARA has been associated with feeding starch-rich diets (Owens et al., 1998; Kleen et al., 2003; Plaizier et al., 2008). Unlike ruminal acidosis, SARA results from continued ingestion of these feeds over a prolonged period rather than sudden exposure without adequate adaptation (Garry and McConnel, 2008). In dairy cattle, which are not well adapted to grain and high-starch diets, the appearance of SARA is common and the ruminal pH is usually low within defined periods (Kleen et al., 2003). The high content of starch in grains increases grain fermentability by rumen microbes, increasing the production of short-chain fatty acids and therefore reducing ruminal pH, which ultimately changes the composition of the rumen microflora (Zebeli and Metzler-Zebeli, 2012). High concentrations of butyric and propionic acids stimulate proliferation of the ruminal papilla epithelium (Garry and McConnel, 2008); if the proliferation of the ruminal papilla epithelium is severe, it can result in parakeratotic changes associated with decreased absorption of VFA and increased susceptibility to damage and inflammation (Steele et al., 2011). Upon induction of SARA, an increased concentration of LPS is observed within several compartments of the ruminant gastrointestinal tract (Gozho et al., 2005; Li et al., 2012). The ruminal wall lesions allow penetration of bacteria and endotoxin with dissemination to the bloodstream (Kleen et al., 2003). Consequently, the presence of systemic LPS may

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[1]Corresponding author: jrodriguez@upei.ca

result in an immunological challenge to the animal, triggering a cascade of events associated with an immunological response within the host (Gozho et al., 2005). Consequences of SARA include feed intake depression, fluctuations in feed intake, reduced diet digestibility, reduced milk yield, reduced milk fat percentage, gastrointestinal damage, liver abscesses, and lameness (Krause and Oetzel, 2006; Plaizier et al., 2008).

The presence of LPS in the systemic circulation could engage the pattern recognition receptors, affecting leukocyte populations and triggering the production of proinflammatory cytokines and acute phase proteins (**APP**). Once in systemic circulation, lipopolysaccharides are recognized by a LPS receptor complex. The LPS receptor complex consists of LPS-binding protein (**LBP**), membrane (m) CD14, the pattern recognition receptor TLR4, and the associated protein myeloid differentiation factor 2 (**MD-2**; da Silva Correia et al., 2001). When engaged by LPS, such as in infections caused by gram-negative bacteria, this complex transduces a signal detected by the myeloid differentiation primary response gene 88 (*MyD88*), resulting in the activation of nuclear factor κ-B, initiating a transcription of a proinflammatory cytokine cascade (Erridge et al., 2002). It might be expected that the presence of endotoxins in the gastrointestinal tract occurs in SARA and the translocation of endotoxins to the systemic circulation triggers innate immune mechanisms involving leukocyte proliferation, activation of LPS/LBP/CD4 and TLR4/MD2 complexes, upregulation of proinflammatory cytokines and production of APP, which are an integral part of the acute phase response (**APR**; Cray et al., 2009).

Results of both experimental induced and field SARA cases have indicated that changes in biochemical profile occur in the course of developing SARA, suggesting a possible relationship between SARA and metabolism (Riuzzi et al., 2009). In dairy cattle suffering from SARA, blood glucose and electrolyte abnormalities have been reported to be associated with the presence of LPS in systemic circulation (Khafipour et al., 2009b). However, the association between the presence of LPS in blood, innate immune response, and biochemical abnormalities in cattle with SARA is yet to be determined.

We hypothesized that experimentally induced SARA in dairy cattle triggers changes in feed intake and body temperature and results in alterations of the leukogram, biochemical profile, and innate immune response. We also hypothesized that those changes may be associated with the presence of ruminal-derived LPS. The objectives of this study were to determine whether SARA induces changes in clinical variables, systemic biochemical profile, and systemic immune response characterized by alteration of the leukogram and expression of CD14 complex on peripheral blood mononuclear cells (**PBMC**), and increased concentrations of APP. The second objective was to determine whether those changes were associated with the presence of ruminal-derived LPS in bloodstream. Subclinical acute ruminal acidosis was induced using 2 models previously reported (Li et al., 2012), a grain-based SARA challenge (**GBSC**) and an alfalfa-pellet SARA challenge (**APSC**).

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

Six nonlactating, nonpregnant, mature (>5 yr) Holstein cows with average initial BW of 620 ± 45.7 kg (mean ± SD) that were fitted with a ruminal and cecal cannula, were used in a replicated Latin Square experimental design with blocking on cows and periods. Cow pairs were randomly assigned to 1 of 3 treatments in 3 different periods. Each experimental period consisted of 4 wk in which all cows received a diet consisting of 70% (DM basis) of forage in the first 3 wk. In wk 4, cows received 1 of 3 diets. One group remained on the control diet whereas the other 2 groups received 1 of 2 SARA-induction diets, GBSC or APSC. Ingredient and nutrient composition of experimental diets are presented in Table 1; further details on the experimental diets were previously described (Li et al., 2012).

Cows were transitioned from the adaptation diet to SARA-induction diets over 3 d. The cows were housed in individual tie-stalls in the Glenlea Dairy Research Unit at the University of Manitoba (Winnipeg, MB, Canada) in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993). On a weekly basis, veterinarians examined cows for heart rate, respiratory rate, and rumen contractions. Rectal temperatures were recorded on sampling days of wk 3 and wk 4. The amounts of TMR offered and refused were recorded daily for each cow.

Blood Collection and Hematological Analyses

Blood samples were collected from the tail vein on d 7 of wk 3, and on d 3 and d 5 of wk 4 of each study period. Blood was collected at 0900 h, before feeding, and at 1500 h, 6 h post feeding. One BD Vacutainer blood-collection tube (BD-Canada, Mississauga, ON, Canada) and one plasma K_2EDTA Vacutainer (Becton Dickinson, Franklin Lakes, NJ) were analyzed by Manitoba Agricultural, Food and Rural Initiatives Vet Diagnostics Services (Winnipeg, MB, Canada) for hematological parameters: red blood cells (**RBC**), packed