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A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation

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ABSTRACT

The effects of a grain-based subacute ruminal acidosis (SARA) challenge on translocation of lipopolysaccharide (LPS) into the peripheral circulation, acute phase proteins in blood and milk, feed intake, milk production and composition, and blood metabolites were determined in 8 lactating Holstein cows. Between wk 1 and 5 of 2 successive 6-wk periods, cows received a total mixed ration ad libitum with a forage to concentrate (F:C) ratio of 50:50. In wk 6 of both periods, the SARA challenge was conducted by replacing 21% of the dry matter of the total mixed ration with pellets containing 50% wheat and 50% barley. Rumen pH was monitored continuously using indwelling pH probes in 4 rumen cannulated cows. Rumen fluid samples were collected 15 min before feed delivery and at 2, 4, 6, 12, 14, 16, 18, and 24 h after feed delivery for 2 d during wk 5 (control) and wk 6 (SARA). Peripheral blood samples were collected using jugular catheters 15 min before feeding and at 6 and 12 h after feeding at the same days of the rumen fluid collections. The SARA challenge significantly reduced average daily pH from 6.17 to 5.97 and increased the duration of rumen pH below pH 5.6 from 118 to 279 min/d. The challenge reduced dry matter intake (16.5 vs. 19 kg/d), milk yield (28.3 vs. 31.6 kg/d), and milk fat (2.93 vs. 3.30%, 0.85 vs. 0.97 kg/d), and tended to increase milk protein percentage (3.42 vs. 3.29%), without affecting milk protein yield (1.00 vs. 0.98 kg/d). The challenge also increased the concentration of free LPS in rumen fluid from 28,184 to 107,152 endotoxin units (EU)/mL. This was accompanied by an increase in LPS in peripheral blood plasma (0.52 vs. <0.05 EU/mL) with a peak at 12 h after feeding (0.81 EU/mL). Concentrations of the acute phase proteins serum amyloid A, haptoglobin, and LPS-binding protein (LBP) in peripheral blood as well as LBP concentration in milk increased (438.5 vs. 167.4, 475.6 vs. 0, 53.1 vs. 18.2, and 6.94 vs. 3.02 μg/mL, respectively) during SARA. The increase in LBP in combination with the increase in LPS in peripheral blood provides additional evidence of translocation of LPS. Results suggest that the grain-based SARA challenge resulted in translocation of LPS into the peripheral circulation, and that this translocation triggered a systemic inflammatory response.
Key words: subacute ruminal acidosis, lipopolysaccharide translocation, acute phase response

INTRODUCTION

Grain-based subacute ruminal acidosis (**SARA**) challenge increases the concentrations of the acute phase proteins serum amyloid A (**SAA**) and haptoglobin (**Hp**) in peripheral blood of cattle (Gozho et al., 2007; Emmanuel et al., 2008; Plaizier et al., 2008). These increases in acute phase proteins, which are part of the acute phase response, indicate that SARA causes inflammation (Horadagoda et al., 1999). This inflammation could be initiated by dietary-induced damage to the gut mucosa or by translocation of immunogenic compounds into circulation, such as free LPS (Horadagoda et al., 1999).

There is substantial evidence that grain-based SARA challenge increases the content of free LPS in the rumen due to the increase in lysis of gram-negative bacteria (Gozho et al., 2007; Nagaraja and Lechtenberg, 2007; Plaizier et al., 2008). This increase in luminal LPS could increase permeability of the gut for LPS (Chin et al., 2006). Also, the barrier function of rumen epithelium may be compromised by the parakeratosis, rumenitis, and abscesses of the rumen wall that result from high rumen acidity (Kleen et al., 2003). Additionally, the high rumen osmolality that is seen during SARA can cause swelling and rupture of ruminal papillae, which will also reduce the barrier function of the rumen. Despite this, no evidence of LPS in the peripheral circulation during SARA has been found (Gozho et al., 2007). There is also inconsistency in detection of LPS in peripheral blood during experimentally induced acute ruminal acidosis (Dougherty et al., 1975; Andersen and Jarlov, 1990; Andersen et al., 1994b). In recent years, the sensitivity and accuracy of bioassays used to detect LPS in low concentrations have been substantially im-

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proved, which makes the conclusion of previous studies regarding the absence of LPS in peripheral circulation questionable. The increases in the concentrations of the acute phase proteins SAA and Hp in peripheral blood do not prove translocation of LPS, as these concentrations can increase due to other inflammatory stimuli. However, because LPS interacts with a specific acute phase protein, LPS binding protein (**LBP**), an increase in LBP in peripheral circulation will provide evidence of the translocation of LPS (Sriskandan and Altmann, 2008).

The main objective of this study was to determine if a grain-based SARA challenge causes translocation of LPS from the gut into peripheral circulation. This was achieved by measuring plasma LPS using a high-sensitivity assay and by monitoring LBP levels in peripheral plasma and milk. The effects of the grain-based SARA challenge on feed intake, milk production and composition, blood metabolites, and SAA and Hp in peripheral blood were also determined.

MATERIALS AND METHODS

Animals, Diets, and Experimental Procedures

Cows used in this study were housed in individual tiestalls in 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). Eight lactating Holstein cows, 4 of which were ruminally cannulated, were used during 2 subsequent 6-wk periods. Cows were on average 84 ± 29 DIM (mean ± SD) with an average milk yield of 35 ± 3.9 kg/d and had an average BW of 615 ± 68 kg at the beginning and 634 ± 50 kg at the end of experiment. Animals were randomly allocated in 2 groups consisting of 2 cannulated and 2 noncannulated cows. During wk 1 to 5 of each 6-wk period, cows received a TMR ad libitum with a forage to concentrate (**F:C**) ratio of 50:50 (Tables 1, 2, and 3), with the intention of allowing for 5 to 10% orts. During wk 6 of both periods, a SARA challenge was conducted by replacing 21% of the DM of the TMR with pellets containing 50% ground wheat and 50% ground barley, resulting in an F:C of 40:60 (Table 1). Cows had unlimited access to fresh water throughout the experiment. Data obtained from wk 5 of each period was considered as control and compared with wk 6 (SARA). Samples of diets, dietary ingredients, and orts were collected and analyzed by wet chemistry as described by Bhandari et al. (2007). The physical characteristics of wheat-barley pellets including dimension, bulk density, and water-holding capacity were determined as described by Giger-Reverdin (2000).

Table 1. Ingredients, nutrient composition, and forage to concentrate ratio (F:C) of the TMR and wheat-barley pellets (WBP)

Item	Control diet	SARA[1] diet	WBP
Ingredients, % of DM			
Alfalfa silage	25.0	20.0	—
Barley silage	25.0	20.0	—
Energy supplement	40.0	31.6	—
Protein supplement	10.0	7.8	—
Ground wheat	—	10.3	50.0
Ground barley	—	10.3	50.0
F:C	50:50	40:60	0:100
Nutrient composition			
DM, %	52.4	60.1	89.2
CP, % of DM	16.9	16.5	14.8
NDF, % of DM	35.7	30.4	11.4
ADF, % of DM	24.3	22.7	5.7
NFC[2], % of DM	32.7	40.4	68.6
Starch, % of DM	26.1	33.4	60.7
Crude fat, % of DM	5.3	4.5	2.2
Ash, % of DM	9.4	8.2	3.0
Ca, % of DM	1.11	0.92	0.22
P, % of DM	0.53	0.52	0.54
K, % of DM	1.91	1.60	0.56
Mg, % of DM	0.32	0.32	0.16
Na, % of DM	0.32	0.26	0.03
Predicted NE_L,[3] Mcal/kg of DM	1.57	1.66	—

[1]Subacute ruminal acidosis.

[2]NFC = 100 – (NDF % + CP % + crude fat % + ash %).

[3]NE_L values were predicted using CNCPS (Cornell Net Carbohydrate and Protein System) software version 5.0.4, Cornell University, Ithaca, NY.

Rumen pH Measurement

Rumen pH was monitored continuously for 4 consecutive days during wk 5 and wk 6 of both experimental periods in 4 rumen cannulated cows using indwelling pH probes as described by Gozho et al. (2006). The pH data were summarized as average pH, time spent below pH 6.0, time spent below pH 5.6, area (time × pH) spent below pH 6.0, and area spent below pH 5.6 for each 24-h period.

Rumen Sampling and Analysis

Rumen fluid samples were collected from the ventral sac of the rumen of rumen cannulated cows 15 min before feed delivery and at 2, 4, 6, 12, 14, 16, 18, and 24 h after feed delivery on 2 consecutive days of each sampling week. Ruminal contents were strained through 4 layers of sterile cheesecloth and divided into 2 portions. The first portion of samples were transferred into 50-mL sterile tubes and kept on ice until transported to the laboratory for the initial processing before LPS determination as described by Gozho et al. (2005). In brief, rumen fluid samples were centrifuged at 10,000 × *g* for 45 min. The supernatant was aspirated gently to prevent its mixing with the pellet and passed through