J. Dairy Sci. 92:3800–3809
doi:10.3168/jds.2009-2178

Relationships between rumen lipopolysaccharide and mediators of inflammatory response with milk fat production and efficiency in dairy cows

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ABSTRACT

The main objective of this study was to evaluate correlative relationships between rumen lipopolysaccharide (LPS) and mediators of acute phase response with milk fat yield and efficiency in dairy cows challenged with graded amounts of barley grain in the diet. An additional aim of the study was to quantify the intercow variation in relation to milk fat production and acute phase response in cows fed graded amounts of grain. Eight primiparous, lactating Holstein cows (60 d in milk) were assigned to 1 of the 4 total mixed rations containing barley grain at 0, 15, 30, and 45% (dry matter basis) in a replicated 4 × 4 Latin square design. Free rumen LPS, plasma acute phase proteins, and milk fat content were quantified in multiple samples collected on d 5 and 7 of the measurement periods shortly before the morning feeding. Results showed markedly greater concentrations of rumen LPS with increasing dietary grain level. The correlative analysis revealed strong negative relationships between rumen LPS and milk fat content and yield. The predictor variable of rumen LPS explained 69% of the variation during the milk fat reduction of the cows. The stronger depression in milk fat percentage was obtained when rumen LPS exceeded a threshold of 5,564 ng/mL, corresponding to a milk fat content of 3.39%. The increase in concentration of rumen LPS was also associated with declines in milk fat yield and 3.5% fat-corrected milk (R^2 = 0.50), as well as milk energy efficiency (R^2 = 0.43). The correlative analysis also indicated that the increase of plasma C-reactive protein (CRP) in response to higher grain feeding was associated with a linear decrease of milk fat content and yield (R^2 = 0.28 to 0.46). Furthermore, the statistical analysis revealed high percentages of intercow variation related to milk fat variables, as well as the responses of rumen LPS and plasma CRP. Taken together, the current results implicate rumen LPS and the host CRP response in the lowering of milk fat content and milk energy efficiency in dairy cows fed high-grain diets. Further research is warranted to understand the mechanism(s) by which rumen LPS and inflammatory responses to LPS lower milk fat synthesis and milk energy efficiency and to develop novel strategies for their prevention.
Key words: milk fat depression, rumen lipopolysaccharide, C-reactive protein, dairy cow

INTRODUCTION

It has long been recognized that when dairy cows are fed high-concentrate/low-forage (**HC/LF**) diets or diets rich in plant-oil supplements containing high amounts of polyunsaturated fatty acids (**FA**), there is a reduction in milk fat content, a syndrome commonly referred to as milk fat depression (**MFD**). The latter disorder has been in the focus of dairy scientists for over a century, with multiple hypotheses being advanced to explain the causes of diet-induced MFD (Davis and Brown, 1970).

Although our knowledge about the causes of MFD has increased tremendously during the last decades, most of the postulates proposed during the years have not fully explained the etiology of MFD (Bauman et al., 2008). One of the hypotheses that has gained the most support recently is that of ruminal biohydrogenation byproducts of long-chain unsaturated FA (Bauman and Griinari, 2001). It was Davis and Brown (1970) who first reported a negative correlation between changes in milk fat yield and concentrations of *trans* FA in the milk. This finding triggered extensive research during the last decades focused predominantly on the interrelationships between rumen biohydrogenation of dietary polyunsaturated FA and mammary synthesis of FA (Bauman et al., 2008).

In most investigations involving plant oil–induced MFD, the *trans*-10, *cis*-12–18:2, an intermediate of the rumen biohydrogenation process, has been identified as a potent suppressor of milk fat synthesis (Davis and Brown, 1970). However, during diet-induced MFD, the amount of *trans*-10, *cis*-12–18:2 in the milk and the magnitude of reduction in milk fat yield do not align

Received March 3, 2009.
Accepted April 9, 2009.
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with the dose–response curve generated with abomasal infusion of this isomer (Piperova et al., 2004; Bauman et al., 2008). This has led several investigators to propose that other rumen unidentified biohydrogenation isomers or products might be involved in the biology of MFD (Piperova et al., 2004; Bauman et al., 2008).

Another interesting line of investigation has indicated that feeding dairy cows diets rich in readily digestible carbohydrates and low in fiber is associated with the release of large amounts of LPS, a cell-wall component of all gram-negative bacteria, in the rumen fluid (Gozho et al., 2007; Emmanuel et al., 2008; Khafipour et al., 2009). It has been increasingly recognized that LPS stimulates the release of proinflammatory cytokines such as tumor necrosis factor (**TNF**)-α, IL-1, and IL-6 by liver macrophages, which in turn activate hepatocytic receptors and initiate the synthesis of acute phase proteins (**APP**; Sweet and Hume, 1996). The LPS also modulates lipid metabolism in different body tissues. For example, pieces of evidence pinpoint suppressive effects of LPS on key enzymes related to de novo FA synthesis such as FA synthetase and acetyl-CoA carboxylase (Pekala et al., 1983; López-Soriano and Williamson, 1994) in the mammary gland and down regulation of lipoprotein lipase (**LPL**) activity (Khovidhunkit et al., 2004). The latter enzyme plays a key role in clearance of circulating triglyceride (**TAG**)-rich chylomicrons and very low density lipoprotein (**VLDL**) as a host defense mechanism to decrease LPS toxicity (Feingold et al., 1992) and is involved in the uptake of FA for incorporation into milk fat (Merkel et al., 2002). Recently, the LPS released in the gastrointestinal tract during feeding of high-grain or high-fat diets has been implicated in the etiology of multiple energy- and lipid-related metabolic disturbances in ruminants (Andersen, 2003; Ametaj et al., 2005), rodents, and humans (Cani et al., 2007; Amar et al., 2008). Nevertheless, the interrelationships between rumen LPS and the host immune responses to LPS during feeding of HC/LF diets with lipid metabolism in the mammary tissue have not yet been established. Based on the aforementioned facts, we hypothesized that free LPS released in the rumen in response to high-grain feeding and its resulting mediators of inflammatory response in plasma might be involved in altering milk fat production and milk energy efficiency in lactating Holstein cows.

To test our hypothesis, we fed lactating dairy cows graded amounts of barley grain and evaluated the relationships between rumen LPS and plasma APP with variables of milk fat production. An additional aim of the study was to quantify the intercow variation in relation to milk production and acute phase response in cows fed graded amounts of grain.

MATERIALS AND METHODS

Animals, Diets, and Experimental Design

All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and cows were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). Because the variables related to rumen LPS and plasma APP have been reported previously (Emmanuel et al., 2008), this article has mainly focused on the effect of diet on milk fat production and efficiency, as well as on the correlative relationships between rumen LPS and plasma APP with milk fat production variables. Eight ruminally cannulated (Ø 100 mm, Bar Diamond, Parma, ID) primiparous Holstein cows were used in this study in a replicated 4 × 4 Latin square design. The experimental period was 21 d, with the first 11 d used for diet adaptation. At the start of the experiment, the cows were at 60 ± 15 d (mean ± SD) postpartum. The cows were housed in tie stalls with free access to water and fed once daily at 0800 h and milked twice at 0500 and 1600 h. To challenge the cows with graded amounts of rumen-fermentable carbohydrates in their diets, a basic ration was supplemented with 0, 15, 30, or 45% (DM basis) barley grain to provide the 4 dietary treatments, which varied from 35.4 to 45.5% in the total content of NFC. The amount of grain in the diet was stepped up or down during the adaptation period. Daily ration was offered as TMR for ad libitum intake to allow about 5% feed refusals. Diets were formulated to meet or exceed the nutrient requirements of a 680-kg lactating cow as per NRC (2001) guidelines. Ingredients and nutrient composition of the TMR are presented in Table 1.

Sample and Data Collection

Blood samples were collected from the coccygeal vein shortly before the morning feeding using 10-mL vacutainer tubes (Becton Dickinson, Franklin Lake, NJ) containing sodium heparin anticoagulant. Blood samples were stored in ice and centrifuged within 20 min at 4°C for 20 min at 3,000 × *g* to separate plasma (Rotanta 460 R, Hettich Zentrifugan, Tuttlingen, Germany). Plasma samples were stored at −20°C until their analysis.

Feed intake was measured by the difference from feed refusals, which were weighed back daily at about 0700 h. Milk samples were collected at 0500 and 1600 h on d 5 and 7 of each experimental period. Milk fat content was measured with mid-infrared spectroscopy (Milko-Scan 605, A/S N Foss Electric, Hillerød, Denmark) by Central Milk Testing Laboratory in Edmonton-Alberta.