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Effects of various starch feeding regimens on responses of dairy cows to intramammary lipopolysaccharide infusion

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ABSTRACT

Endotoxin tolerance (ET) can develop in mammals that have been challenged repeatedly with sublethal amounts of lipopolysaccharide (LPS). Previous research has shown that subclinical ruminal acidosis can increase circulating concentrations of LPS. We investigated whether ET would develop in Holstein cows that were subjected to chronic subacute ruminal acidosis (SARA) or acute SARA followed by intramammary infusion of LPS. Twenty-four cows, both primiparous and multiparous, were assigned to 8 blocks of 3 cows. Cows within blocks were randomly assigned to 1 of 3 treatments: (1) control (diet DM was 24% starch and 35% NDF), (2) high starch (formulated to induce chronic milk fat depression with 29% starch and 32% NDF), and (3) acidosis (designed to cause acute bouts of milk fat depression by short-term feeding of a diet with 32% starch, some of which came from wheat grain, and 30% NDF). Cows on the control and high-starch treatments were fed their respective diets throughout the 24-d trial. The acidosis cows were fed the control diet during most of the experiment, except during two 2-d bouts (d 10 and 11 and 17 and 18 of the experiment) in which a high-starch diet was fed. Cows on the high-starch and acidosis treatments produced milk fat with an altered fatty acid profile indicative of SARA (e.g., increased concentrations of specific *trans*, and odd-, and branched-chain fatty acids), but only cows on the high-starch treatment had milk fat depression. Concentrations of serum amyloid A were elevated in cows on the acidosis treatment, but did not differ between control and high-starch cows. On d 20 of the experiment, all cows were given an intramammary infusion of 10 μg of LPS into 1 mammary quarter 3 h after morning milking. Milk yield and DMI decreased the day of the infusion, but the response was not affected by dietary treatment. No systemic indicators of ET were observed among treatments, but evidence of an ET response at the local level of the mammary gland was observed. Cows fed the control diet had higher concentrations of serum amyloid A in milk 12 and 24 h postinfusion than did cows fed the high-starch diet and higher concentrations than cows on the acidosis treatment at 12 h postinfusion. Our data suggest cows that experienced varying degrees of SARA (based on altered milk fatty acid profile) and subsequent experimental endotoxin mastitis experienced a blunted inflammatory response at the level of the mammary gland, but not a systemic reduction in some inflammatory mediators.

Key words: ruminal acidosis, endotoxin, acute phase response

INTRODUCTION

Subclinical ruminal acidosis in cattle is associated with feeding diets with excess starch. Common signs of SARA include low rumen pH, decreased DMI, diarrhea, deceased milk yield, milk fat depression, and altered milk FA profile (Garrett et al., 1999; Kleen et al., 2003; Colman et al., 2010). Increasing the starch concentration of diets can alter the rumen bacterial population by promoting growth of some gram-positive bacteria at the expense of gram-negative bacteria (Nagaraja et al., 1978). When gram-negative bacteria die they shed LPS from their cell wall (Beutler and Rietschel, 2003), and starch-induced SARA increases concentrations of LPS in rumen fluid and blood (Gozho et al., 2005; Gozho et al., 2006; Emmanuel et al., 2008; Khafipour et al., 2009). Concentrations of circulating acute phase proteins, such as serum amyloid A and haptogloblin (although not all studies were consistent for the different acute phase proteins), also increase with SARA (Gozho et al., 2005; Gozho et al., 2006; Emmanuel et al., 2008; Khafipour et al., 2009), perhaps as part of an inflammatory response to LPS. Mastitis caused by gram-negative bacteria also increase circulating concentrations of LPS (Hogan and Smith, 2003)

Endotoxin tolerance (**ET**) can develop in mammals that have been challenged repeatedly with sublethal amounts of LPS (Beeson, 1947). Tolerant animals experience a blunted inflammatory response to subsequent exposure to LPS (Beeson, 1947; Beutler and Rietschel, 2003), often quantified as decreased produc-

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tion of proinflammatory mediators (Sanchez-Cantu et al., 1989). Endotoxin tolerance is not fully understood, but is thought to be a host defense mechanism to prevent damage from excessive inflammation. However, the attenuated inflammatory response resulting from ET may increase the risk of infections becoming established. Some data suggest that ET can develop in calves, but the response has not been universal to all measurements of inflammation (Elsasser et al., 1996; Bieniek et al., 1998).

We hypothesized that cows experiencing chronic or sporadic bouts of SARA would exhibit ET when subsequently challenged with experimental LPS mastitis. If this occurs, cows with SARA may be more susceptible to subsequent problems with mastitis or other infections because of the potential immunosuppressive effects of ET. The aims of our experiment were to induce chronic SARA and sporadic bouts of SARA in dairy cows by feeding excess starch and then administer LPS into the mammary gland and evaluate local and systemic signs of inflammation.

MATERIALS AND METHODS

Cows and Treatments

All procedures involving animals were approved by The Ohio State University Institutional Animal Care and Use Committee. Twenty-four midlactation Holstein cows averaging 140 (SD = 16) DIM were assigned to 8 blocks (3 cows per block) based on parity (4 blocks of primiparous and 4 blocks of multiparous) and DIM within parity. Cows were moved into the tiestall barn in 4 groups (2 blocks per group) every 2 wk. Cows had free access to water, were fed a TMR once daily (approximately 0330 h) for ad libitum consumption (feed refusal averaged 5.8% of amount fed), and were milked twice daily (0300 and 1500 h). Feed offered and orts were measured daily and milk weights were recorded electronically at each milking. Cows selected for the trial did not have a diagnosed gram-negative bacterial IMI or clinical mastitis during the lactation before LPS challenge, as recorded on barn health sheets.

Cows within each block were randomly assigned to 1 of 3 dietary treatments: (1) control, (2) high starch, and (3) acidosis bouts. The control and high-starch diets differed in starch (essentially all the starch was from corn products), NDF, and forage NDF concentrations (Tables 1 and 2). In a previous experiment (Weiss, 2012), cows fed a diet similar to the high-starch diet had reduced milk fat percentage compared with cows fed a diet similar to the control diet. Cows on those 2 treatments were fed the same diet each day during the 24-d experiment. The high-starch diet was used to elicit longer term effects (i.e., chronic) on rumen fermentation. Cows on the acidosis bout treatment were fed the control diet during most of the experiment, except during two 2-d bouts (d 10–11 and 17–18) when they were fed a diet where 9.7% ground wheat replaced a portion of a wet corn gluten feed product (Tables 1 and 2). The diet fed during the bouts was higher in starch and lower in NDF concentrations than the other diets, and approximately 20% of the starch came from wheat. The acidosis challenge was less severe than other models (e.g., Keunen et al., 2002), but was similar to diets that have induced some short-term changes in rumen fermentation patterns (Colman et al., 2010). This treatment was designed to elicit short-term, abrupt, and repeated changes in rumen fermentation patterns. All diets were formulated to be equal in metabolizable protein (assuming no treatment effects on DMI), vitamins, and minerals and met or exceeded NRC (2001) recommendations.

On d 20 of the experimental period, all cows were given an intramammary infusion of LPS via teat canal into either front mammary quarter using a 34-mm sterile teat infusion cannula (Jorgensen Laboratories Inc., Loveland, CO). The challenge inoculum was 10 μg of LPS diluted in 10 mL of sterile PBS. The LPS was from *Escherichia coli* serotype O111:B4 (Sigma-Aldrich Company, St. Louis, MO). Challenge quarters were determined based on bacteriological and cytological results of quarter foremilk samples taken at d 7, 5, and 3 before challenge. Quarters selected were bacteriologi-

Table 1. Ingredient composition of treatment diets (% of DM)

Item	Treatment diet[1]		
	Control	High starch	Acidosis
Corn silage	44.0	36.0	36.0
Alfalfa silage	11.0	9.0	9.0
Wet corn milling product[2]	25.0	25.0	10.0
Corn grain, ground	9.9	19.9	19.9
Wheat grain, ground	0	0	9.7
Soybean meal, 48% CP	3.90	6.3	8.4
Aminoplus[3]	3.50	1.0	4.2
Animal-vegetable fat	0.62	0.62	0.62
Trace mineralized salt	0.50	0.50	0.50
Limestone	1.10	1.20	1.20
Vitamin-mineral premix[4]	0.48	0.48	0.48

[1]Cows on the control and high-starch treatments were fed their respective diets continuously during the experiment. The acidosis diet was fed to cows on the acidosis treatment for only two 2-d feeding bouts during the experiment.

[2]Cargill Corn Milling, NA, Blair, NE.

[3]Ag Processing Inc., Omaha, NE.

[4]Premix provided 0.2 mg of Se (from sodium selenate), 12 mg of Cu (from copper sulfate), 22 mg of Zn (from zinc sulfate), 3,600 IU of vitamin A, 990 IU of vitamin D, 20 IU of vitamin E, and 0.67 mg of biotin (Rovimix-Biotin, DSM Nutritional Products Inc., Parsippany, NJ) per kilogram of diet DM.