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Feeding High Proportions of Barley Grain Stimulates an Inflammatory Response in Dairy Cows

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ABSTRACT

The objective of this study was to evaluate effects of feeding increasing proportions of barley grain on acute phase response in lactating dairy cows. Eight cannulated primiparous (60 to 140 d in milk) Holstein dairy cows were assigned to 4 diets in a 4 × 4 Latin square experimental design. The experimental period lasted for 21 d, with 11 d of adaptation and 10 d of measurements. Cows were fed the following diets: 1) no barley grain in the diet, 2) 15% barley grain, 3) 30% barley grain, and 4) 45% barley grain, as well as barley and alfalfa silage and alfalfa hay at 85, 70, 55, and 40% [dry matter (DM) basis]. All cows were supplemented with a 15% concentrate mix. Blood and rumen fluid samples were collected on d 1, 3, 5, 7, and 10 of the measurement period, and pH and endotoxin content were measured in rumen samples. Concentrations of serum amyloid A, lipopolysaccharide-binding protein, haptoglobin, and C-reactive protein in plasma were measured by ELISA. Feeding high proportions of barley grain at 0, 15, 30, and 45% of DM was associated with lower feed intake (32.6, 32.9, 27.34, and 25.18 kg/d ± 1.30, respectively), lower ruminal pH (6.8, 6.7, 6.7, and 6.5 ± 0.03, respectively), and higher DM intake (13.33, 15.28, 14.68, and 16.04 ± 0.63 kg/d, respectively) and milk production (27.2, 28.2, 29.0, and 31.0 ± 1.2 kg/d, respectively). Ruminal endotoxin increased in cows receiving 30 and 45% barley grain (5,021, and 8,870 ± 393 ng/mL, respectively) compared with those fed no grain or 15% barley grain (654 and 790 ± 393 ng/mL, respectively). Plasma concentrations of serum amyloid A, lipopolysaccharide-binding protein, and C-reactive protein increased in cows given higher (30 and 45%) proportions of grain. Plasma haptoglobin was not affected by treatments. In conclusion, feeding dairy cows high proportions (30 and 45% DM basis) of barley grain was associated with lower feed intake and rumen pH, increased endotoxin in the rumen fluid, and stimulation of an inflammatory response.

Key words: acute phase protein, barley grain, dairy cow, endotoxin

INTRODUCTION

Ruminant animals are primarily herbivores with little or no starch in their diet. Ruminants themselves do not produce fiber-degrading enzymes but harbor bacteria, fungi, and protozoa that digest fiber. The host provides the microorganisms with a suitable habitat for growth, and the microbes supply protein, vitamins, and short-chain organic acids for the animal. Ruminal microorganisms can ferment starch and sugars, and these nonfibrous materials increase fermentation rate and animal productivity (Nocek and Russell, 1988). Overfeeding cattle with diets rich in starch results in several disorders such as subacute ruminal acidosis, acute ruminal acidosis, fatty liver, laminitis, liver abscesses, displaced abomasum, and bloat (Nocek 1997; Andersen, 2003; Ametaj et al., 2005). The mechanism(s) by which grain increases susceptibility of ruminant animals to metabolic disorders is not clear.

Feeding high amounts of rapidly fermenting carbohydrates decreased rumen pH, altered rumen microbial population, and increased concentration of endotoxin in the rumen fluid (Nocek, 1997; Andersen, 2003). For example, Andersen (2003) reported several fold increases in the concentration of endotoxin in the rumen of cows fed high amounts of grain. Changes in osmotic pressure and ruminal endotoxin resulting from feeding easily fermentable carbohydrates may render the rumen epithelium susceptible to injury, resulting in translocation of rumen endotoxin into the bloodstream (Kleen et al., 2003). Ruminal endotoxin was implicated in the etiology of multiple metabolic disorders like acidosis, fatty liver, laminitis, and sudden death syndrome (Andersen, 2003; Ametaj et al., 2005).

Endotoxin, also known as LPS, is a cellular component of gram-negative bacteria and is an extremely potent toxin. Endotoxin is a strong inducer of acute phase response, which is a nonspecific immune mechanism aimed at restoring disturbed homeostasis. During

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acute phase response, there is alteration in the biosynthetic profile of the liver, resulting in production of proteins known as acute phase proteins (**APP**). The main stimulators of APP production are the inflammation-associated cytokines IL-1, IL-6, tumor necrosis factor (**TNF**)-α, and IFN-γ which are released during inflammatory processes (Gabay and Kushner, 1999). The acute phase response is characterized by leukocytosis, fever, alterations in the metabolism of many organs, as well as changes in the plasma concentrations of various APP.

Two APP, serum amyloid A (**SAA**) and LPS-binding protein (**LBP**), directly participate in the detoxification and removal of endotoxin during an acute phase response. Plasma concentration of SAA increases up to 1,000-fold during an acute phase response and is believed to substitute the apoA-1 fraction of the high-density lipoproteins (Coetzee et al., 1986). The SAA binds to endotoxin monomers, and the complex is removed by liver macrophages. On the other hand, LBP facilitates transfer of endotoxin to macrophages or lipoproteins, which results in neutralization of the effect of endotoxin to induce inflammatory responses (Gallay et al., 1994). Haptoglobin is released during the acute phase response and binds hemoglobin to prevent utilization of Fe, in the free hemoglobin, by bacteria that require Fe for their growth and multiplication (Wassell, 2000). The C-reactive protein (**CRP**) has several functions like opsonization of bacteria and a protective effect against endotoxin by interacting with Fcγ receptors on macrophages (Mold et al., 2002). The objective of this study was to investigate the effects of feeding increasing proportions of barley grain on concentration of endotoxin in the rumen fluid and the subsequent alterations in immune responses as reflected by plasma concentrations of SAA, LBP, haptoglobin, and CRP in lactating dairy cows.

MATERIALS AND METHODS

Animals and Diets

Eight ruminally cannulated primiparous Holstein cows were used in a 4 × 4 Latin square design with 2 cows in each square and 4 periods. The squares represent different treatment groups. The experimental period was 21 d, with 11 d of adaptation and 10 d of measurements. At the start of the experimental period, the cows were at 60 to 140 DIM and weighed from 600 to 700 kg. All cows had the same base ration, which was supplemented with different amounts of barley grain and barley silage for treatment groups receiving 0, 15, 30, or 45% barley grain in their ration. The amount of grain in the diet was increased or decreased during the adaptation period to adjust cows to the new dietary treatment. All experimental procedures were approved by the University of Alberta Animal Policy and Welfare Committee, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). The cows were housed in tie stalls with free access to water and were fed once daily at 0800 h, milked twice at 0500 and 1600 h, and milk yield was recorded electronically. Daily ration was fed as TMR to meet or exceed the requirements of a 680-kg lactating cow as per NRC (2001) guidelines. Estimated protein and energy contents were similar across the different diets. Ingredients and diet composition of the different treatment groups are presented in Table 1. Individual feed intake was recorded daily during the 10 d of the measurement period by the difference between the total daily feed given to each cow with the feed refusals the next morning. All cows remained healthy during the entire experimental period. Health of cows was observed daily based on their daily eating (i.e., DMI) and milking (i.e., milk yield) behavior as well as for clinical signs of disease by the veterinary technician.

Sample Collection

Blood samples were collected from the tail vein on d 1, 3, 5, 7, and 10 of the measurement period at 0800 h before the morning feeding. Blood was collected into 10-mL vaccutainer tubes (Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin anticoagulant. Blood tubes were stored in ice and centrifuged within 20 min at 3,000 × g and 4°C for 20 min to separate plasma. Plasma samples were stored at –20°C until analysis.

Samples from rumen fluid were obtained on d 1, 3, 5, 7, and 10 of the experimental period. Rumen samples were collected through the cannula using a tube fitted with a strainer and a syringe into a 140-mL plastic container. The pH of rumen fluid was determined immediately by a mobile pH meter (Accumet AP61, Fischer Scientific, Ottawa, Ontario, Canada). The samples were centrifuged at 6,000 × g for 15 min, and the supernatant was stored at –20°C until analysis.

Sample Analysis

Concentration of cell-free LPS in the rumen fluid was determined by the pyrochrome *Limulus* amebocyte lysate assay as instructed by the manufacturer (Associates of Cape Cod Inc., East Falmouth, MA). Ten milliliters of rumen fluid samples was centrifuged at 6,000 × g for 15 min, and the supernatant was stored at –20°C. For use in the assay, 1.5 mL of the supernatant was centrifuged again at 10,000 × g for 30 min. The superna-

Table 1. Diet composition and ingredients of experimental diets

Ingredients[1] (% of DM)	0% barley	15% barley	30% barley	45% barley
Alfalfa hay	15.00	15.00	15.00	15.00
Alfalfa silage	12.00	12.00	12.00	12.00
Barley silage	58.00	43.00	28.00	13.00
Rolled barley	0.00	15.00	30.00	14.00
Gluten meal	6.00	6.00	6.00	6.00
Fish meal	1.03	1.03	1.03	1.03
Canola meal	0.98	0.98	0.98	0.98
Dairy premix[2]	0.58	0.58	0.58	0.58
Megalac[3]	1.79	1.79	1.79	1.79
Limestone	0.58	0.58	0.58	0.58
Biofos[4]	0.40	0.40	0.40	0.40
Magnesium oxide	0.35	0.35	0.35	0.35
Sodium bicarbonate	0.76	0.76	0.76	0.76
Vitamin E[5]	0.09	0.09	0.09	0.09
Vitamin D_3[6]	0.17	0.17	0.17	0.17
Molasses	0.35	0.35	0.35	0.35
Hydrogenated tallow	1.87	1.87	1.87	1.87
Nutrient composition (% of DM)				
ME (Mcal/kg of DM)	2.45	2.42	2.40	2.39
CP	16.20	16.40	16.50	16.70
NDF	32.80	30.20	27.60	25.00
ADF	21.80	19.40	17.00	14.60
NFC	35.40	38.80	42.10	45.50
Ca	1.30	1.30	1.20	1.20
P	0.40	0.40	0.50	0.50
DCAD (mEq/kg)	300.00	274.00	248.00	223.00

[1]Fed as TMR.

[2]Contained Ca, 0.1%; P, 0.6%; Na, 11.5%; Mn, 0.3%; K, 0.7%; S, 0.23%; Zn, 5,000 mg/kg; Cu, 1,170 mg/kg; Mn, 3,100 mg/kg; I, 80 mg/kg; Co, 6.2 mg/kg; vitamin A, 1,265,000 IU/kg; vitamin D, 142,000 IU/kg; and vitamin E, 3,800 IU/kg.

[3]Contained 85% fat as fatty acids and 9.6% Ca with a NE_L of 6.52 Mcal/kg.

[4]Contained monocalcium phosphate and dicalcium phosphate in the ratio 2:1.

[5]Contained 5,000 IU/kg.

[6]Contained 500,000 IU/kg.

tant was then passed through a disposable 0.22-μm sterile, pyrogen-free filter (Fischer Scientific, Fairlawn, NJ) and diluted 1,000-fold using pyrogen-free *Limulus* amebocyte lysate reagent water and pyrogen-free test tubes (Associates of Cape Cod Inc.). Commercially available kits (Associates of Cape Cod Inc.) were used for the assay. The method and the quantity of reagents described in the kit were modified to have higher standard ranges of 0.625 to 10 ng/mL. Control standard LPS containing 10 ng of LPS per vial (Associates of Cape Cod Inc.) was used to prepare the standard solutions. Samples were tested in duplicate, and the optical density values were read on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation, Sunnyvale, CA) at a wavelength of 405 nm. Intraassay CV was <10% for all the assays.

Plasma APP

Concentration of SAA in plasma was determined by a commercially available bovine ELISA kit (Tridelta Development Ltd., Greystones Co., Wicklow, Ireland) with mAB specific for SAA coated on the walls of the microplate strips provided. Samples were initially diluted 1:500, and samples with optical density values above the range of the standard curve were diluted further (1:400 or 1:250) and reanalyzed. All samples were tested in duplicate, and the optical density values were read on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation) at 450 nm. The minimum detection limit of the assay was 18.8 ng/mL.

Concentration of LBP in plasma was determined with a commercially available multispecies ELISA kit (Cell Sciences Inc., Norwood, MA). The antibody coated in the walls cross-reacted with bovine LBP. Plasma samples were initially diluted 1:1,000, and samples with optical density values lower than the range of the standard curve were tested with a lower dilution (1:500). Samples were tested in duplicate, and the optical density at 450 nm was measured on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation). Plasma LBP was calculated from a standard curve of known amounts of human LBP.

Concentration of haptoglobin in the plasma was determined by a commercially available bovine ELISA kit (Tridelta Development Ltd.). According to the manufacturer, the minimum detection limit of the assay was 0.25 ng/mL as defined by the linear range of standard curves. All samples were tested in duplicate, and the optical density at 630 nm was measured on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation).

Plasma concentration of CRP was measured using commercial human sandwich ELISA kits (Alpco Diagnostics., Salem, NH) according to the directions of the manufacturer. A human kit for measurement of bovine CRP was used, because there was no specific bovine CRP kit available and because Schroedl et al. (2003) indicated rabbit antihuman CRP antibodies cross-react with bovine CRP. The minimum detectable concentration of the assay was 1.9 ng/mL. All samples were tested in duplicate, and the optical density values were read on a microplate spectrophotometer (Spectramax 190, Molecular Devices Corporation) at 450 nm.

Statistical Analyses

The MIXED procedure of SAS was used to analyze blood variables, rumen pH, and endotoxin as well as DMI and milk yield with a repeated measures design using the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where μ = the population mean; α_i = a population parameter corresponding to treatment i; βj = the fixed effect of time j; $(\alpha\beta)_{ij}$ = the effect of treatment × time interaction; and e_{ijk} = the residual error. The covariance structure of the repeated measurements for each variable was modeled separately according to the lowest values of the fit statistics based on the Bayesian information criteria and an appropriate structure fitted. The PDIFF option was used in each of the comparisons. Significance was declared at $P < 0.05$.

RESULTS

Feed Intake and Milk Production

Feed intake was affected by the quantity of barley grain in the diet ($P < 0.01$; Figure 1A). Cows fed 30 and 45% barley grain (27.34 and 25.18 ± 1.30 kg/d, respectively) had less feed intake than cows fed a 0 or 15% grain diet (32.6 and 32.9 ± 1.30 kg/d, respectively; $P < 0.01$). Feed intake was comparable between the 0% group and the group fed 15% barley grain (32.6 and 32.9 ± kg/d, respectively) diet. Day as well as treatment × day interaction did not affect total feed intake. Dry matter intake was higher for groups of cows fed barley grain at 15, 30, and 45% of the diet DM (15.28, 14.68, and 16.04 ± 0.63 kg/d) compared with those of cows fed no grain (13.33 ± 0.63 kg/d; $P < 0.01$; Figure 1B). Interaction between treatment and day was not significant with respect to DMI.

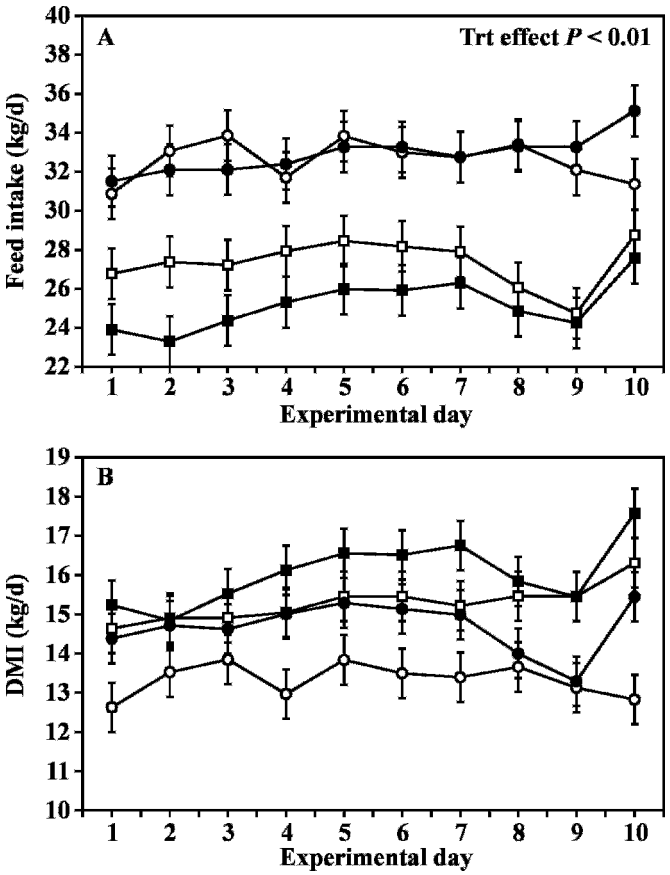

Figure 1. Feed intake (A) and DMI (B) of early lactating (60 to 140 DIM) cannulated Holstein dairy cows (n = 8) fed 0% (○), 15% (●), 30% (□), or 45% (■) barley grain in a 4 × 4 Latin square design with 11 d of adaptation and 10 d of measurement period. Trt = treatment.

Milk yield was affected by the amount of barley grain in the ration ($P < 0.01$; Figure 2). The group of cows fed no grain had the lowest daily milk yield (27.17 ± 1.24 kg/d; $P < 0.01$). The average daily milk yield, in the groups of cows fed 15 and 30% grain, was comparable ($P > 0.05$; 28.18 ± 1.24 and 28.99 ± 1.24 kg/d, respectively), whereas cows fed 45% barley grain had the highest total daily milk yield (31.04 ± 1.24 kg/d). Experimental day affected total daily milk production ($P < 0.05$). No treatment × day interaction was obtained for milk production.

Ruminal Fluid pH

Treatment had an effect in lowering the pH of the ruminal fluid ($P < 0.01$; Figure 3A). The lowest ruminal

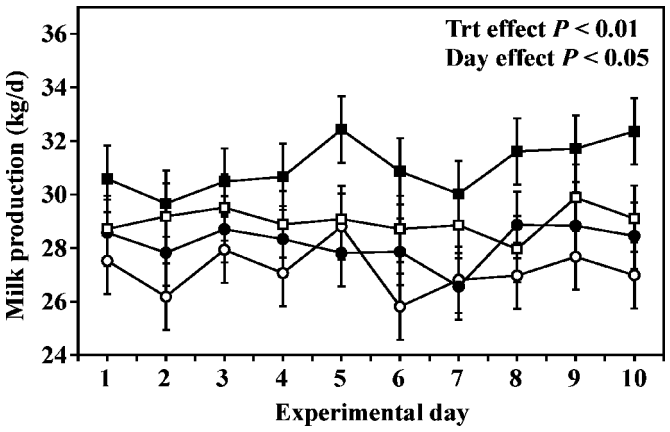

Figure 2. Milk production of early lactating (60 to 140 DIM) cannulated Holstein dairy cows (n = 8) fed 0% (○), 15% (●), 30% (□), or 45% (■) barley grain in a 4 × 4 Latin square design with 11 d of adaptation and 10 d of measurement period. Trt = treatment.

fluid pH was observed in cows fed the 45% grain diet (6.5 ± 0.03), and the highest pH value was obtained for the group fed no grain (6.8 ± 0.03). The other 2 groups fed diets containing 15 and 30% grain had a pH of 6.7 ± 0.03. Day had no effect on ruminal fluid pH, and there was no treatment × day interaction.

Ruminal Fluid Endotoxin

Concentration of endotoxin in the ruminal fluid was affected by the amount of grain in the diet ($P < 0.01$; Figure 3B). Both groups of cows fed 30 and 45% barley grain had higher concentrations of endotoxin in the ruminal fluid (5,021 ± 393 and 8,870 ± 393 ng/mL, respectively) compared with the groups fed 0 or 15% grain (654 ± 393 and 790 ng/mL ± 393; $P < 0.01$). No differences between the groups fed 0 and 15% barley grain were observed ($P > 0.05$). Both day and treatment × day interaction affected the concentration of endotoxin in the rumen ($P < 0.05$ and $P < 0.01$, respectively). Concentration of ruminal endotoxin remained steady in the groups of cows fed 0 and 15% barley grain during the measurement period. On the other hand, the amount of endotoxin in the ruminal fluid of cows fed 30% grain increased continuously, reaching peak values on d 10 of the measurement period. Ruminal endotoxin, in the group of cows fed 45% grain, was high on d 1 of the measurement period and declined slightly by d 10.

Plasma APP

Feeding increasing proportions of barley grain had an effect on plasma concentrations of SAA ($P < 0.01$; Figure 4A). The group of cows fed 30 and 45% barley grain had the highest overall plasma concentration of

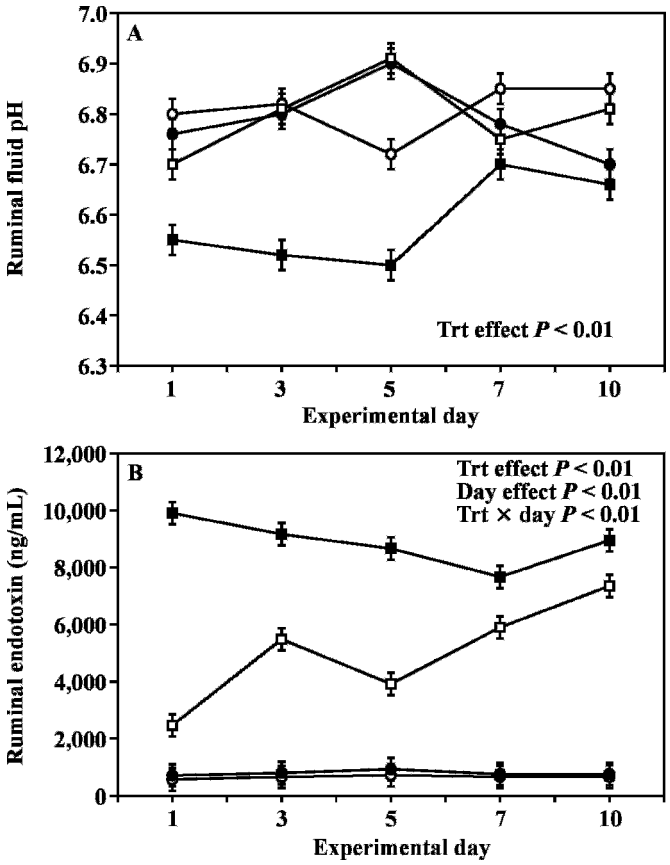

Figure 3. Rumen fluid pH (A) and endotoxin (B) of early lactating (60 to 140 DIM) cannulated Holstein dairy cows (n = 8) fed 0% (○), 15% (●), 30% (□), or 45% (■) barley grain in a 4 × 4 Latin square design with 11 d of adaptation and 10 d of measurement period. Trt = treatment.

SAA (21,825 ± 3,582 and 32,782 ± 3,582 ng/mL, respectively), whereas those fed 0 and 15% grain had the lowest concentrations of SAA (9,255 ± 3,582 and 6,886 ± 3,582 ng/mL, respectively). No differences between the 0% grain group and the group of cows fed 15% were observed ($P > 0.05$). Plasma concentrations of SAA in the cows fed 30 and 45% grain differed from each other ($P < 0.01$). Furthermore, there was an interaction between treatment and day ($P < 0.05$); however, days did not affect plasma concentrations of SAA. The groups of cows fed the lower amounts of grain (0 and 15%) had plasma SAA <10,000 ng/mL. Concentration of SAA in the plasma of cows fed 30% grain ranged from 20 to 30,000 ng/mL. Cows fed 45% grain had high plasma SAA (40,000 ng/mL) on d 1, reaching the lowest value (20,000 ng/mL) on 10 d of the measurement period.

Feeding diets with high proportions of barley grain affected plasma concentrations of LBP ($P < 0.01$; Figure 4B). Concentrations of LBP in plasma were higher in cows fed 45% barley (10,056 ± 697 ng/mL) grain com-

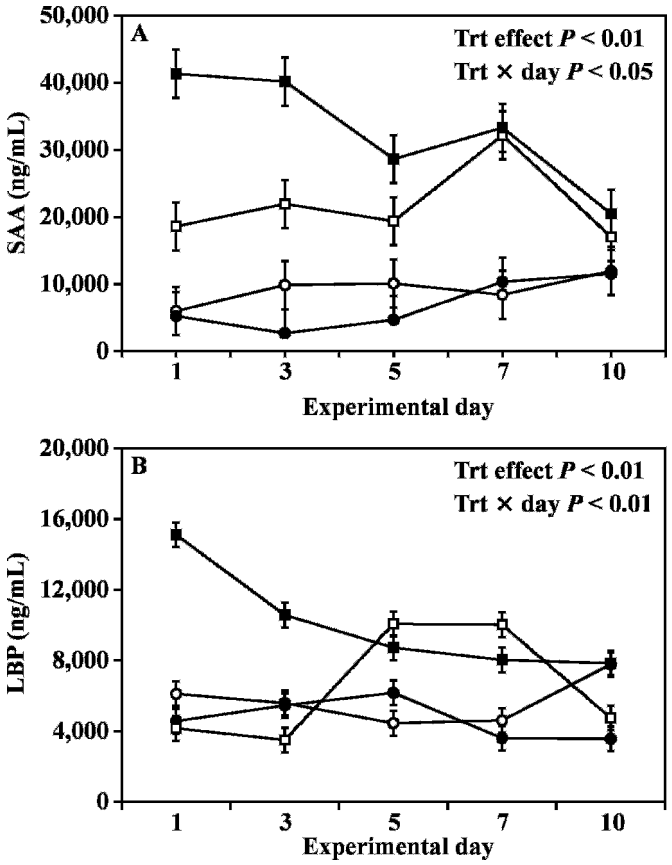

Figure 4. Concentration of serum amyloid A (SAA; A) and LPS-binding protein (LBP; B) in the plasma of early lactating (60 to 140 DIM) cannulated Holstein dairy cows (n = 8) fed 0% (○), 15% (●), 30% (□), or 45% (■) barley grain in a 4 × 4 Latin square design with 11 d of adaptation and 10 d of measurement period. Trt = treatment.

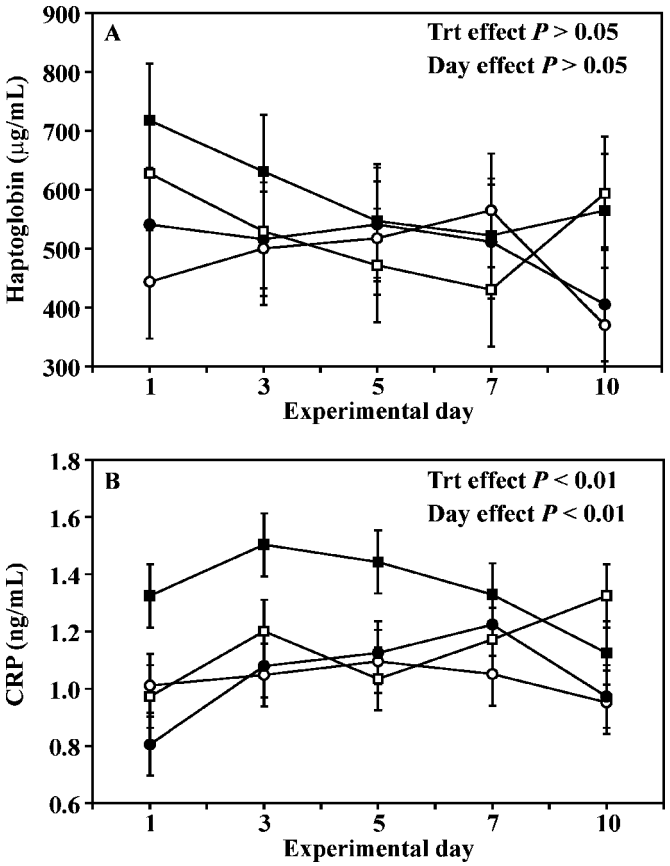

Figure 5. Concentration of haptoglobin (A) and C-reactive protein (CRP; B) in the plasma of early lactating (60 to 140 DIM) cannulated Holstein dairy cows (n = 8) fed 0% (○), 15% (●), 30% (□), or 45% (■) barley grain in a 4 × 4 Latin square design with 11 d of adaptation and 10 d of measurement period. Trt = treatment.

pared with those fed no barley grain (5,707 ± 697 ng/mL) as well as those fed 15% (4,674 ± 697 ng/mL) and 30% barley grain (6,498 ± 697 ng/mL; $P < 0.01$). Plasma concentrations of LBP differed between the groups fed 15 and 30% barley grain ($P < 0.01$). An interaction between treatment and day also was observed ($P < 0.01$). Interestingly, plasma LBP remained unchanged (~6,000 ng/mL) over time in the cows fed 0 and 15% barley grain. In the cows fed 30% grain, there was an increase in plasma LBP from 4,000 ng/mL, at the beginning of the measurement period, to 8,000 ng/mL by d 7 to 10. On the other hand, cows fed 45% grain had high plasma LBP (~16,000 ng/mL) on d 1 and reached the lowest concentration (6,000 ng/mL) by d 10 of the measurement period.

Concentrations of haptoglobin in plasma did not differ among the treatment groups (Figure 5A). There was no effect of day or the treatment × day interaction with respect to plasma concentration of haptoglobin.

The group of cows fed 45% barley grain had higher plasma concentrations of CRP (1.34 ± 0.11 ng/mL) compared with those of cows fed no grain (1.03 ± 0.11 ng/L; $P < 0.001$) or those fed 15 and 30% grain (1.04 ± 0.11 and 1.09 ± 0.11 ng/mL; $P < 0.001$ and $P < 0.001$, respectively; Figure 5B). The day of feeding grain had an effect ($P < 0.05$) on plasma concentration of CRP among treatment groups, whereas no such effect was obtained for the interaction of treatment × day. The group of cows fed the highest proportion of barley grain (45%) in the diet had higher concentrations of CRP compared with other groups ($P < 0.01$). No differences in plasma concentrations of CRP were obtained between the group fed no grain and the groups of cows fed 15 or 30% grain ($P > 0.05$ and $P > 0.05$, respectively) or between the latter 2 groups ($P > 0.05$).

DISCUSSION

Feed Intake, Milk Production, and Ruminal Fluid pH

In the present study, feed intake decreased, whereas DMI increased as the amount of barley grain in the

diet was increased. Two potential mechanisms might explain the feed intake responses of cows to different amounts of grain in the diet: 1) the amount of VFA released in the rumen and 2) translocation of endotoxin from ruminal fluid into blood circulation. First, feeding dairy cows high-grain diets rich in rapidly fermentable carbohydrates is associated with increased release of propionate in the rumen fluid and its absorption into the bloodstream (Sutton et al., 2003). Intraruminal or i.v. infusion of propionate solutions before or after a scheduled meal was associated with depression of feed intake in cattle (Shepherd and Combs, 1998). Rumen fluid analyses, in our experiment, indicated that propionate was higher in cows fed larger amounts of barley grain (data not presented). Therefore, it is possible that absorption of propionate into the blood circulation or its effect on rumen receptors may have contributed to decreased feed intake in cows fed high-grain diets. The second reason for decreased feed intake with increasing the amount of grain in the diet may be due to enhanced release of endotoxin in the rumen fluid, as evidenced in our study, and its translocation into the blood circulation. Increased concentration of endotoxin in the bloodstream and cytokines released after activation of macrophages by endotoxin-like IL-1 and TNF-α suppress feed intake in different species (Porter et al., 1998). The higher DMI with higher-grain diets is related to higher content of DM in cereal grains compared with a forage diet.

Results of our study showed that milk production was higher in cows fed greater amounts of barley grain. Barley grain contains high nonstructural carbohydrates like sugars, starches, and pectins. Nonstructural carbohydrates are degraded rapidly by rumen bacteria to provide high amounts of glucose and propionate. Glucose is essential for synthesis of lactose in the milk. Increasing amounts of either postruminal glucose or ruminal propionate enhance both milk and protein yield in lactating dairy cows when dietary supply of postruminal starch is low such as with grass silage diets (Rigout et al., 2003). Also, because propionate is the major precursor for hepatic glucose production (Danfaer et al., 1995), increased glucose production and release by the liver may favor increased milk production. The increased milk production and lowered feed intake by feeding increasing amounts of barley grain, in the present study, may be due to increased availability of glucose and propionate in the rumen and improved metabolic status due to increased plasma concentrations of these nutrients associated with high-grain feeding. Based on the low number of animals in this experiment and the Latin square experimental design, which extended from 60 to 140 DIM, further research is warranted to confirm this effect of barley on milk production. Unexpectedly, there was a day effect on milk production. The reason for this effect is not clear.

We were able to see differences in ruminal pH by feeding high proportions of grain in the diet. The decreased pH, with increasing amount of grain, is consistent with previous studies reporting energy supplementation at levels >30% of the diet decreases ruminal pH (Mould et al., 1983).

Ruminal Fluid Endotoxin

Results are in agreement with our working hypothesis that feeding increasing proportions of barley grain to lactating dairy cows was associated with increased concentrations of endotoxin in the rumen fluid. Feeding diets containing 30 and 45% barley grain resulted in higher amounts of free endotoxin in the rumen fluid compared with those of cows receiving 0 or 15% barley grain. As expected, no differences in the amount of free endotoxin in the rumen fluid between the groups fed 0 and 15% barley grain were obtained.

Our findings are consistent with previous studies demonstrating enhanced content of endotoxin in the rumen fluid from feedlot or dairy cattle-fed diets containing high proportions of grain (Andersen, 2003). Recently, Gozho et al. (2006) reported a 3-fold increase in the concentration of endotoxin in the rumen fluid when steers were moved from an all-forage diet to a 61% concentrate diet. Interestingly, they failed to detect any changes in the amount of endotoxin in the rumen from an all-forage to 41% concentrate diet. In our study, although the groups of cows fed 0 and 15% barley grain did not differ with respect to concentration of free endotoxin in the ruminal fluid, an increase was observed when the amount of barley grain was enhanced from 0 or 15% to 30 or 45%. The difference in results might be related to the difference in the absolute amount of grain fed to steers vs. dairy cows. Lactating cows are fed greater amounts of grain compared with steers.

Results of this study also showed interesting patterns in the amount of endotoxin in the ruminal fluid in relation with the day of feeding grain diets. Thus, endotoxin in the rumen fluid remained unchanged in cows fed no grain or 15% grain. On the other hand, endotoxin content in the rumen fluid of cows fed 30% barley grain increased from d 1 to 10 of the measurement period and remained from 8,000 to 10,000 ng/mL in the 45% grain group. Understanding the mechanism(s) involved in the release and removal or neutralization of endotoxin in the rumen fluid of dairy cows fed high-grain diets remains to be elucidated in the future.

Plasma APP

Results of this study demonstrated that concentration of SAA, a key APP, increased in plasma of cows fed

higher amounts of barley grain (30 and 45%) compared with cows fed lower amounts (0 or 15%) of barley grain. Our findings are consistent with previous reports of increased plasma concentrations of SAA in dairy cows in which subacute ruminal acidosis was induced by feeding a mixture of wheat and barley grain (Gozho et al., 2006). Serum amyloid A is an APP produced by hepatocytes in response to cytokines like IL-1, IL-6, and TNF-α triggered during infection, inflammation, and tissue injury (Jensen and Whitehead, 1998).

Our study is the first to report an increase in the plasma concentrations of LBP by feeding increasing proportions of barley grain to dairy cows. We observed higher blood concentrations of LBP in groups of cows fed the higher amounts (30 and 45%) of barley grain compared with those fed lower amounts (0 or 15%). Lipopolysaccharide-binding protein is an APP synthesized by hepatocytes in response to IL-1 or IL-6, or both, that binds to endotoxin present in circulation (Tobias et al., 1999). Several studies suggest a protective role for LBP in mediating the host responses to endotoxin. Lipopolysaccharide-binding protein, at low concentrations of endotoxin, activates and amplifies the inflammatory responses to endotoxin, thus potentially serving as a critical component in the activation of the innate immune response against this component of gram-negative bacteria (Gallay et al., 1994).

Interestingly, both SAA and LBP were higher or increased and then decreased toward the end of the experimental period in the higher-grain groups. Statistical processing of data indicated a treatment $\times$ day effect on plasma concentration of both SAA and LBP. The reason for this response is not clear, although we did not find a similar trend with the concentration of endotoxin in the rumen fluid. Therefore, the decline in the concentration of SAA and LBP is not associated with the decrease in the release of endotoxin in the rumen fluid. This might be related to increased mucosal barrier functions in response to translocation of endotoxin as well as involvement of other neutralizing factors such as albumin, transferrin, and lipoproteins in removal of endotoxin from plasma, because the dairy cow is adapted to high-grain diets (Gabay and Kushner, 1999). Based on the plasma responses of SAA and LBP, it can be suggested that dairy cows need at least 3 wk to adapt to high-grain diets.

Research has shown high plasma or milk concentration of CRP in transition cows or in cows with mastitis (Morimatsu et al., 1991). They reported a correlation between plasma CRP and milk yield. It is not clear what the role of CRP is in milk production. The present study, however, is the first to report higher plasma concentration of CRP in cows fed high proportions (45%) of barley grain vs. those fed lower amounts of grain (0, 15, and 30%). Interestingly, cows fed 45% barley grain had higher milk production compared with the other 3 groups. Although no known physiological responses can explain higher plasma CRP in cows fed higher-grain diets, we speculate that the increased plasma CRP in cows fed the highest amount of grain may be due to higher amounts of endotoxin released and translocated into the bloodstream of cows fed higher amounts of grain. Usually, cows with higher milk production tend to be fed higher amounts of grain in the diet. Our data showed a day effect on the concentration of CRP in plasma. This might be related to the amount of endotoxin released in the rumen. It is known that CRP increases up to 1,000-fold within 24 to 48 h of an acute phase stimulus like inflammation, infection, or tissue damage (Volanakis, 2001). Recently, it was demonstrated that CRP protected mice from a lethal dose of endotoxin (Mold et al., 2002). The mechanism by which CRP protects against endotoxin challenge is not yet clear. Mold et al. (2002) showed that CRP-mediated protection against endotoxin shock was associated with enhanced plasma IL-10 and suppression of IL-12. Interleukin-10 inhibits proinflammatory cytokines TNF-α, IL-1, and IL-12 and consequently downregulates the inflammatory response.

CONCLUSIONS

In conclusion, feeding increasing proportions of barley grain to lactating cows was associated with lower feed intake and ruminal fluid pH and higher DMI and milk production. Feeding lactating dairy cows 30 and 45% (DM basis) barley grain increased concentration of endotoxin in the ruminal fluid compared with cows fed 0 and 15% grain in the diet. Also, the higher proportions of barley grain in the ration stimulated an acute phase response with high plasma concentrations of SAA, LBP, and CRP indicating translocation of endotoxin or release of cytokines into the bloodstream. More research is warranted to understand the mechanism(s) that induce an inflammatory response during feeding of high-grain diets as well as on the potential role of endotoxin in the etiology and pathogenesis of multiple metabolic disorders in dairy cows.

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REFERENCES

Ametaj, B. N., B. J. Bradford, G. Bobe, R. A. Nafikov, Y. Lu, J. W. Young, and D. C. Beitz. 2005. Strong relationship between mediators of the acute phase response and fatty liver in dairy cows. Can. J. Anim. Sci. 85:165–175.

Andersen, P. H. 2003. Bovine endotoxicosis: Some aspects of relevance to production diseases. Acta Vet. Scand. 98(Suppl.):141–155.

Canadian Council on Animal Care. 1993. Guide to the Care and Use of Experimental Animals. Vol. 1. 2nd ed. CCAC, Ottawa, Ontario, Canada.

Coetzee, G. A., A. F. Strachan, D. R. van der Westhuyzen, H. C. Hoppe, M. S. Jeenah, and F. C. de Beer. 1986. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. J. Biol. Chem. 261:9644–9651.

Danfaer, A., V. Tetens, and N. Agergaard. 1995. Review and an experimental study on the physiological and quantitative aspects of gluconeogenesis in lactating ruminants. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 111:201–210.

Gabay, C., and I. Kushner. 1999. Acute-phase proteins and other systemic responses to inflammation. N. Engl. J. Med. 340:448–454.

Gallay, P., D. Heumann, D. Le, C. Barras, and M. P. Glauser. 1994. Mode of action of anti-lipopolysaccharide-binding protein antibodies for prevention of endotoxemic shock in mice. Proc. Natl. Acad. Sci. USA 91:7922–7926.

Gozho, G. N., D. O. Krause, and J. C. Plaizier. 2006. Rumen lipopolysaccharide and inflammation during grain adaptation and subacute ruminal acidosis in steers. J. Dairy Sci. 89:4404–4413.

Jensen, L. E., and A. S. Whitehead. 1998. Regulation of serum amyloid A protein expression during the acute-phase response. Biochem. J. 334:489–503.

Kleen, J. L., G. A. Hooijer, J. Rehage, and J. P. T. M. Noordhuizen. 2003. Subacute ruminal acidosis (SARA): A review. J. Vet. Med. A Physiol. Pathol. Clin. Med. 50:406–414.

Mold, C., W. Rodriguez, B. Rodic-Polic, and T. W. Du Clos. 2002. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fcγ R. J. Immunol. 169:7019–7025.

Morimatsu, M., A. Watanabe, K. Yoshimatsu, T. Fujinaga, M. Okubo, and M. Naiki. 1991. Elevation of bovine serum C-reactive protein and serum amyloid P component levels by lactation. J. Dairy Res. 58:257–261.

Mould, F. L., E. R. Orskov, and S. O. Mann. 1983. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis in vivo and dry matter digestion of various roughages. Anim. Feed Sci. Technol. 10:15–30.

Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. J. Dairy Sci. 80:1005–1028.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070–2107.

NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

Porter, M. H., M. Arnold, and W. Langhans. 1998. TNF-α tolerance blocks LPS-induced hypophagia but LPS tolerance fails to prevent TNF-α-induced hypophagia. Am. J. Physiol. 274:R741–R745.

Rigout, S., C. Hurtaud, S. Lemosquet, A. Bach, and H. Rulquin. 2003. Lactational effect of propionic acid and duodenal glucose in cows. J. Dairy Sci. 86:243–253.

Schroedl, W., L. Jaekel, and M. Kruger. 2003. C-reactive protein and antibacterial activity in blood plasma of colostrum-fed calves and the effect of lactulose. J. Dairy Sci. 86:3313–3320.

Shepherd, A. C., and D. K. Combs. 1998. Long-term effects of acetate and propionate on voluntary feed intake by midlactation cows. J. Dairy Sci. 81:2240–2250.

Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86:3620–3633.

Tobias, P. S., R. I. Tapping, and J. A. Gegner. 1999. Endotoxin interactions with lipopolysaccharide-responsive cells. Clin. Infect. Dis. 28:476–481.

Volanakis, J. E. 2001. Human C-reactive protein: Expression, structure, and function. Mol. Immunol. 38:189–197.

Wassell, J. 2000. Haptoglobin: Function and polymorphism. Clin. Lab. 46:547–552.