ABSTRACT: This study investigated the effects of feeding barley grain treated with lactic acid (LA) and heat on the profile of plasma metabolites related to carbohydrate and lipid metabolism and variables related to rumen health and acute phase response. Eight primiparous rumen-fistulated lactating Holstein cows were randomly assigned, in a crossover design, to 1 of the 2 dietary treatments consisting of 32% (DM basis) rolled barley grain steeped in an equal quantity of either tap water alone (CTR) or a 1.0% LA solution and heated at 55°C for 48 h (LAH). Each experimental period was 21 d, with the last 10 d used for measurements. Blood samples were collected on d 1, 3, 5, 7, 9, and 11 before the morning feeding and on the last day of each period at 0, 2, 4, 6, 8, 10, and 12 h postfeeding to measure glucose, lactate, cholesterol, beta-hydroxybutyrate (BHBA), NEFA, haptoglobin (Hp), serum amyloid A (SAA), and tumor necrosis factor-alpha (TNF-α). Also, rumen samples were collected on d 1, 5, and 11 before the morning feeding and on the last day of each period at 0, 4, 8, and 12 h postfeeding on the last day of each period for measuring the concentration of rumen endotoxin. Results of the day-to-day analysis indicated that cows fed the LAH diet had reduced preprandial concentrations of rumen endotoxin (472 vs. 793 ng/mL; P < 0.01) and cholesterol and greater lactate in the plasma; however, treatment had no effect on plasma Hp and TNF-α (P > 0.10). Postprandial responses showed that the LAH diet tended to decrease the concentration of SAA (4.67 vs. 8.50 μg/mL; P = 0.06). Also, there was a treatment by time interaction for rumen endotoxin (P < 0.01), suggesting a role for both the treatment and the time of sampling on this variable. Furthermore, greater concentration of BHBA and a tendency for greater NEFA and reduced concentrations of plasma glucose were observed in cows fed the LAH diet. In conclusion, results indicated that feeding dairy cows barley grain steeped in 1.0% LA and treated with heat modulated the profile of plasma metabolites and acute phase response.

Key words: barley grain, innate immune response, lactic acid and heat, metabolic health

INTRODUCTION

Cows in early lactation often experience a negative energy balance, and one approach to maximize energy intake is to increase the energy density of diets by feeding large amounts of cereal grains. In western Canada, these energy requirements are often met by including increased proportions of barley grain in the diet of lactating dairy cows. However, rapid starch degradation of barley grain is known to increase the release of large amounts of fermentation acids in the rumen fluid, lowering its pH, a process commonly referred to as subacute rumen acidosis (SARA; Yang et al., 1997; Zebeli et al., 2008). It is therefore important to develop feeding strategies that include the processing of rapidly degradable grain such as barley grain to help maintain proper functioning of the rumen and prevent development of a disease state.

Recently, we used low concentrations of lactic acid (LA) to process barley grain fed to dairy cows. The results of this research showed that steeping barley grain in 0.5% LA solution for 48 h decreased the degradation rate in the rumen and increased the amount of
resistant starch of the treated barley (Iqbal et al., 2009). Furthermore, in vivo data indicated that reduced concentrations of VFA (82.1 vs. 65.7 mM) and lactate (205 vs. 140 μM) in the rumen fluid were associated with a reduced risk of SARA (Iqbal et al., 2009).

Because earlier research showed that inclusion of LA in bread affects starch degradation characteristics and glycemic index (Östman et al., 2002a; Svihus et al., 2007), we hypothesized that treating barley grain with a greater concentration of LA at 1.0% and incubating at 55°C for 48 h would improve the rumen fermentation profile and immune status of dairy cows. Therefore, the objectives of this study were to evaluate the effects of feeding rolled barley grain steeped in LA and treated with heat on preprandial and postprandial responses of selected plasma metabolites, rumen endotoxin, and biomarkers of innate immunity in mid- to late-lactation dairy cows.

**MATERIALS AND METHODS**

All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). The data of this research related to the effects on rumen fermentation profile, incidence of SARA, feed intake, and milk production and composition are reported in a companion article (Iqbal et al., 2012). In the present article, data from ruminal fermentation profile are discussed in the context of the changes in the profile of rumen endotoxin and plasma metabolites as well as innate immunity.

**Cows, Diets, and Experimental Design**

Eight ruminally cannulated (inner diameter: 100 mm, Bar Diamond, ID) primiparous Holstein cows at 170 d in milk with an average milk production of 28 kg/d and 680 ± 30 kg BW were used in this study. The cows were assigned, in a paired crossover design, to 1 of the 2 dietary treatments with 2 study periods. Each experimental period was 21 d with 10 d as the adaptation period to the diets and the remaining 11 d as the measurement period. All cows were fed the same basic total mixed ration (TMR) consisting of 15% alfalfa hay, 40% barley silage, and 18% energy and protein supplement. In addition, 31.9% (DM basis) rolled barley grain, steeped in an equal quantity (i.e., in a ratio of 1:1, wt/vol) of either tap water alone (CTR) or a 1.0% LAH solution along with heat treatment at 55°C for 48 h (LAH), was supplemented to the TMR just before the morning feeding at 0800 h. The DM content of rolled barley grain was determined, and it was decreased to 39.7% ± 1.18% or 45.2% ± 2.19% (mean ± SD) after steeping it in tap water or 1.0% LA solution along with heat at 55°C, respectively, from 85.4% ± 1.25%. The LA (DL lactate, 85%, wt/wt) used for steeping the barley grain was purchased from Sigma (Ontario, Canada). The diets were formulated to meet or exceed the requirements of a 680-kg lactating cow producing about 25 kg milk/d with 3.5% fat as per NRC (2001) guidelines. The cows were offered TMR ad libitum intake to allow about 5% feed refusals. The cows were housed in tie stalls with free access to water at all times and were fed once daily at 0800 h. A herd veterinarian monitored cows under experiment for health issues throughout the experimental period.

Diet ingredients were analyzed for concentrations of DM, ash, NDF, ADF, CP, ether extract (EE), and starch after drying samples in a 55°C forced-air oven for 72 h, and then they were ground through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA). The DM concentration was determined by further drying samples to 135°C for 2 h (AOAC, 2002; method 930.15). Ash concentration was determined after burning samples for 5 h at 500°C in a furnace (AOAC, 2002; method 942.05). The methods of Van Soest et al. (1991) were used to determine the NDF and ADF contents of the samples using heat-stable amylase and sodium sulfite in the case of NDF. Crude protein was determined by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy). The values of CP, NDF, starch, and NEf were similar across different diets. The NEf intake was calculated from measured DMI and NEf content of the diets, whereby the latter was determined as the sum of NEf content of individual feeds at 3 times maintenance (NRC, 2001; Iqbal et al., 2012). The ingredients and chemical composition of the diets are presented in Table 1.

**Sample Collection**

Blood samples were collected from the coccygeal vein from each cow on d 11, 13, 15, 17, 19, and 21 [for plasma haptoglobin (Hp), solely on d 11, 15, and 21 of the experimental period] before the morning feeding, whereas for diurnal responses samples were collected on d 21 of the experimental period every 2 h starting from 0800 until 2000 h 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 x g to separate plasma (Rotanta 460 R, Hettich Zentrifugen, Tuttingen, Germany). Plasma samples were stored at −20°C until analysis. For quantitative determination of
Table 1. Ingredients and chemical composition of the experimental diets

| Item                        | Diets
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTR</td>
</tr>
<tr>
<td>Ingredients, % of DM</td>
<td></td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>15.00</td>
</tr>
<tr>
<td>Barley silage</td>
<td>40.00</td>
</tr>
<tr>
<td>Rolled barley grain (water treated)</td>
<td>31.96</td>
</tr>
<tr>
<td>Rolled barley grain (LAH treated)</td>
<td>—</td>
</tr>
<tr>
<td>Canola meal</td>
<td>1.21</td>
</tr>
<tr>
<td>Corn gluten meal</td>
<td>7.81</td>
</tr>
<tr>
<td>Canola oil</td>
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</tr>
<tr>
<td>Biofos²</td>
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</tr>
<tr>
<td>Dairy premix³</td>
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<tr>
<td>Limestone</td>
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<tr>
<td>Sodium bicarbonate</td>
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</tr>
<tr>
<td>Molasses</td>
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</tr>
<tr>
<td>Magnesium oxide</td>
<td>0.18</td>
</tr>
<tr>
<td>Vitamin E (5,000 IU/kg)</td>
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</tr>
<tr>
<td>Vitamin D₃ (500,000 IU/kg)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Nutrient composition, % of DM unless stated

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>CTR</th>
<th>LAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM</td>
<td>57.8</td>
<td>57.8</td>
</tr>
<tr>
<td>Ash</td>
<td>8.12</td>
<td>8.09</td>
</tr>
<tr>
<td>CP</td>
<td>16.6</td>
<td>16.5</td>
</tr>
<tr>
<td>NDF</td>
<td>35.6</td>
<td>35.8</td>
</tr>
<tr>
<td>Forage NDF</td>
<td>30.7</td>
<td>30.7</td>
</tr>
<tr>
<td>ADF</td>
<td>19.3</td>
<td>19.4</td>
</tr>
<tr>
<td>Starch⁴</td>
<td>31.1</td>
<td>32.4</td>
</tr>
<tr>
<td>EE⁵</td>
<td>3.28</td>
<td>3.31</td>
</tr>
<tr>
<td>NFC⁶</td>
<td>36.4</td>
<td>36.3</td>
</tr>
<tr>
<td>NE₇ Meal/kg DM</td>
<td>1.58</td>
<td>1.58</td>
</tr>
</tbody>
</table>

¹CTR = control diet containing rolled barley grain steeped for 48 h in an equal quantity (wt/vol) of tap water; LAH = treatment diet based on rolled barley grain steeped for 48 h in an equal quantity (wt/vol) of tap water containing 1.0% lactic acid and heated at 55°C.
²Contained monocalcium phosphate and dicalcium phosphate in the ratio 2:1 (Champion Feed Services Ltd., Barrhead, Alberta, Canada).
³Contained calcium 0.1%, phosphorus 0.6%, sodium 11.5%, magnesium 0.3%, potassium 0.7%, sulphur 0.23%, zinc 5.00 mg/kg, copper 1.170 mg/kg, manganese 3,100 mg/kg, iodine 80 mg/kg, cobalt 6.2 mg/kg, vitamin A 1,265,000 IU/kg, vitamin D₃ 500,000 IU/kg, vitamin E 3,800 IU/kg.
⁴The contents of soluble starch and resistant starch (McCleary and Monaghan, 2002) were (DM basis) 16.4% and 37.8%, 19.9% and 46.8%, and 8.4% and 64.5% for original rolled barley grain, CTR barley grains, and LAH barley grains, respectively.
⁵EE = ether extract.
⁶Nonfiber carbohydrates = 100 – (% NDF + % CP + % EE + % Ash).
⁷Determined as the sum of NE₇ of individual feeds at 3 times maintenance (NRC, 2001).

The concentration of glucose, beta-hydroxybutyrate (BHB), NEFA, cholesterol, and lactate in the plasma were measured by the method described previously (Ametaj et al., 2009). Quantitative determination of the concentration of plasma Hp was done by commercially available bovine quantitative colorimetric kits (Tridelta Development Ltd., Greystones Co., Wicklow, Ireland) using the methods of Emmanuel et al. (2008). Concentrations of serum amyloid A (SAA) in the plasma were measured by commercially available bovine ELISA kits (Tridelta Development Ltd.) as described previously (Emmanuel et al., 2008).

Plasma tumor necrosis factor-alpha (TNF-α) was measured using commercially available bovine ELISA kits (Bethyl Laboratories, Inc., Montgomery, TX) and as described by the manufacturer. Briefly, standards and samples were incubated in a coated plate, followed by washing and addition of 100 μL of detection antibody and horse radish peroxidase substrate for 1 h and 30 min, respectively. The incubation with each of these reagents was followed by multiple washings (4 times) with wash buffer. The detection antibody solution cross-reacts with the antibodies attached on the walls of the coated wells. The addition of 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB) solution allowed the enzymatic reaction, with the color developed proportional to the amount of anti-TNF-α antibodies present in the solution. The absorbance was measured at 450 nm with a microplate spectrophotometer (Spectramax 340PC384, Molecular Devices Corporation, Sunnyvale, CA). The detection range of TNF-α was between 0.078 and 5 ng/mL.

The concentration of cell-free endotoxin in the rumen fluid was determined by the Pyrochrome Limulus amebocyte lysate assay (Associates of Cape Cod Inc., East Falmouth, MA), using the method described by Emmanuel et al. (2008).

Statistical Analyses

All data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) according to the following model:

\[ Y_{ijklmn} = \mu + S_i + C(S)_j(i) + P_k + T_l + D_m + (TD)_{lm} + e_{ijklmn} \]

where \( Y_{ijklmn} \) = observations for dependent variables, \( \mu \) = overall mean, \( S_i \) = fixed effect of diet sequence \( i \) (\( i = 1 \) to 2), \( C(S)_j(i) \) = random effect of cow \( j \) nested within diet sequence \( i \) (\( j = 1 \) to 8), \( P_k \) = fixed effect of period \( k \) (\( k = 1 \) to 2), \( T_l \) = fixed effect of measurement time \( l \) (\( l = 1 \) to 4 for days and 1 to 7 for hours), \( D_m \) = fixed effect of diet \( m \)
\( l = 1 \text{ to } 2 \), \((TD)_{lm} = \text{fixed effect of diet by time interaction, and } e_{ijklmn} = \text{random residual effect.}\)

Measurements collected at different times on the same cow were considered as repeated measures in the ANOVA, whereby the effects of day and hour were evaluated separately in the model. The \( S_i \) and \( C(S_j(i)) \) terms were included in the model to determine any potential carryover effect. The first-order autoregressive variance-covariance structure of the repeated measures was modeled according to the lowest values of the fit statistics on the basis of the Bayesian information criteria (i.e., smaller is better). Degrees of freedom were approximated by the method of Kenward-Roger (ddfm = kr), and differences at each time point between treatments were conducted with the SLICE option of SAS. Least-squares means and the respective SEM were computed. Significance was declared at \( P \leq 0.05 \), whereas a tendency was considered at \( 0.05 < P \leq 0.10 \).

**RESULTS**

In this study, cows pertaining to the CTR and LAH groups consumed an average of 20.0 and 19.8 kg DM·d\(^{-1}\), respectively (SEM = 0.56; \( P = 0.28 \); Iqbal et al., 2012).

*Plasma Metabolites*

Data showing the responses of preprandial plasma glucose, lactate, and BHBA are presented in Figure 1. Overall, results indicated that cows fed the TMR containing the treated barley grain had a greater concentration of plasma lactate (\( P < 0.05 \)) compared with the CTR group. Furthermore, measurement day alone affected plasma lactate (\( P < 0.01 \)); however, in interaction with dietary treatment this factor did not affect plasma lactate (\( P > 0.10 \)). In addition, preprandial concentrations of glucose and BHBA, measured on different experimental days, were unaffected by treatment (\( P > 0.10 \)). However, data demonstrated an effect of the factor experimental day alone on plasma glucose and BHBA in the present study (\( P < 0.05 \)). In contrast, the factor day of feeding in combination with the dietary treatment did not show an effect on day-to-day variations of plasma glucose and BHBA (\( P > 0.10 \); Figure 1).

Results of the postprandial concentrations of plasma glucose, lactate, and BHBA are shown in Figure 2. Data indicated that cows fed the LAH diet had a decreased concentration of plasma glucose but greater concentration of plasma BHBA compared with the group of cows fed the CTR diet (\( P < 0.05 \)). In contrast, the concentration of plasma lactate was not affected by the dietary treatment (\( P > 0.10 \)). The measurement hour affected postprandial fluctuations of plasma glucose, lactate, and BHBA (\( P < 0.01 \)). In general, concentration of glucose in the plasma was greatest in both groups just before the morning feeding and declined during the first hours after feeding. In contrast, concentrations of plasma BHBA and lactate increased, reaching peak values at 6 h post-feeding. No effect was obtained for the interaction between the treatment and time after feeding for all these variables (\( P > 0.10 \)).

Concentrations of cholesterol and NEFA in the plasma, measured at different experimental days, preprandially, are presented in Figure 3. Data revealed that cows fed the LAH diet tended to have decreased plasma cholesterol (\( P = 0.10 \)). The factor day of sampling alone or in combination with the dietary treatment did not af-
fect plasma cholesterol ($P > 0.10$). In contrast, diet had no influence on preprandial concentration of NEFA in plasma ($P > 0.10$). However, the results indicated an interaction between the factor dietary treatment and measurement day for plasma NEFA ($P < 0.05$).

Data for postprandial concentrations of plasma NEFA and cholesterol are shown in Figure 4. Results indicated that the concentration of plasma NEFA tended to be greater, in particular, during the hours later after the morning feeding, in cows fed the LAH diet ($P = 0.08$). In addition, the factor time of feeding affected plasma NEFA ($P < 0.01$). No effect was observed for the effect sampling time by dietary treatment interaction on diurnal plasma NEFA ($P > 0.10$). Also, the concentration of cholesterol in the plasma, measured at different times after the morning feeding, was not affected by treatment ($P > 0.10$). Furthermore, the factor time after morning feeding alone or in combination with the dietary treatment did not show an effect on postprandial changes in plasma cholesterol ($P > 0.10$) in the present study.

### Rumen Endotoxin and Plasma Acute Phase Reactants

The results of rumen endotoxin and concentration of Hp in the plasma measured preprandially at different days are given in Figure 5. The concentration of rumen endotoxin was reduced in cows fed the LAH diet ($P < 0.05$; Figure 5a). In addition, the factor sampling day alone or in combination with the dietary treatment also affected the concentration of rumen endotoxin ($P < 0.05$) in the present study. In contrast, dietary treatment had no effect on preprandial concentration of Hp in the plasma ($P > 0.10$; Figure 5b). Moreover, this variable was not affected by sampling day alone or by its interaction with dietary treatment ($P > 0.10$).

Data for postprandial concentrations of rumen endotoxin and plasma Hp are shown in Figure 6. An effect
of measurement time on postprandial concentration of rumen endotoxin was observed ($P < 0.01$; Figure 6a). In contrast, the factor dietary treatment alone or in combination with hour of sampling after feeding did not influence postprandial concentration of rumen endotoxin ($P > 0.10$). Treatment of barley grain with LA and heat showed numerically decreased postprandial concentration of Hp in the plasma ($P = 0.13$; Figure 6b). Time after feeding and its interaction with dietary treatment did not affect concentration of Hp in the plasma in this study (Figure 6b).

Postprandial data regarding concentrations of SAA and TNF-α in the plasma are given in Figure 7. Feeding cows with barley grain treated with LAH tended to influence concentrations of plasma SAA ($P = 0.06$) after the morning feeding. In addition, the factor time of feeding affected plasma SAA ($P < 0.01$). Cows fed the LAH diet had reduced concentrations of plasma SAA throughout the day vs. the CTR cows. No differences between the 2 treatment groups were obtained regarding the preprandial concentrations of SAA and TNF-α in the plasma ($P > 0.10$; Figure 8). In the CTR cows plasma SAA ranged between 10,000 and 12,319 ng/mL during the 11-d measurement period, with the peak value on d 5. However, this difference at different days of feeding did not reach significance for plasma SAA between the 2 treatment groups ($P = 0.13$). Results showed no effect of day of feeding on plasma TNF-α ($P > 0.10$). Also, no treatment by day interaction was evidenced for either plasma SAA or TNF-α ($P > 0.10$).

**DISCUSSION**

According to our initial hypothesis the results of the present study showed that the treatment of barley grain with LAH decreased the concentration of preprandial rumen endotoxin and modulated the profile of various plasma metabolites. Indeed, the most important finding of this study was the evidence of decreased concentrations of rumen endotoxin in response to feeding LAH.
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diet, which indicates a better rumen health status and decreased risk of endotoxemia (Emmanuel et al., 2008; Khaefpour et al., 2009). Rumen endotoxin was not different between the 2 treatment groups postprandially (i.e., 0 to 12 h after the morning meal). Endotoxin is released from rapidly growing or dead gram-negative bacteria. Although the reason for this is not understood very well presently, it is speculated that microbiota responses might need more than 12 h to respond to dietary interventions. The beneficial effects of feeding LAH-treated barley grain on reducing rumen endotoxin preprandially can be mainly attributed to the modulatory role of LA and heat treatment on decreasing the fermentability of barley starch in the rumen. Our assumption is supported by other results of this study (Iqbal et al., 2012), whereby feeding cows barley treated with LAH was associated with greater in vivo ruminal pH during the most intensive fermentation phase at 8 h postfeeding (5.92 vs. 5.67 SEM = 0.10; P < 0.01). Moreover, cows fed the diet treated with LAH maintained ruminal pH readings above 5.8 throughout the day (up to 12 h postfeeding; Iqbal et al., 2012), a threshold of ruminal pH often used to indicate SARA (Zebeli et al., 2008). Recently, we showed that ruminal pH < 5.8 for longer than 5.24 h·d⁻¹ should be avoided to prevent development of SARA (Zebeli et al., 2008).

Results also showed that plasma SAA tended to be less in the treated group. The exact mechanisms behind this decrease in the concentration of SAA are not yet clear. However, we hypothesize that decreased concentration of endotoxin in the rumen fluid might have played a role. Endotoxin has been shown to translocate through the rumen and colon tissues (Emmanuel et al., 2007). It is known that SAA is an important component of acute phase response (APR) in cattle associated with lipoproteins, especially with high-density lipoproteins, and its primary role is to bind and neutralize endotoxin that enters into the blood circulation (Levels et al., 2007).

The evidence of decreased concentration of SAA in the plasma of cows fed the LAH-treated barley grain diet is in line with the result of the decreased concentration of rumen endotoxin in those cows. The association between rumen endotoxin and plasma acute phase proteins (APP) was reported previously in dairy cows (Gozho et al., 2007; Emmanuel et al., 2008; Khaefpour et al., 2009) and beef

Figure 6. Postprandial variation of (a) rumen endotoxin and (b) plasma haptoglobin in lactating Holstein cows fed rolled barley grain steeped in an equal volume (wt/vol) of tap water (CTR; diamonds) or in a 1.0% lactic acid solution and heated at 55°C (LAH; squares). Least-squares means ± SEM; n = 8; Trt = effect of treatment; Hour = effect of sampling hour; Trt × Hour = effect of treatment by sampling hour interaction.

Figure 7. Day-to-day variation of plasma serum amyloid A and tumor necrosis factor-alpha (TNF-α) in lactating Holstein cows fed rolled barley grain steeped in an equal volume (wt/vol) of tap water (CTR; diamonds) or in a 1.0% lactic acid solution and heated at 55°C (LAH; squares). Least-squares means ± SEM; n = 8; Trt = effect of treatment; Day = effect of sampling day; Day × Trt = effect of treatment by sampling day interaction.
cattle (Ametaj et al., 2009). In all these studies, the feeding of high-concentrate diets was associated with activation of APR, resulting in greater concentrations of APP, including SAA and Hp, in the systemic blood (Ametaj et al., 2005, 2010). Findings from our group and others suggest that feeding dairy cattle large amounts of cereal grain is associated with an inflammatory state. Indeed, better rumen environment prevented the rise of rumen endotoxin and subsequent activation of APR as reflected by reduced concentrations of plasma Hp and SAA. Previously, we showed that feeding high-grain diets (i.e., barley grain at 30% to 45% of the diet DM) to dairy cows is associated with a 6- to 14-fold increase in the concentration of endotoxin in the rumen fluid and enhanced plasma concentrations of APP related to binding and neutralizing endotoxins like SAA and lipopolysaccharide-binding protein and protection from endotoxin toxicity like C-reactive protein (Emmanuel et al., 2008).

With regard to plasma metabolites data showed that cows fed with the LAH diet had reduced concentration of plasma glucose and greater concentrations of BHBA and lactate in the plasma as well as a tendency for a greater concentration of NEFA compared with their counterparts fed the CTR diet. Changes in those plasma variables reflect the physiological events occurring in the rumen, in response to feeding the treated barley grain. It is speculated that greater BHBA in the plasma, in this study, can be related to greater concentration of butyrate in the rumen fluid, which is metabolized in the rumen epithelium to produce BHBA (Stevens and Stettler, 1966; Weigand et al., 1975; DeFrain et al., 2004). This postulate is supported by the data of rumen fermentation (Iqbal et al. 2012), whereby feeding of rolled barley grain steeped in LAH significantly increased (17.8 vs. 20.9 mM; SEM = 0.93) the concentration of butyrate in the rumen fluid.

The reason for the decreased concentration of plasma glucose in LAH-fed cows could be related to the decreased production of rumen propionate, which serves as the major precursor of glucose via hepatic gluconeogenesis (Reynolds, 2006). Indeed, the results of the fermentation profile of this research showed that feeding cows the LAH diet significantly reduced the concentration of propionate (34.7 vs. 27.4 mM; SEM = 1.22) in the rumen fluid (Iqbal et al., 2012). Moreover, research conducted in human subjects indicated that feeding barley or wheat bread treated with LA lowers the glycemic index (Östman et al., 2002a,b). It was suggested that this is related to reduced starch degradation as a result of interactions between gluten and starch molecules.

Furthermore, the tendency for greater NEFA in the plasma suggests compensative mechanisms of the host to provide energy through lipolysis when glucose is not available. It is known that during periods of decreased glucose and insulin in the plasma, NEFA are mobilized to maintain energy homeostasis by promoting hormone-sensitive lipase activity and lipolysis in the adipose tissue (Webb et al., 1969; Van Harmelen et al., 1999). However, more research is warranted to understand the mechanism(s) behind the metabolic events associated with the effects of feeding LAH-treated barley grain in dairy cows.

The peak concentrations of plasma NEFA occurred immediately after the morning feeding and on d 9, particularly in cows fed the LAH diet. These results are in line with previous reports (Blum et al., 1985; Nielsen et al., 2003; Guo et al., 2007) and indicate the importance of sampling time when using plasma NEFA as an indicator of energy status in dairy cows. The patterns of plasma BHBA during the day (i.e., postprandially) showed the reverse trend compared with those of plasma NEFA, and its concentration was least early after the morning feeding and reached its peak at about 6 h after the meal. This may be explained partly by the fact that the increased concentrations of plasma NEFA in LAH diet leads to their oxidation in liver hepatocytes into ketone bodies and carbon dioxide via the tricarboxylic acid cycle or to their esterification into triacylglycerols, which are exported from the liver in the form of very low density lipoproteins (Roche et al., 2008). Moreover, the
treated cows had decreased glucose, particularly during the hours when BHBA was greater, indicating that increased plasma NEFA and BHBA correspond to reduced plasma glucose. Indeed, the increased concentrations of NEFA in the morning hours may compensate for the relatively low production of BHBA by rumen epithelial cells, which is associated with the reduced feed intake during the night and early morning hours (Ametaj et al., 2009). Thus, the results of NEFA and BHBA suggest that the sampling time should be taken into consideration when evaluating preprandial and postprandial patterns of plasma NEFA and BHBA in dairy cows.

Data from this study showed that cows fed the LAH diet had a tendency for decreased concentrations of plasma cholesterol compared with the control group. We speculate that this effect was due to a shortage of plasma glucose in the cows fed the LAH diet. Indeed, glucose and acetate serve as the major precursors for cholesterol synthesis in the small intestine or liver of dairy cows (Liepa et al., 1978).

It is important to point out that interpretation of daily and diurnal patterns of plasma metabolites should not be limited only to the effects of the diets used. It should be kept in mind that a whole variety of circadian factors, including various enzymes and hormones, also are involved in the regulation of metabolite patterns during the day. Moreover, the short-term (i.e., diurnal) regulation of metabolism reflects both the feeding and feed-regulated signals, including the activity of various hormones like insulin, glucagon, leptin, and ghrelin, which have been shown to exhibit circadian oscillations (Froy, 2010). On the other hand, the long-term concentrations of blood variables are regulated by homeostatic mechanisms and the metabolic status of the host during a determined period of time. For example, the cows in our experiment were mid- to late-lactating cows, which have different nutrient needs compared with early-lactating cows. In conclusion, the interpretation of blood metabolites should take into consideration all the aforementioned factors. However, hormonal regulation of plasma metabolites is beyond the scope of this article, and further information should be pursued elsewhere.

Taken together, the results of this study showed that feeding barley grain steeped in 1.0% LA and treated with heat at 55°C for 48 h reduced rumen endotoxin and plasma SAA and modulated the profile of several plasma metabolites related to carbohydrate and lipid metabolism. Feeding the LAH-treated diet decreased the concentrations of glucose and cholesterol and increased those of BHBA, lactate, and NEFA in the plasma of mid- to late-lactating dairy cows. Further research is warranted to investigate the effects of treatment in periparturient dairy cows and in a larger number of animals.

**LITERATURE CITED**


