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Effects of partial replacement of dietary starch from barley or corn with lactose on ruminal function, short-chain fatty acid absorption, nitrogen utilization, and production performance of dairy cows

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ABSTRACT

In cows fed diets based on corn-alfalfa silage, replacing starch with sugar improves milk production. Although the rate of ruminal fermentation of sugar is more rapid than that of starch, evidence has been found that feeding sugar as a partial replacement for starch does not negatively affect ruminal pH despite increasing diet fermentability. The mechanism(s) for this desirable response are unknown. Our objective was to determine the effects of replacing barley or corn starch with lactose (as dried whey permeate; DWP) on ruminal function, short-chain fatty acid (SCFA) absorption, and nitrogen (N) utilization in dairy cows. Eight lactating cows were used in a replicated 4 × 4 Latin square design with 28-d periods and source of starch (barley vs. corn) and level of DWP (0 vs. 6%, DM basis) as treatment factors. Four cows in 1 Latin square were ruminally cannulated for the measurement of ruminal function, SCFA absorption, and N utilization. Dry matter intake and milk and milk component yields did not differ with diet. The dietary addition of DWP tended to increase ruminal butyrate concentration (13.6 vs. 12.2 mmol/L), and increased the Cl^--competitive absorption rates for acetate and propionate. There was no sugar effect on minimum ruminal pH, and the duration and area when ruminal pH was below 5.8. Minimum ruminal pH tended to be lower in cows fed barley compared with those fed corn (5.47 vs. 5.61). The duration when ruminal pH was below pH 5.8 tended to be shorter (186 vs. 235 min/d), whereas the area (pH × min/d) that pH was below 5.8 was smaller (47 vs. 111) on the corn than barley diets. Cows fed the high- compared with the low-sugar diet had lower ruminal NH_3-N concentration. Feeding the high-sugar diet tended to increase apparent total-tract digestibility of dry matter and organic matters and increased apparent total-tract digestibility of fat. Apparent total-tract digestibility of N tended to be greater in cows fed barley compared with those fed corn, whereas apparent total-tract digestibility of acid-digestible fiber was greater in cows fed corn compared with those fed barley. In conclusion, partially replacing dietary corn or barley starch with sugar upregulated ruminal acetate and propionate absorption, suggesting that the mechanisms for the attenuation of ruminal acidosis when sugar is fed is partly mediated via increased SCFA absorption.
Key words: starch, lactose, short-chain fatty acid absorption, lactation performance

INTRODUCTION

There is potential to use by-products with high sugar content as energy sources in diets for lactating dairy cows (Broderick et al., 2008). The replacement of corn starch with sugar has been reported to induce desirable production responses, including increased DMI (Broderick and Radloff, 2004; Broderick et al., 2008; Penner and Oba, 2009), milk production (Broderick and Radloff, 2004), and milk fat yield (Broderick et al., 2008; Penner and Oba, 2009). Although sugars are rapidly fermented (>500%/h; Weisbjerg et al., 1998), recent studies have demonstrated that increasing dietary sugar content up to 13% of dietary DM by replacing a portion of dietary corn starch with either sucrose (Broderick et al., 2008) or lactose (DeFrain et al., 2004) did not cause a decrease in ruminal pH in lactating cows. In fact, ruminal pH tended to increase (Penner and Oba, 2009) or increased (Martel et al., 2011) when feeding up to 9% sugar as a partial replacement for corn starch. Although several theories have been suggested to explain why feeding more rapidly fermented sugars in place of starch does not depress ruminal pH (Penner et al., 2009a; Oba, 2011), butyrate-induced changes in

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the permeability of ruminal epithelia to short-chain fatty acids (**SCFA**) has received little attention. Feeding lactose increases ruminal butyrate concentrations (Doreau et al., 1987; DeFrain et al., 2004, 2006); during its absorption into blood, up to 90% of ruminal butyrate is metabolized, mainly to BHBA (Bergman, 1990), and several studies (Doreau et al., 1987; DeFrain et al., 2004) have reported elevated plasma BHBA concentrations in dairy cows fed supplemental lactose. Butyrate metabolism during absorption is known to stimulate growth and proliferation of ruminal papillae by increasing cellular mitogenic rate while decreasing apoptosis rate (Mentschel et al., 2001). Morphological surface enlargement resulting from an increase in papillae number and size as diet fermentability and ruminal butyrate concentration increase is well documented (Sakata and Tamate, 1978; Dirksen et al., 1985; Liebich et al., 1987; Baldwin et al., 2004; Naeem et al., 2012). This enhancement of epithelial absorptive area could potentially explain why dietary addition of sugar does not cause an accumulation of SCFA and, subsequently, a decrease in ruminal pH (Ordway et al., 2002; Penner et al., 2009b). However, increasing evidence suggests that functional adaptation in ruminal epithelial cells is characterized by changes in ion transport mechanisms, as diet fermentability increases also play a key role in pH regulation. Sehested et al. (2000) measured short-term adaptation in ruminal absorption of SCFA in cows by feeding additional carbohydrate once daily and measuring epithelial butyrate absorption. In spite of the absence of proliferative changes, butyrate absorption increased as ruminal SCFA concentration increased, possibly implying upregulation of cellular protein-mediated transport (Sehested et al., 2000). Moreover, Penner et al. (2009b) also observed that sheep that were less susceptible to SARA exhibited greater in vitro apical uptake of acetate and butyrate that was mediated via an upregulation in epithelial cell transporter activity. To our knowledge, no study has determined whether dietary inclusion of sugar in dairy cow diets causes changes in SCFA absorption in vivo. It is also not clear if any changes in absorptive function alter the relative proportions of SCFA absorbed via passive diffusion or protein-mediated transport.

Replacing dietary starch with sugar can also alter N utilization via changes in ruminal fermentation. It is well established that carbohydrates digested in the rumen provide energy that drives microbial protein (**MCP**) synthesis (NRC, 2001). Across Canada and the United States, dairy cow diets usually contain barley or corn as the main carbohydrate source. Because sugar is more rapidly fermented in the rumen than starch, substitution of starch with sugar may increase energy availability, thus allowing for more efficient MCP synthesis; however, perusal of the literature indicates that the effects on MCP production of replacing dietary starch with sugar have been equivocal. Intraruminal infusions of sucrose in dairy cattle fed grass silage-based diets (Kim et al., 1999, 2000) or substitution of starch with sugar in continuous culture (Stokes et al., 1991) enhanced MCP production. Conversely, the substitution of starch with sucrose yielded less MCP in dairy cows (Sannes et al., 2002) and in vitro incubations (Hall and Herejk, 2001). These discrepant responses could be attributed to several factors, including differences in the type of sugar (Chamberlain et al., 1993), the forage source (Oelker et al., 2009), and the source of dietary starch that is being replaced with sugar. Corn contains more starch than barley (72 vs. 58%; Huntington, 1997). Also, the rates and extents of ruminal starch degradation differ, with 55 to 70% of corn starch and 80 to 90% of barley starch being degraded in the rumen (Huntington, 1997). Because barley and corn differ in their starch content and ruminal starch digestion, replacing starch with sugar might elicit different responses in ruminal N utilization in lactating cows fed corn or barley as the primary fermentable energy source.

Our objective in the current study was to investigate the effects of partial replacement of barley or corn starch with lactose on ruminal SCFA absorption and ruminal acidosis. The second objective was to delineate the effects of partial replacement of starch from barley or corn with lactose on ruminal fermentation, N utilization, and production performance. We hypothesized that partial replacement of dietary starch with lactose would attenuate ruminal acidosis and improve N utilization and production performance in dairy cows and that the effects would be dependent on the source of starch being partially replaced (i.e., barley or corn).

MATERIALS AND METHODS

Animals and Experimental Design

Eight lactating, multiparous Holstein dairy cows (711 ± 37 kg BW; 109 ± 36 DIM) were used in a replicated 4 × 4 Latin square design with 28-d periods (18 d of dietary adaptation and 10 d of measurements) and a 2 × 2 factorial arrangement of dietary treatments. Four cows in 1 Latin square were ruminally cannulated for the measurement of ruminal fermentation characteristics, SCFA absorption, and N utilization. All cows were housed in individual tiestalls at the Greenbrae Dairy Research Facility (University of Saskatchewan). The University of Saskatchewan Animal Care Committee approved the use of cows for this experiment (UCACS Protocol No. 20040048).

Table 1. Ingredient and chemical composition of diets

Item	Low sugar		High sugar	
	Barley	Corn	Barley	Corn
Ingredient composition, % of diet DM				
Alfalfa hay	15.0	15.0	15.0	15.0
Barley silage	34.4	34.4	34.4	34.4
Soybean hulls	0.25	3.42	0.17	2.60
Barley (rolled)	27.0	—	19.3	—
Corn (rolled)	—	21.9	—	15.8
Whey permeate (dry)	—	—	6.0	6.0
Wheat-based DDGS[1]	10.0	10.0	10.0	10.0
Canola meal	6.2	8.5	9.1	9.3
Corn gluten meal	0.50	0.33	0.58	0.42
Soybean meal	2.5	2.3	1.3	2.3
Fat canola oil	0.60	0.60	0.60	0.60
Mineral-vitamin mix	1.8	1.8	1.8	1.8
Sodium bicarbonate	0.97	0.97	0.97	0.97
Salt	0.37	0.37	0.37	0.37
Limestone	0.23	0.23	0.23	0.23
Dynamate[2]	0.17	0.17	0.17	0.17
Nutrient composition				
DM, %	55.8	56.0	56.3	55.8
OM, % of DM	92.3	92.5	91.8	91.9
CP, % of DM	17.9	17.9	17.9	18.0
Ether extract, % of DM	3.0	3.5	3.0	3.6
ADF, % of DM	18.9	19.6	18.4	19.0
NDF, % of DM	33.4	33.7	32.0	32.2
Starch, % of DM	24.3	24.4	19.9	19.8
Total ESC,[3] % of DM	2.9	3.3	7.6	8.1
NFC,[4] % of DM	38.0	37.5	38.6	38.1
NE_L,[5] Mcal/kg	1.64	1.65	1.64	1.64

[1]Dried distillers grains with solubles.

[2]Dynamate (Eastern Minerals Inc., Henderson, NC) contained 18% K, 11% Mg, and 22% S.

[3]ESC = ethanol-soluble carbohydrates; determined according to Hall et al. (1999) using sucrose as a standard.

[4]NFC = 100 – (%NDF + %CP + %ether extract + %ash).

[5]Estimated using CPM-Dairy (v 3.0.8, Cornell University, Ithaca, NY; University of Pennsylvania, Kennett Square, PA; William H. Miner Agricultural Research Institute, Chazy, NY) using the chemical analysis of feed ingredients.

Experimental Treatments and Feeding Management

The treatment factors were source of starch (barley vs. corn) and dietary inclusion level of dried whey permeate (**DWP**; 0 vs. 6%, DM basis) as a partial replacement for starch. The DWP (Saputo Inc., Tulare, CA) was used as a source of lactose and, according to the manufacturer's specifications, it contained 85.25% water-soluble carbohydrates. Diets were isonitrogenous (18% CP) and contained 3 (low) or 8% (high) total sugar (Table 1). Barley and corn grain were fed as dry rolled, which is the typical processing method used in western Canada. The same batches of DWP, corn, and barley were used throughout the experiment. Cows were fed twice daily at 0900 and 1600 h as a TMR for ad libitum intake. The forage-to-concentrate ratio of the TMR was 50:50 with the forage component of the TMR being a mixture of barley silage and chopped alfalfa hay (Table 1).

Data Collection and Sampling

Feed intake and milk yield and composition were measured using all 8 cows. During each measurement period (d 19 to 28), individual cow feed intake was recorded daily throughout the experiment. Samples of the TMR and orts were collected from d 21 to 23 of each experimental period and were stored at −20°C for later analysis. Cows were milked daily at 0430, 1230, and 1900 h, and milk weights were recorded throughout the experiment. Milk samples were collected on 3 consecutive days (d 21, 22, and 23) from all 3 milkings into vials containing 2-bromo-2-nitropropane-1–2-diol as a preservative. Samples were submitted to the Alberta Central Milk Testing Laboratory (Edmonton, Alberta, Canada) for CP, fat, lactose, and MUN analyses.

Ruminal fermentation characteristics, ruminal SCFA absorption, N balance, and apparent total-tract nutrient digestion were determined using the 4 ruminally

cannulated cows in 1 Latin square. Ruminal pH was measured at 2-min intervals over a 96-h period (d 19 to 23) using the Lethbridge Research Center Ruminal pH Measurement System (Dascor, Escondido, CA) as described by Penner et al. (2006). For the measurement of ruminal SCFA and NH_3-N concentrations, approximately 1,000 mL of ruminal digesta was collected from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula at 0900, 1500, and 2100 h on d 23, 0300, 1200, and 1800 h on d 24, and 0000 and 0600 h on d 25, such that the collected samples were representative of a 24-h feeding cycle. The ruminal contents were strained through 4 layers of cheesecloth. Two 10-mL subsamples of ruminal fluid were then collected and mixed with chilled 25% (wt/vol) metaphosphoric acid (H_2PO_4) or 1% H_2SO_4 and stored at −20°C for later determination of SCFA and NH_3-N, respectively. At the same time points as ruminal digesta sampling, blood samples were collected via a jugular vein catheter into 10-mL vacutainer tubes containing lithium heparin (Becton Dickinson, Franklin Lakes, NJ). Blood samples were then centrifuged at 1,500 × *g* for 15 min at 4°C. Plasma samples were subsequently pooled by cow for each 24-h feeding cycle and then stored at −20°C until analysis for BHBA, glucose, insulin, and plasma urea nitrogen (**PUN**).

To determine apparent total-tract nutrient digestibility and N balance, 4-d total collections of urine and feces were conducted from 0800 h on d 19 to 0800 h on d 23 as described by Plaizier et al. (2000). Briefly, total urine output was collected using indwelling Bardex Foley bladder catheters (26 French, 75-cc ribbed balloon, lubricious-coated; C. R. Bard Inc., Covington, GA). Catheters were inserted at 0900 h on d 18, and were then connected to urine-collection tubing when urine collections were initiated at 0900 h on d 19. Urine was collected into 20-L carboy polyethylene containers into which 200 mL of H_2SO_4 had been added to achieve urine pH of less than 3. The acidification of urine was necessary to prevent microbial degradation and the loss of volatile NH_3-N. Daily urinary output was weighed, mixed thoroughly, and a 5% subsample of the daily output was drawn, pooled for each cow during each collection period, and stored at −20°C until analysis for total N. In addition, a 2-mL aliquot of urine was diluted with 8 mL of distilled water and stored at −20°C for later determination of urea-N. Feces were collected into large steel trays, which were positioned over the gutter behind each stall. Daily fecal output for each cow was determined by weighing and feces were then mixed thoroughly before 2.5% of daily output was sampled and stored at −20°C for later chemical analysis. During each 4-d total collections period, TMR and orts samples were collected daily and stored at −20°C for later analysis.

On d 28, the temporarily isolated and washed reticulo-rumen (**WRR**) technique, as described by Care et al. (1984), was used to determine SCFA absorption. Briefly, reticulo-ruminal contents were evacuated through the cannula and were stored in insulated and covered plastic containers. After evacuation, the reticulo-rumen was washed 3 times with lukewarm water (10 L per wash at 38°C), followed by 4 washes using a buffer solution (8 L per wash at 38°C). The wash buffer contained 100 mmol/L of NaCl, 25 mmol/L of $NaHCO_3$, and 30 mmol/L of sodium acetate. Osmolality of the wash buffer was 310 mOsm/kg and its pH was adjusted to 6.2 by the addition of HCl. After each wash, the wash buffer was removed from the reticulo-rumen using a wet-dry vacuum. Isolation of the reticulo-rumen was achieved by placement of a custom-made esophageal-occluding device (University of Leipzig, Leipzig, Germany) in the distal esophagus. Once placed in the distal esophagus, an inflatable cuff on the occluding device prevented the passage of saliva into the reticulo-rumen but allowed its aspiration into a plastic container using a vacuum pump (UN86KT.45P; KNF Neuberger Inc., Trenton, NJ). To prevent the passage of experimental buffer from the reticulo-rumen, an indwelling Bardex Foley bladder catheter (26 French, 75-cc ribbed balloon, lubricious-coated; C. R. Bard Inc.) was placed in the omasal orifice before the balloon was inflated. Following isolation of the reticulo-rumen, a final wash (8 L of wash buffer) was conducted and the remaining buffer was removed completely from the reticulo-rumen. Subsequently, 20 L of prewarmed (38°C) low- and high-Cl^- experimental buffers containing Cr-EDTA (1 mmol/L) as a volume marker (see Table 2 for the chemical composition of experimental buffers) were sequentially incubated in the reticulo-rumen. To provide some background to this experimental approach using a low- and high-Cl^- buffer, past research has shown inhibitory effects of Cl^- on SCFA absorption (Gäbel et al., 1991; Sehested et al., 1999; Aschenbach et al., 2009). The inclusion of Cl^- at ≥40 mmol/L inhibited acetate and propionate (but not butyrate) absorption from the reticulo-rumen in sheep (Aschenbach et al., 2009), with the inhibited portion accounting for $SCFA^-/HCO_3^-$ exchange, as both $SCFA^-$ and Cl^- serve as suitable substrates. Ruminal Cl^- concentration is usually low, ranging from 16 to 20 mmol/L (Duffield et al., 2004; Shen et al., 2012). Therefore, in our study, measuring WRR disappearance rates using a low-Cl^- buffer gave estimates of total absorption rates for acetate, propionate, and butyrate (i.e., SCFA absorption via lipophilic diffusion and transporter-mediated pathways). Conversely, measur-

ing WRR disappearance rates using a high-Cl^- buffer gave estimates of the uninhibitable SCFA absorption rates (i.e., Cl^--independent absorption). Thereafter, Cl^--competitive SCFA absorption (i.e., bicarbonate-dependent SCFA absorption via the $SCFA^-/HCO_3^-$ exchange system) was estimated as the difference between total and Cl^--independent absorption rates of SCFA. The order in which the experimental buffers were incubated was randomly assigned and balanced for residual effects. Before incubation of the second experimental buffer, what remained of the first experimental buffer was removed completely from the reticulo-rumen. This was followed by another wash (8 L of wash buffer) to prevent buffer carryover. To ensure mixing in the reticulo-rumen, incubated buffer was continuously gassed with 100% CO_2 through the use of tubing fitted with an air stone. A 15-mL sample of each experimental buffer was collected before incubation and stored −20°C for later determination of osmolality. Experimental buffer samples (35 mL) were also collected into 50-mL centrifuge tubes containing 7 mL of 25% (wt/vol) H_2PO_4 before incubation and at 5 and 50 min after incubation in the rumen. Samples were immediately stored at −20°C for later Cr and SCFA analyses. Following collection of the last sample, residual ruminal buffer was vacuumed out and reticulo-ruminal contents were transferred back into the rumen.

Sample Analyses

After the experiment, frozen TMR and fecal samples were thawed overnight at room temperature, pooled per collection period for each cow, and subsequently dried in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and fecal samples were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, England). Samples were analyzed for DM (AOAC, 1990; method 930.15), OM (AOAC, 1990; method 942.05), CP using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05), ether extract (AOAC, 1990; method 920.39), and ADF and NDF (Van Soest et al., 1991). Amylase and sodium sulfite were used for NDF determination. Total starch in ground feed samples was determined using the Megazyme Total Starch Assay Kit (McCleary et al., 1997; Megazyme International Ireland Ltd., Wicklow, Ireland), whereas total ethanol-soluble carbohydrates were determined as described by Hall et al. (1999) and Dubois et al. (1956). Frozen urine samples were thawed overnight at room temperature and then analyzed for N using the macro-Kjeldahl procedure (AOAC, 1990; method 976.05). The plasma and dilute urine samples were analyzed for urea-N by the diacetyl monoxime method (Marsh et al., 1957) using a colorimetric urea-N kit (Stanbio Urea Nitrogen Kit, Procedure No. 0580; Stanbio Laboratory, Boerne, TX). The concentration of glucose in plasma was determined colorimetrically using the glucose oxidase method (Procedure No. 1070; Stanbio Laboratory). Plasma insulin was measured using a commercial bovine ELISA kit (Mercodia AB, Uppsala, Sweden). To quantify plasma BHBA, a coupled enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutyrate dehydrogenase (No. H6501; Roche, Mississauga, Ontario, Canada) and reduction of NAD to NADH was measured using a plate reader at 340 nm. Milk samples were analyzed for fat, CP, lactose, and MUN using infrared spectroscopy (MilkoScan 605; Foss Electric, Hillerød, Denmark; AOAC, 1990; method 972.16).

Ruminal fluid and buffer samples were analyzed for SCFA using GC as described by Khorasani et al. (1996) with minor modifications. Briefly, samples were thawed and centrifuged (6,000 × *g*, 10 min, 4°C) before 1.5 mL of supernatant was transferred to microcentrifuge tubes for additional centrifugation (14,000 × *g*, 10 min, 4°C). A 1-mL subsample of the resultant supernatant was mixed with 200 μL of isocaproic acid as an internal standard. Separation of SCFA was conducted on an Agilent GC system (Agilent 6890 Series, Agilent Technologies, Waldbronn, Germany) using a column (30.0 m × 320 μm × 0.25 μm; model 7HM-G009–11, Zebron, Phenomenex, Torrance, CA) flow rate of 35 mL/min. Column conditions were an initial temperature of 90°C held for 0.1 min before an increase of 10°C/min to 170°C. Injector temperature was set at 170°C, whereas detector temperature was 250°C. Ruminal samples for NH_3-N analysis were thawed and centrifuged (18,000

Table 2. Composition of experimental buffers

Item	Buffer	
	Low chloride	High chloride
Ingredient, mmol/L		
Ca gluconate	2	—
Mg gluconate	2	—
Na gluconate	5	—
Ca chloride	—	2
Mg chloride	—	2
Na chloride	—	5
Potassium acetate	20	20
Sodium acetate	40	40
Mannitol	84	—
Choline chloride	—	40
Sodium propionate	25	25
Butyric acid	15	15
Lactic acid	5	5
$NaHCO_3$	20	20
Cr-EDTA	1	1
Chloride concentration, mmol/L	0	49
Osmolality, mOsm/kg	332	332
pH	6.2	6.2

× *g*, 10 min, 4°C), with the resultant supernatant analyzed for NH_3-N using the phenol-hypochlorite assay (Broderick and Kang, 1980). Ruminal buffers were also centrifuged (6,000 × *g*, 10 min, 20°C) and the supernatant used for Cr analysis by atomic absorption spectroscopy (iCE 3000 series, Thermo Fisher Scientific Inc., Waltham, MA) as described by Williams et al. (1962). An osmometer (model 3250, Advanced Instruments Inc., Norwood, MA) was used to measure osmolality of thawed experimental buffers.

Calculations and Statistical Analyses

The recorded ruminal pH data was summarized daily as minimum, mean, and maximum pH. Ruminal acidosis was considered to occur when ruminal pH was <5.8. The duration (min/d) and total area (pH × min) that ruminal pH was <5.8 were also calculated (Penner et al., 2006).

The Cr concentration of samples collected following 5 and 50 min of incubation were used to determine the actual volume of ruminal buffer using the equation described by Care et al. (1984). The absorption rates of acetate, propionate, and butyrate were then calculated according to the following formulae: absolute SCFA absorption rate (mmol/h) = $[(V_{5min} \times C_{5min}) - (V_{50min} \times C_{50min})] \times 60/45$, and fractional SCFA absorption rate (%/h) = $[(V_{5min} \times C_{5min} - V_{50min} \times C_{50min})/(V_{5min} \times C_{5min})] \times 60/45 \times 100$, where V = volume of buffer and C = concentration of acetate, propionate, or butyrate at the respective time of sampling (Gäbel et al., 1993). To decipher the mechanisms involved in SCFA absorption, total SCFA absorption was SCFA absorption that was measured with the low-Cl^- buffer and Cl^--insensitive absorption was SCFA absorption that was measured with the high-Cl^- buffer. Chloride-competitive SCFA absorption was then calculated as total SCFA absorption minus Cl^--insensitive SCFA absorption. All data on nutrient digestibilities, N balance, ruminal pH, SCFA absorption, and blood metabolites were analyzed as a 4 × 4 Latin square using the PROC MIXED procedure of SAS (SAS Institute, 2004). Production data for the 8 cows were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2004) for a replicated 4 × 4 Latin square design. The model included the following independent variables: cow, period, type of grain (barley vs. corn), level of dietary sugar (low vs. high), and the type of grain × level of dietary sugar interaction. Period and dietary treatment were considered as fixed, whereas cow was considered as random. The interaction term was removed from the model when $P > 0.15$. Treatment differences were considered significant when $P \leq 0.05$ and trends when $0.05 < P \leq 0.10$.

RESULTS

Dietary Characteristics

Dietary ingredient and chemical compositions are presented in Table 1. The 6% inclusion level of DWP targeted a total sugar concentration of approximately 7% based on previous studies showing improved lactation performance with dietary sugar levels ranging between 4.5 and 8.7% (Penner and Oba, 2009; Penner et al., 2009a). Nonstructural carbohydrate analysis of TMR showed that partial replacement of an equivalent amount of barley or corn starch with 6% DWP (DM basis) increased total sugar from 2.9 and 3.3% (DM basis) in low-sugar diets to 7.6 and 8.1% in high-sugar diets, respectively. The starch content of the low-sugar diets was 24.4% compared with 19.9% for the high-sugar diets and there were marginal deviations in total nonstructural carbohydrate concentration (within ±0.6 percentage units of the targeted 38.0%; Table 1). These results indicate that the 4 diets conformed to our experimental plan.

Production Parameters

There was no grain type or sugar effect ($P > 0.05$) for DMI, milk yield, ECM, and feed efficiency (Table 3). Although no diet effect ($P \geq 0.75$) on milk fat content was observed, milk protein content tended to be higher ($P = 0.09$) in cows fed the barley diet compared with those fed the corn diet. Increasing dietary sugar content tended to decrease ($P = 0.09$) milk lactose concentration. No diet effect ($P \geq 0.22$) on milk fat, protein, and lactose yields was noted. Cows fed the barley diet had a greater ($P = 0.046$) MUN concentration compared with those fed the corn diet, whereas increasing the sugar content of the diet decreased ($P < 0.01$) MUN concentration.

Ruminal Fermentation Characteristics and Ruminal pH

Ruminal NH_3-N concentration was lower ($P < 0.01$) in cows fed the high-sugar compared with those fed the low-sugar diet (Table 4). Although total SCFA concentration was not affected by treatment ($P \geq 0.11$), increasing dietary sugar content increased ruminal molar proportions of butyrate ($P = 0.02$) and valerate ($P < 0.01$), whereas it decreased the ruminal molar proportions of isobutyrate ($P < 0.01$) and isovalerate ($P = 0.02$). Feeding barley compared with corn increased ruminal molar proportion of valerate, and the increase was larger in cows fed the low-sugar compared with

Table 3. Dry matter intake and milk yield and composition of cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 8)

	Low sugar		High sugar			*P*-value[1]		
Item	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
DM intake, kg/d	28.2	29.7	29.7	29.8	0.96	0.28	0.28	0.36
Milk yield, kg/d	41.0	40.2	40.5	40.9	2.36	0.95	0.79	0.36
ECM,[2] kg/d	41.6	40.6	40.8	41.3	2.18	0.92	0.66	0.22
Feed efficiency[3]	1.44	1.37	1.38	1.38	0.054	0.39	0.22	0.14
Milk fat, %	3.51	3.54	3.49	3.51	0.130	0.76	0.75	0.99
Milk fat yield, kg/d	1.41	1.39	1.38	1.42	0.079	0.99	0.82	0.31
Milk protein, %	3.40	3.36	3.35	3.27	0.095	0.17	0.09	0.91
Milk protein yield, kg/d	1.38	1.32	1.35	1.33	0.073	0.73	0.22	0.51
Milk lactose, %	4.57	4.58	4.52	4.55	0.061	0.09	0.43	0.76
Milk lactose yield, kg/d	1.88	1.87	1.83	1.87	0.119	0.75	0.99	0.23
MUN, mg/dL	18.0	17.5	17.0	16.1	0.83	<0.01	0.046	0.49

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.
[2]Energy-corrected milk = [0.327 × milk yield (kg)] + [12.95 × fat yield (kg)] + [7.2 × protein yield (kg)] (Orth, 1992).
[3]Feed efficiency = ECM/DMI.

those fed the high-sugar diet (interaction; $P = 0.04$). Ruminal acetate-to-propionate ratio did not differ ($P > 0.05$) with diet.

No effects of sugar or grain source on daily mean ($P \geq 0.17$) and maximum ruminal pH were noted ($P \geq 0.79$; Table 5). In addition, the dietary inclusion of sugar had no effect ($P \geq 0.55$) on the duration and area when ruminal pH <5.8. Daily minimum ruminal pH tended to be higher ($P = 0.06$) in cows fed corn compared with those fed barley; however, the duration ($P = 0.08$) and area ($P = 0.07$) when ruminal pH <5.8 tended to be higher in cows fed barley compared with those fed corn.

Blood Metabolites

Increasing dietary sugar content tended to reduce PUN ($P = 0.07$); however, diet had no effect ($P \geq 0.13$) on plasma glucose, insulin, and BHBA (Table 6).

Ruminal SCFA Absorption

Both total and Cl^--insensitive absolute absorption rates (mmol/h; Table 7) and fractional rates (%/h; Table 8) for acetate, propionate, and butyrate did not differ ($P \geq 0.32$) with diet. However, increasing dietary sugar content increased Cl^--competitive absolute absorption rate (mmol/h) for acetate ($P = 0.02$) and tended to increase ($P = 0.08$) that of propionate. The Cl^--competitive fractional absorption rate was higher for acetate ($P = 0.02$) and tended to be higher for propionate ($P = 0.09$) on the high- compared with the low-sugar diet. However, both absolute and fractional Cl^--competitive absorption rates for butyrate did not differ ($P \geq 0.16$) with diet.

Apparent Nitrogen Balance and Total-Tract Nutrient Digestibility

No diet effect ($P \geq 0.16$) was observed on N intake, urinary and fecal N excretion, and apparent N balance

Table 4. Ruminal fermentation characteristics of cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar			*P*-value[1]		
Variable	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
Ammonia-N, mg/dL	13.1	14.9	11.5	10.8	1.06	<0.01	0.43	0.11
Total SCFA,[2] mmol/L	111	110	112	105	3.6	0.42	0.11	0.18
Molar proportion								
Acetate	59.9	60.5	58.9	60.4	1.36	0.45	0.18	0.48
Propionate	23.3	22.0	23.0	20.7	1.81	0.50	0.17	0.66
Butyrate	10.4	11.2	12.1	12.4	0.54	0.02	0.26	0.62
Isobutyrate	0.94	0.92	0.78	0.81	0.034	<0.01	0.84	0.48
Valerate	1.75	1.63	1.82	1.76	0.027	<0.01	<0.01	0.04
Isovalerate	1.34	1.33	1.11	1.22	0.057	0.02	0.43	0.33
Acetate:propionate	2.67	2.76	2.69	2.93	0.231	0.48	0.25	0.58

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.
[2]SCFA = short-chain fatty acids.

Table 5. Ruminal pH of cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar		SEM	*P*-value[1]		
Variable	Barley	Corn	Barley	Corn		Sugar (S)	Grain (G)	S × G
Ruminal pH								
Daily minimum	5.47	5.52	5.48	5.71	0.134	0.18	0.06	0.20
Daily mean	6.03	6.09	6.05	6.17	0.142	0.42	0.17	0.57
Daily maximum	6.65	6.63	6.63	6.67	0.128	0.79	0.94	0.58
Duration pH <5.8, min/d	476	253	392	217	198.2	0.55	0.08	0.81
Area pH <5.8, pH × min/d	114	51	108	43	53.7	0.82	0.07	0.98

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.

(Table 9). Feeding a high-sugar diet tended to increase apparent total-tract DM ($P = 0.10$) and OM ($P = 0.08$) digestibility and increased ($P = 0.02$) apparent total-tract fat digestion. Apparent total-tract N digestibility tended to be higher ($P = 0.09$) when cows were fed barley compared with corn, whereas feeding corn compared with barley increased apparent ADF digestibility ($P = 0.04$).

DISCUSSION

Increasing concentrate feeding or the fermentation of carbohydrates in the rumen can increase SCFA production, thus increasing energy supply and productivity in high-yielding dairy cows (Broderick, 2003). However, it also increases the risk of ruminal acidosis due to the accumulation of protons in the rumen, which can potentially have negative effects on animal health and production performance (Broderick, 2003; Ferraretto et al., 2013). Therefore, the induction of ruminal acidosis has been a major concern when considering partial substitution of dietary starch with sugar in lactating cow diets. Given its faster rate of ruminal fermentation compared with starch, it is logical to expect that the dietary inclusion of sugar could result in an accumulation of SCFA in the rumen (Firkins et al., 2008; Oba, 2011). However, feeding lactose (3 vs. 8% total dietary sugar) as a partial replacement for barley or corn starch in our study did not result in a decrease in ruminal pH or changes in the duration and area when pH was below 5.8. Similar to our findings, ruminal pH did not change when cows were fed up to 13% of dietary DM as lactose or sucrose (Broderick and Radloff, 2004; DeFrain et al., 2004; Broderick et al., 2008). In fact, feeding up to 5% sucrose as a partial replacement for corn starch tended to increase (Penner and Oba, 2009) or increased (Martel et al., 2011) ruminal pH, thus implying that supplemental sugar could potentially attenuate ruminal acidosis. Thus, a major objective of our study was to delineate if diet-induced changes in ruminal absorption of SCFA (as measured under WRR conditions) could explain why the dietary inclusion of sugar as a partial substitute for starch could attenuate ruminal acidosis.

Gäbel et al. (1991) suggested that changes in ruminal SCFA profiles when more fermentable diets are fed could increase the permeability of ruminal epithelia to SCFA. Ruminal concentration of butyrate typically increases when lactose is fed to dairy cows (Doreau et al., 1987; DeFrain et al., 2004, 2006). As expected, feeding lactose in the current study also resulted in a tendency for an increase in ruminal butyrate concentration. Butyrate is a potent modulator of numerous cellular processes and functions in the gut (Guilloteau et al., 2010). Therefore, it is plausible that increased butyrate supply could trigger rapid functional changes in ruminal epithelial tissue that are geared toward increasing SCFA absorption (Etschmann et al., 2009; Penner et al., 2011). It has been established that the absorption

Table 6. Blood metabolites of cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar			*P*-value[1]		
Variable	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
PUN,[2] mg/dL	19.3	17.8	16.7	17.0	1.61	0.07	0.47	0.29
Plasma glucose, mg/dL	64.3	64.0	64.1	64.5	2.13	0.90	0.95	0.76
Plasma insulin, μg/L	1.12	1.11	1.09	1.56	0.293	0.18	0.15	0.13
Plasma BHBA, mg/dL	10.9	11.0	11.7	11.2	0.81	0.45	0.80	0.71

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.
[2]Plasma urea N.

Table 7. Absolute ruminal absorption rate of short-chain fatty acids (SCFA) in cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar			*P*-value[1]		
Variable	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
Total absorption,[2] mmol/h								
Acetate	564	513	615	604	74.0	0.36	0.68	0.79
Propionate	258	244	265	261	47.9	0.74	0.81	0.89
Butyrate	163	153	169	170	22.6	0.63	0.86	0.81
Total SCFA[3]	984	910	1,048	1,035	141	0.48	0.74	0.82
Cl^--insensitive absorption,[4] mmol/h								
Acetate	556	525	454	548	64.4	0.56	0.63	0.37
Propionate	264	250	215	238	29.9	0.32	0.88	0.54
Butyrate	166	157	141	172	19.1	0.80	0.55	0.32
Total SCFA	984	933	810	958	112.1	0.52	0.67	0.40
Cl^--competitive absorption,[5] mmol/h								
Acetate	9.3	−12.3	160.4	55.3	40.73	0.03	0.15	0.33
Propionate	−6.6	−6.4	49.6	23.4	29.00	0.09	0.57	0.56
Butyrate	−2.6	−3.8	27.9	−2.5	10.30	0.16	0.16	0.19
Total SCFA	0.12	−22.5	237.8	76.2	73.58	0.04	0.24	0.37

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.
[2]Total absorption = SCFA absorption measured with the low-chloride buffer.
[3]Total SCFA = acetate + propionate + butyrate.
[4]Cl^--insensitive absorption = SCFA absorption measured with the high-chloride buffer.
[5]Cl^--competitive absorption = total absorption − Cl^--insensitive absorption.

of SCFA contributes substantially to the regulation of ruminal pH, with estimates of over 50% of protons in the rumen being removed through SCFA absorption (Gäbel et al., 1991; Allen, 1997; Gäbel et al., 2002). Notably, the buffering capacity arising from SCFA absorption is predicted to increase with diets with greater fermentability or proportions of concentrate (Bannink et al., 2012). Passive diffusion and protein-mediated transport are thought to be the key modes of absorption of undissociated SCFA (i.e., HSCFA) and dissociated SCFA (i.e., $SCFA^-$), respectively (Aschenbach et al., 2011). Although both mechanisms could potentially

Table 8. Fractional ruminal absorption rates of short-chain fatty acids (SCFA) in cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar			*P*-value[1]		
Variable	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
Total absorption,[2] %/h								
Acetate	49.4	47.3	52.3	56.8	6.56	0.34	0.85	0.60
Propionate	52.1	51.1	52.6	56.3	9.26	0.68	0.85	0.73
Butyrate	58.1	56.8	58.7	64.6	8.68	0.61	0.78	0.66
Total SCFA[3]	51.4	49.7	53.3	57.9	7.43	0.46	0.83	0.64
Cl^--insensitive absorption,[4] %/h								
Acetate	48.3	48.1	41.2	51.0	6.19	0.72	0.43	0.41
Propionate	54.9	54.8	47.3	53.8	6.99	0.52	0.63	0.61
Butyrate	57.8	58.1	51.1	64.3	7.29	0.97	0.36	0.38
Total SCFA	51.4	51.2	44.2	53.7	6.47	0.70	0.46	0.44
Cl^--competitive absorption,[5] %/h								
Acetate	0.796	−1.286	13.82	5.298	3.7202	0.03	0.19	0.41
Propionate	−1.481	−1.656	10.55	4.386	6.1773	0.11	0.54	0.56
Butyrate	−0.823	−1.513	10.08	−0.893	3.8241	0.17	0.16	0.21
Total SCFA	0.004	−1.533	9.09	4.158	3.9397	0.09	0.43	0.68

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.
[2]Total absorption = SCFA absorption measured with the low-chloride buffer.
[3]Total SCFA = acetate + propionate + butyrate.
[4]Cl^--insensitive absorption = SCFA absorption measured with the high-chloride buffer.
[5]Cl^--competitive absorption = total absorption − Cl^--insensitive absorption.

Table 9. Nitrogen balance and apparent total-tract nutrient digestibility of cows not supplemented (low sugar) or supplemented (high sugar) with dried whey permeate as a partial replacement for starch in diets containing dry-rolled barley or corn as the principal source of starch (n = 4)

	Low sugar		High sugar			*P*-value[1]		
Variable	Barley	Corn	Barley	Corn	SEM	Sugar (S)	Grain (G)	S × G
N intake, g/d	806	819	846	815	42.0	0.46	0.70	0.37
Urinary excretion								
Total, kg/d	38.7	41.3	38.0	38.8	4.60	0.70	0.68	0.83
Total N, g/d	301	307	326	303	15.9	0.16	0.28	0.08
Total N, % of N intake	37.7	37.6	38.5	37.8	2.30	0.79	0.82	0.87
Urea-N, g/d	225	225	247	219	15.3	0.30	0.10	0.10
Urea-N, % of urinary N	74.7	73.3	75.8	71.9	1.80	0.90	0.08	0.38
Fecal excretion								
DM, kg/d	9.25	9.26	8.94	8.76	0.551	0.16	0.75	0.71
N, g/d	233	244	237	244	14.2	0.80	0.32	0.82
N, % of N intake	29.0	29.8	27.9	30.2	0.86	0.60	0.04	0.26
Total N excretion								
g/d	534	551	563	547	26.8	0.08	0.94	0.04
% of N intake	66.7	67.4	66.5	67.9	2.81	0.92	0.60	0.84
Milk N								
g/d	223	211	219	214	20.5	0.92	0.34	0.68
% of N intake	27.4	25.8	25.9	26.0	1.88	0.41	0.34	0.33
Apparent N-balance, g/d	48.8	57.0	64.1	53.8	15.19	0.70	0.95	0.56
Productive N[1], g/d	272	268	283	267	30.4	0.81	0.66	0.79
Apparent total-tract digestibility, %								
DM	67.0	67.8	69.3	68.9	1.16	0.12	0.85	0.55
OM	67.7	68.2	70.5	69.6	1.14	0.09	0.87	0.54
N	70.7	70.2	72.1	69.8	0.90	0.49	0.09	0.23
Ether extract	85.0	83.9	88.1	88.5	1.48	0.03	0.82	0.62
NDF	43.3	49.6	45.3	45.4	1.87	0.53	0.12	0.13
ADF	39.8	45.7	40.0	42.4	1.68	0.38	0.04	0.31

[1]Sugar = effect of the addition of dried whey permeate (as a source of lactose); grain = effect of the source of grain; and S × G = interaction.

remove protons from ruminal fluid, passive diffusion relies on the movement of undissociated SCFA and a portion of the protons are expected to be recycled back to the ruminal fluid as a strategy to regulate intracellular pH (Aschenbach et al., 2011). Conversely, using the "temporarily isolated and WRR technique" in sheep, Gäbel et al. (1991) showed the existence of an anion ($SCFA^-$, Cl^-, HCO_3^-) exchange system in ruminal epithelial cells and suggested that an increase in net HCO_3^- secretion and SCFA absorption by the $SCFA^-$/HCO_3^- exchangers (Gäbel et al., 1991) would help to stabilize ruminal pH. The functionality of the $SCFA^-$, Cl^-, HCO_3^- exchange system was later confirmed by Sehested et al. (1999) and Aschenbach et al. (2009).

In the present study, no lactose effect was noted on total and Cl^--insensitive absorption of acetate, propionate, and butyrate. However, increasing dietary lactose content led to an increase in Cl^--competitive absorption of acetate and propionate, suggesting an upregulation of carrier-mediated transport of dissociated acetate and propionate ($acetate^-/HCO_3^-$ and $propionate^-/HCO_3^-$ exchange). Upregulation of Cl^--competitive absorption of acetate and propionate could have increased the influx of HCO_3^- into the rumen in the current study. This presumption is supported by our observation that increasing dietary lactose content did not cause a decrease in ruminal pH despite the predicted greater rate of fermentation relative to starch. Epithelial cell-derived HCO_3^- plays a critical role in ruminal pH regulation as it reacts with luminal protons (H^+) to form CO_2 and water (Aschenbach et al., 2009, 2011; Connor et al., 2010). Aschenbach et al. (2011) estimated that the anion exchange system in high-yielding dairy cows could contribute a comparable amount of HCO_3^- to the rumen as saliva; Bannink et al. (2012) estimated that the contribution of HCO_3^- from the ruminal epithelium increases with increasing diet fermentability and the proportion of dietary concentrate, such that the expected supply of HCO_3^- from the epithelium is greater than that from saliva. Although speculative, the fact that Cl^--competitive absorption of acetate and propionate increased on the high-sugar diet is suggestive of a functional adaptation mechanism that not only increases absorption of acetate and propionate, but also prevents the expected fall in ruminal pH due to the buffering effect of epithelial cell-derived HCO_3^-. This functional adaptation could involve an increase in the activity or number of transport proteins, including putative anion transporter-1 (PAT), anion exchanger-2 (AE), and downregulated-in-adenoma (DRA), which are bicarbonate (HCO_3^-)-exchange proteins that are thought to be involved in SCFA transport across the ru-

minal epithelium in ruminants (Bilk et al., 2005). Diet-dependent functional and metabolic changes in ruminal epithelial cells have also been reported in other studies. Connor et al. (2010) noted increased gene expression for DRA in calves as diet fermentability increased, whereas Gäbel et al. (1991) reported an increase in both SCFA absorption and HCO_3^- secretion under WRR conditions in concentrate- compared with hay-fed sheep. Storeheier et al. (2003) showed that the documented seasonal decrease in ruminal surface area (decreased papillae length and density) in Arctic reindeer is counteracted by an increase in SCFA transporter activity when they consume high-sugar diets (lichens that contain mannose, glucose, and galactose) in winter. Therefore, besides morphological adaptation, an increase in Cl^--competitive uptake of acetate and propionate as observed in our study is a mechanism that could possibly prevent a deleterious decrease in ruminal pH when feeding high-sugar diets. Other SCFA-transport systems reported in literature (Penner et al., 2009b), including the HCO_3^--independent and nitrate-sensitive uptake of acetate and HCO_3^--independent uptake of butyrate by epithelial tissue, could also be involved but could not be assessed with the methodology that was used in the current study.

In contrast to acetate and propionate, no upregulation of Cl^--competitive absorption of butyrate was observed when feeding a high-lactose diet. When compared with acetate and propionate, passive diffusion is considered to be the major pathway for the ruminal absorption of butyrate, primarily because of its higher lipophilicity and steeper diffusion gradient due to its more extensive metabolism by ruminal epithelium (Aschenbach et al., 2011). Therefore, the marginal contribution of carrier-mediated transport to total absorption of butyrate could possibly explain our findings. Aschenbach et al. (2009) also noted that a Cl^- concentration of up to 80 mmol/L did not inhibit butyrate absorption under WRR conditions. This further implies that Cl^--competitive absorption might not be of significance for butyrate uptake.

As a result of the positive correlation between the rate of carbohydrate fermentation and sequestration of preformed AA and NH_3-N into microbial protein in the rumen (Nocek and Russell, 1988; Russell et al., 1992), feeding sugar has the potential to improve the efficiency of ruminal N utilization in dairy cows. In the current study, partially replacing barley and corn starch with lactose resulted in a decrease in ruminal NH_3-N concentration. Others reported similar findings following dietary inclusion of lactose (DeFrain et al., 2004, 2006) and sucrose (Sannes et al., 2002). Evidence has been noted that some ruminal bacterial species that ferment nonstructural carbohydrates have a preference for preformed AA as precursors for protein synthesis (Oh et al., 1999). Therefore, an increase in the sequestration rate of preformed AA into microbial protein would limit their ruminal deamination into NH_3-N in cows fed supplemental sugar, which could partly explain the decrease in ruminal NH_3-N concentration (Hristov et al., 2005). Additionally, an increase in dietary readily fermentable energy supply could also reduce ruminal NH_3-N concentration by enhancing the use of NH_3-N for microbial growth, especially by fibrolytic bacteria (Russell et al., 1992).

Apart from the decrease in ruminal NH_3-N following the dietary addition of lactose in our study, a decrease in PUN and MUN concentrations was also noted. Others (Charbonneau et al., 2006; DeFrain et al., 2006) also reported similar findings that are suggestive of an improvement in ruminal N utilization (NRC, 2001). A decrease in ruminal NH_3-N could possibly result in a decrease urinary urea-N (**UUN**) excretion by reducing endogenous urea-N production or increasing the proportion of PUN that is recycled to the gastrointestinal tract (Reynolds and Kristensen, 2008). This is supported by work from Sannes et al. (2002) and Broderick et al. (2008), who observed a decrease in both ruminal NH_3-N concentration and UUN excretion when feeding sucrose to cows. However, partial replacement of starch with lactose in the present study did not result in a decrease in UUN excretion. Therefore, it is possible that feeding cows the high- compared with the low-sugar diet in our study did not result in a substantial decrease in endogenous urea-N production or an increase in the repartitioning of PUN toward recycling to the gastrointestinal tract rather than excretion as UUN.

Lactation responses reported in studies that have been conducted to determine the effects of increasing dietary sugar content by replacing starch are equivocal. In the current study, substituting dietary starch with lactose had no effect on milk and milk protein yield, supporting previous studies (DeFrain et al., 2006; Penner and Oba, 2009). However, feeding an incremental amount of sucrose (up to 10% total dietary sugar) resulted in a quadratic change in yields of milk and milk protein that mirrored changes in DMI (Broderick and Radloff, 2004). In the current study, we did not observe a sugar effect on DMI and this, perhaps, partly explains the similar yields of milk and milk protein in cows fed the low- compared with the high-sugar diet. Similarly, others also reported no changes in both DMI and yields of milk and milk protein after partially replacing corn starch with lactose (DeFrain et al., 2006) and sucrose (Penner and Oba, 2009). Increased milk fat secretion has been reported with the feeding of lactose (Schingoethe and Skyberg, 1981) and sucrose (Golombeski et al., 2006; Penner and Oba, 2009). This response

has been attributed to an increase in ruminal butyrate production and its extensive metabolism by ruminal epithelial cells during absorption to form BHBA, which is a precursor for milk FA synthesis. This is supported by the reported positive correlation that exists between plasma BHBA and milk fat yield (Penner and Oba, 2009). In the current study, feeding lactose resulted in a tendency for an increase in ruminal butyrate concentration. However, no sugar effect on milk fat yield was noted, possibly because plasma BHBA concentration was similar across diets.

Given its slower rate and extent of ruminal starch degradation (Patton et al., 2012), we anticipated a less dramatic decrease in ruminal pH when feeding corn relative to barley. Therefore, the higher daily minimum pH and the shorter duration and smaller area when pH was below 5.8 when feeding corn compared with barley were consistent with our expectations. In agreement with our findings, Khorasani et al. (2001) reported the postprandial decrease in ruminal pH to be slower and steadier for corn than barley. McCarthy et al. (1989) and Overton et al. (1995) also reported total SCFA concentration to be lower and average ruminal pH to be higher when dietary corn was replaced with barley. The lower ruminal pH when cows were fed barley instead of corn in our study could partly explain the decrease in apparent ruminal fiber digestion. A ruminal pH of 6.2 has been suggested as the minimum pH for optimum fiber digestion (Van Soest, 1994), and previous in vitro studies have reported that populations of the principal fiber-digesting bacteria, such as *Fibrobacter* spp. and *Ruminococcus albus*, decline rapidly when pH falls below 6.0 (Russell and Wilson, 1996). In the present study, mean ruminal pH was closer to 6.2 and the severity of ruminal acidosis (i.e., the duration and area when pH was <5.8) was lower in cows fed corn compared with those fed barley, thus contributing to the observed differences in ruminal fiber digestion. McCarthy et al. (1989) and Overton et al. (1995) reported similar findings.

Research results on the relationship between dietary starch source (barley vs. corn) and milk production and composition are also equivocal (Silveira et al., 2007). An increase in DMI when cows are fed corn compared with barley resulted in an increase in the amount of substrates available for synthesis of milk and milk components (McCarthy et al., 1989; Overton et al., 1995). In the present study, feeding barley did not compromise DMI, possibly explaining the similar milk yields across diets. Our results are in agreement with Yang et al. (1997) and Sadri et al. (2009), who did not observe changes in either DMI or milk yield when feeding either barley or corn. Changes in ruminal fermentation patterns when cows are fed corn compared with barley starch could also alter milk composition and compositional yields (Gozho and Mutsvangwa, 2008). In our study, milk composition was comparable on the corn and barley diets with the exception of a tendency for increased milk protein content with barley.

CONCLUSIONS

Results from this study indicate that partially replacing dietary corn or barley starch with lactose (as DWP) had no effect on ruminal pH in dairy cows. This response was partly attributed to an upregulation of ruminal Cl^--competitive absorption of acetate and propionate. Our results also indicate that despite the improvement in some indicators of N utilization, including ruminal NH_3-N concentration when cows were fed up to 6% of dietary DM as DWP (lactose), no increase in milk production could be observed. Feeding barley compared with corn in our study resulted in a greater severity of ruminal acidosis, and had no effect on Cl^--competitive absorption of acetate and propionate. Although milk production did not differ, feeding barley compared with corn resulted in a tendency for an increase in milk protein content in this study.

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