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Selection of Barley Grain Affects Ruminal Fermentation, Starch Digestibility, and Productivity of Lactating Dairy Cows

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ABSTRACT

The objective of this study was to evaluate the effects of 2 lots of barley grain cultivars differing in expected ruminal starch degradation on dry matter (DM) intake, ruminal fermentation, ruminal and total tract digestibility, and milk production of dairy cows when provided at 2 concentrations in the diet. Four primiparous ruminally cannulated (123 ± 69 d in milk; mean ± SD) and 4 multiparous ruminally and duodenally cannulated (46 ± 14 d in milk) cows were used in a 4 × 4 Latin Square design with a 2 × 2 factorial arrangement of treatments with 16-d periods. Primiparous and multiparous cows were assigned to different squares. Treatments were 2 dietary starch concentrations (30 vs. 23% of dietary DM) and 2 lots of barley grain cultivars (Xena vs. Dillon) differing in expected ruminal starch degradation. Xena had higher starch concentration (58.7 vs. 50.0%) and greater in vitro 6-h starch digestibility (78.0 vs. 73.5%) compared with Dillon. All experimental diets were formulated to supply 18.3% crude protein and 20.0% forage neutral detergent fiber. Dry matter intake and milk yield were not affected by treatment. Milk fat concentration (3.55 vs. 3.29%) was greater for cows fed Dillon compared with Xena, but was not affected by dietary starch concentration. Ruminal starch digestion was greater for cows fed high-starch diets compared with those fed low-starch diets (4.55 vs. 2.49 kg/d), and tended to be greater for cows fed Xena compared with those fed Dillon (3.85 vs. 3.19 kg/d). Ruminal acetate concentration was lower, and propionate concentration was greater, for cows fed Xena or high-starch diets compared with cows fed Dillon or low-starch diets, respectively. Furthermore, cows fed Xena or high-starch diets had longer duration that ruminal pH was below 5.8 (6.6 vs. 4.0 and 6.4 vs. 4.2 h/d) and greater total tract starch digestibility (94.3 vs. 93.0 and 94.3 vs. 93.0%) compared with cows fed Dillon or low-starch diets, respectively. These results demonstrate that selection of barley grain can affect milk fat production and rumen fermentation to an extent at least as great as changes in dietary starch concentration.
Key words: lactating dairy cow, barley grain, starch digestibility, ruminal fermentation

INTRODUCTION

Barley grain varies in chemical composition; it ranges from 7.5 to 15% CP (LaFrance and Watts, 1986), from 17.3 to 32.1% NDF (Ovenell-Roy et al., 1998), from 45.9 to 62.8% starch (Ovenell-Roy et al., 1998), and from 20.0 to 64.4 %/h for the rate of in situ DM degradation (Khorasani et al., 2000). Yang et al. (1997a,b) compared hulled and hull-less barley grain, and reported that milk production was not affected by treatment despite the distinctive differences in chemical and physical characteristics of the barley grains. However, in the companion study, which compared 2 lots of barley cultivars, Xena and Dillon, cows fed Xena had higher total tract starch digestibility, milk yield, milk protein and lactose concentrations and a tendency to have lower milk fat compared with cows fed Dillon (Silveira et al., 2007). Although that study demonstrated that selection of barley grain affects productivity of lactating dairy cows, the treatment effects on ruminal pH and fermentation, nutrient digestibility in the rumen, and the efficiency of microbial protein production were not evaluated. We hypothesized that lower milk fat content for cows fed Xena resulted from greater starch digestion in the rumen and lower ruminal pH. We also hypothesized that selection of barley grain could affect rumen fermentation and productivity to a similar extent as altering the proportion of grain in the diet, the most common practical approach to changing ruminal fermentation and milk production. The objective of our study was to understand the mechanism by which Xena treatments increased milk production by evaluating rumen fermentation, ruminal and total tract digestion, and microbial protein production of lactating dairy cows. Two dietary concentrations of barley grains were used so that these

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Table 1. Nutrient composition of steam rolled barley grains used to formulate experimental diets

Item	Cultivar	
	Dillon	Xena
DM, %	89.6	87.7
CP, % DM	10.1	12.6
Soluble protein, % CP	2.0	2.3
ADF, % of DM	8.1	3.7
NDF, % of DM	27.0	19.0
Ash, % of DM	2.9	2.8
Starch, % of DM	50.0	58.7
NFC, % of DM	55.9	62.7
Test weight, kg/hL	61.5	75.3
In vitro 6-h starch digested, % of starch	73.5	78.0

effects were evaluated at high and low dietary starch concentrations.

MATERIALS AND METHODS

Cows, Diets, and Treatments

The study was conducted at the Agriculture and Agri-Food Canada Research Centre in Lethbridge (Alberta, Canada), and cows were cared for according to the guidelines of the Canadian Council on Animal Care. Four multiparous Holstein cows in early lactation (46 ± 14 DIM, mean ± SD) that were surgically fitted with ruminal and duodenal cannulas and 4 primiparous Holstein cows in early to mid lactation (123 ± 69 DIM) that were fitted with ruminal cannulas were used. The ruminal cannulas measured 10 cm in diameter and were constructed of soft plastic (Bar Diamond, Parma, ID). Duodenal cannulas were T-shaped and were placed proximal to the common bile and pancreatic duct, approximately 10 cm distal to the pylorus. The multiparous and primiparous cows were separately assigned to one of two 4 × 4 Latin squares balanced for carryover effects. Both squares were conducted simultaneously. Treatments were 2 dietary starch concentrations (30 vs. 23% dietary DM) and 2 lots of steam-rolled barley grain cultivars (Dillon vs. Xena). At the beginning of the experiment, BW of the multiparous cows averaged 628 ± 35 kg and BCS was 3.0 ± 0.40; BW of the primiparous cows averaged 628 ± 55 kg and BCS was 2.8 ± 0.30. Treatment periods were 16 d with the final 6 d used to collect samples and data.

Two lots of barley grain cultivars Xena and Dillon were obtained from a local grain company. These lots were selected because of their distinctive differences in physical and chemical characteristics (Table 1), and were the same lots used in the companion study (Silveira et al., 2007). All experimental diets were formulated to contain 18.3% CP and 20.0% forage NDF (Table 2), and to meet or exceed the other nutrient requirements of the cows (NRC, 2001). Diets were fed as a TMR. Throughout the experiment, cows were housed in tie stalls with continuous access to water, fed 3 times daily (0600, 1200, and 1800 h) for ad libitum intake, and milked in their stalls twice daily (0600 and 1700 h). Animals were allowed to exercise once daily (0900 h) for 2 h.

Data and Sample Collection

Body weight was recorded immediately before the start of the first period and on the last day of each period. Body condition score was determined by a trained investigator 1 d before the start of each period and on the last day of each period, using a 5-point scale (1 = thin to 5 = fat; Wildman, 1982). The amount of feed offered and orts were weighed and recorded daily. Representative samples of all dietary ingredients, TMR, and orts (approximately 12.5% of feed refused, as-fed basis) were sampled daily during the collection period. The orts samples were pooled and 1 sample was retained per cow per period. The DM content of barley silage and alfalfa hay was determined weekly to adjust allocation of forages to maintain a consistent forage-to-concentrate ratio on a DM basis. Milk yield was measured daily and averaged over the collection period. Milk was sampled at every milking on d 13, 14, 15, and 16 of each period.

Ruminal pH was measured continuously for 3 d (d 14 to 16) using the Lethbridge Research Centre ruminal pH measurement system (Penner et al., 2006). Ruminal pH readings were taken every 30 s and averaged every 60 s. Ruminal digesta samples (250 mL per site) were obtained from 4 locations within the rumen (reticulum, dorsal and ventral sac, and the fiber mat), composited, and squeezed through a nylon mesh (1-mm pore size) at 0600, 1800, and 2400 h on d 13, at 1400 h on d 14, and at 0900 and 2100 h on d 15. Five milliliters of filtrate was preserved by adding 1 mL of 25% (wt/vol) HPO_3 to determine VFA concentrations and 5 mL of filtrate was preserved by adding 1 mL of 1% (wt/vol) H_2SO_4 to determine ammonia concentration. The samples were stored at −20°C until the analyses.

Duodenal digesta flow and fecal flow of nutrients were estimated using $YbCl_3$ (GFS Chemicals, Inc., Powell, OH) as an external marker. Ammonium sulfate labeled with ^{15}N ($[^{15}NH_4]_2SO_4$, 10.6 atom % ^{15}N; Isotec-Sigma-Aldrich, St. Louis, MO) was used as a ruminal microbial marker. During d 7 to 16, the marker solution containing Yb and ^{15}N was continuously infused into the rumen of cows via the ruminal cannula using an automatic pump. Daily amounts infused were 1.5 g of Yb and 180 mg of ^{15}N dissolved in 550 mL of water for

Table 2. Ingredient and nutrition composition of experimental diets (% of dietary DM)

	High starch		Low starch	
Item	Dillon	Xena	Dillon	Xena
Ingredients				
Barley silage	31.9	31.9	31.9	31.9
Alfalfa hay	8.7	8.7	8.7	8.7
Concentrate mix[1]	18.4	17.9	18.1	17.8
Barley grain	40.8	41.5	27.5	27.9
Urea	0.2	—	0.04	—
Beet pulp	—	—	13.8	13.7
Nutrients				
Forage NDF	20.0	20.0	20.0	20.0
NDF	33.5	30.8	35.2	33.2
CP	18.6	18.1	18.2	18.4
Starch	28.6	32.0	21.7	24.0
Barley grain starch, % of dietary starch	71.3	76.1	63.4	68.2
Ether extract	2.0	1.8	1.9	1.8
NE_L,[2] Mcal/kg	1.68	1.74	1.64	1.67
Particle size separation				
First (top) sieve (19-mm)	22.7	20.9	18.3	20.3
Second sieve (8-mm)	23.8	30.0	30.4	28.3
Third sieve (1.18-mm)	47.9	44.3	46.1	44.8
Collection pan	5.5	4.9	5.3	6.6

[1]Concentrate mix contained 1.3% canola oil, 26.4% heat-processed canola meal (Alberta Gold, Canbra Foods, Lethbridge, Alberta, Canada), 25.3% heat- and xylose-treated soybean meal (Soy Pass, LignoTech, Rothschild, WI), 3.5% premix of vitamins and minerals, 3.7% calcium diphosphate, 2.3% sodium bicarbonate, 2.3% limestone, 23.9% corn gluten meal, and 11.3% Megalac (Arm & Hammer Animal Nutrition Group, Church & Dwight, Princeton, NJ).

[2]Estimated from diet formulation (CPM-Dairy, version 3.0.7).

each animal. Ruminal (800 mL from the dorsal, middle, and ventral parts of the rumen), duodenal (300 mL), and fecal samples (100 g) were collected at 0600, 1200, 1800, and 2400 h on d 13; 0800, 1400, and 2000 h on d 14; 0200, 0900, 1600, and 2100 h on d 15; and at 0400 h on d 16. Twelve subsamples were pooled per cow per period providing representative ruminal, duodenal, and fecal samples that accounted for diurnal variation. Ruminal samples were immediately squeezed through nylon mesh (1-mm pore size). Ruminal particles were then blended (400 g of ruminal particles plus 400 mL of 0.9% NaCl) in a Waring blender (Waring Products Division, New Hartford, CT) for 1 min and then squeezed through a nylon mesh (1-mm pore size). Filtrates obtained by squeezing the ruminal samples and the filtrate obtained from squeezing the blended homogenate were combined and centrifuged ($800 \times g$ for 15 min at 4°C) to remove protozoa and remaining fine feed particles, and the supernatant was centrifuged ($27{,}000 \times g$ for 30 min at 4°C) to obtain a mixed ruminal bacteria pellet. Microbial pellets were pooled by period for each cow, freeze-dried, and ground using a ball mill (Mixer Mill MM2000; Retsch, Haan, Germany) to determine the ratio of ^{15}N to N, starch, and OM. Duodenal samples were pooled by cow within each period, mixed using a blender (model MX-9100, Toshiba, Tokyo, Japan) and freeze-dried. Fecal samples were collected from the rectum of each cow, dried in a forced-air oven at 55°C, and pooled by cow within each period. Dried duodenal and fecal samples were ground through a 1-mm screen (Thomas-Wiley, Philadelphia, PA).

Sample Analysis

Diet ingredients, orts, duodenal digesta, and fecal samples were analyzed for concentrations of DM, ash, NDF, ether extract (**EE**), CP, and starch. The DM concentration was determined by drying samples at 135°C for 2 h (AOAC, 1990). Ash concentration was determined after 5 h at 500°C in a furnace. The NDF concentrations were determined by the Van Soest method with amylase and sodium sulfite (Van Soest et al., 1991). Crude protein was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; Handbook of Food Analytical Chemistry, 2005), ^{15}N enrichment in rumen bacterial pellets and duodenal samples were also determined by flash combustion (Carlo Erba Instruments) with isotope ratio mass spectrometry (VG Isotech, Middlewich, UK). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide; glucose concentration was measured (Raabo and Terkildsen, 1960) using a glucose oxidase/peroxidase enzyme (Sigma No. P7119), and dihydrochloride (Sigma No. F5803). Absorbance was determined with a microplate reader

(SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Ether extract was determined using a Goldfisch extraction apparatus (Labconco, Kansas City, MO; Rhee, 2005). Ytterbium concentration in duodenal and fecal samples was determined using inductively coupled plasma optical emission spectroscopy according to AOAC (1990) with modification such that no KCl was used during sample digestion.

Milk fat, protein, and lactose, and SCC concentrations were measured (AOAC, 1996) with infrared spectroscopy by Edmonton-Alberta DHIA (MilkoScan 605), and MUN (mg/dL) was determined with an automated infrared Fossomatic 400 Milk Analyzer (Foss North America, Brampton, Ontario, Canada). Ruminal and duodenal ammonia concentrations were determined by the method described by Fawcett and Scott (1960). Ruminal VFA concentrations were determined by gas chromatography (Varian 3700; Varian Specialities, Ltd., Brockville, Ontario, Canada) using a 15-m fused silica column (DB-FFAP column; J&W Scientific, Folsom, CA).

The TMR and orts were analyzed for particle size distribution using the Penn State Particle Separator (Nasco, Fort Atkinson, WI) to determine the extent of sorting, which was expressed as a sorting index. The sorting index was calculated as the actual intake/expected intake for each portion retained on the individual sieves. Expected intake was calculated as the particle size distribution of the TMR (%, as fed basis) × actual as-fed intake. Actual intake was calculated as the amount of feed offered × particle size distribution in the TMR (%, as-fed basis) – the amount feed refused × the particle size distribution in the orts samples (%, as-fed basis). A sorting index of 1 indicates no sorting, a sorting index of <1 indicates sorting against, and >1 indicates sorting for, particles on the particular screen.

Statistical Analysis

All data were analyzed using the fit model procedure of JMP (version 5.1, SAS Institute Inc., Cary, NC) according to the following model:

$$Y_{ijkl} = \mu + S_i + C(S)_{ij} + P_k + T_l + e_{ijklm}$$

where μ = overall mean; S_i = fixed effect of square (i = 1 to 2); $C(S)_{i(j)}$ = random effect of cow nested in squares (j = 1 to 8); P_k = fixed effect of period (k = 1 to 4); T_l = fixed effect of treatment (l = 1 to 4); and e_{ijklm} = residual, assumed to be normally distributed.

The square × treatment interaction was originally included in the model, but it was subsequently removed because the interaction was not significant for any of the response variables. Outliers were removed according to the jackknife distance analysis and orthogonal contrasts were made to evaluate the effect of dietary starch concentration, barley grain and their interactions. Treatment effect and its tendency were considered significant at $P < 0.05$ and $P < 0.10$, respectively.

RESULTS

The DMI was not affected by dietary starch concentration or by barley grain treatment, and averaged 20.0 kg/d (Table 3). Sorting index, BW change, and BCS change were not affected by treatment either. Milk yield was not affected by treatment, and averaged 31.0 kg/d. Milk fat concentration (3.55 vs. 3.29%) was greater for cows fed Dillon compared with Xena, but was not affected by dietary starch concentration. Consequently, 4% FCM tended to be higher for cows fed Dillon compared with Xena (28.7 vs. 27.7 kg/d). The concentrations of milk protein and lactose, SCC, and MUN were not affected by treatment.

High-starch diets tended to decrease mean ruminal pH (6.10 vs. 6.17; Table 4), and increased the duration that pH was below 5.8 (6.4 vs. 4.2 h/d) and the area below pH 5.8 (5,715 vs. 3,375 pH × s) compared with low-starch diets. However, mean ruminal pH was not affected by barley grain treatment, although cows fed Xena had longer duration that ruminal pH was below 5.8 (6.6 vs. 4.0 h/d) and that ruminal pH was between 5.5 and 5.2 (1.97 vs. 1.18 h/d) and tended to have lower minimum pH (5.45 vs. 5.59) and greater standard deviation for daily pH measurements (0.32 vs. 0.28) compared with cows fed Dillon.

Ruminal acetate molar proportion was lower (60.1 vs. 62.0 and 60.2 vs. 61.8 mol/100 mol; Table 4), and propionate molar proportion was greater (24.4 vs. 22.1 and 24.3 vs. 22.2 mol/100 mol) for cows fed Xena or high-starch diets compared with cows fed Dillon or low-starch diets, respectively. Total VFA molar concentration was not affected by treatment.

Apparent total tract digestibilities of DM, OM, EE, and CP were not affected by treatment (Table 5). Total tract starch digestibility was higher for cows fed high-starch (94.3 vs. 93.0%) or Xena (94.3 vs. 93.0%) diets compared with low-starch or Dillon diets, respectively. Dillon treatments had greater total tract NDF digestibility compared with Xena treatments (57.9 vs. 52.3%).

Organic matter truly digested in the rumen and OM flow to the duodenum and apparently digested in the intestines were not affected by treatment (Table 6). Cows fed Xena tended to have more starch truly digested in the rumen (3.85 vs. 3.19 kg/d) compared with cows fed Dillon diets. Cows fed low-starch diets shifted the site of starch digestion from the rumen to the intestines; true starch digestibility in the rumen was lower

Table 3. The effects of dietary starch concentration and barley grain on performance of lactating dairy cows

	High starch		Low starch			*P*-value[1]		
Item	Dillon	Xena	Dillon	Xena	SE	Starch	BG	INT
DMI, kg/d	20.1	20.1	19.9	20.2	0.57	0.71	0.49	0.41
NE_L intake,[2] Mcal/d	39.5	37.2	37.4	37.7	1.47	0.46	0.36	0.20
Sorting index[3]								
First (top) sieve (19-mm)	0.90	0.74	0.83	0.87	0.056	0.55	0.19	0.04
Second sieve (8-mm)	0.99	1.27	1.07	1.05	0.112	0.55	0.27	0.20
Third sieve (1.18-mm)	0.99	1.05	1.02	1.03	0.018	0.98	0.10	0.24
Collection pan	0.93	0.86	0.84	0.85	0.075	0.50	0.68	0.55
Yield, kg/d								
Milk	30.5	30.6	31.1	31.8	1.62	0.14	0.57	0.59
Milk fat	1.09	0.97	1.08	1.06	0.060	0.23	0.02	0.12
Milk protein	0.97	0.97	0.99	1.01	0.044	0.09	0.78	0.61
Milk lactose	1.41	1.41	1.44	1.47	0.080	0.13	0.56	0.56
4% FCM	28.7	26.7	28.7	28.6	1.40	0.12	0.09	0.12
Milk composition, %								
Milk fat	3.59	3.22	3.51	3.35	0.172	0.78	0.01	0.31
Milk protein	3.2	3.21	3.21	3.18	0.083	0.87	0.79	0.49
Milk lactose	4.62	4.62	4.63	4.63	0.075	0.57	0.94	0.94
SCC, 10^3/mL	346	322	351	238	183.0	0.70	0.52	0.67
MUN, mg/dL	13.3	12.6	12.5	12.2	0.70	0.08	0.15	0.47
BW change, kg/d	0.74	0.48	–0.37	0.68	0.229	0.14	0.22	0.02
BCS change, /16 d	0.06	0.03	0	0.09	0.086	1.00	0.72	0.48

[1]Starch = effect of dietary starch concentration; BG = effect of barley grain, Dillon vs. Xena; INT = interaction of dietary starch concentration and barley grain.

[2]NE_L intake was calculated from DMI and measured NE_L content of diets; digestible energy (DE) content of diets was first calculated from nutrient components actually digested in the total tract, and then converted to NE_L according to the NRC (2001): ME_P (Mcal/kg) = 1.01 × DE (Mcal/kg) − 0.45; NE_L (Mcal/kg) = [0.703 × ME_P (Mcal/kg)] − 0.19, where ME_P = ME at production levels of intake.

[3]Sorting index = actual intake/expected intake (<1 sorting against, >1 sorting for, and =1 no sorting).

Table 4. The effects of dietary starch concentration and barley grain on ruminal pH and ruminal fermentation

	High starch		Low starch			*P*-value[1]		
Item	Dillon	Xena	Dillon	Xena	SE	Starch	BG	INT
Ruminal pH								
Daily minimum pH[2]	5.56	5.38	5.63	5.52	0.122	0.18	0.06	0.65
Daily mean pH	6.15	6.05	6.19	6.15	0.087	0.09	0.13	0.41
Daily maximum pH[2]	6.73	6.71	6.74	6.78	0.073	0.28	0.85	0.40
Daily SD	0.29	0.33	0.28	0.32	0.026	0.58	0.06	0.90
pH<5.8, h/d	4.69	8.16	3.40	5.00	1.551	0.05	0.03	0.40
pH<5.5, h/d	1.78	2.78	1.10	1.68	0.783	0.08	0.12	0.66
pH<5.2, h/d	0.45	0.44	0.06	0.1	0.215	0.07	0.95	0.90
pH between 5.8 and 5.5, h/d	2.91	5.39	2.29	3.34	0.998	0.16	0.08	0.44
pH between 5.5 and 5.2, h/d	1.33	2.34	1.04	1.61	0.613	0.17	0.04	0.54
Area <5.8, pH × s/d	4,779	6,651	2,826	3,923	1,850.2	0.05	0.20	0.73
Area <5.5, pH × s/d	1,419	1,702	560	881	632.5	0.10	0.54	0.97
Area <5.2, pH × s/d	266	125	1	43	136.5	0.22	0.72	0.51
VFA								
Total VFA, m*M*	132	130	132	134	3.563	0.54	0.80	0.42
VFA molar proportions, mol/100 mol								
Acetate	61.4	59.0	62.5	61.1	1.387	<0.01	<0.01	0.37
Propionate	22.7	25.8	21.4	22.9	1.782	<0.01	<0.01	0.26
Isobutyrate	0.90	0.83	0.80	0.78	0.019	<0.001	0.02	0.24
Butyrate	11.6	10.8	11.9	11.9	0.629	0.14	0.34	0.44
Isovalerate	1.52	1.45	1.48	1.30	0.077	0.19	0.11	0.48
Valerate	1.45	1.72	1.41	1.59	0.0946	0.30	0.01	0.60

[1]Starch = effect of dietary starch concentration; BG = effect of barley grain, Dillon vs. Xena; INT = interaction of dietary starch concentration and barley grain.

[2]95% confidence interval; mean ± 2 × SD.

Table 5. The effects of dietary starch concentration and barley grain on total tract digestibility (%) of 8 lactating dairy cows

	High starch		Low starch			*P*-value[1]		
Nutrient	Dillon	Xena	Dillon	Xena	SE	Starch	BG	INT
DM	68.6	65.9	66.1	66.3	1.29	0.38	0.31	0.24
OM	70.5	67.9	67.9	68.3	1.29	0.37	0.39	0.23
Ether extract	76.1	73.1	71.1	71.4	2.04	0.12	0.52	0.43
CP	73.1	70.2	71.5	71.4	1.84	0.85	0.28	0.31
NDF	59.0	50.9	56.8	54.5	2.94	0.77	0.04	0.02
Starch	94.1	94.4	91.8	94.2	0.93	0.05	0.04	0.11

[1]Starch = effect of dietary starch concentration; BG = effect of barley grain, Dillon vs. Xena; INT = interaction of dietary starch concentration and barley grain.

(49.2 vs. 70.2%), but intestinal starch digestibility was greater (42.2 vs. 23.2%) compared with high-starch diets.

Cows fed Dillon (6.92 vs. 6.32 kg/d) or low-starch diets (6.90 vs. 6.34 kg/d) had higher NDF intake compared with cows fed Xena or high-starch diets, respectively. Cows fed low-starch diets also had a tendency to have more NDF digested in the rumen compared with high-starch diets (1.99 vs. 1.41 kg/d).

True ruminally digested organic matter (**TRDOM**), N intake, duodenal passage of ammonia N and NAN, microbial N produced daily, microbial efficiency (g of microbial N/kg of TRDOM) and N digested in the total tract were not affected by treatment (Table 7). Cows fed high-starch (11.5 vs. 8.7 mg/dL) or Dillon (11.3 vs. 8.9 mg/dL) diets had higher rumen ammonia concentrations compared with cows fed low-starch or Xena diets. Duodenal passage of nonammonia nonmicrobial N (**NANMN**) was higher for cows fed Xena diets compared with cows fed Dillon when expressed as a quantity (176 vs. 100 g/d), as a percentage of N intake (26.1 vs. 15.9%), and as a percentage of duodenal NAN flow (27.9 vs. 19.0%).

DISCUSSION

Milk yield was not affected by barley starch availability in the rumen or the total digestive tract. This finding is contrary to our previous observation that cows fed Xena had higher yields of milk containing higher concentrations of protein and lactose (Silveira et al., 2007). The primary purpose of the current study was to evaluate the digestion parameters associated with the milk response of cows fed Xena, a barley grain cultivar with high expected ruminal degradability. Failure to observe a consistent milk yield effect between these 2 studies might be partly explained by the use of low-producing cows in the present study. Cows used in the previous study were 94 ± 29 DIM (mean ± SD), with DMI and milk yield averaging 22.3 and 38.4 kg/d, respectively. For cows used in the current study, NE_L and MP intake averaged 37.9 Mcal/d and 2,582 g/d, respectively, which are far greater than requirements to produce the average milk yield of 31.0 kg/d that was attained. Thus, milk production in the present study may not have been limited by energy intake or MP supplied from diets; rather, the limitation may have been the milk potential of the cows.

We hypothesized that cows fed Xena would have lower ruminal pH compared with cows fed Dillon, and that selection of barley grain can affect ruminal fermentation and productivity of cows to a similar extent as altering dietary starch concentration. The duration of ruminal pH below 5.8, ruminal VFA profile, and total tract starch digestibility were affected by both dietary starch concentration and barley grain treatment. In addition, it is noteworthy that milk fat yield and concentration were decreased for cows fed Xena vs. Dillon, but not for cows fed high-starch diets vs. low-starch diets. These results demonstrate that selection of barley grain can affect ruminal fermentation and milk production to an extent at least as great as dietary starch concentration.

Rumen Fermentation and Milk Fat Depression

Milk fat depression was observed for cows fed Xena. Although fatty acid composition was not determined in this study, Xena treatments increased the duration that ruminal pH was below 5.8 and tended to decrease the daily minimum pH, which could have caused an accumulation of *trans* $C_{18:1}$, and led to milk fat depression (Gaynor et al., 1995; Griinari et al., 1998). However, milk fat was not depressed for cows fed high-starch diets, although this diet increased the duration that ruminal pH was below 5.8; thus, low rumen pH itself may not explain the milk fat depression observed in our study. It is noteworthy that the fluctuation in rumen pH, measured as the standard deviation of ruminal pH measurements, tended to be greater for cows fed Xena compared with cows fed Dillon ($P < 0.06$), but was not affected by dietary starch concentration. Greater

Table 6. The effects of dietary starch concentration and barley grain on digestibility of DM, OM, starch, and NDF of 4 ruminally and duodenally cannulated lactating dairy cows

	High starch		Low starch			*P*-value[1]		
Item	Dillon	Xena	Dillon	Xena	SE	Starch	BG	INT
DM								
Intake, kg/d	20.7	20.9	20.3	20.7	0.94	0.31	0.28	0.72
Apparently digested in total tract								
kg/d	14.1	13.1	13.6	13.4	1.02	0.84	0.44	0.61
%	67.7	64.8	64.9	64.3	2.65	0.55	0.54	0.67
OM								
Intake, kg/d	19.3	18.8	19.3	19.1	0.871	0.64	0.30	0.70
Apparently digested in the rumen								
kg/d	4.61	5.58	5.87	5.33	0.546	0.18	0.53	0.06
%	24.8	30.4	30.2	26.9	3.16	0.46	0.54	0.03
Truly digested in the rumen								
kg/d	10.8	11.6	10.8	11.0	0.69	0.61	0.38	0.53
%	56.1	57.9	60.1	55.9	3.11	0.68	0.64	0.28
Passage to the duodenum, kg/d	13.7	13.3	13.4	14.8	1.11	0.25	0.38	0.13
Apparently digested in the intestines								
kg/d	7.86	7.02	6.99	7.25	0.822	0.60	0.64	0.35
% of intake	42.1	36.5	36.4	38.0	3.36	0.45	0.46	0.20
% of duodenal passage	55.0	50.5	51.8	55.4	3.56	0.80	0.90	0.28
Apparently digested in total tract								
kg/d	13.5	12.6	12.9	12.9	0.96	0.72	0.48	0.59
%	69.6	66.7	66.6	66.4	2.67	0.54	0.60	0.65
Starch								
Intake, kg/d	6.19	6.79	4.71	5.20	0.275	<0.001	<0.001	0.46
Digested in the rumen								
kg/d	4.16	4.93	2.21	2.77	0.271	<0.001	0.06	0.72
%	67.8	72.6	46.1	52.2	4.37	0.005	0.27	0.89
Passage to the duodenum, kg/d	2.03	1.87	2.50	2.41	0.269	0.05	0.54	0.87
Digested in the intestines								
kg/d	1.60	1.45	2.05	2.07	0.289	0.03	0.73	0.63
% of intake	25.2	21.1	43.7	40.6	4.23	0.004	0.37	0.90
% of duodenal passage	74.9	74.2	81.3	85.8	4.80	0.02	0.52	0.39
Digested in total tract								
kg/d	5.76	6.37	4.26	4.85	0.325	<0.001	<0.001	0.92
%	92.9	93.6	89.8	93.3	1.03	0.15	0.09	0.23
NDF								
Intake, kg/d	6.69	6.00	7.16	6.64	0.340	<0.001	<0.001	0.37
Digested in the rumen								
kg/d	1.45	1.36	2.36	1.61	0.345	0.07	0.15	0.25
%	21.3	24.3	32.7	23.9	6.28	0.22	0.49	0.19
Passage to the duodenum, kg/d	5.07	4.65	4.80	5.04	0.563	0.85	0.78	0.33
Digested in the intestines								
kg/d	2.12	2.81	1.54	2.00	0.725	0.63	0.88	0.39
% of intake	30.1	19.7	21.7	29.4	8.90	0.91	0.82	0.18
% of duodenal passage	42.7	30.9	32.1	33.6	9.55	0.44	0.33	0.22
Digested in total tract								
kg/d	3.8	3.17	3.9	3.59	0.504	0.49	0.17	0.57
%	56.4	52.5	54.4	53.3	4.52	0.91	0.60	0.76

[1]Starch = effect of dietary starch concentration; BG = effect of barley grain, Dillon vs. Xena; INT = interaction of dietary starch concentration and barley grain.

fluctuation in ruminal pH may indicate a more pulsatile nutrient supply from the rumen (Oba and Allen, 2000), because ruminal pH affects the rate of VFA absorption. This pattern is consistent with the observation that cows fed Xena had greater plasma insulin concentration compared with cows fed Dillon (Silveira et al., 2007). Milk fat depression observed for cows fed Xena, but not for cows fed high-starch diets, can be at least partly attributed to the glucogenic theory (Gaynor et al., 1995). However, the recent work using the hyperinsulinemic-euglycemic clamp (Corl et al., 2006) does not support the glucogenic-insulin theory for diet-induced milk fat depression.

Daily mean ruminal pH was not affected by treatment although the amount of starch truly digested in the rumen varied from 2.25 to 4.94 kg/d among treatments. All experimental diets were formulated to contain the same concentration of forage NDF, which stimulates chewing and rumination, and increases saliva secretion (NRC, 2001). It is interesting that daily mean

Table 7. The effects of dietary starch concentration and barley grain on N metabolism of 4 ruminally and duodenally cannulated lactating dairy cows

	High starch		Low starch			*P*-value[1]		
Item	Dillon	Xena	Dillon	Xena	SE	Starch	BG	INT
TRDOM,[2] kg/g	10.8	10.8	11.6	11.0	0.69	0.39	0.61	0.54
N intake, g/d	627	594	620	620	23.5	0.34	0.14	0.12
Ammonia in the rumen, mg/dL	13.6	9.3	9.0	8.4	4.89	<0.001	<0.001	<0.001
Passage to the duodenum								
NAN								
g/d	548	588	567	581	31.3	0.82	0.35	0.67
% of intake	87.6	98.5	91.8	94.9	5.16	0.94	0.15	0.40
NANMN[3]								
g/d	112	149	87	163	22.9	0.77	0.02	0.32
% intake	17.6	25.3	14.1	26.9	4.39	0.78	0.03	0.49
% of duodenal NAN	21.9	26.9	16.0	28.9	4.85	0.57	0.04	0.29
Microbial N								
g/d	426	438	480	418	48.2	0.67	0.55	0.37
% of duodenal NAN	78.1	73.1	84.0	71.1	4.85	0.58	0.04	0.29
g/kg of TRDOM	44.7	43.0	41.4	40.3	3.56	0.26	0.57	0.91
NAN digested in the intestines								
g/d	376	392	383	394	36.1	0.90	0.72	0.96
% of duodenal passage	68.2	66.9	70.1	67.4	3.53	0.76	0.61	0.86
N apparently digested in total tract								
g/d	455	398	436	433	29.4	0.69	0.16	0.20
%	72.3	67.2	70.3	69.3	3.41	0.92	0.30	0.42
MP intake[4]								
g/d	2,422	2,732	2,549	2,708	179.6	0.66	0.09	0.53

[1]Starch = effect of dietary starch concentration; BG = effect of barley grain, Dillon vs. Xena; INT = interaction of dietary starch concentration and barley grain.

[2]TRDOM = true ruminally degraded OM.

[3]NANMN = nonammonia nonmicrobial nitrogen.

[4]MP intake (g/d) was calculated from the measured flows of NANMN and microbial N and NAN digestibility in the intestines assuming that 80% of microbial N is true protein according to NRC (2001).

ruminal pH averaged 6.1, which is the pKa of bicarbonate, the primary salivary buffer. These observations indicate that salivary buffer played a dominant role in maintaining similar daily mean ruminal pH across treatments. However, milk fat depression observed for cows fed Xena, despite no differences in daily mean ruminal pH, indicates that mean rumen pH does not necessarily reflect effects of ruminal fermentation on physiological responses including milk fat depression. This finding emphasizes the importance of continuous measurement of ruminal pH. Although barley grain treatment did not affect mean ruminal pH, it affected milk fat production and the duration that rumen pH was below 5.8, which can be determined only by continuous measurement of ruminal pH.

Digestibility and N Metabolism

Digestibility. We found that cows fed low-starch diets, in which barley grain was replaced by beet pulp, decreased starch digestibility in the rumen. In agreement with our observation, Ipharraguerre et al. (2002) and Voelker and Allen (2003) found that starch digestibility in the rumen decreased when fibrous byproducts (soyhulls and beet pulp, respectively) were added to diets of lactating dairy cows. Voelker and Allen (2003) attributed the linear reduction in true ruminal starch digestibility caused by the increased substitution of high moisture corn with beet pulp to the decreased amylotic enzyme activity in the rumen and to increased starch passage rate caused by greater ruminal fill, which may also have occurred in our study. Alternatively, starch from dietary ingredients other than barley grain may have been more resistant to microbial degradation in the rumen because barley grain only provided 73.7 and 65.8% of total dietary starch, respectively for high- and low-starch diets. Although true ruminal starch digestibility was 20 percentage units greater for cows fed high-starch diets compared with low-starch diets, the difference in apparent total tract starch digestibility was far less, indicating that compensatory postruminal starch digestion occurred for cows fed low-starch diets. Previous research has also shown that, unless enzyme activities limit intestinal starch digestion, compensatory starch digestion in the intestines occurs when less ruminally fermentable grains are fed (Knowlton et al., 1998; Ying and Allen, 1998; Callison et al., 2001).

Starch digestibility in the rumen was not affected by barley grain although Xena had a greater 6-h in vitro

starch digestibility than Dillon (78.0 vs. 73.5% starch). Ruminal starch digestibility is also affected by passage rate (Nocek and Tamminga, 1991). Thus, lack of significant effects of barley grain treatment on ruminal starch digestibility might have been due to faster passage rate of Xena from the rumen, although passage rate was not measured in this study. However, kernel density was greater for Xena compared with Dillon (75.3 vs. 61.5 kg/hL), which may have increased kernel passage from the rumen due to higher specific gravity (Murphy et al., 1989). The amount of starch digested in the rumen was greater for Xena treatments, which is consistent with the lower minimum pH and the longer duration of pH below 5.8.

Apparent total tract NDF digestibility was lower for cows fed Xena, which could be explained by the lower ruminal pH and higher starch concentrations of Xena treatments because low ruminal pH and presence of starch negatively affect NDF digestion (Grant and Mertens, 1992). However, ruminal NDF digestion was not affected by barley grain treatment; thus, the reasons for decreased total tract NDF digestibility for Xena treatments are not known.

N Metabolism. In the companion study, milk protein yield was greater for cows fed Dillon (Silveira et al., 2007); therefore, we expected that barley grain treatment would affect N metabolism. Although MP intake tended to be greater for cows fed Xena compared with those fed Dillon, microbial N flow to the duodenum and microbial efficiency were not affected by treatment in the current study. Cows fed Xena or high-starch diets had longer duration that ruminal pH was below 5.8. Lower pH can cause energy spilling by using additional energy to maintain intracellular pH and decreasing the energy available for microbial growth (Strobel and Russell, 1986). But, barley grain treatment did not affect microbial efficiency in the current study.

Greater ruminal ammonia concentration for the high-starch diets was not expected. These higher ruminal ammonia concentrations were probably due to greater protein degradability for barley grain compared with beet pulp; barley grain protein has a greater soluble fraction, as well as a faster rate of degradation for the potentially degradable fraction, compared with beet pulp protein (NRC, 2001). Similarly, the greater ruminal ammonia concentration and the decreased duodenal flow of NANMN for Dillon treatments may have resulted from the urea supplementation of the Dillon barley that was used to make the experimental diets isonitrogenous. It is well documented that urea supplementation increases ruminal ammonia concentration (Cameron et al., 1991).

CONCLUSIONS

The duration that ruminal pH was below 5.8 was longer, ruminal propionate concentration was greater, and ruminal acetate concentration was lower for cows fed Xena or high-starch diets compared with cows fed Dillon or low-starch diets, respectively. Milk fat yield and concentration were decreased for cows fed Xena, but were not affected by dietary starch concentration. The 2 lots of barley cultivars evaluated in this study affected ruminal fermentation, digestibility, and milk production to an extent as great as the changes in dietary starch concentration. A more extensive evaluation is required to establish whether the lots of barley grain used in this study are representative of these particular cultivars, and to determine the range in digestion characteristics that occurs among other barley cultivars used in dairy rations. This study demonstrates that selection of barley grain lots for quality is an important management tool to optimize milk production.

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