Effects of method of barley grain processing and source of supplemental dietary fat on duodenal nutrient flows, milk fatty acid profiles, and microbial protein synthesis in dairy cows

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ABSTRACT

The objective of this study was to determine the interactive effects of barley grain processing and source of supplemental fat on ruminal nutrient digestion, duodenal nutrient flow, microbial protein synthesis, and milk fatty acid (FA) profiles in dairy cows. Four Holstein cows (656 ± 28 kg of BW; 80 ± 12 d in milk) fitted with permanent ruminal and duodenal cannulas were used in a 4×4 Latin square design with a 2×2 factorial arrangement of experimental treatments, in which barley was either dry-rolled (DRB) or pelleted (PB) and supplemented with full-fat canola seed (canola) or full-fat flaxseed (flaxseed). Cows fed DRB consumed 1.8 to 3.5 kg/d more dry matter compared with those fed PB. Mean ruminal pH was lower and the duration (min/d) and area (pH $\times$ min) of total ruminal acidosis (RA; ruminal pH <5.8), mild RA ($5.8 >$ ruminal pH >5.5), and severe RA ($5.5 >$ ruminal pH >5.2), and the duration of acute RA (ruminal pH <5.2) were greater in cows fed PB compared with those fed DRB. Milk yield was unaffected by diet. Milk fat content and yield were greater in cows fed DRB than in cows fed PB. Cows fed PB had greater duodenal flows of C18:1 *trans*, C18:1 *cis*, C18:2 *trans*-9,*trans*-12, C18:2 *trans*-9,*cis*-12, C18:2 *trans*-11,*cis*-15, and C18:2 *trans*-10,*cis*-12 compared with those fed DRB. Duodenal flows of C18:1 *cis*, C18:1 *trans*, total C18:1, C18:2n6, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-10,*cis*-12, C18:2 *trans*-11,*trans*-13, total conjugated linoleic acid (CLA), and C18:3n3 were greater in cows fed flaxseed compared with those fed canola. Milk concentrations of C18:3n3 and total CLA tended to be greater in cows fed PB compared with those fed DRB. When compared with cows fed canola, milk concentrations of C18:2 *trans*-11,*cis*-15, C18:2 *cis*-9,*trans*-11, C18:2 *trans*-11,*trans*-13, total CLA, and C18:3n3 were greater in cows fed flaxseed. Ruminal digestion of neu-

tral detergent fiber and acid detergent fiber was greater in cows fed DRB compared with those fed PB. Duodenal flow of ammonia-N tended to be greater in cows fed PB compared with those fed DRB. Duodenal flow of nonammonia nonmicrobial N was greater in cows fed flaxseed compared with those fed canola; however, barley grain processing did not affect duodenal nonammonia nonmicrobial N flow. Duodenal flow of microbial nonammonia N and microbial efficiency were not affected by diet. In summary, barley grain processing and source of fat altered ruminal FA biohydrogenation, and this was reflected in changes in duodenal FA flow and milk FA profiles; however, ruminal microbial protein synthesis was unaltered.

Key words: canola, flax, processed barley, milk fatty acid composition

INTRODUCTION

Numerous studies have examined nutritional manipulation of bovine milk fatty acid (FA) composition to improve its healthfulness and appeal to consumers (Mansbridge and Blake, 1997). The primary goal of these studies has been to decrease the SFA content of milk, especially myristic (C14:0) and palmitic (C16:0) acids, which have been demonstrated to have undesirable hypercholesterolemic effects and to increase the risk of coronary heart disease (Berner, 1993), at the same time enhancing the milk fat content of those long-chain PUFA, which might have human health benefits (Parodi, 1997). Among the PUFA, specific isomers of conjugated linoleic acid (CLA; particularly C18:2 *cis*-9,*trans*-11), oleic (C18:1), linoleic (C18:2n6), and linolenic (C18:3n3) can potentially improve human health (Parodi, 1997). Milk CLA is derived from 2 main sources: (1) CLA produced from incomplete ruminal biohydrogenation (BH) of dietary C18:2n6 and C18:3n3 and (2) de novo mammary synthesis of CLA by desaturation of vaccenic acid (C18:1 *trans*-11), which is also an intermediate of incomplete ruminal BH (Griinari et al., 2000). Therefore, any factors affecting the rate and

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extent of ruminal BH of dietary unsaturated FA can potentially affect the milk content of CLA.

Ruminal pH (Van Nevel and Demeyer, 1996), and source and amount of dietary PUFA (Beam et al., 2000) have been demonstrated to quantitatively affect the rate and extent of ruminal BH. Dietary factors that alter ruminal pH and fermentation may alter BH pathways of PUFA in the rumen, leading to ruminal accumulation and increased postruminal flow of PUFA, especially CLA and C18:1 *trans*-10 (Loor et al., 2003). Decreasing pH has been shown to inhibit lipolysis and BH of unsaturated FA in vitro (Van Nevel and Demeyer, 1996), and to increase the flow of CLA isomers from a continuous-culture system (Qiu et al., 2004). Feeding full-fat canola seed (Khorasani and Kennelly, 1998) or canola seed and flaxseed (Ward et al., 2002) to dairy cows increased milk content of PUFA. In western Canada and some regions of the United States, dairy cow diets typically contain barley as the main source of energy, and it is fed either in a dry-rolled or pelleted form. Such processing has been shown to alter the dietary content of ruminally fermentable carbohydrate, with pelleting decreasing particle size and, thus, shifting the site of carbohydrate digestion from postruminal sites to the rumen (Kiran and Mutsvangwa, 2007). These differences in the rates and extents of ruminal starch degradation might change ruminal pH, which, in turn, would alter milk FA profiles via changes in ruminal BH pathways of dietary FA.

Providing supplemental fat in the diet has been reported to have inhibitory effects on ruminal bacterial growth (Jenkins, 1993; Doreau and Ferlay, 1995), with more pronounced effects when unsaturated rather than saturated fats are added to the diet (Doreau and Ferlay, 1995). This inhibition of ruminal bacterial growth is important because microbial protein (MCP) derived from ruminal fermentation is the main component of MP reaching the small intestine in lactating dairy cows and, qualitatively, it has an essential AA profile that closely matches essential AA requirements for milk protein synthesis (NRC, 2001). Full-fat canola seed and flaxseed are extensively used in lactating cow diets in western Canada and the northern United States. Because of differences in their degree of unsaturation and rumen protein degradation, the dietary inclusion of canola or flaxseed could have differential effects on ruminal fermentation, thus potentially influencing MCP synthesis and duodenal flow of N. However, limited information is available on how concomitant changes in dietary content of ruminally fermentable carbohydrate and source of supplemental fat might influence ruminal fermentation, duodenal FA flows, and milk FA composition. The objective of this study, therefore, was

to determine how interactions between the method of barley grain processing (dry rolling vs. pelleting) and source of supplemental dietary fat (full-fat canola seed vs. full-fat flaxseed) might alter ruminal fermentation, duodenal FA flow, and milk FA composition in dairy cows.

MATERIALS AND METHODS

Animals and Experimental Design

All cows that were used in this study were cared for and handled in accordance with the Canadian Council of Animal Care regulations, and all procedures were approved by the University of Saskatchewan Animal Care Committee (Saskatoon, SK, Canada; UCACS protocol no. 20040048). Four multiparous Holstein cows [2.5 ± 0.5 parity, 656 ± 28 kg of BW, and 80 ± 12 DIM (means $\pm$ SD) at the beginning of the experiment] that were fitted with ruminal and duodenal cannulas were used in a 4×4 Latin square design with 28-d periods and a 2×2 factorial arrangement of dietary treatments. Ruminal cannulas were 10 cm in internal diameter (Cannula #1C; Bar Diamond Inc., Parma, ID). Duodenal cannulas were simple T-shaped, and were inserted proximal to the common bile and pancreatic duct, approximately 10 cm distal to the pylorus. Each experimental period consisted of 14 d of dietary adaptation and 14 d of data collection. Throughout the experiment, cows were housed individually in tie-stalls (2.6-m length $\times$ 1.32-m width) in the Metabolism Wing at the Greenbrae Dairy Research Facility (University of Saskatchewan). The experiment was conducted between May and September 2007 and the temperature in the Metabolism Wing ranged from 18 to 24°C.

Experimental Treatments and Feeding Management

Treatment factors were (1) barley processing [i.e., dry-rolled barley (**DRB**) vs. pelleted barley (**PB**)] as the main source of ruminally fermentable starch and (2) source of supplemental fat [i.e., full-fat canola seed (canola) vs. full-fat flaxseed (flaxseed)] as the main source of dietary fat. Diets were formulated to be iso-nitrogenous and isolipidic. Complete ingredient and chemical compositions of the experimental diets are presented in Table 1. Experimental diets were fed twice daily at 0900 and 1600 h as TMR for ad libitum intake. The forage:concentrate ratio of the TMR was 50:50. The forage component of the TMR was a mixture of barley silage and chopped alfalfa hay. Barley silage contained 35.5% DM and its chemical composition (DM basis) was: OM, 90.9%; CP, 11.3%; NDF, 54.7%;

Table 1. Ingredient and chemical composition of TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed

Item	Canola		Flax	
	DRB	PB	DRB	PB
Ingredient, % of DM				
Barley silage	32.5	32.5	32.5	32.5
Alfalfa hay	17.2	17.2	17.3	17.3
Barley grain	29.8	29.8	29.4	29.4
Canola seed	8.13	8.13	—	—
Flaxseed	—	—	8.30	8.30
Soybean meal	3.55	3.55	3.75	3.75
Molasses	0.40	0.40	0.40	0.40
Canola meal	3.49	3.49	3.49	3.49
Corn gluten meal	0.96	0.96	0.96	0.96
Wheat dried distillers grains	1.78	1.78	1.78	1.78
Dynamate ¹	0.18	0.18	0.18	0.18
Salt, Co-I ²	0.33	0.33	0.33	0.33
Mineral-vitamin supplement ³	1.66	1.66	1.66	1.66
Chemical composition				
DM, %	57.5	58.3	57.4	58.8
Starch, % of DM	23.0	22.6	24.7	23.7
CP, % of DM	17.4	17.3	17.2	17.0
NDF, % of DM	34.8	35.2	34.8	34.7
ADF, % of DM	21.0	21.4	21.0	20.9
Crude fat, % of DM	5.32	5.33	5.24	5.29

¹The Mosaic Co., Plymouth, MN. Contained 18% K, 11% Mg, and 22% S.

²Salt, cobalt, and iodine mix.

³Contained (per kilogram of premix; DM basis): 330,000 IU of vitamin A, 60,000 IU of vitamin D, 1,000 IU of vitamin E, 16% Ca, 8.5% P, 6.3% Na, 4.5% Mg, 2,100 mg of Zn, 1,500 mg of Mn, 535 mg of Cu, 12 mg of Se, and 45 mg of I.

ADF, 35.8%; and ether extract (**EE**), 3.42%. Cows had free access to water provided through individual water bowls.

Sample Collection

During the 14-d data collection period (d 15 to 28), feed intake was recorded daily, TMR samples were collected 3 times weekly, and orts were collected daily and composited by cow proportionally to the weight of feed refused each day and stored at -20°C for later analysis. Cows were milked twice daily at 0500 and 1500 h, and milk weights were recorded during the 14-d data collection period. Milk samples were collected into plastic vials with or without a preservative (2-bromo-2-nitropropane-1,2-diol) on d 23, 25, and 26 from morning and afternoon milking. Milk samples were then pooled daily based on milk yield. Pooled samples with preservative were analyzed for CP, lactose, and fat using a near infrared analyzer (Foss System 4000; Foss Electric A/S, Hillerød, Denmark; AOAC, 1990) by the Provincial Milk Testing Laboratory (Saskatchewan Agriculture, Food and Rural Revitalization, Regina, SK, Canada). Milk samples without preservative were stored at -20°C until FA analysis.

To quantify digesta flow to the duodenum, Yb and Cr were used as solid and liquid fraction markers,

respectively, whereas microbial protein synthesis was measured using ^{15}N as the ruminal microbial marker. For Yb and ^{15}N , a marker solution was prepared by dissolving 4.88 g of $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ (2.2 g of Yb) and 7.6 g of $(^{15}\text{NH}_4)_2\text{SO}_4$ (182 mg of $^{15}\text{N}/\text{d}$; 10% atom percentage of ^{15}N ; Cambridge Isotope Laboratories, Andover, MA) in 2 L of deionized water as described by Gozho et al. (2009). For the Cr complex of EDTA, a marker solution was prepared using the method described by Binnerts et al. (1968) and infused at a rate of 2.7 g of Cr-EDTA/d. Just before marker infusions into the rumen were initiated on d 22, samples of whole ruminal contents and whole duodenal digesta were taken from each cow to determine background concentrations of Cr and Yb, and ^{15}N natural abundance. At the beginning of the infusions, priming doses equal to one-half of the daily doses of Yb, Cr, and ^{15}N were administered via the ruminal cannula. Thereafter, between d 22 and 28, marker solutions were then continuously infused using an automatic peristaltic pump (model 205U; Watson Marlow, Cornwall, UK) into the rumen at a rate of 1 L/d.

Sampling of ruminal contents and duodenal digesta began at 0700 h on d 27 and was conducted every 2 h until 0500 h on d 28, to represent the 24-h feeding cycle. At each sampling, 300 mL of duodenal digesta was taken and kept frozen at -20°C for later analy-

sis. For ruminal contents, 1,000 mL were collected by manually taking 250 mL from the cranial ventral, caudal ventral, central, and cranial dorsal rumen through the cannula. After each sampling, ruminal contents were processed immediately to isolate mixed ruminal bacteria, as described by Gozho et al. (2009). Briefly, ruminal contents were squeezed through 4 layers of cheesecloth, and the filtrate was retained. A 5-mL aliquot of strained ruminal fluid was mixed with 1 mL of 1% H₂SO₄ and stored at -20°C for later determination of ruminal NH₃-N. The solid particles that were retained on the cheesecloth were mixed with 300 mL of a sterile physiological saline solution, homogenized in a blender for 60 s to dislodge particle-associated bacteria, and then the blended mixture was squeezed through 4 layers of cheesecloth to obtain a supernatant. The filtrate and homogenate fractions were mixed in equal amounts (300 mL) and used to isolate mixed ruminal bacteria. The mixed ruminal fluid mixture was then centrifuged at 1,000 × *g* for 5 min at 4°C to remove protozoa and residual feed particles. The supernatant was subsequently centrifuged at 20,000 × *g* for 30 min at 4°C to obtain a mixed ruminal bacterial pellet, which was kept frozen at -20°C until later analysis. Starting on d 25 of each experimental period, ruminal pH was measured continuously for 3 consecutive days as described by Penner et al. (2006). Ruminal pH readings were taken every 30 s, stored in a data logger (model M1b-pH-1KRTD; Dascor Inc., Escondido, CA) and downloaded daily.

Sample Analyses

At the end of the trial, frozen TMR and Orts subsamples were thawed overnight at room temperature, pooled per collection period for each cow, and were then analyzed for DM by drying in an oven at 60°C for 48 h (AOAC, 1990; method 930.15). Dried TMR and Orts were then ground through a 1-mm screen using a Christy-Norris mill (Christy and Norris Ltd., Chelmsford, UK) and analyzed for OM by combustion in a muffle furnace at 550°C for at least 8 h (AOAC, 1990; method 942.05), CP by the macro-Kjeldahl procedure (AOAC, 1990; method 976.05) using a Kjeltac 2400 autoanalyzer (Foss Analytical, Hillerød, Denmark) to determine N content, and EE (AOAC, 1990; method 920.39). Neutral detergent fiber and ADF were analyzed sequentially according to the procedure of Van Soest et al. (1991). Sulfite and heat-stable α -amylase were used for NDF analysis. Ground TMR and Orts were also analyzed for total starch (AOAC, 1990; method 996.11) using a commercial kit (Megazyme International Ireland Ltd., Wicklow, Ireland). Whole duodenal digesta samples

were thawed at room temperature and composited by animal and period, and mixed thoroughly before being separated into liquid duodenal digesta (LDD) and solid duodenal digesta (SDD) fractions as described by Gozho et al. (2009). Samples of LDD and SDD were freeze dried, and then ground through a 1-mm screen using a Christy-Norris mill. The concentrations of Cr and Yb in LDD and SDD were determined by atomic absorption spectrophotometry (Perkin Elmer 2300; Perkin-Elmer Corp., Norwalk, CT) and atomic emission spectroscopy (Varian Spectra 220 atomic absorption spectrometer; Varian Australia Pty Ltd., Mulgrave, Victoria, Australia), respectively, as described by Gozho et al. (2009). Concentrations of Yb and Cr in LDD and SDD were then used to physically recombine DM from the freeze-dried LDD and SDD in the correct proportions to reconstitute the duodenal true digesta (DTD) reaching the duodenum using the double-marker method (France and Siddons, 1986). Reconstituted DTD samples were analyzed for OM, CP, EE, NDF, ADF, and total starch using procedures already described. For ammonia nitrogen (NH₃-N) analysis, DTD samples were prepared using 10 mL of pH 2.2 Na-citrate buffer as described by Reynal and Broderick (2005), and NH₃-N determination was conducted using a phenol-hypochlorite assay (Broderick and Kang, 1980). Enrichment of ¹⁵N in NAN of DTD samples was determined by combustion to N₂ gas in an elemental analyzer and continuous flow isotope ratio-mass spectrometry. Bacterial pellet samples were thawed at room temperature and composited by animal and period. Pooled bacterial pellets were freeze dried and then ground. Ground bacterial samples were analyzed for OM, CP, and ¹⁵N enrichment using procedures already described. Ruminal fluid samples for ruminal NH₃-N determination were thawed, centrifuged for 10 min at 18,000 × *g* to obtain a clear supernatant, and then analyzed using a phenol-hypochlorite assay (Broderick and Kang, 1980).

Pooled milk samples without preservative were thawed at 38°C in a water bath (AOAC, 1990). Lipids for FA analysis were extracted from pooled milk, TMR, and reconstituted DTD samples according to Bligh and Dyer (1959), with minor modifications as described by AlZahal et al. (2009). Extracted FA were methylated using sodium methoxide (NaOCH₃) as a catalyst, and quantification of FA methyl esters was performed using a GLC (Agilent 6890N; Agilent Technologies Inc., Palo Alto, CA) equipped with a split/splitless injector, a flame ionization detector at 250°C (Agilent Technologies Inc.), and a capillary column (CP-Sil 88 column; 100 m, 0.25 mm, 0.2- μ m of film thickness, Varian Inc., Mississauga, Ontario, Canada), with GLC conditions as described by AlZahal et al. (2009). Identification of

FA peaks was by comparison of retention times with known FA methyl ester standards (Nu-Chek Prep Inc., Elysian, MN). The individual isomers of C18:1 and C18:2 FA were identified as described by AlZahal et al. (2009). Fatty acid composition was expressed as grams per 100 g of total FA.

Calculations and Statistical Analysis

The ruminal pH data were summarized as minimum, mean, and maximum pH over a 24-h cycle. The degree and incidence of ruminal acidosis was determined from the pH data using 3 pH thresholds of 5.8, 5.5, and 5.2. Ruminal acidosis (**RA**) was considered to occur when ruminal pH was <5.8 (total RA). The pH profiles were further classified as mild RA when 5.8 > ruminal pH > 5.5, severe RA when 5.5 > ruminal pH > 5.2, and acute RA when ruminal pH < 5.2 (Penner et al., 2006). The duration (min/d) and total area (pH × min) that ruminal pH was below each threshold were calculated.

The ¹⁵N enrichment (expressed as atom percent excess, **APE**) in DTD and bacterial samples was calculated as $^{15}\text{N-APE} = \frac{^{15}\text{N-atom percentage in sample} - ^{15}\text{N-atom percentage in background sample}}{^{15}\text{N-atom percentage in background sample}}$. The mean of 16 observations for background ¹⁵N abundance was 0.36903%. Microbial NAN flow to the duodenum was calculated as total NAN flow × (DTD ¹⁵N APE/mixed bacterial ¹⁵N APE). The NAN content of DTD samples was calculated as total N – NH₃-N. Flows of other nutrients to the duodenum were calculated by multiplying DM flow by their concentration in DTD. Apparent ruminal digestion of nutrients was calculated as nutrient intake – duodenal flow of nutrient, and true duodenal digestion as nutrient intake – (duodenal flow – microbial DM flow) (Reynal and Broderick, 2005). Similarly, the flows of nonammonia nonmicrobial N (**NANMN**), RUP, and RDP at the duodenum were also calculated as described by Reynal and Broderick (2005) as follows: NANMN flow = total NAN flow – microbial NAN flow, RUP flow = total CP flow – microbial CP flow, and RDP supply = total CP intake – RUP flow.

All data were analyzed as a 4 × 4 Latin square using the Proc Mixed procedure of SAS (SAS Institute, 2004). For DMI, duodenal nutrient and microbial flow, and ruminal nutrient digestion, the statistical model used included the following terms: method of barley grain processing and source of supplemental fat, which were considered fixed, the interaction between method of barley grain processing and source of supplemental fat, the random effects of period and cow, and the residual error. Data on milk yield, milk composition, ruminal pH, and ruminal NH₃-N concentrations were analyzed, accounting for repeated measures as recommended by Wang and Goonewardene (2004) for the

analysis of animal experiments. Data for these repeated measurements were analyzed by including in the model a REPEATED model statement, as well as terms for time (hour or day), and interactions (method of barley grain processing × time, source of supplemental fat × time, and time × method of barley grain processing × source of supplemental fat) in the model described previously. When processing × time or oilseed × time interactions were significant, the slice option for the LSMEANS statement in PROC MIXED was used to determine which time period means were different. Significance for all models was declared at $P \leq 0.05$, and trends at $0.05 < P \leq 0.10$. When a significant interaction occurred between method of barley processing and source of supplemental fat, least squares means were separated by the Tukey honestly significant difference (HSD) test (SAS Institute, 2004).

RESULTS

Diet Characteristics

The ingredient composition and chemical analysis of experimental TMR are presented in Table 1. Diets were formulated to be isonitrogenous and isolipidic, and chemical analysis indicates that this was achieved (Table 1). The mean total dietary fat was 5.3%, and this falls within the desired range of ≤6% total dietary fat. Oilseed contents (g per 100 g of FA) of C16:0, C16:1, C18:0, C18:1, C18:2n6, and C18:3n3 were 5.44, 0.23, 1.77, 57.1, 22.9, and 11.7, respectively, for canola and 6.28, 0.15, 3.32, 16.7, 15.2, and 55.3, respectively, for flaxseed. Thus, of the major dietary PUFA those are substrates for ruminal BH, the content of C18:3n3 was greater in flaxseed compared with canola, and this was reflected in the dietary FA contents (Table 2).

Ruminal Fermentation Characteristics

As expected, the severity of ruminal acidosis was greater in cows fed PB compared with those fed DRB (Table 3). The minimum ($P < 0.01$) and mean ($P = 0.01$) ruminal pH were lower in cows fed PB compared with those fed DRB. Also, the duration (min/d) and area (pH × min) of total RA, mild RA, severe RA, and the duration of acute RA were greater ($P < 0.01$) in cows fed PB compared with those fed DRB. The source of supplemental fat had no effects ($P > 0.05$) on any of the measures of ruminal acidosis. Averaged across all sampling times, ruminal NH₃-N concentration was greater ($P = 0.02$) in cows fed DRB compared with those fed PB diets, but was not affected by the source of oilseed (Table 3).

Table 2. Fatty acid profile (g/100 g of total FA) of TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed

Item	Canola		Flax	
	DRB	PB	DRB	PB
C10:0	0.052	0.045	0.040	0.052
C12:0	0.059	0.062	0.071	0.078
C14:0	0.256	0.262	0.330	0.318
14:1 <i>cis</i> -9	0.020	0.021	0.020	0.025
C15:0	0.106	0.117	0.139	0.129
C16:0	10.1	10.1	13.2	12.5
C16:1 <i>cis</i> -9	0.263	0.262	0.191	0.171
C18:0	4.24	3.91	5.24	5.19
C18:1 <i>cis</i> -9	43.3	43.7	16.6	16.6
C18:1 <i>cis</i> -11	3.69	3.69	1.50	1.49
C18:2n-6	26.1	25.6	26.0	25.1
C18:3n-3	9.47	9.95	35.2	36.7
C20:0	0.731	0.712	0.332	0.365
C20:1 <i>cis</i> -11	0.045	0.086	0.058	0.064
C20:2n-6	0.405	0.339	0.171	0.187
C22:0	0.525	0.519	0.329	0.393
C20:3n-3	0.117	0.116	0.155	0.175
C24:0	0.384	0.380	0.320	0.382
C26:0	0.102	0.101	0.120	0.129

Nutrient Intakes, Ruminal Digestibilities, and Duodenal Nutrient Flows

Compared with those on PB diets, cows fed the DRB diets consumed 1.8 to 3.5 kg/d more ($P = 0.02$) DM

(Table 4). The source of supplemental fat had no effect ($P = 0.41$) on DMI. Intakes of OM ($P = 0.02$), NDF ($P = 0.007$), ADF ($P = 0.03$), and EE ($P = 0.008$) were greater, and that of starch tended to be greater ($P = 0.08$) in cows fed DRB compared with

Table 3. Ruminal pH and ammonia nitrogen (NH₃-N) concentrations in dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	<i>P</i> -value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
Minimum pH	5.65	4.92	5.30	5.06	0.11	<0.01	0.52	0.13
Mean pH	6.13	5.73	5.99	5.77	0.11	0.05	0.66	0.48
Maximum pH	6.59	6.67	6.74	6.67	0.13	0.92	0.79	0.78
Total ruminal acidosis ³								
Duration, min/d	140.0	827.2	414.4	741.8	106.2	<0.01	0.42	0.19
Area, pH × min	26.6	380.2	104.5	337.7	79.9	0.02	0.81	0.55
Mild ruminal acidosis ⁴								
Daily episodes, no.	5.1	5.7	4.9	6.8	1.2	0.25	0.68	0.54
Duration, min/d	139.8	349.4	199.3	277.9	49.7	<0.01	0.89	0.16
Area, pH × min	26.9	194.1	80.5	178.0	29.9	<0.01	0.51	0.31
Severe ruminal acidosis ⁵								
Daily episodes, no.	0.3	7.3	2.1	5.0	0.65	<0.01	0.85	<0.01
Duration, min/d	60.7	256.2	146.3	220.5	43.6	0.02	0.40	0.17
Area, pH × min	10.8	100.1	22.5	105.9	25.8	0.03	0.71	0.82
Acute ruminal acidosis ⁶								
Daily episodes, no.	0.7	3.0	0.5	4.4	1.1	0.05	0.56	0.78
Duration, min/d	6.0	221.7	11.8	243.5	74.8	0.06	0.88	0.96
Area, pH × min	0.0	86.0	1.4	53.9	29.9	0.13	0.72	0.70
Ruminal NH ₃ -N, mg/dL	19.9	13.9	17.2	15.5	1.3	0.02	0.65	0.27

¹Values are least squares means obtained from 4 ruminally cannulated cows.

²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); BP × OS = interaction.

³Total ruminal acidosis = pH ≤ 5.8.

⁴Mild ruminal acidosis = 5.8 ≥ pH ≥ 5.5.

⁵Severe ruminal acidosis = 5.5 ≥ pH ≥ 5.2.

⁶Acute ruminal acidosis = pH ≤ 5.2.

Table 4. Nutrient intakes, duodenal nutrient flows, and nutrient disappearance in the rumen in dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	<i>P</i> -value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
DM								
Intake, kg/d	24.6	21.1	24.5	22.7	0.9	0.02	0.41	0.36
Duodenal flow, kg/d	14.5	13.9	15.5	14.7	0.6	0.23	0.14	0.90
Apparently digested in the rumen								
kg/d	10.1	7.3	9.1	8.0	0.87	0.06	0.86	0.32
% of DMI	40.4	33.4	36.7	34.9	2.8	0.15	0.70	0.37
OM								
Intake, kg/d	22.5	19.5	22.8	21.0	0.9	0.02	0.30	0.45
Duodenal flow, kg/d								
Total OM	14.4	13.5	15.4	14.5	0.60	0.16	0.12	0.94
Microbial OM	1.62	2.18	2.02	2.25	0.15	0.01	0.07	0.39
Truly digested in the rumen								
kg/d	11.4	10.3	11.3	11.1	0.8	0.41	0.65	0.55
% of OM intake	49.7	52.9	49.5	53.0	2.6	0.22	0.99	0.96
Starch								
Intake, kg/d	5.65	4.80	6.00	5.38	0.37	0.08	0.24	0.77
Duodenal flow, kg/d	0.58	0.40	0.68	0.48	0.05	0.003	0.07	0.77
Apparently digested in the rumen								
kg/d	5.08	4.40	5.38	4.90	0.38	0.17	0.32	0.80
% of starch intake	89.9	90.8	89.1	91.1	1.2	0.11	0.79	0.56
NDF								
Intake, kg/d	8.63	7.38	8.85	8.10	0.34	0.007	0.10	0.35
Duodenal flow, kg/d	5.05	5.08	5.03	4.83	0.38	0.81	0.71	0.76
Apparently digested in the rumen								
kg/d	3.63	2.28	3.85	3.25	0.34	0.02	0.12	0.30
% of NDF intake	41.8	31.7	43.1	39.9	3.5	0.09	0.20	0.34
ADF								
Intake, kg/d	4.95	4.30	5.15	4.75	0.22	0.03	0.13	0.53
Duodenal flow, kg/d	3.03	2.93	3.00	2.83	0.18	0.47	0.74	0.84
Apparently digested in the rumen								
kg/d	1.93	1.40	2.13	1.93	0.23	0.14	0.14	0.47
% of ADF intake	38.7	32.3	41.3	40.3	3.6	0.33	0.19	0.48
Ether extract (EE)								
Intake, kg/d	1.28	1.12	1.29	1.14	0.05	0.008	0.66	0.89
Duodenal flow, kg/d	1.02	1.35	1.34	1.27	0.15	0.32	0.38	0.16
Apparently digested in the rumen								
kg/d	0.26	−0.24	−0.04	−0.12	0.18	0.07	0.50	0.16
% of EE intake	21.1	−25.0	−3.59	−11.2	14.2	0.04	0.61	0.10

¹Values are least squares means obtained from 4 ruminally cannulated cows.

²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); BP × OS = interaction.

those fed PB, reflecting observed differences in DMI (Table 4). Duodenal flows of DM ($P \geq 0.14$), OM ($P \geq 0.12$), NDF ($P \geq 0.71$), and ADF ($P \geq 0.47$) were unaffected by method of barley grain processing or source of supplemental fat (Table 4). The amounts of OM truly digested in the rumen tended to be greater ($P = 0.09$) in cows fed DRB compared with those fed PB. The source of supplemental fat had no effect on ruminal digestibility of NDF and ADF, when expressed as absolute amounts ($P \geq 0.12$) or as a proportion of intake ($P \geq 0.19$). Ruminal digestibility of NDF was greater ($P = 0.02$) in cows fed DRB compared with those fed PB when expressed in absolute amounts, but only tended to be greater ($P = 0.09$) when NDF digestibility was expressed as a proportion of NDF intake.

Ruminal ADF digestibility, expressed both in absolute amounts or as a proportion of ADF intake, was not affected by method of barley grain processing (Table 4).

Cows fed DRB consumed 12 to 18% more ($P = 0.08$) starch compared with those fed PB (Table 4). Apparent ruminal starch digestibility, when expressed in absolute amounts ($P = 0.17$) or as a proportion of starch intake ($P = 0.11$), was unaffected by method of barley grain processing. Duodenal flow of starch was greater ($P = 0.003$) in cows fed DRB compared with those fed PB. Intake of EE was greater ($P = 0.008$) in cows fed DRB compared with those fed PB; however, the source of supplemental fat had no effect ($P = 0.66$) on EE intakes and duodenal flow of EE was similar ($P \geq 0.32$) across dietary treatments (Table 4).

Table 5. Nitrogen intakes, duodenal total N and N fraction flows, and microbial N flows estimated using ^{15}N in dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	<i>P</i> -value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
N intake, g/d	685	582	674	605	30	0.02	0.85	0.57
RDP supply								
g/d	3,158	2,673	2,628	2,495	239	0.22	0.17	0.47
% of DMI	12.9	12.6	10.7	10.9	0.8	0.92	0.04	0.80
Flow to the duodenum								
Total N, g/d	394	446	517	462	18.2	0.94	0.004	0.02
Total N, % of N intake	57.9	77.3	77.1	77.3	4.37	0.04	0.04	0.04
NH ₃ -N, g/d	3.00	4.38	4.23	4.50	0.47	0.06	0.10	0.16
NAN, g/d	391	442	513	458	18	0.90	0.004	0.02
NAN, % of N intake	57.5	76.6	76.4	76.5	4.3	0.04	0.04	0.04
NANMN, ³ g/d	180	155	253	206	20	0.10	0.01	0.59
NANMN, % of NAN	46.9	35.4	49.3	44.9	3.7	0.06	0.14	0.36
NANMN, % of N intake	26.4	27.5	37.6	34.9	3.9	0.85	0.04	0.64
RUP								
g/d	1,123	965	1,583	1,286	125	0.10	0.01	0.59
% of DMI	4.60	4.75	6.46	5.80	0.61	0.69	0.04	0.53
Microbial N								
g/d	267	287	260	252	22.5	0.78	0.37	0.54
% of total NAN	68.7	64.6	50.7	55.1	5.4	0.97	0.03	0.45
Microbial efficiency								
g of NAN/kg of OMTDR ⁴	25.3	28.0	23.9	22.7	3.2	0.81	0.32	0.56

¹Values are least squares means obtained from 4 ruminally cannulated cows.²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); and BP × OS = interaction.³NANMN = nonammonia, nonmicrobial nitrogen.⁴OMTDR = OM truly digested in the rumen.

Nitrogen Intake, Duodenal Flow of N Fractions, and Ruminal Microbial Protein Synthesis

In parallel with observed changes in DMI, cows fed DRB diets consumed 11 to 18% more N compared with those fed PB diets ($P = 0.02$; Table 5). The method of barley grain processing did not affect ($P = 0.94$) duodenal flow of total N; however, when compared with cows fed DRB, cows fed PB with canola had a greater (+52 g/d) total N flow to the duodenum, but not with flaxseed (−55 g/d; interaction, $P = 0.02$). Duodenal flow of NH₃-N tended ($P = 0.06$) to be greater in cows fed PB compared with those fed DRB diets. Across dietary treatments, duodenal flow of NH₃-N was 4 g/d, accounting for <1% of total duodenal N flow (Table 5). Nonammonia N reaching the duodenum was unaffected ($P = 0.90$) by method of barley grain processing. However, compared with cows fed DRB, NAN reaching the duodenum in cows fed PB was 13% greater with diets containing canola, but 12% lower with diets containing flaxseed (interaction, $P = 0.02$; Table 5). Duodenal flow of NANMN, expressed as absolute amounts ($P = 0.01$) or as a proportion of NAN ($P = 0.03$) or N intake ($P = 0.02$), were greater in cows supplemented with flaxseed compared with those fed canola; however, the method

of barley grain processing did not affect ($P \geq 0.42$) duodenal NANMN flow (Table 5).

The supply of RDP was unaffected ($P \geq 0.17$) by dietary treatment (Table 5). Duodenal flow of RUP tended ($P = 0.10$) to be greater in cows fed DRB diets compared with those fed PB diets. The source of supplemental fat influenced duodenal flow of RUP, with cows that were supplemented with flaxseed having 391 g/d more of RUP reaching the duodenum than those supplemented with canola ($P = 0.01$; Table 5). Also, when expressed as a proportion of DMI, RUP supply was greater ($P = 0.04$) for cows supplemented with flaxseed compared with those supplemented with canola. Duodenal flow of microbial NAN ($P = 0.78$), and microbial efficiency [expressed as grams of microbial NAN per kilogram of OM truly digested in the rumen (**OMTDR**); $P = 0.81$] were not affected by method of barley grain processing (Table 5). Similarly, duodenal flow of microbial NAN ($P = 0.37$), and microbial efficiency ($P = 0.32$) were not affected by the source of supplemental fat; however, when expressed as a proportion of total duodenal NAN flow, microbial NAN flow to the duodenum was 14% greater ($P = 0.03$) in cows fed diets supplemented with canola compared with those with flaxseed (Table 5).

Duodenal Flow of FA

Duodenal flows (g/d) of C13:0 *iso*, C16:0 *iso*, and C18:0 were lower ($P < 0.05$), whereas those of C14:0, C15:0 *anteiso*, C17:0 *anteiso*, C18:1 *cis*, C18:1 *trans*, and total C18:1 were greater ($P < 0.05$) in cows fed PB compared with those fed DRB (Table 6). The duodenal flow of total SFA was lower ($P = 0.01$), whereas that of total MUFA was greater ($P = 0.0007$) in cows fed PB compared with those fed DRB. Cows fed PB had greater ($P < 0.05$) duodenal flows of C18:2 *trans*-9, *trans*-12, C18:2 *trans*-9, *cis*-12, C18:2 *trans*-11, *cis*-15, and CLA *trans*-10, *cis*-12 compared with those fed DRB (Table 6). Of the C18:1 FA, duodenal flow of *cis*-9, *cis*-11, *cis*-13, *trans*-5, *trans*-6+7+8, *trans*-9, *trans*-10, *trans*-11, *trans*-12, and *trans*-13+14 were greater ($P < 0.05$) in cows fed PB compared with those fed DRB (Table 6).

Duodenal flows of C13:0 *iso*, C13:0 *anteiso*, C14:1 *cis*-9, C15:0 *anteiso*, C16:0, C17:0 *anteiso*, C18:1 *cis*, C18:1 *trans*, total C18:1, C18:2, total CLA, and C18:3n-3 were greater ($P < 0.05$), whereas that of C18:0 tended to be lower ($P = 0.07$) in cows supplemented with flaxseed compared with those supplemented with canola (Table 6). Feeding flaxseed increased ($P < 0.05$) duodenal flows of total MUFA and PUFA compared with feeding canola. Duodenal flows of C18:2 *trans*-9, *cis*-12, C18:2 *trans*-11, *cis*-15, CLA *cis*-9, *trans*-11, CLA *trans*-10, *cis*-12, CLA *trans*-11, *trans*-13, and CLA *trans*, *trans* were greater ($P < 0.05$) in cows fed flaxseed compared with those fed canola (Table 6). Of the C18:1 FA, duodenal flows of *cis*-12, *cis*-13, *trans*-11, *trans*-12, *trans*-13+14, and *trans*-16 were greater ($P < 0.05$), whereas those of *cis*-11, *trans*-5, *trans*-6+7+8, and *trans*-9 were lower ($P < 0.05$), in cows fed flaxseed compared with those fed canola (Table 6).

Milk Production and Composition

Milk yield was not affected by method of barley grain processing ($P = 0.60$) or source of supplemental fat ($P = 0.54$; Table 7). Milk fat content ($P < 0.01$) and milk fat yield ($P < 0.04$) were greater in cows fed DRB than in cows fed PB. Milk protein and lactose contents ($P \geq 0.23$) and yields ($P \geq 0.12$) were unaffected by the method of barley grain processing and source of supplemental fat. However, MUN concentration was greater ($P < 0.01$) in cows fed DRB compared with those fed PB. Feeding PB decreased ($P < 0.05$) milk concentrations of C16:0 *iso* and C16:1 *cis*-9, and tended ($0.05 < P < 0.10$) to decrease milk concentrations of C14:0, C18:0, C17:0 *anteiso*, C18:0 *anteiso*, and total SFA (Table 8). Milk concentrations of C18:1 *cis* and *trans* FA were largely unaffected by the method of barley grain pro-

cessing, except that milk concentration of C18:1 *cis*-13 was greater ($P = 0.004$) in cows fed PB compared with those fed DRB. Milk concentrations of both nonconjugated and conjugated C18:2 FA were unaffected by the method of barley grain processing. Supplementing the PB diet with flaxseed tended to result in a greater milk concentration of total CLA compared with feeding DRB, whereas supplementing the PB diet with canola tended to result in a lower milk concentration of total CLA compared with feeding DRB (interaction effect; $P = 0.06$). When FA were grouped based on their origin, milk concentrations of de novo synthesized FA (i.e., <C16), preformed FA (i.e., >C16), and FA derived from both de novo synthesis and the diet (i.e., C16 + C16:1) were unaffected ($P \geq 0.23$) by the method of barley grain processing (Table 8).

Milk concentrations of C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C13:0 *anteiso*, C15:0 *anteiso*, total CLA, and C18:3n-3 were greater ($P < 0.05$), whereas those of C17:0 *anteiso* and C18:1 *cis* were lower ($P < 0.05$) in cows fed flaxseed compared with those fed canola (Table 8). Of the C18:1 FA, milk concentrations of *cis*-9, *trans*-4, *trans*-5, *trans*-6+7+8, and *trans*-9 were lower ($P < 0.05$), whereas those of *cis*-12, *trans*-11, *trans*-12, *trans*-13+14, and *trans*-16 were greater ($P < 0.05$) in cows fed flaxseed compared with those fed canola (Table 8). When compared with cows fed canola, milk concentrations of C18:2 *trans*-11, *cis*-15, CLA *cis*-9, *trans*-11, and CLA *trans*-11, *trans*-13 were greater in cows fed flaxseed (Table 8). When FA were grouped based on their origin, the milk concentration of <C16 was greater ($P = 0.02$) in cows supplemented with flaxseed compared with those fed canola; however, milk concentrations of >C16 and C16 + C16:1 were unaffected by the source of oilseed. The milk concentration of total PUFA was greater ($P = 0.005$) in cows fed flaxseed compared with those fed canola.

DISCUSSION

A comprehensive characterization of ruminal pH using a continuous ruminal pH measurement system indicated that the severity of ruminal acidosis was greater in cows fed PB compared with those fed DRB. These differences were expected because more extensive processing of barley, such as pelleting, would result in a more rapid and extensive ruminal carbohydrate fermentation (Kiran and Mutsvangwa, 2007), and increase the risk of ruminal acidosis. Similarly, Yang et al. (2000, 2001) reported that increasing the extent of barley grain processing increased the severity of ruminal acidosis in dairy cows fed barley-based diets. The lower ruminal $\text{NH}_3\text{-N}$ concentration that we observed in cows fed PB compared with those fed DRB likely

Table 6. Flow of FA (g/d) in duodenal digesta of dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	<i>P</i> -value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
C10:0	2.71	3.04	4.68	4.09	0.29	0.58	0.0005	0.09
C12:0	2.42	2.89	2.46	2.83	0.23	0.09	0.97	0.81
C13:0	0.50	0.82	0.75	0.85	0.11	0.08	0.24	0.35
C13:0 <i>iso</i>	0.57	0.48	0.75	0.59	0.05	0.03	0.02	0.43
C13:0 <i>anteiso</i>	0.56	0.54	0.80	0.72	0.06	0.45	0.01	0.63
C14:0	5.87	8.00	6.34	8.22	0.52	0.004	0.53	0.82
C14:0 <i>iso</i>	1.25	1.10	1.22	1.25	0.09	0.51	0.56	0.39
C14:1 <i>cis</i> -9	1.23	1.44	2.27	1.98	0.17	0.77	0.001	0.13
C15:0	8.50	9.79	9.39	10.6	0.97	0.22	0.40	0.98
C15:0 <i>iso</i>	3.54	3.32	4.18	3.66	0.33	0.29	0.17	0.67
C15:0 <i>anteiso</i>	7.96	10.2	11.0	13.1	0.89	0.04	0.009	0.95
C16:0	141.2	147.5	190.2	167.3	8.6	0.36	0.003	0.12
C16:0 <i>iso</i>	3.04	2.07	3.35	2.00	0.39	0.02	0.77	0.65
C16:1 <i>cis</i> -9	4.07	4.82	5.05	4.18	0.49	0.91	0.74	0.13
C17:0	4.98	6.25	5.71	6.19	0.59	0.17	0.59	0.52
C17:0 <i>iso</i>	2.20	2.34	2.58	2.57	0.29	0.83	0.32	0.79
C17:0 <i>anteiso</i>	0.62	0.91	0.92	1.19	0.08	0.009	0.008	0.87
C18:0	944.1	766.1	867.8	580.8	74.9	0.008	0.07	0.40
C18:1 <i>cis</i>								
Total	85.1	145.9	106.9	128.3	10.5	0.006	0.84	0.10
<i>cis</i> -9	67.7	115.5	84.4	100.9	8.97	0.01	0.91	0.13
<i>cis</i> -11	10.3	19.2	8.74	12.8	1.64	0.004	0.03	0.14
<i>cis</i> -12	6.23	9.89	12.1	11.4	1.13	0.12	0.005	0.04
<i>cis</i> -13	0.89	1.42	1.70	3.15	0.19	0.001	0.0004	0.04
C18:1 <i>trans</i>								
Total	128.0	255.9	230.7	395.4	25.5	0.0006	0.002	0.44
<i>trans</i> -4	2.62	4.21	4.69	2.67	0.45	0.64	0.57	0.007
<i>trans</i> -5	1.68	2.77	1.33	1.59	0.21	0.01	0.006	0.09
<i>trans</i> -6+7+8	12.7	25.4	8.24	19.7	2.26	0.001	0.05	0.77
<i>trans</i> -9	8.16	13.6	6.68	11.0	0.86	0.0009	0.04	0.50
<i>trans</i> -10	10.7	75.3	10.1	90.9	17.3	0.001	0.58	0.56
<i>trans</i> -11	35.6	55.5	73.7	106.7	16.8	0.08	0.01	0.62
<i>trans</i> -12	13.3	18.7	21.0	26.7	1.5	0.005	0.0006	0.94
<i>trans</i> -13+14	29.4	47.9	74.1	105.7	5.2	0.001	<0.0001	0.24
<i>trans</i> -16	14.0	12.5	30.8	30.6	3.3	0.81	0.0005	0.86
C18:1 total	213.1	401.9	337.5	523.7	32.9	0.0007	0.006	0.97
C18:2 nonconjugated								
<i>trans</i> -9, <i>trans</i> -12	0.43	0.86	0.74	4.52	0.96	0.06	0.07	0.12
<i>trans</i> -9, <i>cis</i> -11	0.57	1.05	1.16	3.56	0.49	0.03	0.02	0.09
<i>cis</i> -9, <i>trans</i> -12	1.12	1.11	1.31	1.34	0.13	0.95	0.14	0.89
<i>trans</i> -11, <i>cis</i> -15	1.95	7.61	12.5	45.7	6.27	0.02	0.007	0.06
<i>cis</i> -9, <i>cis</i> -12 (C18:2n-6)	20.9	25.5	48.0	26.0	9.2	0.37	0.17	0.18
C18:2 conjugated								
<i>cis</i> -9, <i>trans</i> -11	0.52	0.50	0.74	0.90	0.11	0.48	0.02	0.39
<i>trans</i> -10, <i>cis</i> -12	0.14	0.17	0.24	0.27	0.03	0.32	0.01	0.88
<i>trans</i> -11, <i>trans</i> -13	0.58	0.69	1.76	1.69	0.15	0.89	0.0003	0.55
<i>trans</i> , <i>trans</i> ³	0.79	1.21	1.36	1.57	0.10	0.008	0.001	0.24
C18:2 ⁴	24.9	36.2	63.7	81.2	11.3	0.14	0.002	0.72
CLA ⁵	2.04	2.58	4.10	4.43	0.270	0.15	0.0003	0.69
C18:3n-3	4.80	8.76	11.75	14.54	2.847	0.27	0.05	0.84
C19:0	0.64	0.84	1.42	1.57	0.155	0.23	0.001	0.87
C20:0	17.4	14.7	8.41	6.69	0.82	0.01	<0.0001	0.44
C20:1 <i>cis</i> -11	0.46	0.49	0.51	0.57	0.058	0.35	0.21	0.75
C20:2n-6	0.63	0.69	0.87	0.93	0.185	0.75	0.24	0.97
C20:3n-3	0.60	0.96	1.27	1.81	0.164	0.03	0.003	0.58
C20:3n-6	0.41	0.53	0.64	0.68	0.111	0.47	0.14	0.74
C20:4n-6	0.18	0.28	0.35	0.43	0.04	0.06	0.003	0.72
C22:0	6.43	6.53	5.88	5.45	0.34	0.60	0.03	0.40
C22:3n-3	1.42	1.43	1.02	1.04	0.19	0.95	0.07	0.99
C23:0	0.26	0.37	0.33	0.35	0.053	0.25	0.62	0.42
C24:0	4.63	4.63	5.67	5.34	0.290	0.44	0.004	0.45
C26:0	7.19	5.69	9.31	5.78	0.975	0.01	0.18	0.21

Continued

Table 6 (Continued). Flow of FA (g/d) in duodenal digesta of dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	P-value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
Total SFA	1,166.6	998.1	1,143.1	831.2	81.8	0.01	0.22	0.35
Total MUFA	217.6	407.2	343.1	528.5	33.3	0.0007	0.006	0.95
Total PUFA	35.0	51.4	83.7	105.0	13.6	0.13	0.003	0.83

¹Values are least squares means obtained from 4 ruminally cannulated cows.

²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); BP × OS = interaction.

³Total of unresolved *trans*-9,*trans*-11 and *trans*-10,*trans*-12.

⁴Sum of C18:2 FA excluding conjugated linoleic acid (CLA) isomers.

⁵Total CLA.

reflects reduced microbial proteolytic activity as a result of a more acidic ruminal environment with PB (Van Soest, 1994). Conversely, a lower ruminal NH₃-N concentration may also be as a result of a greater level of utilization of NH₃-N as a result of a greater proportion of ruminally fermentable carbohydrate in PB diets (Hristov et al., 2001). In agreement with our observations, Yang et al. (2000, 2001) also reported a lower ruminal NH₃-N concentration in cows fed more extensively processed barley compared with those fed coarsely rolled barley. Across dietary treatments, ruminal NH₃-N concentrations ranged from 13.9 to 19.9 mg/dL, and mean ruminal NH₃-N concentrations at all sampling times postfeeding were >5 mg/dL (data not shown), which has been suggested to be optimum for maximum microbial protein synthesis (Satter and Slyter, 1974).

In the present study, cows fed DRB consumed more DM compared with those fed PB. Lower DMI in dairy cows fed more extensively processed barley have been reported previously (Yang et al., 2000), and are likely mediated via increased acidity in the rumens of cows fed PB compared with those fed DRB. On the other hand, dietary inclusion of canola and flaxseed as sources of supplemental fat did not alter DMI, supporting the

findings of a study by Ward et al. (2002). Our results show that milk yield was not affected by method of barley grain processing. Yang et al. (2000, 2001) reported improved milk yields in cows fed more extensively processed barley compared with those fed coarsely rolled barley, a response that was partly explained by the greater DMI of cows fed more extensively processed barley. Besides greater DMI, the positive lactational response to cereal grain processing is also mediated in part via increased ruminal and total-tract starch digestion, thus resulting in greater ruminal VFA concentrations and net energy absorption from the rumen, and increased MCP flow to the duodenum (Theurer et al., 1999). In the present study, DMI, proportions of dietary starch intake that were digested in the rumen, and duodenal flows of microbial N were not altered by the method of barley grain processing, thus possibly explaining the lack of responses observed. Milk yield was not affected by source of supplemental fat, supporting previous findings (Ward et al., 2002). Milk protein content and yield did not change due to the method of barley grain processing and source of supplemental fat. Yang et al. (2000, 2001) observed greater milk protein contents in cows fed more extensively processed barley compared with those fed coarsely rolled barley, a re-

Table 7. Milk yield and milk composition in dairy cows fed TMR based on dry-rolled (DRB) or pelleted barley (PB) supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	P-value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
Milk yield, kg/d	33.4	36.3	36.5	36.4	2.4	0.60	0.54	0.56
Fat, %	3.34	2.94	3.44	2.72	0.11	<0.01	0.72	0.33
Fat, kg/d	1.11	1.05	1.24	0.97	0.07	0.04	0.73	0.17
Protein, %	2.85	2.97	2.99	3.10	0.08	0.34	0.23	0.94
Protein, kg/d	0.94	1.06	1.08	1.09	0.05	0.23	0.12	0.29
Lactose, %	4.13	4.23	4.20	4.26	0.60	0.36	0.57	0.78
Lactose, kg/d	1.38	1.53	1.54	1.53	0.09	0.50	0.46	0.46
MUN, mg/dL	6.35	4.87	6.13	5.78	0.16	<0.01	0.16	0.03

¹Values are least squares means obtained from 4 ruminally cannulated cows.

²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); BP × OS = interaction.

Table 8. Milk FA concentrations (g/100 g of FA) in dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	<i>P</i> -value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
C4:0	3.13	2.90	3.30	3.39	0.34	0.80	0.27	0.58
C6:0	1.63	1.63	2.14	1.96	0.11	0.30	0.002	0.29
C8:0	1.21	1.23	1.56	1.47	0.07	0.61	0.003	0.40
C10:0	2.26	2.26	3.01	2.76	0.15	0.43	0.006	0.45
C11:0	0.045	0.039	0.054	0.080	0.009	0.26	0.02	0.09
C12:0	2.46	2.39	3.04	2.86	0.127	0.36	0.003	0.66
C13:0	0.166	0.142	0.170	0.214	0.017	0.57	0.05	0.08
C13:0 <i>iso</i>	0.021	0.023	0.029	0.026	0.003	0.80	0.14	0.50
C13:0 <i>anteiso</i>	0.059	0.052	0.067	0.080	0.005	0.65	0.008	0.10
C14:0	9.48	9.11	10.1	9.16	0.304	0.06	0.31	0.38
C14:0 <i>iso</i>	0.080	0.064	0.081	0.067	0.009	0.14	0.84	0.91
C14:1 <i>cis</i> -9	0.90	0.89	0.88	1.16	0.09	0.17	0.20	0.14
C15:0	1.06	0.92	0.97	1.15	0.09	0.81	0.41	0.10
C15:0 <i>iso</i>	0.178	0.190	0.196	0.148	0.016	0.26	0.46	0.09
C15:0 <i>anteiso</i>	0.48	0.45	0.51	0.54	0.03	0.94	0.04	0.16
C16:0	21.4	21.3	21.6	19.6	0.9	0.25	0.41	0.27
C16:0 <i>iso</i>	0.28	0.24	0.27	0.24	0.01	0.05	0.77	0.57
C16:1 <i>cis</i> -9	1.41	1.57	1.28	1.66	0.08	0.007	0.78	0.15
C17:0	0.59	0.60	0.55	0.62	0.03	0.22	0.75	0.37
C17:0 <i>iso</i>	0.42	0.50	0.44	0.51	0.041	0.11	0.77	0.96
C17:0 <i>anteiso</i>	0.16	0.18	0.11	0.15	0.01	0.09	0.04	0.61
C18:0	14.1	14.2	14.6	9.91	1.20	0.09	0.15	0.08
C18:0 <i>iso</i>	0.053	0.067	0.037	0.060	0.013	0.17	0.39	0.74
C18:0 <i>anteiso</i>	0.164	0.229	0.116	0.189	0.033	0.06	0.21	0.91
C18:1 <i>cis</i>								
Total	25.5	26.9	21.9	20.8	1.0	0.82	0.0002	0.09
<i>cis</i> -9	24.2	25.5	20.7	19.2	1.0	0.85	0.0003	0.07
<i>cis</i> -11	0.85	0.89	0.44	0.75	0.14	0.26	0.10	0.38
<i>cis</i> -12	0.38	0.41	0.60	0.67	0.05	0.31	0.0006	0.78
<i>cis</i> -13	0.11	0.14	0.10	0.19	0.01	0.004	0.23	0.11
C18:1 <i>trans</i>								
Total	7.80	6.98	7.39	12.9	1.66	0.19	0.13	0.09
<i>trans</i> -4	0.074	0.089	0.045	0.055	0.009	0.21	0.008	0.78
<i>trans</i> -5	0.074	0.109	0.050	0.060	0.140	0.13	0.03	0.38
<i>trans</i> -6+7+8	0.87	0.81	0.45	0.76	0.12	0.32	0.08	0.15
<i>trans</i> -9	0.83	0.79	0.52	0.70	0.07	0.37	0.02	0.18
<i>trans</i> -10	2.09	1.57	0.50	2.68	0.89	0.38	0.80	0.17
<i>trans</i> -11	1.51	1.45	2.15	4.07	0.62	0.17	0.03	0.14
<i>trans</i> -12	0.70	0.69	0.86	1.06	0.05	0.08	0.0004	0.07
<i>trans</i> -13+14	1.17	1.00	1.85	2.65	0.20	0.16	0.001	0.05
<i>trans</i> -16	0.47	0.46	0.98	0.93	0.05	0.52	<0.0001	0.68
C18:1 total	33.3	33.9	29.3	33.7	1.6	0.14	0.21	0.25
C18:2 nonconjugated								
<i>trans</i> -11, <i>cis</i> -15	0.32	0.21	0.67	1.16	0.23	0.44	0.03	0.24
<i>cis</i> -9, <i>cis</i> -12 (C18:2n-6)	1.97	2.06	1.74	2.25	0.22	0.22	0.92	0.37
C18:2 conjugated								
<i>cis</i> -9, <i>trans</i> -11	0.61	0.56	0.73	1.46	0.19	0.11	0.02	0.07
<i>trans</i> -9, <i>cis</i> -11	0.049	0.044	0.034	0.070	0.013	0.26	0.67	0.14
<i>trans</i> -10, <i>cis</i> -12	0.016	0.015	0.014	0.022	0.0004	0.45	0.66	0.36
<i>trans</i> -11, <i>trans</i> -13	0.038	0.031	0.062	0.070	0.0005	0.83	0.0002	0.81
<i>trans</i> , <i>trans</i> ³	0.13	0.14	0.13	0.21	0.03	0.10	0.22	0.22
C18:2 ⁴	2.30	2.27	2.40	3.41	0.37	0.22	0.13	0.20
CLA ⁵	0.84	0.79	0.97	1.83	0.22	0.10	0.03	0.07
C18:3n-3	0.74	0.66	1.24	2.01	0.16	0.07	0.0009	0.03
C18:3n-6	0.034	0.028	0.031	0.027	0.003	0.18	0.60	0.76
C19:0	0.053	0.041	0.056	0.092	0.014	0.43	0.08	0.12
C20:0	0.245	0.258	0.191	0.153	0.017	0.37	0.0007	0.09
C20:1 <i>cis</i> -11	0.161	0.161	0.088	0.092	0.008	0.83	<0.0001	0.83
C20:2n-6	0.052	0.057	0.045	0.052	0.004	0.17	0.17	0.76
C20:3n-3	0.058	0.070	0.066	0.072	0.009	0.37	0.58	0.75
C20:3n-6	0.061	0.076	0.069	0.077	0.009	0.15	0.56	0.63
C20:4n-3	0.038	0.034	0.061	0.061	0.005	0.42	<0.0001	0.42
C20:4n-6	0.100	0.128	0.094	0.105	0.017	0.26	0.39	0.62

Continued

Table 8 (Continued). Milk FA concentrations (g/100 g of FA) in dairy cows fed TMR based on dry-rolled (DRB) or pelleted (PB) barley supplemented with canola or flaxseed¹

Item	Canola		Flax		SEM	P-value ²		
	DRB	PB	DRB	PB		BP	OS	BP × OS
C20:5n-3	0.044	0.046	0.068	0.067	0.004	0.87	0.0005	0.74
C22:0	0.060	0.062	0.058	0.050	0.006	0.54	0.17	0.31
C22:1 <i>cis</i> -13	0.014	0.014	0.012	0.018	0.001	0.02	0.31	0.02
C22:4n-3	0.019	0.021	0.019	0.026	0.003	0.20	0.42	0.46
C22:4n-6	0.025	0.033	0.022	0.025	0.003	0.13	0.14	0.38
C22:5n-3	0.074	0.072	0.082	0.080	0.006	0.75	0.23	0.98
C23:0	0.021	0.023	0.023	0.028	0.002	0.17	0.19	0.72
C24:0	0.035	0.031	0.032	0.037	0.004	0.95	0.63	0.27
C26:0	0.024	0.030	0.029	0.031	0.004	0.38	0.48	0.55
Summation by source ⁶								
<C16	23.2	22.3	26.1	25.1	0.9	0.32	0.02	0.93
16:0 and 16:1	23.1	23.1	23.2	21.5	0.8	0.36	0.39	0.33
>C16	53.8	54.6	50.8	53.5	1.4	0.23	0.16	0.50
Total SFA	59.8	59.2	63.3	55.5	2.0	0.07	0.97	0.11
Total MUFA	35.8	36.6	31.5	36.7	1.6	0.10	0.22	0.20
Total PUFA	4.39	4.28	5.17	7.84	0.59	0.06	0.005	0.04

¹Values are least squares means obtained from 4 ruminally cannulated cows.

²BP = barley grain processing (dry rolled vs. pelleted); OS = source of supplemental fat in the diet (canola vs. flax); BP × OS = interaction.

³Total of unresolved *trans*-9,*trans*-11 and *trans*-10,*trans*-12.

⁴Sum of C18:2 FA, excluding conjugated linoleic acid (CLA) isomers.

⁵Total CLA.

⁶Fatty acids classified based on their source: <C16 are derived from de novo synthesis, >16 are preformed FA that are extracted from the blood by the mammary gland, and C16 + C16:1 are derived from both de novo synthesis and blood extraction.

sponse likely attributable to increased duodenal flow of MP (Theurer et al., 1999). However, in the present study, duodenal N flow was unaltered by diet and this could partly explain why milk protein was not changed. Milk urea N was greater in cows fed DRB compared with those fed PB, reflecting dietary differences in ruminal NH₃-N concentrations, as MUN is largely derived from NH₃-N that is absorbed from the rumen (NRC, 2001).

It is well established that FA of dietary origin undergo extensive lipolysis and BH in the rumen, resulting in the synthesis of a wide range of BH intermediates that can flow out of the rumen and be absorbed at the small intestine (Chilliard et al., 2007). In most dairy cow diets, C18:2n6 and C18:3n3 are the major PUFA and their ruminal BH results in the synthesis of various C18:1-*trans*, C18:1-*cis*, and nonconjugated and conjugated C18:2 isomers (Chilliard et al., 2007). Of the many factors that influence the extent of ruminal BH of FA, ruminal starch availability and the source of supplemental lipid appear to have the most profound effects (Chilliard et al., 2007). In the present study, shifting starch digestion from the small intestine to the rumen by feeding PB compared with DRB resulted in a decrease in the duodenal flow of C18:0, increases in duodenal flow of various BH intermediates (C18:1-*trans*, C18:1-*cis*, and some nonconjugated and conjugated C18:2 isomers) and numerical increases in

the duodenal flow of C18:2n6 (+11.3 to 17.5 g/d) and C18:3n3 (+2.79 to 3.96 g/d). Of particular significance, duodenal flows of C18:1 *trans*-10 and C18:1 *trans*-11, which are key C18:1-*trans* FA that play a role in mammary FA synthesis (Lor et al., 2005), increased 6- to 8-fold when PB was fed. When taken together, these responses in duodenal FA flow when PB was fed in place of DRB are indicative of impaired ruminal BH of dietary PUFA. These perturbations in ruminal BH pathways when more ruminally fermentable starch is fed arise from a more acidic ruminal environment and changes in ruminal microbial populations (Glasser et al., 2008). In the current study, the depression in ruminal pH was more severe in cows fed PB compared with those fed DRB. Previous studies indicated that depressed pH altered BH pathways of unsaturated FA, such that an accumulation of C18:1 *trans*-10 and CLA isomers occurred (Van Nevel and Demeyer, 1996; Qui et al., 2004). Besides increased ruminal acidity altering ruminal BH pathways, changes in ruminal bacterial populations can also contribute to the observed responses in duodenal FA flows when more ruminally fermentable starch is fed (Lor et al., 2004). Although we did not assess changes in ruminal bacterial populations in the current study, Nielsen et al. (2006) reported that feeding more ruminally fermentable starch stimulated the growth of *Megasphaera elsdenii*, which can convert C18:2n6 to C18:2 *trans*-10,*cis*-12 and C18:1 *trans*-10

(Bauman and Griinari, 2001). Based on the increase in the duodenal flow of C18:1 *trans*-10 that we observed in cows fed PB compared with those DRB, we can surmise that feeding more ruminally fermentable starch likely shifted microbial populations in the rumen.

Both milk fat content and yield decreased when cows were fed PB in place of DRB, and this milk fat depression can be attributed to the increase in ruminal acidity in cows fed PB disrupting ruminal BH pathways of FA. Of the BH intermediates that have been demonstrated to play a role in milk FA synthesis, C18:1 *trans*-10 (Bauman and Griinari, 2001) and C18:2 *trans*-9,*cis*-11 (Perfield et al., 2007) have been shown to be potent inhibitors of milk FA synthesis in dairy cows, a response that is likely mediated via reduced activity or mRNA abundance (or both) for several key lipogenic enzymes in the mammary gland (Piperova et al., 2000; Peterson et al., 2003). Also, C18:1 *trans*-11 has been observed to have inhibitory effects on the activity of acetyl-CoA carboxylase and FA synthase in cultured bovine mammary epithelial cells (Jayan and Herbein, 2000), suggesting that C18:1 *trans*-11 might also play a regulatory role in mammary FA synthesis. In cows that exhibited milk fat depression in the current study (i.e., cows that were fed PB), duodenal flows of C18:1 *trans*-10, C18:1 *trans*-11, and C18:2 *trans*-9,*cis*-11 were elevated, suggesting that these *trans*-FA were likely responsible for depressing milk fat. However, despite the observed milk fat depression and associated increases in duodenal flows of key C18:1 and C18:2 isomers in cows fed PB compared with DRB, the milk concentrations of these C18:1 and C18:2 isomers remained largely unchanged. This is in contrast to previous studies in cows reporting milk fat depression with concomitant increases in milk concentrations of these C18:1 and C18:2 isomers (Nielsen et al., 2006; Chilliard et al., 2007; Sterk et al., 2011), and the reasons for this discrepancy are unclear. Others (Bauman and Griinari, 2001) have reported that C18:2 *trans*-10,*cis*-12, another key BH intermediate in dairy cows fed high-concentrate diets with or without supplemental vegetable oil, can also be a potent inhibitor of milk FA synthesis. However, in the present study, we did not observe any changes in duodenal flow of C18:2 *trans*-10,*cis*-12, thus suggesting that other BH intermediates were responsible for the observed milk fat depression.

Responses in duodenal flows and milk profiles of FA are also dependent on the FA composition of the supplemental lipid fed (Chilliard et al., 2007). In the present study, dietary FA profiles were altered by including canola or flaxseed as the main sources of supplemental lipid as they vary in their contents of FA, primarily C18:3n3 and, to a lesser degree, C18:2n6. By design, therefore, dietary intakes of C18:3n3 were greater for

cows fed flaxseed compared with those fed canola (data not presented). When compared with canola, supplementation with flaxseed increased duodenal flows of C18:3n3 and various BH intermediates, including C18:1 *trans*, C18:1 *cis*, and C18:2 *cis*-9,*trans*-11 and C18:2 *trans*-10,*cis*-12, which are the primary CLA isomers that are of biological importance. However, duodenal flow of C18:0, the major end product of complete ruminal BH of PUFA, decreased when flaxseed was fed in place of canola. Collectively, these data demonstrate that ruminal BH of dietary PUFA was disrupted to a greater extent when the diet contained greater levels of C18:3n3 and this is in agreement with results of other studies (Loor et al., 2005; Sterk et al., 2011). Milk fat contents of C18:3n3 and the various ruminal BH intermediates, notably C18:1 *trans*, C18:1 *cis*, and C18:2 *cis*-9,*trans*-11 and C18:2 *trans*-10,*cis*-12, were greater in cows fed flaxseed compared with those fed canola and this reflects the observed responses in duodenal flows of these FA as they are extracted from the blood and directly incorporated into milk fat. Ward et al. (2002) also reported similar changes in milk FA profiles in a feeding experiment in which flaxseed was compared with canola as supplemental sources of lipid.

Another major objective of this study was to determine how interactions between method of barley grain processing and source of dietary supplemental fat alter ruminal microbial protein synthesis and duodenal flow of nitrogenous fractions. Our results indicate that duodenal flow of microbial NAN and microbial efficiency were not affected by method of barley grain processing. We had hypothesized that processing barley grain by either dry rolling or pelleting would alter the supply of ruminally fermentable carbohydrate and, thus, affect the efficiency of N capture for MCP synthesis. Previous studies investigating the effect of dietary carbohydrate content in dairy cows indicated that a greater dietary concentration of NSC (Hoover and Stokes, 1991) or increasing ruminal starch digestion by steam flaking (compared with dry rolling) of sorghum (Poore et al., 1993) or corn (Plascencia and Zinn, 1996) grains increases the utilization of ruminal NH₃-N for microbial protein synthesis and results in greater MCP production. In the present study, supplying more degradable starch by feeding PB compared with DRB had no effects on microbial N supply or microbial efficiency. The lack of effects of barley grain processing on ruminal MCP production or efficiency confirms previous findings from studies using similar barley-based diets in dairy cows (Yang et al., 2000). Similar to findings in the present study, Yang et al. (2000) did not report any changes in ruminal starch or ruminal OM digestion as the extent of barley grain processing was increased, thereby suggesting that a

quantitative difference in ruminal energy supply for microbial growth might not have existed. Microbial efficiencies calculated in the present study ranged from 23 to 28 g of microbial NAN/kg of OMTDR, and are in close agreement with published values obtained with dairy cows fed similar barley-based diets (22 to 28 g of microbial NAN/kg of OMTDR; Yang et al., 2000). Russell and Wilson (1996) observed inhibitory effects on microbial protein synthesis when ruminal pH was <6.2. In the present study, mean ruminal pH ranged from 5.73 to 6.13; however, ruminal microbial synthesis and microbial efficiency were unaffected, supporting results from previous studies (Yang et al., 2000). We also observed that the duodenal flow of microbial NAN and microbial efficiency were not affected by source of supplemental fat; however, when expressed as a proportion of total duodenal NAN flow, microbial NAN flow to the duodenum was 14% greater in cows fed canola compared with those fed flaxseed, possibly reflecting numerically greater total NAN flows in cows fed canola. In addition, RDP supply in cows fed canola was 354 g/d greater than that of cows fed flaxseed. Reynal and Broderick (2005) observed that a greater RDP supply was accompanied by an increase in duodenal microbial NAN flow.

Ruminal digestibility of NDF was greater in cows fed DRB compared with those fed PB. These negative effects of more extensive barley grain processing on ruminal fiber digestion were not surprising because pelleting increased ruminal acidity when compared with dry rolling. In cows fed PB, mean ruminal pH ranged from 5.73 to 5.77, which is lower than the ruminal pH of 6.2 that has been suggested as the minimum ruminal pH for optimum fiber digestion (Van Soest, 1994). Although ruminal bacterial populations were not measured in the present study, Russell and Wilson (1996) demonstrated that populations of the main fiber-digesting bacteria such as *Fibrobacter* spp. and *Ruminococcus albus* declined rapidly when pH fell below 6.0 in an in vitro fermentation system. Previous studies have reported depressed fiber digestion in cows subjected to ruminal acidosis using a grain-challenge model (Krajcarski-Hunt et al., 2002) or when ruminal starch digestion was increased by steam-flaking sorghum grain (Poore et al., 1993) or choice of cereal grain (barley vs. corn; McCarthy et al., 1989). Surprisingly, contrary to our observations on ruminal NDF digestion, apparent ADF digestibility was unaffected by the method of barley grain processing. The reasons for this discrepancy are unclear; however, it is noteworthy that, numerically, ruminal ADF digestibility was greater in cows fed DRB compared with those fed PB. Herrera-Saldana et al. (1990) also reported changes in ruminal NDF digestion

without concomitant changes in ruminal ADF digestion when ruminal starch digestion was manipulated.

In the present study, cows fed DRB consumed 12 to 18% more starch compared with those fed PB, which is reflective of the observed changes in DMI. Surprisingly, ruminal starch digestion was unaffected by the method of barley grain processing. Because pelleting decreases particle size, it had been anticipated that cows fed PB would exhibit a greater extent of ruminal starch disappearance compared with those fed DRB. The lack of effect of barley grain processing on ruminal starch digestion is not consistent with the observed changes in ruminal acidity that were observed, and the reasons for these discrepancies are unclear. It is noteworthy that cows fed DRB digested 10 to 15% (in absolute amounts) less starch in their rumens compared with those fed PB, and the magnitude of this difference in ruminal starch digestibility is similar to that in starch intake. In addition, duodenal flows of starch was greater in cows fed DRB compared with those fed PB. In agreement with the present study, Yang et al. (2000) did not observe any effects of grain processing on ruminal starch digestion in dairy cows fed barley grain processed to varying degrees, ranging from coarsely rolled to flat rolled; however, Yang et al. (2001) reported greater ruminal starch digestion when dairy cows were fed flat-rolled barley compared with coarsely rolled barley. Across dietary treatments, around 90% of dietary starch intake was digested in the rumen. Previous studies using similar barley-based diets observed lower ruminal starch digestibility coefficients, ranging from 38 to 50% (Yang et al., 2001) and 61 to 71% (Yang et al., 2000), possibly reflecting differences in, among other things, the extents of barley grain processing, level of feed intakes, and ruminal environmental conditions.

CONCLUSIONS

In summary, results indicate that changing the availability of ruminally fermentable carbohydrate and the source of supplemental fat altered duodenal FA flows and milk FA composition. No interactions were observed between method of barley processing and source of supplemental fat for duodenal flow of C18:2n6, C18:3n3, and most of the major ruminal BH intermediates. However, interactions or tendencies were observed for milk fat proportions of C18:3n3, total C18:1 *trans*-FA, C18:2 *cis*-9,*trans*-11, and total CLA, with major differences in milk proportions of these FA between cows fed DRB and PB with full-fat flaxseed as the source of supplemental fat, with smaller differences between DRB and PB with full-fat canola. Also, this study indicated that, in general, the method of

barley grain processing altered nutrient digestion in the rumen, with cows fed PB exhibiting lower nutrient digestibilities compared with cows fed DRB. However, nutrient flows to the duodenum were largely unaffected by the method of barley grain processing. The source of supplemental fat and method of barley grain processing had no effects on efficiency of MCP synthesis or duodenal flow of microbial protein, and their interactions between these factors were not significant.

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