Effect of flaxseed physical form on digestibility of lactation diets fed to Holstein steers

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ABSTRACT

Four multicannulated (rumen, duodenum, and ileum) Holstein steers (459.7 ± 46.4 kg of initial body weight) were used in a 4×4 Latin square design to determine the effect of flaxseed processing method on ruminal fermentation and digestibility. Treatments were based on inclusion of (1) 7.5% linseed meal (control), (2) 10% whole flaxseed, (3) 10% rolled flaxseed, or (4) 10% ground flaxseed on a dry matter (DM) basis, and were formulated to mimic typical high-producing dairy cow lactation diets. The control diet contained linseed meal in a proportion to provide crude protein (CP) equal to the amount of CP contributed by the flaxseed in the other treatments. Diets were fed for ad libitum intake and contained 30% corn silage, 17% chopped alfalfa hay, 6% sugar beet pulp, and 47% concentrate (comprising ground corn, supplemental protein, trace minerals and vitamins, and either flaxseed or linseed meal (DM basis). Diets were formulated to contain 17% CP, 34% neutral detergent fiber, 21% acid detergent fiber, and 4% fatty acid (DM basis). Periods were 14 d long and consisted of 7 d of adaptation and 7 d of sample collection. Dry matter intake (as a % of body weight) was similar (2.41 ± 0.17) for all treatments. The inclusion of flaxseed, regardless of processing method, tended to decrease total-tract organic matter digestibility relative to the linseed control, but no differences in CP intake, duodenal CP flow (bacterial, apparent feed, or total), ileal CP flow, fecal CP output, microbial efficiency, or CP digestibility (apparent ruminal, true ruminal, small intestine, large intestine, or total tract) were observed between treatments. Method of processing did not alter ruminal pH, ammonia, or volatile fatty acids production. The ground flaxseed treatment had the fastest rate of in situ DM degradation (11.25%/h), followed by the control (7.46%/h), rolled flaxseed (4.53%/h), and whole flaxseed (0.57%/h) treatments. Degradability of CP and fat followed the same pattern as DM degradability for processed flaxseed. In situ degradation rates

of alfalfa hay neutral detergent fiber and acid detergent fiber tended to be fastest for the ground flaxseed treatment. Taken together, the digestibility, fermentation, and in situ data indicate that rolling and grinding are both acceptable methods of processing flaxseed. The in situ data strongly support the need for processing flaxseed before inclusion in lactation diets.

Key words: dairy steer, digestibility, flaxseed, physical form

INTRODUCTION

Flaxseed can be used as a source of supplemental fat and protein for dairy cows (Mustafa et al., 2003). Nonetheless, supplementation of nonprotected fat in excess of 7% of dietary DM can decrease DMI and reduce digestibility in ruminants (Merchen, 1988; Doreau et al., 1991; Jenkins, 1993). Lipids can reduce fiber digestibility but the degree depends on the type and nature of the lipid (Ward et al., 2002). The reduction in DMI is associated with changes in ruminal fermentation, gut motility, palatability, release of gut hormones, and the oxidation of fat in the liver (Allen, 2000). Processing oil seeds increases ruminally unprotected PUFA, which can have detrimental effects on ruminal fermentation, including altering the microbial population or hindering microbial fiber digestion (Palmquist and Jenkins, 1980; Moore et al., 1986); however, processing methods such as rolling or grinding also generally increase the availability of nutrients to the animal. Gonthier et al. (2004) concluded that flaxseed supplementation (12.6% of DM) improved total-tract digestibility with no detrimental effects on rumen function. Those authors also concluded that micronization increased RUP for flaxseed. Previous studies recommend the processing of flaxseed in beef finishing diets (Maddock et al., 2006) and lactating dairy cow diets (da Silva et al., 2007) compared with whole flaxseed but have not measured the effects of processing on digestion. Therefore, the objective of this study was to evaluate the effects of physical form of flaxseed on the site and extent of digestion, ruminal fermentation, and in situ degradation. Our focus was to assess relative differences of processing and method from altering the physical form of flaxseed.

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MATERIALS AND METHODS

Animals and Diets

All procedures were approved by the North Dakota State University (NDSU) Animal Care and Use Committee. Four Holstein steers (459.7 ± 46.4 kg of initial BW) with ruminal, duodenal, and ileal cannulas were used in a 4×4 Latin square design to determine the effect of flaxseed processing (whole, rolled, or ground) on ruminal fermentation and digestibility of nutrients. Steers were housed in individual pens (3.0×3.7 m) at the NDSU Animal Nutrition and Physiology Center (Fargo). During the sample collection periods, steers were kept in individual metabolism stalls (1.0×2.2 m). Steers were fed completely mixed diets at 0700 and 1900 h daily and were allowed unlimited access to water. Diets were offered for ad libitum intake to ensure an approximate daily 5% rate of feed refusals.

All diets (Table 1) were formulated to meet NRC (2001) requirements for a lactating dairy cow. Steers were fed basal diets of 47% concentrate, 30% corn silage, 17% alfalfa hay, and 6% beet pulp shreds (DM basis). Alfalfa hay was chopped in a tub-grinder (HayBuster, model H1100 Tilt, DuraTech Industries International Inc., Jamestown, ND) with a 3.8-cm screen. Corn silage and alfalfa hay used for the study were from the same harvest. The concentrate portion of the diets containing the flaxseed or linseed meal was mixed before the initiation of the study by the NDSU Northern Crops Institute Feed Mill (Fargo, ND). Dietary treatments included (1) 7.5% linseed meal (control), (2) 10% whole flaxseed, (3) 10% rolled flaxseed, or (4) 10% ground flaxseed. The control diet contained solvent-extracted linseed meal (Cargill, West Fargo, ND) at proportions equal to the amount of protein contributed by the flaxseed in the other treatments. The rolled flaxseed was processed using a roller-grinder (2-pair stacked rollers, 23×30.5 cm, top pair = 0.42 corrugations/cm, bottom pair = 0.84 corrugations/cm; differential drive reduction of 1.5:1, fast to slow; motor size = 25 hp; model K, Roskamp Champion, Waterloo, IA). The ground flaxseed was processed using a hammermill (96.5 cm diameter, split-screen design, down-swing side = 4.8-mm screen hole, up-swing side = 5.6-mm screen hole; motor size = 50 hp; model E-38095 TF, Bliss Industries, Ponca City, OK). Chromic oxide was included in the diets as an external marker from d 4 to 14 at 0.25% of the DM to determine digesta flows at the duodenum. Chromic oxide was premixed into the concentrate by using a Hobart mixer (model H-600, Hobart Manufacturing Co., Troy, OH).

Diets were mixed in advance using a Davis paddle mixer (model S-20, H. C. Davis Sons Mfg. Co. Inc.,

Bonner Springs, KS) in sufficient quantities to last 3 to 4 d. The total mixed diets were stored in barrels in a walk-in freezer (-20°C), with each day's rations moved to a walk-in cooler (4°C) for 12 h before feeding twice daily at 0700 and 1900 h. Diet intakes were recorded and amounts were adjusted daily for fluctuations in DMI to include 5% daily refusal.

Sample and Data Collection

Each experimental period was 14 d long including 7 d for adaptation to the diet and 7 d for sample collection. For each animal,orts were collected on d 8 to 14, weighed, composited, and mixed by hand. Samples of duodenal (200 g) and ileal (100 g) chyme were collected on d 11 to 13 in a manner to achieve a sampling point in time of every other hour between the feedings (0700 and 1900 h). Samples were composited within steer and period for the experiment. Fecal trays were placed behind the steers for total fecal collection from d 10 through 14. Fecal output was weighed and subsampled (10% of wet weight), and composited across days within steer and stored at -20°C . Composite samples were mixed in a rotary mixer (model H-600, Hobart Manufacturing Co.) and subsampled. Fecal samples were dried in a forced-air oven for 48 h (50°C ; model SB-350, Grieve Corp., Round Lake, IL). Chyme samples were freeze-dried and stored at -20°C .

On d 14, ruminal fluid samples were collected at -2 , 0, 2, 4, 6, 8, 10, and 12 h relative to the morning feeding. After the collection at -2 h, the rumen was dosed with 200 mL of a CoEDTA solution (20 g of Co/L) via a catheter-tipped, 50-mL syringe with a tube attached, to determine the ruminal liquid dilution rate (Uden et al., 1980). Ruminal fluid samples (200 mL) were drawn using a suction strainer, and pH was recorded using a pH meter and combination electrode (model 2000, Beckman Instruments Inc., Fullerton, CA). A 3-mL sample of ruminal fluid was retained, and 1 mL of 25% (wt/vol) HPO_3 was added to the sample in a 12×75 -mm culture tube. Samples were stored at -20°C until analysis for NH_3 and VFA.

In situ bags were incubated from d 8 to 13. Dacron bags (10×20 cm, 50 ± 15 μm pore size, Ankom, Fairport, NY) containing 5-g samples of ground alfalfa hay were incubated in the rumen to determine the effect of flax processing on rate and extent of forage digestion. The same kind of bags containing 5-g samples of flaxseed and linseed meal (physical form equal to treatment physical form) were also incubated in the rumen to examine digestibility differences between processing methods. Duplicate samples were incubated within lingerie bags, initially placed below ruminal mat layer in the ventral sac, in each steer for 0, 2, 5, 9, 12,

Table 1. Ingredients and chemical composition of experimental diets of Holstein steers fed flaxseed

Item	Diet ¹			
	CON	WF	RF	GF
Composition ² (% of DM)				
Corn silage	30.0	30.0	30.0	30.0
Alfalfa hay	17.0	17.0	17.0	17.0
Beet pulp, shreds	6.0	6.0	6.0	6.0
Corn, ground	30.3	26.6	26.6	26.6
Linseed meal	7.5	—	—	—
Whole flaxseed	—	10.0	—	—
Rolled flaxseed	—	—	10.0	—
Ground flaxseed	—	—	—	10.0
Sunflower meal	1.8	3.4	3.4	3.4
Feather meal	3.0	2.6	2.6	2.6
Limestone ³	1.3	1.3	1.3	1.3
Corn gluten meal	0.8	0.7	0.7	0.7
Blood meal	0.8	0.7	0.7	0.7
Sodium bicarbonate ³	0.6	0.6	0.6	0.6
Magnesium oxide ³	0.4	0.4	0.4	0.4
Urea	0.2	0.3	0.3	0.3
Dairy Balancer II ³	0.3	0.3	0.3	0.3
Salt	0.1	0.1	0.1	0.1
Rumensin 80 ⁴	0.007	0.007	0.007	0.007
Chromic oxide	0.3	0.3	0.3	0.3
Analyzed dietary nutrients (% of DM)				
CP	18.0	17.8	17.5	17.4
NDF	34.6	36.4	35.4	36.0
ADF	18.0	19.5	18.8	19.1
FA	4.4	4.9	5.0	5.3

¹CON = control (linseed meal), WF = whole flaxseed, RF = rolled flaxseed, and GF = ground flaxseed.

²Forage-to-concentrate ratio for all rations was 53:47; NE_L (Mcal/kg): CON = 1.6, WF, RF, and GF = 1.7.

³Dairy Balancer II (Nutrition Professionals Inc., Madison, WI; vitamins A, D, and E, microminerals, and selenium).

⁴Rumensin 80 (monensin; Elanco Animal Health, Greenfield, IN).

24, 34, 48, 72, and 120 h. After removal, all bags were rinsed with water to remove large particulate matter. In situ bags were then rinsed in a top-loading washing machine (General Electric, Louisville, KY). Using the delicate cycle, the machine was filled with 45 L of cold water. The bags were agitated for 1 min, drained, and spun for 2 min. This cycle was repeated 5 times.

In Situ Flaxseed Preparation

Preparation of flaxseed for in situ degradability evaluation included removal of chaff and other impurities with a gravity table (Forsberg Inc., Thief River Falls, MN). Smaller impurities were then separated from the flaxseed using U.S.A. standard testing sieves (VWR Scientific, West Chester, PA), arranged (top to bottom) as follows: No. 10 (2 mm), No. 16 (1.19 mm), and catch pan. The sieves were shaken with a Ro-Tap shaker (Testing Sieve Shaker, model no. 9221, The W. S. Tyler Company, Cleveland, OH). Flaxseed underwent size reduction in a roller mill, with rollers aligned with minimum opening without binding, or were ground in a small batch grinder (model no. MG, Urschel Laboratories, Valparaiso, IN), with the

cutting head (model no. 66900, Urschel Laboratories) set at a height of 0.05 m in a 1.22-m column. The horizontal separators were 0.6 mm thick with a 1.0-mm opening between separators. Whole flaxseed remained unprocessed, and alfalfa hay was ground in a Wiley mill (2-mm screen; Arthur H. Thomas, Philadelphia, PA) for in situ analysis. All physical forms of flaxseed were compared for particle size (Table 2) using a particle size analyzer (Micron Powder Systems, Hosokawa Micron, Summit, NJ). Samples (20 g) were vacuumed (61.65 mmHg; 60 s) through with USA Standard Test Sieves (Hosokawa Micron Powder Systems, 600 μ m-30 mesh and 850 μ m-20 mesh).

Laboratory Analysis

Orts and fecal samples were composited by animal, mixed in a rotary mixer (model H-600, Hobart Manufacturing Co.), and subsampled (10% of wet weight). Feed, Orts, and fecal samples were subsequently dried in a 55°C forced-air oven (model SB-350, The Grieve Corporation, Round Lake, IL) for 48 h. Dried samples were ground in a Wiley mill (2-mm screen; Arthur H. Thomas). Samples were composited within each of the

Table 2. Effect of flaxseed processing on nutrient composition and particle size of flaxseed and linseed meal used in the study

Item	Linseed meal	Processing method		
		Whole	Rolled	Ground
Composition (% of DM)				
Ash	6.4	3.6	3.6	3.6
CP	38.0	24.8	25.1	25.2
NDF	20.9	36.3	33.3	34.3
ADF	12.4	18.3	18.1	18.1
Ether extract	4.3	36.3	37.2	32.8
Particle size (% remaining)				
>850 μm	48.5	99.0	90.4	8.5
>600 μm	71.5	99.5	96.5	30.0

4 periods for analysis. Intestinal chyme samples were lyophilized and ground in a Wiley mill with a 1-mm screen. Diet, ruminal, duodenal, ileal, and fecal samples were analyzed for DM, ash, N, ether extract (methods 930.15, 940.05, 984.13, 945.16, respectively, AOAC International, 1997), as well as NDF (assayed with thermostable α -amylase and sodium sulfite expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentration by the method of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, NY). Intestinal and fecal samples were also analyzed for Cr (Czarnocki et al., 1961). Sample Cr and nutrient concentrations were used to estimate site of digestion and relative digestibility (Merchen, 1988).

Ruminal fluid was thawed and centrifuged ($10,000 \times g$; 10 min). Three milliliters of supernatant was mixed with 0.75 mL of 25% (wt/vol) meta-phosphoric acid and centrifuged again ($10,000 \times g$; 10 min). Ruminal VFA concentrations were determined by GLC (Hewlett Packard 5890A Series II GC, Wilmington, DE) and separated on a capillary column (Nukol, Supelco, Bellefonte, PA) using 2-ethyl butyric acid as the internal standard (Goetsch and Galyean, 1983). A small portion (0.6 g) of the lyophilized duodenal samples was reconstituted to 3% (wt/vol) in 0.1 N HCl, vortexed, and centrifuged at $20,000 \times g$ for 20 min (Hannah et al., 1991). Supernatant from the duodenal and first ruminal centrifugation were used for $\text{NH}_3\text{-N}$ analysis (Broderick and Kang 1980). Cobalt concentration was determined using atomic absorption spectrometry (AAnalyst 800; PerkinElmer, Shelton, CT).

Ruminal bacteria were isolated from a 4-kg sample of ruminal contents by blending with a Waring blender (model 37BL19 CB6; Waring Products, New Hartford, CT) and strained through 2 layers of cheesecloth. Feed particles and protozoa in ruminal samples were removed via centrifugation at $1,000 \times g$ for 10 min. Bacteria were separated from supernatant by centrifuging at $20,000 \times g$ for 20 min. Isolated bacteria were frozen and analyzed

for DM (lyophilized), ash, N (as previously described), and purines (Zinn and Owens, 1986). Diet digestibility was determined by analyzing duodenal, ileal, and fecal samples for chromium concentrations (Fenton and Fenton, 1979).

Calculations

A corrected daily chromium dosage was calculated to take into account the amount of chromium recovered in feces and that removed through duodenal and ileal sampling. Fecal recovery of chromium was calculated by multiplying the measured fecal flow rate (kg/d) by the concentration of chromium in the fecal sample. The amount of chromium removed with duodenal or ileal samples was calculated by multiplying the mass of a sample by its DM and chromium concentrations. Duodenal flow rate was calculated by dividing the concentration of chromium measured at the duodenum into the sum of chromium recovered in feces and amounts of chromium removed at the duodenum and ileum. Ileal flow was calculated similarly to determine flow to the large intestine.

Microbial OM and N leaving the abomasum were calculated using purines as microbial markers (Zinn and Owens, 1986). Organic matter fermented in the rumen was OM intake minus the difference between the amount of total OM reaching the duodenum and microbial OM reaching the duodenum. Nonmicrobial N escape to the small intestine was calculated by subtracting microbial N from total N and thus includes any endogenous and ammonia N contributions. Fluid dilution rate was calculated using regression analysis of the natural logarithm of Co concentrations over time (Warner and Stacy, 1968).

Statistical Analysis

The statistical model used for all analyses included the main effects of diet, period (fixed effect), and steer

(random effect). Data were analyzed as a 4×4 Latin square using PROC MIXED of SAS (version 9.1, SAS Institute, Cary, NC). The model included period, animal, and diet: $Y_{ijk} = \mu + T_i + P_j + c_k + e_{ijkl}$, where Y_{ijk} = dependent variable, μ = overall mean, T_i = fixed effect of treatment i ($i = 1, \dots, 4$), P_j = fixed effect of period j ($j = 1, \dots, 4$), c_k = random effect of steer k ($k = 1, \dots, 4$) $N \sim (0, \sigma_c^2)$, and e_{ijk} = random residual $N \sim (0, \sigma_e^2)$. Means were expressed as least squares means and considered different at $P \leq 0.05$. Preplanned orthogonal contrasts compared control versus flaxseed, whole flaxseed versus processed flaxseed, and ground flaxseed versus rolled flaxseed.

In situ disappearance data were fitted to the model designed by Ørskov and McDonald (1979) using the nonlinear procedures of SAS (SAS Institute Inc.). The equation was $p = a + b(1 - e^{-ct})$, where p is disappearance, a is the rapidly degraded fraction, b is the slowly degraded fraction, c is the disappearance rate, and t is time.

RESULTS AND DISCUSSION

Particle Size and Nutrient Composition of Flaxseed

Particle size data are presented in Table 2. Rolling and grinding flaxseed resulted in 90.4 and 8.5%, respectively, of particles $>850 \mu\text{m}$ compared with the whole flaxseed, which had 99% of particles $>850 \mu\text{m}$. The particle size of the control sample was 48.5% $>850 \mu\text{m}$. The whole, rolled, and ground flaxseed had 99.5, 96.5, and 30.0%, respectively, of particles $>600 \mu\text{m}$. Our intent when processing the rolled flaxseed was to coarsely open the seed coat to allow ruminal microbes access to the inner-seed meal; therefore, particle size measurement may not be accurate in revealing the potential for efficiency of degradation. Nutrient composition of processed flaxseed appeared similar (Table 2). Numerically, NDF was slightly greater for whole flaxseed, and fat level was greater for rolled flaxseed. As expected, linseed meal had increased ash and CP values but lower fiber and fat levels. Lardy and Anderson (2009) reported the proximate analysis of flaxseed to include 22.8% CP, 35% lipid, and 8% ADF. Flaxseed ranges in protein values from 18.8 to 24.4% (Daun and Przybylski, 2000).

Diets and Intake

Dietary CP ranged from 17.37% for the ground flaxseed diet to 17.95% for the control diet (Table 1). Dry matter intake was similar among treatments ($11.92 \pm 0.86 \text{ kg/d}$; $P = 0.59$; data not shown). These results are

in agreement with Petit (2002) and Ward et al. (2002), who found that untreated flaxseed supplementation at levels up to 15% of the dietary DM did not affect DMI of Holstein cows. Formaldehyde-treated flaxseed fed up to 15% of dietary DM had no effect on DMI (Petit, 2002). Feeding other oilseeds such as canola or sunflower have resulted in similar findings (Khorasani et al., 1991; Chichlowski et al., 2005).

Dry matter intake expressed as a percentage of BW (2.41 ± 0.17 , data not shown; $P = 0.78$) was also similar for steers fed flaxseed that was processed. Even though supplemental dietary fat from plant sources can lower intake, our research found no significant effects of adding 4.1% fat from flaxseed when supplemented according to NRC (2001) guidelines with a 53:47 forage:concentrate ratio.

Digestion and Fermentation

Treatment had no effect on apparent and true OM digestion in the rumen ($P \geq 0.43$; Table 3). Our results are in agreement with Gonthier et al. (2004), who compared raw, micronized, and extruded flaxseed. Large intestine, small intestine, and total-tract OM digestibilities were also similar for all treatments ($P \geq 0.15$; Table 3). However, linseed meal tended ($P = 0.06$; Table 3) to have greater total-tract OM digestibility when compared with all physical forms of flaxseed. Gonthier et al. (2004) reported that diets with flaxseed improved postruminal OM and total-tract OM digestibility (expressed as a percent of passage to duodenum and percent of intake, respectively) compared with a control diet containing no flaxseed.

In another Canadian trial comparing ground and whole flaxseed, ground flaxseed tended ($P = 0.07$) to increase OM digestibility (da Silva et al., 2007). Pires et al. (1997) found that grinding cottonseed increased total-tract digestibility of OM when compared with whole-raw, whole-roasted, or ground-roasted cottonseed and a control. Scott et al. (1991) compared roasting, extrusion, and reducing particle size of soybeans by utilizing treatments of rolled-raw, finely ground-raw, roasted, and extruded soybeans and found that apparent digestibility of nutrients in the entire digestive tract were greatest for rolled-raw soybeans. Tice et al. (1993) compared whole-raw, whole-roasted, cracked-roasted, and ground-roasted soybeans to a control diet that contained no soybeans and found no differences in total-tract digestibility of OM. In the control diet, urea and casein provided 59% of the CP equivalent.

In our study, we adjusted the control diet to account for the protein contribution from flaxseed; flaxseed (linseed) meal was included in the formulation to provide concentrations of amino acids similar to the other diets.

Table 3. Effect of flaxseed processing on OM intake, flow, and digestion in dairy lactation diets offered to Holstein steers

Item	Physical form of flaxseed ¹				SEM ²	<i>P</i> -value ³	Contrast ⁴		
	CON	WF	RF	GF			1	2	3
Intake (kg/d)	10.76	10.28	11.12	11.43	0.78	0.61	0.80	0.23	0.74
Intake (g/kg of BW)	21.8	21.2	21.9	22.9	1.6	0.23	0.88	0.43	0.59
Duodenal flow (kg/d)									
Bacterial	1.18	1.37	1.68	1.67	0.22	0.19	0.08	0.19	0.97
Apparent feed	3.16	3.22	2.93	3.24	0.64	0.87	0.94	0.72	0.49
Total	3.58	3.85	3.87	4.11	0.66	0.74	0.38	0.73	0.63
Ileal flow (kg/d)	2.49	2.68	2.90	2.92	0.42	0.44	0.19	0.37	0.93
Fecal output (kg/d)	1.96	2.06	2.09	2.27	0.24	0.41	0.24	0.45	0.35
Digestibility (% of intake)									
Apparent ruminal	67.07	63.64	65.12	64.28	5.19	0.65	0.29	0.62	0.79
True ruminal	78.00	76.46	80.08	78.62	4.10	0.43	0.83	0.16	0.51
Small intestine	9.91	11.06	8.76	10.31	2.09	0.64	0.93	0.36	0.41
Large intestine	4.98	5.80	7.27	5.66	1.75	0.30	0.21	0.51	0.19
Total tract	81.96	80.20	81.14	80.25	1.58	0.15	0.06	0.47	0.27

¹Physical forms of flaxseed include CON = control (linseed meal), WF = whole flaxseed, RF = rolled flaxseed, and GF = ground flaxseed.

²n = 4 observations per treatment.

³Probability value for the *F*-test of overall treatment.

⁴Contrasts include 1 = control versus the average of whole, rolled, and ground flaxseed; 2 = whole flaxseed versus the average of rolled and ground flaxseed; and 3 = rolled flaxseed versus ground flaxseed.

Other studies have reported that protein digestion is not changed by adding fat to the diet (Grainger et al., 1961; Boggs et al., 1987). Our study is in agreement with these findings as we found no differences in CP intake (by design), duodenal CP flow (bacterial, apparent feed, or total), ileal CP flow, fecal CP output, microbial efficiency, or CP digestibility (apparent ruminal, true ruminal, small intestine, large intestine, or total tract; $P \geq 0.44$; Table 4). Unlike Tice et al. (1993), who fed whole soybeans, we did not observe reduced bacterial protein flow to the duodenum with the different treatments. Aldrich et al. (1995) reported similar findings with steers fed raw and roasted soybeans. Coppock et al. (1985) reported a decrease in CP digestibility for whole, acid-delinted cottonseed compared with rolled, acid-delinted cottonseed. They attributed the greater loss of nutrients from the whole cottonseed diet to passage of more intact seeds in the feces. We did not investigate feces for whole flaxseeds. Gonthier et al. (2004) found that extruded flaxseed had greater ruminal and lower postruminal CP digestibility; however, they found that total-tract CP digestibility of heat-treated flaxseed was similar to ground flaxseed. Increases in total-tract CP digestibility have also been reported with ground flaxseed compared with whole flaxseed (da Silva et al., 2007). Scott et al. (1991) reported lower total-tract CP digestibility for ground raw soybeans compared with extruded soybeans. In our study, flaxseed contributed approximately 14.5% of the total dietary CP and, with greater inclusion levels, it is possible that CP digestibility may be affected by processing flaxseed, but this was not detected in the present study.

The findings from the present study provide indirect evidence that microbial efficiency was unaffected by the addition of fat in the form of flaxseed to the diet when microbial efficiency (g of microbial N/kg of OM truly fermented) was used as an indicator of microbial activity ($P = 0.96$; Table 4). Because no differences were noted in CP and OM digestibility, one might expect no differences in microbial efficiency. When Tice et al. (1993) investigated roasted soybean processing, no differences in microbial efficiency were detected due to either added fat or processing. However, Gonthier et al. (2004) found that microbial efficiency was reduced with raw, micronized, and extruded (12.7% of diet DM) flaxseed compared with a control diet containing no flaxseed when nutrient requirements were met with greater levels of the basal ingredients.

In our study, flaxseed processing had no effect on total-tract NDF or ADF digestion with inclusion levels of 10% of dietary DM (Table 5). No differences ($P \geq 0.38$) in NDF and ADF intake, duodenal flow, ileal flow, fecal output, or digestibility were noted. Grinding, roasting, and extruding soybeans has led to decreased NDF digestibility (Scott et al., 1991). Sullivan et al. (1993) reported no fiber differences with cottonseed, whereas Pires et al. (1997) reported increases in NDF digestibility with the grinding of cottonseed. In contrast, Reveneau et al. (2005) reported no differences in NDF digestibility when altering particle sizes or delinting cottonseed. da Silva et al. (2007) reported decreased ADF digestibility with ground flaxseed compared with whole flaxseed. Micronized flaxseed reduced ruminal NDF fermentation and increased postruminal NDF di-

Table 4. Effect of flaxseed processing on CP intake, flow, and digestion in dairy lactation diets offered to Holstein steers

Item	Physical form of flaxseed ¹				SEM ²	P-value ³	Contrast ⁴		
	CON	WF	RF	GF			1	2	3
Intake (kg/d)	2.06	1.94	2.09	2.14	0.16	0.70	0.99	0.28	0.80
Duodenal flow (kg/d)									
Bacterial	0.62	0.58	0.68	0.70	0.10	0.59	0.65	0.23	0.85
Apparent feed	0.76	0.77	0.70	0.75	0.14	0.90	0.87	0.63	0.62
Total	1.37	1.35	1.38	1.45	0.22	0.94	0.87	0.67	0.70
Ileal flow (kg/d)	0.57	0.58	0.61	0.62	0.08	0.86	0.53	0.59	0.95
Fecal output (kg/d)	0.48	0.48	0.48	0.52	0.06	0.72	0.65	0.56	0.42
Microbial efficiency ⁵	11.76	12.50	12.39	12.40	2.12	0.96	0.63	0.94	0.99
Digestibility (% of intake)									
Apparent ruminal	33.65	31.15	33.78	32.94	9.18	0.96	0.82	0.65	0.88
True ruminal	63.66	60.59	66.18	65.10	6.27	0.44	0.92	0.14	0.76
Small intestine	38.73	39.10	36.89	38.40	6.14	0.94	0.85	0.68	0.71
Large intestine	4.63	5.32	6.18	4.42	1.23	0.47	0.50	0.99	0.17
Total tract	77.02	75.57	76.85	75.76	2.10	0.55	0.36	0.50	0.39

¹Physical forms of flaxseed include CON = control (linseed meal), WF = whole flaxseed, RF = rolled flaxseed, and GF = ground flaxseed.

²n = 4 observations per treatment.

³Probability value for the *F*-test of overall treatment.

⁴Contrasts include 1 = control versus the average of whole, rolled, and ground flaxseed; 2 = whole flaxseed versus the average of rolled and ground flaxseed; and 3 = rolled flaxseed versus ground flaxseed.

⁵Grams of microbial N/kg of OM truly fermented; truly fermented OM = OM intake – apparent feed OM flow at the duodenum.

gestion, but did not affect total-tract NDF digestibility (Mustafa et al., 2002). Our results are in contrast to those of Gonthier et al. (2004), who reported greater NDF digestibility with flaxseed inclusion (12.7% of dietary DM) for which they attributed greater digestibilities of the flaxseed fiber constituents than those of the basal diet. However, in their study, total-tract ADF digestibilities (percentage of intake) were unaffected ($P = 0.97$).

No time by treatment interactions were apparent for ruminal pH, ammonia, and VFA concentration (data not shown; $P \geq 0.54$). Flaxseed processing had no effect on ruminal pH, ammonia, or VFA concentration ($P \geq 0.40$; Table 5). These results are in agreement with our nutrient digestion and microbial efficiency results. They indicate similar fiber digestion and similar VFA concentrations among treatments, indicative of similar ruminal fermentation among steers fed processed flaxseed. Gonthier et al. (2004) also reported no differences in the average values of ruminal pH, $\text{NH}_3\text{-N}$, or total VFA. Other studies evaluating processed oilseeds have shown no effect on ruminal pH (Scott et al., 1991; Reveneau et al., 2005), $\text{NH}_3\text{-N}$ (Pires et al., 1997), and total VFA production (Pires et al., 1997; Reveneau et al., 2005).

Most studies that reported differences in individual VFA also reported differences in ruminal fiber digestion (Scott et al., 1991; Pires et al., 1997; Gonthier et al., 2004). The data from the microbial efficiency and the rumen fermentation parameters indicate that microbial activity was likely not influenced by the flax processing treatments utilized in this study. This may be due

to the relatively low inclusion levels of flaxseed in the diet (10% of diet DM). In addition, rumination may have provided further processing of the flaxseed by the animals used in the study (Welch and Hooper, 1993).

We anticipated relative differences of the digestive measures among treatments from Holstein steers to be similar to that of dairy cows fed mid-lactation diets. Multiple citations in the literature report on the use of Holstein steers or nonlactating Holstein cows to compare the effects of a variety of fat sources on digestion and fermentation (Elliott et al., 1994, 1997). The demands of milk synthesis increase DMI in lactating cows beyond that of growing steers or nonlactating cows, which may result in a shift in the site to post-ruminal digestion, although direct comparisons in the literature are lacking. In work conducted in beef cattle, lactation resulted in greater DMI compared with non-pregnant controls, but OM digestion was not different, even though the passage rate of indigestible ADF was greater (Vanzant et al., 1991). Based on the published literature, we are confident that the relative treatment differences identified in this study are applicable to diets for lactating cows.

In Situ Degradability of Linseed Meal and Flaxseed

We detected differences ($P \leq 0.03$) in DM soluble percentage, slowly degradable portion, degradation rate, and extent of degradability for steers fed linseed meal, ground flaxseed, rolled flaxseed, or whole flaxseed DM among dietary treatments. Figure 1 indicates the percent in situ disappearance over time for linseed meal, as

Table 5. Effect of flaxseed processing on intake, flow, and digestion of NDF and ADF, pH, ammonia, and VFA in dairy lactation diets offered to Holstein steers

Item	Physical form of flaxseed ¹				SEM ²	<i>P</i> -value ³	Contrast ⁴		
	CON	WF	RF	GF			1	2	3
NDF									
Intake (kg/d)	4.16	4.15	4.41	4.65	0.31	0.51	0.43	0.27	0.54
Duodenal flow (kg/d)	0.87	0.97	0.81	0.93	0.12	0.59	0.74	0.37	0.36
Ileal flow (kg/d)	1.05	1.14	1.23	1.24	0.18	0.38	0.15	0.38	0.94
Fecal output (kg/d)	1.10	1.12	1.16	1.24	0.12	0.51	0.39	0.35	0.43
Total-tract digestibility (% of intake)	73.73	72.94	73.79	73.11	2.23	0.96	0.79	0.77	0.74
ADF									
Intake (kg/d)	2.21	2.28	2.39	2.49	0.15	0.49	0.28	0.36	0.60
Duodenal flow (kg/d)	0.49	0.54	0.44	0.50	0.06	0.61	0.92	0.31	0.42
Ileal flow (kg/d)	0.65	0.70	0.73	0.75	0.11	0.62	0.27	0.59	0.78
Fecal output (kg/d)	0.65	0.67	0.69	0.73	0.07	0.52	0.34	0.41	0.45
Total-tract digestibility (% of intake)	70.76	70.47	71.23	70.11	2.58	0.97	0.94	0.93	0.65
pH	6.64	6.59	6.67	6.60	0.09	0.91	0.85	0.66	0.59
Ammonia (mM)	7.23	8.28	7.82	7.70	0.48	0.56	0.27	0.40	0.86
VFA									
Acetate (mol/100 mol)	63.56	63.19	62.57	63.28	0.88	0.88	0.60	0.81	0.58
Propionate (mol/100 mol)	18.21	17.43	18.01	18.09	0.50	0.70	0.54	0.33	0.91
Isobutyrate (mol/100 mol)	1.99	2.00	2.09	1.96	0.12	0.89	0.86	0.89	0.46
Butyrate (mol/100 mol)	2.16	2.06	2.13	2.05	0.12	0.89	0.57	0.82	0.66
Isovaleric (mol/100 mol)	11.00	11.73	11.35	10.70	0.43	0.40	0.21	0.31	0.59
Valeric (mol/100 mol)	3.38	3.58	3.83	3.61	0.27	0.71	0.36	0.68	0.58
Total (mM)	112.29	113.66	109.01	116.40	6.12	0.86	0.92	0.90	0.41
Acetate:propionate	3.63	3.76	3.62	3.63	0.14	0.89	0.81	0.45	0.94
Acetate:butyrate:propionate	3.75	3.87	3.74	3.75	0.14	0.89	0.83	0.46	0.96

¹Physical forms of flaxseed include CN = control (linseed meal), WF = whole flaxseed, RF = rolled flaxseed, and GF = ground flaxseed.²n = 4 observations per treatment.³Probability value for the *F*-test of overall treatment.⁴Contrasts include 1 = control versus the average of whole, rolled, and ground flaxseed; 2 = whole flaxseed versus the average of rolled and ground flaxseed; and 3 = rolled flaxseed versus ground flaxseed.

Table 6. In situ degradation kinetics of linseed, flaxseed, and alfalfa fractions in Holstein steers offered dairy lactation diets

Item	Diet treatment ¹					<i>P</i> -value ²	Contrast ³		
	CON	WF	RF	GF	SEM		1	2	3
Processed flaxseed									
DM									
Soluble (%)	30.4	−0.7	5.8	33.1	3.4	<0.01	<0.01	<0.01	<0.01
Slowly degradable (%)	56.8	94.8	82.8	55.4	3.5	<0.01	<0.01	<0.01	<0.01
Degradation rate (%/h)	7.46	0.57	4.53	11.25	0.49	<0.01	<0.01	<0.01	<0.01
Extent degradability (%)	87.2	94.1	88.6	88.5	2.4	0.03	0.55	0.08	0.62
CP									
Soluble (%)	31.7	−7.4	2.7	38.1	4.2	<0.01	<0.01	<0.01	<0.01
Slowly degradable (%)	64.5	98.7	95.8	58.7	3.7	<0.01	<0.01	<0.01	<0.01
Degradation rate (%/h)	7.58	1.03	5.09	11.43	0.47	<0.01	<0.01	<0.01	<0.01
Extent degradability (%)	96.2	91.3	98.6	96.7	1.0	<0.01	0.18	<0.01	0.07
Fat									
Soluble (%)	71.3	−11.2	22.5	60.3	9.2	<0.01	0.02	<0.01	0.01
Slowly degradable (%)	26.2	100.0	77.5	39.2	8.9	<0.01	0.02	<0.01	<0.01
Disappearance rate (%/h)	13.78	0.80	5.24	17.97	1.35	<0.01	<0.01	<0.01	<0.01
Extent disappearance (%)	97.4	88.8	100.0	99.5	1.2	<0.01	0.02	<0.01	0.17
Chopped alfalfa									
DM									
Soluble (%)	26.0	25.8	26.3	33.9	4.4	0.53	0.17	0.95	0.97
Slowly degradable (%)	52.4	52.9	53.4	47.2	3.0	0.50	0.16	0.99	0.81
Degradation rate (%/h)	6.32	5.90	5.90	6.20	0.78	0.97	0.87	0.84	0.71
Extent degradability (%)	78.4	78.7	79.7	81.1	2.0	0.63	0.27	0.85	0.57
CP									
Soluble (%)	38.2	38.9	34.8	44.4	6.2	0.71	0.33	0.75	0.69
Slowly degradable (%)	53.0	52.4	56.9	48.3	5.4	0.73	0.38	0.70	0.62
Degradation rate (%/h)	10.16	9.68	9.96	9.67	1.08	0.98	0.84	0.78	0.90
Extent degradability (%)	91.2	91.3	91.7	92.7	1.3	0.48	0.17	0.84	0.62
NDF									
Soluble (%)	−6.3	−5.8	−4.1	−6.0	2.0	0.86	0.79	0.82	0.47
Slowly degradable (%)	71.0	71.9	71.7	73.6	3.8	0.95	0.60	0.89	0.88
Degradation rate (%/h)	4.93	4.44	4.40	6.42	0.64	0.07	0.02	0.71	0.46
Extent degradability (%)	64.8	66.2	67.6	67.5	2.5	0.77	0.60	0.99	0.39
ADF									
Soluble (%)	−5.5	−4.1	−2.9	−7.2	2.3	0.37	0.16	0.95	0.31
Slowly degradable (%)	64.2	65.7	65.7	69.5	4.2	0.76	0.34	0.88	0.78
Degradation rate (%/h)	4.27	3.85	3.82	5.75	0.59	0.06	0.01	0.73	0.49
Extent degradability (%)	58.7	61.6	62.8	62.3	2.5	0.60	0.64	0.77	0.24

¹In situ treatments of processed flaxseed [CON = control (linseed meal); GF = ground flaxseed; RF = rolled flaxseed; and WF = whole flaxseed] was equal to the steers' dietary treatment for a given period, and all steers for all periods also had incubated chopped alfalfa in situ bags.

²Significance of the *F*-test.

³Contrasts include 1 = control versus the average of whole, rolled, and ground flaxseed; 2 = whole flaxseed versus the average of rolled and ground flaxseed; 3 = rolled flaxseed versus ground flaxseed.

well as whole, rolled, and ground flaxseed. Linseed meal and ground flaxseed had the greatest soluble percent (A fraction) for DM, CP, and fat (Table 6). The high solubility of these 2 treatments was likely the result of the small particle size. As the particle size of flaxseed increased, soluble percentages decreased, with the percentage of whole flaxseed not being different (-0.70 ± 3.40) from zero because of the high portion of the flaxseed meal and ground flaxseed that had already degraded or disappeared. The flaxseed meal and ground flaxseed had much lower slowly degradable portions compared with whole flaxseed and rolled flaxseed. These values may be exaggerated relative to the fit of the model for whole flaxseed. The ground flaxseed treatment had the fastest in situ DM degradation rate (11.25%/h; $P <$

0.01), followed by linseed meal (7.46%/h; $P < 0.01$), rolled flaxseed (4.53%/h; $P < 0.01$), and whole flaxseed (0.57%/h; $P < 0.01$; Table 6). Degradability of CP and fat followed the same pattern as DM degradability for in situ processed flaxseed. The results from the whole flaxseed diet indicate a strong linear degradation rate. Our in situ study was conducted to 120 h in an attempt to reach a plateau on the rate of degradation for whole flaxseed. We did not approach the plateau at 120 h but the nonlinear model did converge. If one were to assume a 5%/h ruminal passage rate, about 10% of whole flax would be effectively degraded in the rumen. Other studies that evaluated oilseed processing have reported similar in situ results. Reveneau et al. (2005) reported an increase in degradation rates for

Figure 1. In situ disappearance (DM) for processed flaxseed in Holstein steers offered dairy lactation diets (contrasts: linseed meal vs. the average of whole, rolled, and ground flaxseed, $P < 0.01$; whole flaxseed vs. the average of rolled and ground flaxseed, $P < 0.01$; rolled flaxseed vs. ground flaxseed, $P < 0.01$). In situ treatments of processed flaxseed [CON = control (linseed meal); GF = ground flaxseed; RF = rolled flaxseed; and WF = whole flaxseed] was equal to the steers' dietary treatment for a given period, and all steers for all periods also had incubated chopped alfalfa in situ bags.

conventional small, whole cottonseed pellets compared with whole cottonseed. However, Mustafa et al. (2002) reported reductions in degradation rates of micronized flaxseed versus whole flaxseed.

In Situ Degradability of Alfalfa Hay

To determine if flaxseed oil had an effect on ruminal fiber degradability, we incubated alfalfa hay in situ (Table 6). Doreau et al. (1991), Ferlay et al. (1992), and Khorasani et al. (1992) concluded that no differences existed for ruminal in situ DM degradation of hay samples among treatments with diets containing different levels of fat. Our study results concur. Dry matter and CP degradation kinetics of chopped alfalfa hay (16.4% CP, 51.7% NDF, and 32.8% ADF; DM basis) were not different ($P \geq 0.48$) for all dietary treatments. The slowly degradable fraction of alfalfa hay DM ranged from 47 to 52% ($P = 0.50$), whereas the extent of degradation ranged from 78 to 81% ($P = 0.63$). The slowly degradable CP of alfalfa hay ranged from 48 to 57% ($P = 0.73$), and the extent of degradation ranged from 91 to 93% ($P = 0.48$).

We anticipated a faster release of lipid from processed flaxseed compared with the whole flaxseed treatment, which may alter fiber degradation. However, such results were not apparent in our study. In fact, the ground flaxseed treatment tended ($P \leq 0.07$) to have the fastest rates of degradation for both NDF and ADF. These observations further indicate that our diets were not high

enough in lipid to hinder fiber digestion, but instead the rate of digestion of the ground flaxseed may have caused a rapid release of CP to the rumen microorganisms, aiding in fiber digestion in the rumen (Jenkins, 1993). These results agree with Scott et al. (1991), who reported no in situ differences for alfalfa hay DM and NDF degradation rates for diets containing rolled raw, ground raw, extruded, and roasted soybeans, and dried distillers grains.

CONCLUSIONS

Dry matter intake expressed as a percentage of BW was similar for the flaxseed treatments and the control. Although we hypothesized that whole flaxseed would be less digestible, no major differences were detected for the digestion and ruminal fermentation of diets supplemented with similar levels of whole, rolled, and ground flaxseed and flaxseed meal. It is reasonable to expect differences to be relative among treatments but caution is warranted when applying data from steers directly to lactating dairy cows. Nevertheless, we conclude that whole flaxseed has very low rate of ruminal degradation and either rolling or grinding is an acceptable way of mechanically processing flaxseed for use in lactation diets.

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