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Rumen fermentation and nutrient flow to the omasum in Holstein cows fed extruded canola seeds treated with or without lignosulfonate[1]

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ABSTRACT - Four multiparous Holstein cows averaging 548 kg of body weight and 74 d in lactation were used in a Latin square design with four 21-d experimental periods to determine effects of feeding extruded *versus* non-extruded canola seed, with or without 50 g/kg lignosulfonate on rumen fermentation, nutrient flow to the omasum, and degradability of dry matter (DM) and N of each diet. The DM effective degradability increased with extrusion and lignosulfonate treatment had no effect. The effective degradability of N was similar between diets. Lignosulfonate treatment of extruded *versus* non-extruded canola seeds decreased ruminal and total tract apparent digestibility of organic matter. The lowest apparent ruminal and highest intestinal digestibilities of protein, expressed as a percentage of N intake were observed for cows fed extruded canola seeds without lignosulfonate. Lignosulfonate treatment and extrusion had no effect on pH and concentrations of ammonia N and volatile fatty acids in the rumen. Results suggest that extruded canola seed untreated with formaldehyde may stimulate efficiency of microbial protein synthesis and is an effective means of increasing the availability of protein in the small intestine without affecting the total tract apparent digestibility of protein.

Key Words: chemical treatment, digestion, heat treatment, oilseed

Introduction

Heat treatment is commonly used to protect oilseeds from ruminal degradation (Mustafa et al., 2002; Abu-Ghazaleh et al., 2002a,b). Kennelly (1996) suggested that the application of heat to highly fat products such as oilseeds can denature the protein matrix surrounding the fat droplets, thus preventing the access of ruminal bacteria to fatty acids, protecting the dietary fat from ruminal biohydrogenation. Extrusion is a method to heat-processed oilseeds that has the potential of reducing ruminal crude protein (CP) degradability (Petit et al., 1999) and N solubility (Chouinard et al., 1997a) as shown for full fat soybeans. Moreover, extrusion results in a slow-release of linoleic acid from full-fat soybeans in the rumen (Peterson et al., 2002), with little effect on ruminal fermentation (Scott et al., 1991). However, previous results have shown that extrusion of canola seeds has no effect on total tract apparent digestibility, but it decreases milk fat concentration (Neves et al., 2009), which may result in a potential negative effect of extruding canola seeds on ruminal digestion.

Lignosulfonate, which is a by-product of the wood industry, decreases ruminal degradability of CP and increases the rumen undegradable CP concentration of full fat soybeans by up to 173% (Petit et al., 1999). However, there is little information on the effect of lignosulfonate treatment on ruminal degradability of ground canola seeds. Results from a previous experiment (Neves et al., 2009) showed that extrusion of canola seeds increased milk fat concentration of trans11-18:1 to a lower extent with, than without, lignosulfonate treatment (113 *versus* 150%) and that concentrations of cis9, trans11 conjugated linoleic acid and polyunsaturated fatty acids were similar between treatments. However, the concentration of trans11-18:1 increased with extrusion of full fat soybeans but not with lignosulfonate treatment and concentrations of cis9, trans11 conjugated linoleic acid and polyunsaturated fatty acids tended ($P = 0.08$ and $P = 0.06$, respectively) to increase with lignosulfonate treatment of full fat soybeans (Neves et al., 2007). Taken together, these results suggest that it is possible to modify the milk fatty acid composition by feeding extruded oilseeds, but the effect of lignosulfonate

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treatment on the milk fatty acid profile seems to differ depending on the type of oilseed treated. Enhanced comprehension of ruminal metabolism and nutrient utilization of extruded oilseeds treated with lignosulfonate will help to manipulate milk fatty acid profile. Although research has been conducted with supplementing canola to alter ruminal fermentation and N metabolism in the gastrointestinal tract, none could be found on effects of treating ground canola seeds with both extrusion and lignosulfonate.

The objectives of this experiment were to determine the effects of extrusion and lignosulfonate treatment of ground canola seeds on ruminal fermentation, ruminal degradability, and nutrient flow to the omasum in early-lactating dairy cows.

Material and Methods

Four rumen-fistulated multiparous Holstein cows averaging 548.1±66.9 kg of body weight and 74±12 days in lactation were assigned to a 4 × 4 Latin square design to determine the effects of extrusion and lignosulfonate treatment of canola seed on rumen fermentation, digestibility in the rumen, the intestine and the total tract, and degradability of dry matter (DM) and N of each diet. Each experimental period consisted of 14 d of adaptation to the diets and 7 d for sample collection. Cows were housed in tie stalls and fed individually (8 a.m. and 4 p.m.). Cows were weighed on the first and last days of each experiment period. A full description of the four diets was given by Neves et al. (2009). Briefly, the four total mixed diets (Table 1) consisted of supplements based on non-extruded canola seeds, extruded canola seeds, non-extruded canola seeds treated with 50 g/kg DM lignosulfonate or extruded canola seeds treated with 50 g/kg DM lignosulfonate.

Feed intake was recorded daily and adjusted for 100 g/kg orts as fed. Samples of each diet were collected daily from day 15 to 20, frozen, and pooled on a period basis. Composite samples were mixed thoroughly and subsampled for chemical analyses. Fecal grab samples (100 g) obtained via rectal palpation and spot samples (ca. 500 mL) of the omasal digesta leaving the rumen were collected and accumulated for four consecutive days at 8, 12, 16 and 20 hours of each experimental period for days 16, 17, 18, and 19, respectively. The omasal sampling technique was similar to that of Huhtanen et al. (1997). Samples of feces and omasal digesta were kept at -20 ^{o}C for further analyses. Indigestible neutral detergent fiber (iNDF) was the marker to measure omasal flow (Huhtanen et al., 1994). On day 21, rumen fluid samples were collected at 0, 2, 4, 6, and 8 hours from different sites within the rumen to obtain a total of around 200 mL. Ruminal contents were filtered with cheesecloth and the ruminal fluid pH was measured immediately (HI 931000 pH meter; Hanna Instruments, Ronchi di Villafranca, PD, Italy). Samples were acidified to pH 2 with 1 ml of 20% H_2SO_4 and frozen at -20 ^{o}C for determination of volatile fatty acids (VFA) and ammonia N concentrations. Another sample of ruminal

Table 1 - Ingredient and chemical composition of total mixed diets of Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diets							
Ingredient (g/kg DM)	NEC	EXC	NEL	EXL				
Corn silage	570	570	570	570				
Ground corn grain	91	91	91	91				
Soybean meal (480 g/kg CP, solvent)	181	181	181	181				
Mineral and vitamin supplement[1]	18	18	18	18				
Canola seeds	140	-	-	-				
Extruded canola seeds	-	140	-	-				
Canola seeds with lignosulfonate	-	-	140	-				
Extruded canola seeds with lignosulfonate	-	-	-	140				
Chemical analysis					SEM	E	L	I
Dry matter (g/kg fresh weight)	525	534	527	536	2.7	<0.01	0.38	0.58
Crude protein (g/kg DM)	178	175	174	173	1.1	0.08	0.02	0.63
Ether extract (g/kg DM)	67b	77a	73ab	70ab	2.6	0.19	0.83	0.03
Neutral detergent fiber (g/kg DM)	410	412	419	402	5.1	0.16	0.88	0.10
Acid detergent fiber (g/kg DM)	243	241	244	246	2.1	0.95	0.14	0.47
Organic matter (g/kg DM)	927	928	927	927	1.4	0.98	0.90	0.71
NE_L, Mcal/kg DM[2]	1.63	1.60	1.56	1.61				

a,b Means within rows and lignosulfonate treatment with different letters differ (P<0.05).
SEM = standard error of the mean; NE_L = net energy of lactation; E = extrusion, L = lignosulfonate; I = interaction.
[1] Contained: Ca - 270 g/kg; P - 80 g/kg; Mg - 20 g/kg; S - 20 g/kg; Fe - 2200 mg/kg; Zn - 2800 mg/kg; Cu - 800 mg/kg; F - 801 mg/kg of F; I - 60 mg/kg; Co - 50 mg/kg; Se - 40 mg/kg; vitamin A - 216,000 IU/kg; vitamin D_3 - 67,600 UI/kg; vitamin E - 500 IU/kg.
[2] Calculated using published values of feed ingredients (NRC, 2001).

contents (ca. 3 kg) was collected from each cow from different sites within the rumen before the 08:00 h feeding and processed according to the procedure of Cecava et al. (1990) to determine microbial protein synthesis as described by Ushida et al. (1985).

Ruminal in situ incubations were carried out using three rumen fistulated multiparous cows fed the non-extruded untreated canola seeds. The diet was fed *ad libitum* for two weeks before the incubation trial. Samples of each of the four canola treatments were milled through a 2-mm screen and subsamples (6 g) were then placed in nitrogen-free polyester bags (10 × 20 cm) with a pore size of 50±15 µm (R1020 Ankom products; Ankom, Fairport, NY, USA). Samples of each canola treatment were incubated in the rumen of each cow for 72, 48, 36, 24, 12, 8, 6, 4, 2, and 0 h. The bags were inserted in reverse order of incubation period, so that they could all be removed at the same time (NRC, 2001). All bags were incubated in duplicate for each time point of incubation except for 72 h, when bags were incubated in triplicate to have enough residue for chemical analyses. All bags were attached to a stainless-steel weight of 540 g, and placed in the ventral sac of the rumen. Once the bags were removed from the rumen, they were immersed in 20-L buckets containing cold water, then washed in an automatic washing machine (40 min, 4 cycles; 5 × 1-min wash, 2-min spin) until the rinse water was clear. The bags (with residues) were then frozen.

Degradation of dry matter (DM) and N were calculated using the following first-order model with a lag of t_0 based on the model of McDonald (1981):

$p = a$ if $t < or = to$,

$p = a + b\ [1 - e^{(-c(t-t_0))}]$ if $t > to$,

where p = percentage disappearance at time t, a = the intercept representing the portion of DM or N solubilized at time 0, b = the fraction of DM or N that is potentially degradable in the rumen, c = the constant rate of disappearance of fraction b, and t = time on incubation. The nonlinear parameters a, b, c, and t_0 were estimated by an iterative least squares procedure of the software SAS (Statistical Analyses System, version 5.0), and best-fit values were chosen with the Secant method using the convergence criterion (10^{-8}) of SAS (Statistical Analyses System, version 5.0).

Values of effective degradability of DM (EDDM) and total N (EDTN) were calculated using the equation of Dhanoa et al. (1999) stated in terms of a, b, c, t_0, and k (the rumen outflow rate at 8% h^{-1}).

$EDMM$ or $EDTN = a + bc\ (e^{(-k.t)}) / (c + k)$

Dry matter of the diets was determined in a forced-ventilation oven according to the procedure 934.01 (AOAC, 1990). Total mixed diets were ground to pass through 1-mm screen Wiley mill before analyses of N, ether extract (EE), acid detergent fiber (ADF), and neutral detergent fiber (NDF). Total N determination used a Tecnal TE–036/1 (Piracicaba, São Paulo, Brazil) following procedure 990.03 of AOAC (1990). Concentrations of NDF and ADF, including those of residual ash were measured according to the nonsequential procedures of Van Soest et al. (1991) with the use of amylase but without sodium sulfite in the neutral detergent solution. Ether extraction in diets was conducted with Tecnal TE–044/1 (Piracicaba São Paulo, Brazil) according to the method No. 7.060 of AOAC (1990). Indigestible NDF was used as an internal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986; Dann et al., 2007). Preparation of feed, orts and fecal residues from *in situ* incubation followed by iNDF extraction was similar to the technique described by Ellis et al. (1984) for preparation of indigestible NDF (i.e., NDF remaining after 144 h of *in situ* incubation). Coefficients of apparent digestion of dietary components were determined by comparing dietary indigestible NDF concentration (corrected for that in orts) with fecal or omasal digesta indigestible NDF concentration as outlined by Cochran et al. (1986). Apparent digestibility in the small intestine was calculated as the difference between 100 and apparent digestibility in the rumen. Isolation of rumen bacteria was carried out following the procedure of Cecava et al. (1990).

All results were analyzed as a 4 × 4 Latin square design balanced for residual effect using the MIXED procedure of SAS (Statistical Analyses System, version 8.02) and with a 2 × 2 factorial arrangement of treatments. Data on *in sacco* degradability, intake, omasal flow, ruminal, intestinal and total tract apparent digestibility, and ruminal fermentation parameters were analyzed using the following general model:

$Y_{ijk} = \mu + C_i + P_j + T_k + e_{ijk}$

where: Y_{ijk} = the dependent variable, μ = overall mean, C_i = random effect of cow (i = 1 to 4), P_j = fixed effect of period (k = 1 to 4), T_k = fixed effect of treatment (k = non-extruded untreated canola seeds, extruded untreated canola seeds, non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate and extruded canola seeds treated with 50 g/kg DM of lignosulfonate), and e_{ijk} = random residual error. Data on ruminal pH, ammonia concentration and VFAs were analyzed as repeated measurements. The compound symmetry was used as the covariance structure. Treatments were compared to provide factorial contrasts: 1) non-extruded *versus* extruded canola, 2) with *versus* without lignosulfonate, and 3) the interaction between extrusion and lignosulfonate treatment. Significance was declared at $P<0.05$ and a trend was accepted at $P\leq0.10$, unless otherwise stated.

Results and Discussion

There was a trend (P = 0.09) for an interaction between extrusion and lignosulfonate for the rapidly (*a*) degradable fraction of DM; extruding canola seeds with lignosulfonate increased the rapidly degradable fraction of DM and there was no effect of extrusion in the absence of lignosulfonate (Table 2). Adding lignosulfonate to canola seeds had no effect on the slowly (*b*) degradable fraction and the rate of degradation of DM. On the other hand, extrusion of canola seeds decreased the slowly (*b*) degradable fraction of DM with no effect on the rate of degradation.

There was a significant interaction between extrusion and lignosulfonate for the rapidly (*a*) degradable fraction of N and there was a trend (P = 0.08) for the slowly (b) degradable fraction of N. The lowest (*a*) and highest (*b*) values of N were obtained for extruded canola seeds treated with lignosulfonate. The rate of N degradation was similar between diets.

The fact that extrusion increased the percentage of DM solubilized at initiation of ruminal incubation and decreased the percentage of DM potentially degradable in the rumen is in agreement with results previously reported by Petit et al. (1997) for canola seeds. The percentage of DM solubilized at initiation of ruminal incubation was increased with extrusion in the presence of lignosulfonate while the percentage of CP solubilized at initiation of ruminal incubation was decreased. These results may suggest that the higher fraction of DM solubilized at initiation of ruminal incubation was not due to higher solubilization of the protein fraction but to a higher release of the lipid fraction. Processes such as extrusion have been shown to increase the release of oil from soybeans (Mohamed et al., 1988). Moreover, Chouinard et al. (1997b) reported a greater accumulation of *trans* intermediates isomers in milk fat of cows fed extruded compared with micronized soybeans and roasted soybeans, which may result from greater release of fat in the rumen that led to lipolysis and biohydrogenation of fatty acids by rumen microorganisms (Griinari & Bauman, 1999).

Extrusion has been used to protect the protein of leguminous grains, such as peas, lupins and soybeans (Poncet & Rémond, 2002) from ruminal degradation, but the effect on oilseeds rich in lipids has seldom been observed. Ferlay et al. (1992) found no effect of extrusion at 140 ^{o}C on protein degradability of rapeseeds. Moreover, Deacon et al. (1988) found that extrusion increased the *in situ* soluble fraction of protein from canola seed although there were no differences in ruminal DM and N degradabilities between non-extruded *versus* extruded canola seed. Several researchers have reported that extrusion is less effective than other heat treatments such as roasting in protecting oilseeds from ruminal degradation (Pena et al., 1986; Reddy et al., 1994).

Lignosulfonate, which is a by-product of the wood industry, decreased the percentage of N solubilized at initiation of ruminal incubation of extruded *versus* non-extruded canola seeds. Previous results have shown that lignosulfonate decreases ruminal degradability and increases the rumen undegradable CP concentration of full fat soybeans (Petit et al., 1999). Moreover, according to Windschitl & Stern (1988), the effective ruminal CP degradability of soybean meal treated with 50 g/kg DM lignosulfonate and heated at 90-95 °C for 45 min was significantly decreased when compared with untreated soybean meal. Similarly, treatment of canola screenings with 50 g/kg DM lignosulfonate and heat at 100 °C for 60 min (von Keyserlingk et al., 2000) and of canola meal with

Table 2 - *In sacco* degradability of total mixed diets of Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diet					P value		
In sacco degradability	NEC	EXC	NEL	EXL	SEM	E	L	I
Dry matter								
Fraction A	0.31	0.34	0.28	0.36	0.01	0.001	0.63	0.09
Fraction B	0.66	0.63	0.69	0.61	0.02	0.01	0.64	0.14
Fraction C	0.10	0.09	0.08	0.08	0.01	0.72	0.20	0.75
ED	0.64	0.67	0.63	0.65	0.01	0.05	0.20	0.80
Nitrogen								
Fraction A	0.24a	0.24a	0.27a	0.18b	0.01	0.01	0.29	0.02
Fraction B	0.75	0.76	0.72	0.80	0.02	0.05	0.86	0.08
Fraction C	0.09	0.07	0.07	0.11	0.02	0.46	0.72	0.13
ED	0.64	0.59	0.59	0.64	0.04	0.94	0.99	0.31

a,b Means within rows and lignosulfonate treatment with different letters differ (P<0.05). All trends (i.e., P≤0.10) referred to in the text.
SEM = standard error of the mean; fraction A = rapidly degradable (soluble) fraction; fraction B = potentially degradable fraction; fraction C = fractional rate of degradation (h^{-1}); ED = effective degradability at 8% outflow rate per hour; E = extrusion; L = lignosulfonate; I = interaction.

50 g/kg DM lignosulfonate and heat at 100 °C for 60 to 120 min (McAllister et al., 1993) effectively reduced degradation of DM and CP in the rumen. Rumen protection of CP usually parallels fat, as a protein-rich matrix surrounds the fat droplets of oilseeds (Khorasani et al., 1992). This led us to hypothesize that protection of protein against ruminal degradability with lignosulfonate treatment could overcome the negative effects of a greater release of oil with extrusion on ruminal digestion. Protection of protein against ruminal degradability on the diet of extruded canola seeds treated with 50 g/kg DM of lignosulfonate is corroborated by extruded canola seeds treated with lignosulfonate that resulted in the lowest fraction of N solubilized at initiation of ruminal incubation. As a result, N duodenal flow was higher for cows fed extruded *versus* non-extruded canola seeds without lignosulfonate. This is in agreement with the greater N flow in the duodenum observed for cows fed diets with protein of lower ruminal degradability (Zerbini et al., 1988).

Daily intake and total omasal flow of DM averaged, respectively, 14.5 kg and 8.96 kg and they were similar between treatments (Table 3). There was a significant interaction between extrusion and lignosulfonate and there was a trend (P = 0.06), respectively, for ruminal digestibility of DM and intestinal digestibility of DM, expressed as a percentage of DM intake. Cows fed extruded canola untreated with lignosulfonate had the lowest DM digestibility in the rumen and the highest DM intestinal digestibility, expressed as a percentage of intake. Digestibility in the intestine, expressed as a percentage of DM omasal flow, tended (P = 0.05) to decrease with lignosulfonate treatment.

The lack of an extrusion effect on feed intake agrees with Bayourthe et al. (2000) for cows fed extruded *versus* whole canola seeds. Similarly, lignosulfonate treatment of soybean meal had no effect on dry matter intake of cows (Windshitl & Stern, 1988; Mansfield & Stern, 1994; Wright et al., 2005).

The lignosulfonate treatment could contribute to decreasing the amount or rate of release of fat in the rumen, which will overcome the negative effect of extrusion on ruminal digestibility and result in similar ruminal and total tract apparent digestibility of OM for cows fed non-extruded canola seeds treated or not with lignosulfonate *versus* extruded canola seeds treated with lignosulfonate. However, extrusion of rapeseeds fed at 145 g/kg DM (Ferlay et al., 1992) and lignosulfonate treatment of soybean meal and soybean hulls (Mansfield & Stern, 1994) had no effect on ruminal digestibility, duodenal flow, and total tract apparent digestibility of OM. The conditions of treatment may be responsible for discrepancies between experiments. For example, the effect of extrusion on release of oil in the rumen differs according to the temperature used for treating as shown for soybeans (Chouinard et al., 1997a; Petit et al., 1999). The lack of a lignosulfonate effect on intestinal digestibility of OM agrees with results of Windshitl & Stern (1988) for cows fed soybean meal.

Table 3 - Intake, omasal flows, ruminal, intestinal and total tract apparent digestibility (TTAD) in Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diet					P value		
	NEC	EXC	NEL	EXL	SEM	E	L	I
Dry matter (DM)								
Intake (kg/day)	14.3	14.7	14.5	14.6	0.2	0.29	0.89	0.46
Total duodenal flow (kg/day)	8.46	10.26	8.93	8.19	0.78	0.52	0.33	0.14
Ruminal digestibility (g/g of intake)	039a	0.27b	0.35ab	0.41a	0.03	0.44	0.23	0.04
Intestinal digestibility (g/g of intake)	0.29	0.38	0.29	0.26	0.03	0.34	0.07	0.06
Intestinal digestibility (g/g of duodenal flow)	0.46	0.51	0.44	0.43	0.02	0.38	0.05	0.21
TTAD (g/g of intake)	0.68	0.65	0.64	0.66	0.01	0.95	0.36	0.10
Organic matter								
Intake (kg/day)	13.45	13.81	13.18	13.29	0.94	0.81	0.69	0.90
Total duodenal flow (kg/day)	6.84a	8.36b	7.21b	6.72b	0.29	0.13	0.07	0.01
Ruminal digestibility (g/g of intake)	0.48	0.39	0.46	0.50	0.03	0.35	0.23	0.08
Intestinal digestibility (g/g of intake)	0.22	0.28	0.20	0.19	0.03	0.37	0.10	0.19
Intestinal digestibility (g/g of duodenal flow)	0.41	0.45	0.36	0.37	0.03	0.45	0.12	0.63
OMADR (kg/day)	6.61	5.45	5.97	6.57	0.52	0.61	0.66	0.14
OMTDR (kg/day)	8.44	8.76	8.34	8.84	0.71	0.58	0.99	0.90
OMTDR (g/g of intake)	0.62	0.63	0.63	0.66	0.04	0.70	0.58	0.83
TTAD (g/g of intake)	0.70a	0.67b	0.67b	0.69ab	0.01	0.59	0.27	0.03

a,b Means within rows and lignosulfonate treatment with different letters differ (P<0.05).
SEM = standard error of the mean; OMADR = organic matter apparently digested in the rumen (OM intake – OM duodenal flow); OMTRD = organic matter truly digested in the rumen (OMADR + microbial OM flow), assuming the ash content of microbial DM was 0.1; E = extrusion; L = lignosulfonate; I = interaction.

The total tract apparent digestibility was similar between treatments. Organic matter intake was similar between treatments (Table 3). There was a significant interaction between extrusion and lignosulfonate for total OM omasal flow, and cows fed extruded canola untreated with lignosulfonate had the highest flow. Ruminal digestibility of organic matter, expressed as a percentage of organic matter intake, tended (P = 0.08) to be lower for cows fed extruded canola untreated with lignosulfonate as shown by the trend for the interaction between extrusion and lignosulfonate. Treating canola with lignosulfonate tended (P = 0.10) to decrease OM intestinal digestibility, expressed as a percentage of OM intake.

Organic matter intestinal digestibility, expressed as a percentage of omasal flow, OM apparently digested in the rumen, expressed in kilogram per day, and OM truly digested in the rumen, expressed in kilogram per day and as a percentage of organic matter intake, were similar between diets. Total tract apparent digestibility of OM, expressed as a percentage of intake, decreased with extrusion in the absence of lignosulfonate, while extrusion had no effect with the lignosulfonate treatment.

Ether extract intake, expressed in kilogram per day, was significantly higher for cows fed extruded canola untreated with lignosulfonate (Table 4). Extrusion and lignosulfonate treatment had no effect on total omasal flow, ruminal, intestinal and total tract apparent digestibility, expressed as a percentage of intake and intestinal digestibility, expressed as a percentage of omasal flow, of ether extract.

Nitrogen intake was similar between treatments (Table 5). The highest omasal N flow and microbial N flow, expressed in grams per day, were observed for cows fed extruded canola untreated without lignosulfonate as shown by the significant interaction between extrusion and lignosulfonate. Non-microbial N flow, expressed in g per day, was similar between diets. Duodenal N flow, expressed as a percentage of intake, tended (P = 0.05) also to reach the highest value for cows fed extruded untreated canola seeds.

There was an interaction between extrusion and lignosulfonate for microbial efficiency, expressed in g of N per kg of organic matter apparently or truly digested in the rumen, and the highest values were obtained for cows fed extruded untreated canola seeds. The lowest apparent ruminal and highest intestinal digestibilities, expressed as a percentage of N intake, were observed for cows fed extruded untreated canola seeds. True ruminal digestibility, intestinal digestibility, expressed as a percentage of omasal flow, and total tract apparent digestibility of N were similar among treatments.

Microbial N flow to the duodenum and efficiency of microbial protein synthesis have been shown to decrease when treating soybean meal with lignosulfonate (Windshitl & Stern, 1988). In the present experiment, treating extruded canola seeds with lignosulfonate may have protected seeds against degradability and/or fat release in the rumen as discussed previously, while extruded canola seeds with no formaldehyde had no protection, which led to higher microbial N and total N flow to the duodenum and lower N apparent ruminal digestibility. Increased efficiency of microbial protein synthesis also was found with extruded *versus* non-extruded canola seeds untreated with formaldehyde. According to Murphy et al. (1987), increased efficiency of microbial protein synthesis is a result of fat supplementation of the diet and results of the present experiment suggest that fat from extruded *versus* non-extruded canola seeds untreated with formaldehyde was more available in the rumen. The higher N duodenal flow with extruded *versus* non-extruded canola seeds untreated with formaldehyde was a result of higher microbial N flow in the duodenum as there was no difference in non-microbial N flow in the duodenal between diets, which resulted in improved microbial protein synthesis.

Table 4 - Intake, omasal flows, ruminal, intestinal and total tract apparent digestibility (TTAD) in Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diet					P value		
	NEC	EXC	NEL	EXL	SEM	E	L	I
Ether extract								
Intake (kg/day)	1.03b	1.22a	1.13ab	1.10ab	0.03	0.04	0.73	<0.01
Total duodenal flow (kg/day)	0.23	0.32	0.24	0.22	0.07	0.67	0.56	0.48
Ruminal digestibility (g/g of intake)	0.77	0.74	0.78	0.80	0.05	0.89	0.58	0.68
Intestinal digestibility (g/g of intake)	0.13	0.16	0.10	0.11	0.04	0.63	0.40	0.94
Intestinal digestibility (g/g of duodenal flow)	0.56	0.53	0.40	0.51	0.06	0.54	0.24	0.36
TTAD (g/g of intake)	0.91	0.88	0.91	0.91	0.01	0.39	0.50	0.30

a,b Means within rows and lignosulfonate treatment with different letters differ (P<0.05).
SEM = standard error of the mean; E = extrusion; L = lignosulfonate; I = interaction.

Table 5 - Nitrogen intake, omasal N, microbial N and non-microbial N flow (g/d), and N digestibility in Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diet					P value		
	NEC	EXC	NEL	EXL	SEM	E	L	I
Nitrogen intake (g/day)	434	437	416	420	32	0.92	0.60	0.99
N duodenal flow (g/day)	291b	371a	317b	293b	13	0.07	0.09	<0.01
Non-microbial N flow (g/day)	134	107	126	117	33	0.60	0.97	0.80
Microbial N flow (g/day)	157b	263a	191b	176b	23	0.09	0.28	0.04
N flow/N intake	0.68	0.85	0.75	0.70	0.05	0.28	0.44	0.05
Microbial efficiency								
g of N/kg OMADR	25.2b	51.3a	32.7ab	27.4b	4.5	0.06	0.11	0.01
g of N/kg OMTDR	19.3b	30.7a	22.8ab	19.0b	2.2	0.12	0.10	0.01
N digestibility								
Apparent ruminal (g/100 g of intake)	32.0a	15.0b	25.0a	30.0a	0.04	0.17	0.32	0.02
True ruminal[1]	0.69	0.76	0.70	0.72	0.07	0.51	0.88	0.72
Intestinal digestibility (g/100 g of intake)	43.0b	58.0a	47.0b	43.0b	0.04	0.18	0.17	0.04
Intestinal digestibility (g/100 g of duodenal flow)	63.0	68.0	61.0	61.0	0.02	0.35	0.16	0.37
TTAD (g/100 g of intake)	75.0	73.0	72.0	73.0	0.01	0.79	0.37	0.25

a,b Means within rows and lignosulfonate treatment with different letters differ (P<0.05).
SEM = standard error of the mean; OMADR = organic matter apparently digested in the rumen (OM intake – OM duodenal flow); OMTDR = organic matter truly digested in the rumen (OMADR + microbial OM flow), assuming the ash content of microbial DM as 0.1; E = extrusion; L = lignosulfonate; I = interaction; TTAD = total tract apparent digestibility.
[1] True ruminal N digestibility: 1 – [(N flow – microbial N)/N intake].

There was a shift in digestibility of N from the rumen to the small intestine with extruded *versus* non-extruded canola seeds untreated with formaldehyde, which may increase the amount of rumen bypass protein available to the cows. This agrees with results of Solanas et al. (2005), who reported that residues from ruminal incubation of extruded *versus* non-extruded feeds such as soybeans and lupins had higher intestinal N digestibility. However, there was no difference in intestinal N digestibility between extruded *versus* non-extruded canola seeds treated with the formaldehyde treatment. This disagrees with von Keyserlingk et al. (2000), who observed an increase in intestinal CP disappearance of lignosulfonate-treated canola screenings when compared with untreated canola screenings, although the total tract apparent digestibility of CP was not affected by the 50 g/kg DM lignosulfonate treatment, as observed in the present experiment.

The lack of an extrusion effect on ruminal digestibility of ether extract is in agreement with results of Bauchart et al. (1990), who observed similar duodenal flows of linoleic acid at the duodenum between diets containing raw *versus* extruded rapeseeds. Moreover, Gonthier et al. (2004) found no effect of extrusion of flaxseed on ruminal digestibility, post-ruminal digestibility and total tract apparent digestibility of fatty acids. Similarly, Scott et al. (1991) found no effect of extrusion of soybeans on the total tract apparent digestibility of fatty acids.

There was no interaction between extrusion and lignosulfonate and between treatment and sampling time for any parameters of ruminal fermentation (Table 6). Ruminal pH, logpH, concentrations of ammonia, total volatile fatty acids (VFA), and molar proportions of VFA were similar between treatments. Treatment with lignosulfonate tended (P = 0.09) to decrease the acetate-to-propionate ratio in the rumen.

The lack of an extrusion effect on ruminal pH, total VFA, and molar proportions of individual VFA disagrees with results of Khorasani & Kennelly (1998), who reported trends for higher ruminal pH and lower total VFA concentrations and molar percentages of propionate with an increased amount of canola seeds from 0 to 145 g/kg DM in the diet of dairy cows.

Changes in the proportions of propionate and acetate in the rumen with canola seed supplementation (Leupp et al., 2006) have been attributed to a decrease in ruminal fiber digestion. However, although there was a trend towards lower acetate-to-propionate ratio with the lignosulfonate treatment in the present experiment, there was no difference in the total tract apparent digestibility of fiber between diets (Neves et al., 2009). Lignosulfonate has been shown to decrease protein degradability in the rumen (Windschitl & Stern, 1988), which decreases ammonia N in the rumen (Zerbini et al., 1988). However, in the present experiment, effective degradability of N was not affected by lignosulfonate or extrusion, which may explain that ammonia N concentration in the rumen was similar between treatments.

Table 6 - Ruminal pH and concentrations of ammonia and total volatile fatty acids (VFAs) and molar proportions of individual VFA of Holstein cows fed non-extruded untreated canola seeds (NEC), extruded untreated canola seeds (EXC), non-extruded canola seeds treated with 50 g/kg DM of lignosulfonate (NEL) or extruded canola seeds treated with 50 g/kg DM of lignosulfonate (EXL)

	Diet					P value		
	NEC	EXC	NEL	EXL	SEM	E	L	I
Rumen variables								
pH	6.28	6.34	6.12	6.26	0.08	0.23	0.17	0.66
Log pH	0.80	0.80	0.79	0.80	0.01	0.24	0.17	0.61
Ammonia (mg/L)	156.6	161.6	151.1	148.7	6.5	0.85	0.21	0.60
Total VFA (mmol/L)	89.5	92.3	96.9	101.8	8.5	0.67	0.36	0.91
VFAs (mmol/mol)								
Acetic acid	51.35	51.44	55.06	55.77	6.26	0.95	0.53	0.96
Propionic acid	24.42	22.98	27.88	29.83	3.64	0.94	0.18	0.65
iso-Butyric acid	0.26	0.25	0.24	0.44	0.11	0.39	0.45	0.35
n-Butyric acid	9.97	8.52	10.45	11.68	1.88	0.95	0.35	0.49
iso-Valeric acid	2.75	2.75	2.14	2.88	0.31	0.26	0.46	0.26
n-Valeric acid	0.78	0.96	1.13	1.14	0.29	0.74	0.37	0.78
Acetate:propionate	2.51	2.40	2.30	2.04	0.16	0.25	0.09	0.65

SEM = standard error of the mean; E = extrusion; L = lignosulfonate; I = interaction; VAF = volatile fatty acid.

Conclusions

Fat supplementation from canola seeds at 140 g/kg DM in the form of extruded *vs* non-extruded and untreated *vs* treated with 50 g/kg DM lignosulfonate had no effect on the flows of organic matter and ether extract to the duodenum. Moreover, results suggest that extruded canola seed untreated with formaldehyde may stimulate efficiency of microbial protein synthesis and is an effective means of increasimg the availability of protein in the small intestine without affecting the total tract apparent digestibility of protein.

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