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Heat- and Lignosulfonate-Treated Canola Meal as a Source of Ruminal Undegradable Protein for Lactating Dairy Cows

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ABSTRACT

This experiment used 18 lactating Holstein cows in a 3 × 3 Latin square replicated 6 times to determine the effectiveness of processing with moist heat or moist heat combined with lignosulfonate (LSO_3) for increasing the ruminal undegradable fraction of canola meal for use as a protein supplement for lactating dairy cows. Diets were formulated to be isonitrogenous and contained one of 3 forms of canola meal; untreated canola meal (UCM), heat-treated canola meal (HTCM) or heat- and LSO_3-treated canola meal (LSO_3CM). Total collection of urine and feces was taken from each cow during the last 5 d of each 42-d experimental period. Milk production was greater for cows fed the LSO_3CM diet (36.6 kg/d) than for cows fed the UCM diet (34.8 kg/d) but did not differ from cows fed the HTCM diet (35.3 kg/d). Digestibility of crude protein was lower for cows supplemented with LSO_3CM and they had reduced concentrations of ruminal ammonia N, blood urea N, and milk urea N compared with cows supplemented with UCM or HTCM. Dry matter intake and apparent digestibilities of neutral and acid detergent fiber were increased in cows fed the LSO_3CM diet. Urinary N excretion (as % of N intake) was reduced in cows fed the LSO_3CM diet. These results indicate that moist heat combined with LSO_3 treatment of canola meal was effective in increasing the proportion of crude protein digested in the lower digestive tract of lactating cows and was therefore used more effectively as a source of protein than UCM or HTCM.
(**Key words:** lactating dairy cow, lignosulfonate, canola meal, ruminal undegradable protein)

Abbreviation key: BUN = blood urea nitrogen, **HTCM** = heat-treated canola meal, **LSO_3** = lignosulfonate, **LSO_3CM** = heat- and LSO_3-treated canola meal, **UCM** = untreated canola meal.

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INTRODUCTION

High-producing dairy cows require sufficient protein in the diet to optimize microbial growth and fiber digestion in the rumen, and adequate amounts of essential AA to be available in the small intestine to provide for their increased metabolic and lactation demands (NRC, 2001; Cant et al., 2003). Canola meal is a readily available protein supplement used extensively in ruminant rations in Canada (Christensen and McKinnon, 1989). Relative to the composition of milk protein, canola meal has an excellent balance of AA (Piepenbrink and Schingoethe, 1998). However, canola meal is not an effective source of AA because of its extensive degradation in the rumen, as indicated by previous work where the effective ruminal degradabilities of the protein component of canola meal ranged from 44.3 (Kendall et al., 1991) to 74% (McAllister et al., 1993).

The use of canola meal as a source of AA for milk production is therefore limited by the breakdown of AA in the rumen. The addition of lignosulfonate (**LSO_3**) to canola screenings (von Keyserlingk et al., 2000) in an in situ study showed that LSO_3 treatment was effective in reducing ruminal degradation of DM and CP with a corresponding increase in disappearance from the lower gastrointestinal tract. It was shown in situ that heat treatment and the addition of LSO_3 (Wright, 1998) can reduce the proportion of RDP, thereby increasing the availability of essential AA to the mammary gland for milk synthesis. The combination of heat treatment and LSO_3 treatment has been effective in reducing the ruminal degradability of canola meal without affecting its digestibility (McAllister et al., 1993; Stanford et al., 1995). The treatment of soybean meal with LSO_3 decreased degradability of soybean meal in vivo (Windschitl and Stern, 1988a) and in vitro (Windschitl and Stern, 1988b). Moreover, diets supplemented with LSO_3-treated soybean were used more effectively for milk production than diets supplemented with untreated soybean meal (Nakamura et al., 1992). Given these positive production results, the objective of this experiment was to determine the effectiveness of heat

and LSO_3 treatment of canola meal on the productive performance of lactating cows fed canola meal as the major source of RUP.

MATERIALS AND METHODS

Canola Meal Treatments

Commercially available solvent-extracted canola meal was either left untreated (**UCM**), processed with the addition of 2% water and heated for 120 min at 100°C (**HTCM**), or processed with the addition of 5% LSO_3 (Lignotech USA, Inc., Rothschild, WI) and heated for 120 min at 100°C (**LSO_3CM**). The heat treatment was applied at a commercial feed manufacturing facility (Agro Pacific Industries Ltd., Chilliwack, BC, Canada) using moist heat in a hydrothermal cooker (Amandus Kahl Nachf, Hamburg, Germany). Excess moisture was removed by forced air drying at ambient temperature. Samples for the 3 treated canola meals were analyzed for ADF and NDF using the modified filter bag method of Van Soest et al. (1991). Samples were analyzed for ADIN and N (AOAC, 2000; method 993.13) using a LECO FP-428 N analyzer (Leco Corp., St. Joseph, MI). Dry matter contents of the treated canola meals were determined by drying in a forced air oven at 105°C for 24 h. The in situ nylon bag procedure (de Boer et al., 1987) was used to estimate 12-h ruminal disappearance of DM and CP of the canola meals.

Lactation Study

Eighteen multiparous Holstein cows, averaging 60 ± 7 d in milk were used in the production trial. Cows were housed in a free-stall barn and individual intakes were measured using Calan feeding doors (American Calan Inc., Northwoods, NH). Cows were blocked according to expected level of milk production, calving date, BW, and parity. Cows within each block were then randomly assigned to one of 3 treatment groups in a 3 × 3 Latin square, replicated 6 times. Experimental periods were 42 d. The cows were cared for according to the standards set by the Canadian Council on Animal Care (1993).

Animals were fed isonitrogenous diets consisting of 30% corn silage, 20% grass silage, and 50% concentrate (DM basis; Table 1) plus one of 3 canola meal supplements: UCM, HTCM, or LSO_3CM. The diets fed to each treatment group were prepared once daily as a TMR and fed in equal portions at 0830 and 1530 h. Orts were removed daily before the 0830 h feeding and the amount of TMR offered to individual cows was adjusted to maintain approximately 10% orts. Feed intakes were measured daily for each cow, averaged by week, and corrected for DM content of the respective diets and orts to calculate individual DMI. Body weights for each cow were recorded at 1000 h on 3 consecutive days at the beginning of each treatment period and at the conclusion of the experiment. The 3 diets were sampled 3 times per week and samples for each diet composited weekly and frozen. Samples of orts were taken twice weekly from each cow and composited weekly for each diet. Samples of the forage and concentrate components of the diet were taken every 2 wk over the duration of the trial. The DM content of the feed samples was determined by drying samples in a forced air oven at 60°C until constant weight was achieved. The dried samples were then ground (1-mm screen) and stored before determination of NDF, ADF, and N by methods previously described.

Table 1. Ingredients and composition of the 3 experimental diets (% DM basis) fed to dairy cows.

	Diet[1]			
	UCM	HTCM	LSO_3CM	SE
Ingredient				
Corn silage	30.0	30.0	30.0	. . .
Grass silage	20.0	20.0	20.0	. . .
Barley	25.0	24.4	23.5	. . .
UCM	20.0	0	0	. . .
HTCM	0	20.6	0	. . .
LSO_3CM	0	0	21.5	. . .
Soybean meal	3.7	3.7	3.7	. . .
Limestone	0.95	0.95	0.95	. . .
Salt	0.25	0.25	0.25	. . .
Mineral-vitamin mix[2]	0.10	0.10	0.10	. . .
CP	17.3[ab]	17.2[b]	17.5[a]	0.1
NDF	32.5[b]	33.2[b]	34.4[a]	0.4
ADF	19.8[b]	20.3[ab]	20.8[a]	0.2

[a,b]Means within rows with different letters differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat-treated canola meal; LSO_3CM = canola meal treated with heat and lignosulfonate.

[2]Mineral-vitamin mixture (DM basis) = 40,000 mg/kg Mn; 40,000 mg/kg Zn; 16,000 mg/kg Fe; 12,000 mg/kg Cu; 640 mg/kg I; 240 mg/kg Se; 160 mg/kg Co; 4000 KIU/kg vitamin A; 800 KIU/kg vitamin D; and 10 KIU/kg vitamin E.

Milk yield was recorded twice daily for all cows. Milk samples were collected from each cow for 4 consecutive milkings during the second, third, and fifth week of each treatment period. These samples were analyzed for fat, protein, and lactose by infrared spectroscopy (BC DHIS Laboratory, Chilliwack, BC, Canada; AOAC, 2000; method 972.160). Milk urea N was determined on defatted milk samples using a colorimetric diagnostic kit (Crocker, 1967; Sigma Diagnostics, St. Louis, MO, procedure 535) utilizing a Kodak Ektachem DT 60 analyzer (Clinical Products Division, Eastman Kodak Co., Rochester, NY).

Ruminal fluid samples were collected by intubation from each cow between 1030 and 1130 h, approximately 2 h after the morning feeding on d 21 and 35 of each treatment period. Ruminal pH was determined at time

of sampling. An aliquot of each sample was frozen at –10°C for the determination of ammonia N (Fawcett and Scott, 1960). The remainder of each sample was acidified to pH 2.0 with 50% H_2SO_4, centrifuged, and the supernatant frozen until analysis of VFA (Erwin et al., 1961). Concentrations of VFA were determined using a Shimadzu gas chromatograph equipped with a capillary column (30 m × 0.25 mm i.d. Stablwax-DA). Injection port temperature was set at 170°C, column temperature was programmed from 120 to 180°C at the rate of 10°C/min, and the internal standard was isocaproic acid (0.70 g in 200 mL of water).

Blood samples (20 mL) were taken from each cow by jugular venipuncture between 1030 and 1130 h on d 21 and 25 of each treatment period. Hematocrit was determined and blood was centrifuged and plasma frozen for later colorimetric quantification of blood urea N (**BUN**) (Crocker, 1967; Sigma Diagnostics, St. Louis, MO, procedure 535) and glucose (Vitros Chemistry Products, Rochester, NY, procedure GLU DT) utilizing a Kodak Ektachem DT 60 analyzer (Clinical Products Division, Eastman Kodak Co.).

Digestibility Trial

For the last 5 d of each treatment period, cows were confined to metabolism stalls for the total collection of urine and feces. The procedure and sampling protocol for the digestibility trial followed those outlined by Dinn et al. (1998).

Statistical Analysis

Statistical analysis was via least squares ANOVA, following the GLM procedure of SAS (SAS Institute, 1999). Treatment effects were considered to be significant at $P < 0.05$. The model used for this experiment was $Yijkl = \mu + \alpha k + \tau j + \beta l + \gamma i(k) + \varepsilon ijl(k)$, where μ = overall mean, αk = effect of square (k = 1,...,6), τj = effect of treatment (j = 1,...,3), βl = effect of period (l = 1,...,3), $\gamma i(k)$ = effect of cow within square (i = 1,...,3) and $\varepsilon ijl(k)$ = experimental error.

RESULTS

Dry matter content of the HTCM and the LSO_3CM supplements were slightly lower than for the UCM supplement (Table 2). The NDF and ADF contents and the ADIN as a percentage of N were all significantly greater ($P < 0.05$) for LSO_3CM compared with the UCM and HTCM diets. Ruminal disappearance of DM and CP following 12 h of incubation was less for the LSO_3CM compared with the UCM and HTCM supplements (Table 2). Chemical analysis of the total diets reflected a slight increase in the CP, NDF, and ADF contents of the diet containing LSO_3CM compared with the diet containing UCM (Table 1).

Table 2. Chemical composition and in situ ruminal disappearance of DM and CP (12-h incubation) from canola meal treatments.

	Supplement[1]			
	UCM	HTCM	LSO_3CM	SE
DM	88.8[a]	86.3[b]	86.4[b]	0.4
CP	35.8	35.2	35.1	0.3
NDF	24.0[b]	25.0[b]	33.4[a]	1.5
ADF	18.1[b]	18.8[b]	22.1[a]	0.8
ADIN	1.8[b]	2.0[b]	4.1[a]	0.5
Ruminal disappearance, %				
DM	63.5[a]	64.2[a]	42.6[b]	1.4
CP	71.3[a]	71.7[a]	29.9[b]	3.2

[a,b]Means within columns with different superscripts differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat-treated canola meal; LSO_3CM = canola meal treated with heat and lignosulfonate.

The DMI by cows fed the diet containing LSO_3CM was greater ($P < 0.05$) than the DMI of cows fed the diet supplemented with UCM or HTCM (Table 3). There was a trend toward higher BW gain in cows fed the diet containing LSO_3CM (Table 3). Milk yield was higher for cows fed diets containing LSO_3CM compared with those fed diets containing UCM (36.6 vs. 34.8 kg/d), whereas 4% FCM yield was greater for cows fed the LSO_3CM diet compared with that from cows fed the HTCM diet (Table 3). Milk composition was not influenced by diet except that MUN levels were lower ($P < 0.05$) in milk from cows fed diets containing LSO_3CM supplement compared with cows fed diets containing UCM or HTCM (Table 3).

There was no effect of diet on ruminal pH or total quantity of VFA in ruminal fluid, but cows consuming

Table 3. Intake, weight gain, milk yield, and milk composition resulting from canola meal supplements.

	Diet[1]			
	UCM	HTCM	LSO_3CM	SE
DMI, kg/d	24.6[b]	25.1[b]	26.4[a]	0.2
DMI, % of BW	3.70	3.77	3.92	0.04
BW gain, kg/d	0.158	0.125	0.337	0.093
Milk, kg/d	34.8[b]	35.3[ab]	36.6[a]	0.6
4% FCM, kg/d	33.8[ab]	33.4[a]	35.2[b]	0.6
Milk composition, %				
Fat	3.80	3.68	3.82	0.06
Protein	3.25	3.28	3.31	0.04
Lactose	4.41	4.50	4.54	0.05
Total solids	12.70	12.69	12.92	0.12
MUN, mg/dL	15.68[a]	15.34[a]	13.86[b]	0.27

[a,b]Means within rows with different letters differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat-treated canola meal; LSO_3-CM = canola meal treated with heat and lignosulfonate.

Table 4. Ruminal fermentation variables from dairy cows fed diets containing canola meal supplements.

Parameter	Diet[1] UCM	HTCM	LSO_3CM	SE
Ruminal pH	6.79	6.83	6.84	0.03
Ammonia N, mg/dL	11.24[a]	9.45[ab]	8.00[b]	0.73
Total VFA, m*M*	100.6	95.7	95.1	2.6
VFA, mol/100 mol				
Acetate	59.5[b]	59.4[b]	60.4[a]	0.2
Propionate	22.1[a]	22.2[a]	21.3[b]	0.3
Isobutyrate	1.07[a]	1.06[a]	0.95[b]	0.01
Butyrate	12.7	12.6	12.8	0.1
Isovalerate	2.32[a]	2.30[a]	2.10[b]	0.04
Valerate	1.85	1.87	1.79	0.03
Caproate	0.50[b]	0.53[b]	0.64[a]	0.03
Acetate:propionate	2.73[b]	2.70[b]	2.88[a]	0.04
Acetate + butyrate:propionate	3.32[b]	3.28[b]	3.49[a]	0.05

[a,b]Means within rows with different letters differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat-treated canola meal; LSO_3CM = canola meal treated with heat and lignosulfonate.

the diet containing the LSO_3CM supplement had lower quantities of ammonia N in ruminal fluid than cows fed the diet containing UCM ($P < 0.05$; Table 4). Ruminal fluid from cows fed the diet supplemented with LSO_3CM had higher proportions of acetate and caproate and lower proportions of propionate, isobutyrate, and isovalerate compared with cows fed diets supplemented with UCM or HTCM ($P < 0.05$; Table 4). Ratios of acetate:propionate and acetate+butyrate:propionate in ruminal fluid of cows fed the diet supplemented with LSO_3CM were greater ($P < 0.05$) than among cows supplemented with UCM or HTCM.

The type of canola meal supplement did not influence ($P > 0.05$) hematocrit or blood glucose levels, but cows fed the diet supplemented with LSO_3CM had lower ($P < 0.05$) BUN levels (16.7 mg/dL) than cows fed UCM or HTCM diets (18.6 and 18.2 mg/dL, respectively).

The type of supplement in the diet did not influence the apparent digestibility of DM but the digestibility of CP was lower ($P < 0.05$) and the digestibility of the ADF and NDF fractions was higher for cows fed the diet supplemented with LSO_3CM compared with the other 2 diets (Table 5). The percentage of dietary N lost in the feces was higher but the percentage lost in the urine was lower for cows fed the diet supplemented with LSO_3CM ($P < 0.05$; Table 6).

Table 5. Apparent digestibilities of nutrients in diets containing canola meal supplements fed to lactating dairy cows.

Apparent digestibility	Diet[1] UCM	HTCM	LSO_3CM	SE
DM	69.6	69.1	69.3	0.4
CP	73.6[a]	73.0[a]	70.9[b]	0.4
NDF	50.1[b]	50.9[b]	54.0[a]	0.6
ADF	45.2[b]	45.0[b]	48.3[a]	0.9

[a,b]Means within rows with different letters differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat-treated canola meal; LSO_3CM = canola meal treated with heat and lignosulfonate.

Table 6. Utilization of N, as a percentage of N intake, in dairy cows fed diets containing different canola meal supplements.

Measurement	Diet[1] UCM	HTCM	LSO_3CM	SE
	(% of N intake, DM basis)			
Fecal N	26.4[b]	27.0[b]	29.1[a]	0.4
Urinary N	38.3[a]	36.9[a]	31.6[b]	1.1
Milk N	25.8	25.9	25.4	0.9
Retained N[2]	9.6	10.1	13.8	1.5

[a,b]Means within rows with different letters differ ($P < 0.05$).

[1]UCM = Untreated canola meal; HTCM = heat treated canola meal; LSO_3CM = canola meal treated with heat and lignosulfonate.

[2]Retained N = 100 [intake N − (fecal N + urinary N + milk N)] / intake N.

DISCUSSION

The treatment of canola meal with LSO_3 and moist heat resulted in a 1.8-kg/d increase in milk yield compared with cows fed the UCM supplement. The supplementation of high-producing dairy cows with an increased amount of RUP has been shown to increase the flow of essential AA to the small intestine as required for milk synthesis (Baker et al., 1995; Wright et al., 1998). Studies using soybean meal (Lundquist et al., 1986; Keery and Amos, 1993) reported no improvement in milk yield of cows fed diets supplemented with soybean treated to reduce ruminal degradability. However, these results were not unexpected considering that the diets containing untreated soybean meal were likely not limiting in protein. The discrepancy in response to increased RUP between our work and that previously undertaken with soybean meal may also be attributable to the more suitable balance of AA associated with canola meal (Cant et al., 2003).

In our study, we observed a significant increase in milk production and this increase was not accompanied by a dilution of milk protein percentage. Milk composition was not influenced by increasing the quantity of RUP available to the small intestine, an observation that supports the earlier results of Khorasani et al. (1996) but differs from those of Sloan et al. (1988), who reported that increased dietary RUP reduced milk fat percentage. The lack of response in milk protein content to an increase in available RUP has been previously reported (Khorasani et al., 1996).

The 29% reduction in ruminal ammonia N concentration in cows fed the LSO_3CM compared with the UCM supplement provides further evidence that this diet was not degraded as extensively in the rumen. The reduc-

tion in ruminal ammonia N concentration also resulted in a 10% reduction in BUN and a 12% reduction in MUN when cows were fed the LSO_3CM. This response agrees with the observations of Baker et al. (1995), who reported a lower proportion of nonprotein nitrogen in the milk of cows when fed increasing proportions of RUP. The relatively low BUN and MUN levels for cows fed the LSO_3CM supplement suggests that there was less wastage of N (Baker et al., 1995) compared with cows fed the diet containing UCM. The BUN and MUN levels for cows fed the diet supplemented with HTCM were not appreciably lower than cows fed the diet containing UCM, indicating that the ruminal protection of canola meal was not effective with heat treatment alone.

Changes in the molar proportions of ruminal VFA among cows fed diets containing LSO_3CM, although statistically significant, were relatively minor from a practical perspective. Moreover, they appeared to reflect increased apparent digestibilities of the NDF and ADF components of the diets supplemented with LSO_3CM compared with the other 2 treatments. This finding of increased apparent digestibility of NDF and ADF for diets supplemented with LSO_3CM compared with those supplemented with UCM or HTCM indicated that the ammonia N levels were not limiting fiber digestion as they were in the previous studies of Stern (1984) and Windschitl and Stern (1988b). The increase in fecal N losses for cows fed the LSO_3CM supplement reflected the lower protein degradability associated with feeding that supplement. Owens and Bergen (1983) suggested that a slight depression in total tract N digestion might be necessary to maximize the supply of digestible N to the small intestine. In addition, they suggested that N retention, rather than fecal N loss, must be used as an index for the feeding value of treated protein. Similar to the present study, Wohlt et al. (1991) reported a decrease in urinary N as a percentage of absorbed N, from 42% for cows fed soybean meal to 38% for cows fed a fish meal supplement of lower ruminal degradability. Improvement in the efficiency of dietary N use is economically important for dairy producers from 3 aspects. First, protein is a relatively expensive component of the diet, second, there is a metabolic cost for disposing of excess ammonia N as urea, and third, there is an environmental cost for disposing of excess N in the manure. Decreased N concentration in the manure (feces and urine) from dairy cows fed diets lower in CP has been shown to result in decreased ammonia emission and decreased N volatilization as a fraction of the N excreted (Paul et al., 1998).

CONCLUSIONS

Treating canola meal with 5% LSO_3 and heating for 2 h was effective in decreasing its ruminal degradability, thereby increasing the amount of AA available in the lower digestive tract. This increase in RUP resulted in increased DMI, milk yield, and efficiency of N use in lactating dairy cows supplemented with LSO_3CM.

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