Use of lignosulfonate to decrease the rumen degradability of canola meal protein

McAllister, T. A., Cheng, K.-J., Beauchemin, K. A., Bailey, D. R. C., Pickard, M. D. and Gilbert, R. P. 1993. **Use of lignosulfonate to decrease the rumen degradability of canola meal protein.** Can. J. Anim. Sci. **73**: 211–215. Treatment of canola meal (CM) with 5 or 10% lignosulfonate (LSO_3) for 1 or 2 h at 100°C increased borate-insoluble CP and neutral-detergent-insoluble nitrogen and decreased in vitro ammonia concentration compared with untreated or heated CM. Acid-detergent-insoluble nitrogen was increased in 2% xylose and 10% LSO_3 CM heated for 2 h. Ruminal degradability of CP was reduced by heating CM ($P < 0.01$), and this effect was more pronounced ($P < 0.001$) with LSO_3 or xylose.

Key words: Cattle, canola meal, lignosulfonate, rumen undegradable protein

McAllister, T. A., Cheng, K.-J., Beauchemin, K. A., Bailey, D. R. C., Pickard, M. D. et Gilbert, R. P. 1993. **Utilisation du lignosulfonate pour diminuer la dégradabilité ruminale des protéines du tourteau de canola.** Can. J. Anim. Sci. **73**: 211–215. Le traitement du tourteau de canola avec 5 ou 10% de lignosulfonate (LSO_3), complété par une exposition de une ou deux heures à 100°C, a accru la teneur en PB insoluble au borate ainsi que l'azote insoluble au détergent neutre et diminué les concentrations ammoniacales in vitro par comparaison au tourteau non traité ou au tourteau simplement chauffé. L'azote insoluble au détergent acide était accru dans le tourteau traité avec 2% de xylose ou 10% de lignosulfonate et chauffé pendant 2 h. La dégradabilité ruminale de la PB était diminuée par le chauffage du tourteau ($P < 0{,}01$), l'effet étant plus prononcé ($P < 0{,}001$) en présence de LSO_3 ou de xylose.

Mots clés: Bovin, tourteau de colza canola, lignosulfonate, protéines non dégradables dans le rumen

Rapidly growing ruminants and lactating dairy cattle rely on both microbial protein and rumen-undegradable feed protein digested in the small intestine to satisfy their amino acid requirements. Canola meal (CM) has a high concentration of protein, which is digested mainly in the rumen. Researchers have previously attempted to decrease the rumen degradability of CM protein through treatment with heat, acids, sodium hydroxide or formaldehyde. Some of these treatments have been successful but require extensive modification of existing canola crushing plants or the handling of hazardous chemicals. Thus, there is a need to develop methods that decrease the rumen degradability of CM protein without involving the use of hazardous chemicals or high processing temperatures.

Windschitl and Stern (1988) showed that treatment with lignosulfonate (LSO_3), followed by heating at 90–95°C for 45 min decreases the rumen degradability of soybean meal protein. The objective of the present study was to evaluate the effectiveness of LSO_3 for decreasing the rumen degradability of the crude protein in CM.

Solvent-extracted CM (200 g) was left untreated (U-CM), treated with water, or treated with 5 or 10% (wt wt^{-1}) LSO_3 (CM-5% LSO_3; CM-10% LSO_3) (20% reducing sugar, Lignotech, Rothschild, Wisconsin). CM was also treated with 2% (wt wt^{-1}) xylose (CM-2% xyl) (Aldrich, Milwaukee, Wisconsin) to compare treatment with a known sugar to that of sugars in LSO_3. The DM of a sample (3 g) of CM was determined by drying at 105°C for 24 h, and sufficient water or mixtures of water and LSO_3 or water and xylose were added to increase the moisture concentration of CM to 25%. CM was thoroughly mixed with each

solution and heated for 1 or 2 h at 100°C in a convection air oven. CP was measured by the Kjeldahl method as described by the Association of Official Analytical Chemists (AOAC) (1984).

Solubility of CP was estimated in borate–phosphate buffer as described by Krishnamoorthy et al. (1982), neutral-detergent-insoluble nitrogen (NDIN) as outlined by Goering and van Soest (1970) but without sodium sulfite and acid-detergent-insoluble nitrogen (ADIN) according to the methods of AOAC (1984). Insoluble nitrogen was expressed as a percentage of total nitrogen and insoluble CP was expressed as a percentage of total protein.

Inoculum was prepared as described by McAllister et al. (1992) from ingesta and rumen fluid collected from a ruminally cannulated Holstein cow fed 40% concentrate, containing 8% CM, and 60% alfalfa hay.

LSO_3 was added to U-CM and to CM after heating, to make their reducing sugar content equal to that of CM treated with LSO_3 or xylose. Triplicate CM samples, containing 15 mg N, were incubated at 39°C with 20 mL of rumen inoculum. Fermentation was terminated at 24 h by adding 1 mL of 5% mercuric chloride. Vials containing no CM were incubated for 24 h to correct for NH_3 production from rumen inoculum. Ammonia concentration was determined as described by Weatherburn (1967).

Data were analyzed using the general linear model (GLM) procedure and means were compared using the least-square-mean linear hypothesis test (Statistical Analysis System (SAS) Institute, Inc. 1989).

Three ruminally cannulated Holstein steers weighing approximately 500 kg were fed three diets: 80% concentrate (1:1 barley: wheat) and 20% alfalfa hay cubes, 100% alfalfa hay cubes, or ad libitum barley straw supplemented with hay cubes (0.5% of body weight) in a 3 × 3 Latin square design. Steers were fed twice daily (08:00 and 16:00) and were adapted to their diets for 21 d during each period. During each period, nylon bags (53-μm pore size) containing 2.5 g of U-CM, 1- or 2-h heated CM-H_2O, CM-5% LSO_3, CM-10% LSO_3 and CM-2% xyl were incubated in the rumen of each steer. Two bags of each type of CM were removed after 2, 6, 12, 24 and 48 h of rumen incubation. Bags incubated in autoclaved rumen fluid were used to estimate the extent of DM disappearance without microbial digestion. Washing of the bags and estimation of DM disappearance were performed as described by McAllister et al. (1990). CP was estimated in CM residues to assess protein disappearance and was not corrected for microbial contamination. The equation of Orskov and McDonald (1979)

$$p = a + b(1 - e^{-ct})$$

was used to estimate a (rapidly soluble fraction), b (slowly degradable fraction) and c (fractional rate constant at which b is degraded), with the constraint that $a + b \leq 100\%$, by an iterative nonlinear regression procedure of SAS (1989). Effective rumen degradability of CP (EDCP) was estimated using the equation of Orskov and McDonald (1979):

$$\text{EDCP} = a + (bc)/(k + c)$$

EDCP was calculated with an estimated solid outflow from the rumen (k) of 5% h^{-1} (Windschitl and Stern 1988).

In situ data were analyzed as a 3 × 3 Latin square with period, steer and diet in the model. Analysis of variance for treatment of CM was estimated by the (GLM) procedure with the repeated-measure option of SAS (1989). Contrasts were performed within diet to examine the main effects of heat, LSO_3 and xylose on effective CP degradability.

CP content tended to be lower in CM-10% LSO_3 than in other forms of CM (Table 1). Treatment of CM with LSO_3 and 2% xyl increased insoluble CP compared with that in U-CM or heated CM. This result was accentuated by increasing the concentration of LSO_3 from 5% to 10%, whereas increasing the heating time increased insoluble CP only slightly.

Table 1. Crude protein (CP), borate-insoluble CP, acid-detergent-insoluble nitrogen (ADIN), neutral-detergent-insoluble nitrogen (NDIN), and in vitro NH_3 concentration of untreated and treated canola meal

Treatment	CP (%)	Insoluble CP (%)	NDIN (%)	ADIN (%)	NH_3 (mg dL^{-1})
Untreated	37.4	77.6	16.8	6.7	4.0*a*
1 h heat + H_2O	37.1	78.4	21.8	6.8	3.4*ab*
2 h heat + H_2O	37.9	80.6	24.1	6.5	2.5*c*
1 h heat + 5% LSO_3	36.6	84.7	26.9	6.6	2.6*bc*
2 h heat + 5% LSO_3	37.7	86.9	34.4	7.6	2.0*cd*
1 h heat + 10% LSO_3	34.7	91.2	32.6	7.2	2.0*cd*
2 h heat + 10% LSO_3	35.7	91.5	45.6	12.4	1.3*d*
1 h heat + 2% xylose	36.9	89.0	38.4	17.0	2.2*cd*
2 h heat + 2% xylose	36.8	92.4	47.9	22.5	1.4*d*
SEM	ND	ND	ND	ND	0.3

*a–d*Means within a column with different letters differ ($P < 0.05$).
ND, not determined.

NDIN content of 1- and 2-h heated CM was increased compared with U-CM. NDIN values of CM heated for 1 h at 100°C were about 60% lower than those measured in CM heated for 1 h at 127°C (Moshtaghi Nia and Ingalls 1992). Treatment of CM with LSO_3 or xylose further increased NDIN, and, unlike insoluble CP, the increase in NDIN was enhanced by heating LSO_3 or xylose-treated CM for 2 h rather than 1 h. ADIN content of 1-h heated LSO_3-treated CM was virtually unchanged compared with U-CM. However, ADIN content was increased in CM-2% xyl and CM-10% LSO_3 heated for 2 h.

Heating of CM for 2 h at 100°C reduced ($P < 0.05$) in vitro NH_3 concentration compared with U-CM or CM heated for 1 h. Khorasani et al. (1989) reported that heating CM at 105°C for 20 h reduced in vitro accumulation of NH_3. Although consistently lower, NH_3 concentrations in LSO_3 and xylose-treated CM samples heated for 2 h and for 1 h were not different ($P > 0.05$) within a treatment. In vitro NH_3 concentration was not different ($P > 0.05$) between CM-10% LSO_3 and CM-2% xyl.

The present study indicates that treatment of CM with 5 or 10% LSO_3 followed by heating for 1 h at 100°C increases the insoluble CP and NDIN content of CM without a substantial increase in ADIN. A reduction in in vitro NH_3 concentration suggests that 5% LSO_3 can be used to increase the resistance of CM protein to microbial digestion, likely through denaturation and the formation of primary Maillard products. Furthermore, this resistance is accomplished without the formation of the intestinally indigestible terminal Maillard products associated with ADIN. However, because of the negative relationship between ADIN content and protein digestibility, care must be taken to avoid overprotection of CM with high concentrations of LSO_3 and extended periods of heating.

EDCP of U-CM and treated CM was lower ($P < 0.05$) in steers fed concentrate than in steers fed hay or straw (Table 2), and EDCP of untreated and 2-h heated CM was lower ($P < 0.05$) in steers fed straw than in those fed hay. Rumen-undegradable protein values (calculated as 100 minus EDCP) of U-CM protein in steers fed straw, hay and concentrate were 28, 25 and 37%, respectively. Rumen-undegradable protein values of U-CM in steers fed straw and hay were close to 28%, as reported by the National Research Council (1989), but that in steers fed concentrate was substantially greater. Proteolytic activity per unit of bacterial biomass has been shown to

Table 2. Effective rumen degradability of crude protein (EDCP) of treated canola meal in steers fed straw, hay and concentrate diets in experiment 2

Treatment	Diet (% EDCP)			SEM
	Straw	Hay	Concentrate	
1. Untreated	72.3*b*	74.9*a*	62.5*c*	0.2
2. 1 h heat + H_2O	68.5*a*	72.3*a*	57.8*b*	0.9
3. 2 h heat + H_2O	67.2*b*	70.2*a*	53.6*c*	0.1
4. 1 h heat + 5% LSO_3	64.1*a*	65.6*a*	49.9*b*	0.2
5. 2 h heat + 5% LSO_3	56.3*a*	59.1*a*	40.4*b*	0.8
6. 1 h heat + 10% LSO_3	59.9*a*	59.5*a*	40.5*b*	0.7
7. 2 h heat + 10% LSO_3	51.7*a*	50.0*a*	29.0*b*	1.2
8. 1 h heat + 2% xylose	59.9*a*	61.0*a*	45.4*b*	0.3
9. 2 h heat + 2% xylose	54.0*a*	48.5*a*	32.3*b*	1.3
Contrasts				
1 vs. 1/2 (2 & 3)	$P < 0.01$	$P < 0.01$	$P < 0.01$	
2 & 3 vs. 4 & 5	$P < 0.01$	$P < 0.01$	$P < 0.01$	
4 & 5 vs. 6 & 7	$P < 0.02$	$P < 0.01$	$P < 0.01$	
4 & 5 vs. 8 & 9	$P < 0.05$	$P < 0.01$	$P < 0.01$	
6 & 7 vs. 8 & 9	$P < 0.46$	$P < 0.99$	$P < 0.08$	

*a–c*Means within a row with different letters differ ($P < 0.05$).

be lower in steers fed concentrate than in steers fed forage (McAllister et al. 1993), which, combined with a reduction in the digestion of protein associated with cell-wall carbohydrates, may account for the decrease in the EDCP of CM in concentrate-fed steers.

Heat treatment of CM at 100°C for 1 or 2 h, compared with U-CM, decreased ($P < 0.01$) EDCP regardless of diet. Similarly, heat treatment of CM for 10 min at 125° or 145°C reduced in situ CP disappearance of CM (McKinnon et al. 1991). However, higher processing temperatures are less desirable because of the decomposition of glucosinolates (Pickard et al. 1989) and the formation of indigestible protein (McKinnon et al. 1991).

Treatment with 5% LSO_3 and heat decreased ($P < 0.006$) EDCP compared with heating without LSO_3. Increasing the concentration of LSO_3 to 10% caused a further decline ($P < 0.02$) in EDCP compared with treatment with 5% LSO_3. The 2% xyl treatment resulted in a decrease ($P < 0.05$) in the EDCP compared with 5% LSO_3 treatment, but there was no difference ($P > 0.05$) in EDCP between CM-10% LSO_3 and CM-2% xyl (Table 2). These results are in agreement with those of Windschitl and Stern (1988) and confirm that reducing sugars, mainly xylose, are likely the reactive component in LSO_3.

Windschitl and Stern (1988) found that EDCP (calculated with an estimated solid outflow rate of 5% h^{-1}) of untreated soybean meal, compared with soybean meal treated with 5% LSO_3 and heated at 90–95°C for 45 min, was reduced from 70.6% to 40.6%. In the present experiment, when averaged across diets, treatment of CM with 5% LSO_3 and heat at 100°C for 1 h reduced the EDCP value of U-CM from 69.9% to 59.9%. This reduction was a consequence of a decrease in both the soluble CP (*a* fraction) and the rate of CP degradation (*c*). Comparison of our EDCP data with those of Windshitl and Stern (1988) suggest that LSO_3 is more effective at decreasing rumen degradability of soybean meal protein than CM protein. This may be because CM has a lower lysine content than soybean meal and the epsilon-amino groups of lysine are a primary reactive site for aldehydes during the Maillard reaction.

In conclusion, LSO_3 treatment was effective at decreasing the in situ digestion of CM protein without a substantial increase in indigestible protein as estimated by ADIN. Furthermore, this decrease in the degradability of CM was accomplished by heating at 100°C, a temperature that is obtainable without modification of existing canola crushing plants. Further research is required to examine the effect of LSO_3 treatment on the intestinal digestibility of CM CP escaping rumen degradation, particularly its effect on the availability of lysine and other essential amino acids.

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Association of Official Analytical Chemists. 1984. Official methods of analysis. 14th ed. AOAC, Arlington, VA.

Goering, H. K. and van Soest, P. J. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). Agric. Res. Serv. USDA, Washington, DC, Agric. Handbook 379.

Khorasani, G. R., Robinson, P. H. and Kennelly, J. J. 1989. Effect of chemical treatment on in vitro and in situ degradation of canola meal crude protein. J. Dairy Sci. **72**: 2074–2080.

Krishnamoorthy, U., Muscato, T. V., Snifffen, C. J. and Van Soest, P. J. 1982. Nitrogen fractions in selected feedstuffs. J. Dairy Sci. **65**: 217–225.

McAllister, T. A., Rode, L. M., Major, D. J., Cheng, K.-J. and Buchanan-Smith, J. G. 1990. Effect of ruminal microbial colonization on cereal grain digestion. Can. J. Anim. Sci. **70**: 571–579.

McAllister, T. A., Phillippe, R. C., Rode, L. M. and Cheng, K.-J. 1993. Effect of the protein matrix on the digestive characteristics of cereal grains, amylolytic and proteolytic activity in the rumen. J. Anim. Sci. **71**: 205–212.

McKinnon, J. J., Olubobokun, J. A., Christensen, D. A. and Cohen, R. D. H. 1991. The influence of heat and chemical treatment on ruminal disappearance of canola meal. Can. J. Anim. Sci. **71**: 773–780.

Moshtaghi Nia, S. A. and Ingalls, J. R. 1992. Effect of heating on canola meal protein degradation in the rumen and digestion in the lower gastrointestinal tract of steers. Can. J. Anim. Sci. **72**: 83–88.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th ed. NAS–NRC National Academy Press, Washington, DC.

Orskov, E. R. and McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. (Camb.) **92**: 499–503.

Pickard, M. D., Youngs, C. G., Wetter, L. R. and Boulter, G. S. 1989. Processing of canola seed for quality meal. Pages 3–4 in D. R. Clandinin, ed. Canola meal for livestock and poultry. Canola Council of Canada, Winnipeg, MB.

Statistical Analysis System Institute, Inc. 1989. SAS/STAT user's guide. Version 6, 4th ed. Vol. 2, Cary, NC. 846 pp.

Weatherburn, M. W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Anal. Chem. **39**: 971–974.

Windschitl, P. M. and Stern, M. D. 1988. Evaluation of calcium lignosulfonate-treated soybean meal as a source of rumen protected protein for dairy cattle. J. Dairy Sci. **71**: 3310–3322.

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