THE EVALUATION OF HEAT AND LIGNOSULFONATE TREATED CANOLA MEAL

AS SOURCES OF RUMEN UNDEGRADABLE PROTEIN

FOR LACTATING COWS

by

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ABSTRACT

Two studies were conducted to evaluate the effectiveness of moist heat and lignosulfonate (LSO_3) in increasing the rumen undegradable fraction of canola meal for use as a protein supplement for dairy cattle. In the first study, the in situ nylon bag and mobile nylon bag techniques were utilized to determine the dry matter (DM) and crude protein (CP) degradability of canola meal treatments in the rumen, intestines and total tract. Treatments consisted of untreated canola meal and canola meal heated at 100°C, with or without 5% LSO_3 (wt wt^{-1}), for 0, 30, 60, 90 or 120 min. Results indicated that treatment of canola meal with or without 5% LSO_3 and heated at 100°C for 120 min was effective in reducing ($P<0.05$) the rumen degradation of DM and CP compared to untreated canola meal. The corresponding intestinal CP disappearances for untreated, heat treated, and LSO_3 plus heat treated canola meal following 8 h rumen pre-incubation were 15.9, 23.5 and 34.2%, respectively. The shift in degradability from the rumen to the intestines was accomplished without reducing total tract disappearance.

The second study was undertaken to evaluate the effects of feeding diets containing three canola meal protein supplements, varying in rumen degradability, on nutrient digestibility and animal performance. Eighteen lactating multiparous Holstein cows were randomly assigned to treatment sequences in a 3 x 3 Latin Square design, replicated six times. Total mixed rations were formulated to be isonitrogenous and to contain approximately 30% corn silage, 20% grass silage, 24% barley, 4% soybean meal, 1% mineral and vitamin mix and 21% of one of the following canola meal sources: 1) untreated (U-CM); 2) heat treated (HT-CM); or 3) LSO_3 plus heat treated

(LSO$_3$-CM) on a DM basis.   Milk production and dry matter intake (DMI) were measured daily during each 42 d experimental period.   Milk, blood and rumen fluid samples were taken during the third and fifth weeks.   Total collections of urine and feces from nine cows occurred during the last 5 d of each experimental period.   Milk production was greater (P<0.05) for cows fed LSO$_3$-CM diet (36.6 kg d^{-1}) than U-CM diet (34.8 kg d^{-1}), but did not differ from HT-CM diet (35.4 kg d^{-1}).   Cows supplemented with LSO$_3$-CM showed reduced (P<0.05) apparent CP digestibility and concentrations of rumen ammonium N, blood urea nitrogen and milk urea nitrogen compared to cows supplemented with U-CM and HT-CM.   DMI and apparent digestibilities of neutral and acid detergent fibre were increased (P<0.05) in cows fed LSO$_3$-CM diet when compared to cows fed U-CM and HT-CM diets.   The urinary excretion of N, as a percentage of N intake, was reduced (P<0.05) in cows fed LSO$_3$-CM diet relative to cows fed U-CM and HT-CM diets.

The results of these studies showed that treatment of canola meal with 5% LSO$_3$ followed by heating at 100°C for 120 min was an effective means of increasing the proportion of protein digested in the intestines.   When supplemented in the diets of lactating cows, the protein in LSO$_3$-CM was used more efficiently and was more effective as a rumen undegraded protein source than was the protein in HT-CM or U-CM.

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LIST OF SYMBOLS, NOMENCLATURE AND ABBREVIATIONS

Nomenclature

LSO_3	calcium-sodium lignosulfonate
$NH_4^+ N$	ammonium nitrogen

Abbreviations

ADF	acid detergent fibre
ADIN	acid detergent insoluble nitrogen
AA	amino acid
BUN	blood urea nitrogen
CM	canola meal
CP	crude protein
d	day
dl	decalitre
DM	dry matter
DMI	dry matter intake
FCM	fat corrected milk
h	hour
HT-CM	heat and water treated canola meal
LSO_3-CM	heat and lignosulfonate treated canola meal
min	minute
mM	millimolar
MUN	milk urea nitrogen
NEL	net energy for lactation
N	nitrogen
NDF	neutral detergent fibre
NPN	non-protein nitrogen
RDP	rumen degradable protein
RUP	rumen undegradable protein
SBM	soybean meal
SCC	somatic cell counts
SE	standard error
TMR	total mixed ration
U-CM	untreated canola meal
VFA	volatile fatty acid
wt	weight

I. GENERAL INTRODUCTION

As a consequence of their foregut fermentation, a ruminant's amino acid (AA) requirements are provided by two major sources. One is microbially synthesized protein of good quality in terms of its AA content (Storm and Orskov 1983) and digestibility (Storm et al. 1983), while the other comes from dietary protein that escapes rumen fermentation. Digestion of this mixture of microbial protein and undegraded dietary protein in the abomasum and small intestine yields the AA supply to the ruminant. Early research demonstrated that cattle fed purified diets with only non-protein nitrogen as a nitrogen (N) source gained 65% of the weight of the cattle fed conventional energy ingredients and protein supplements (Oltjen 1969). When lactating dairy cattle were fed protein free diets, they produced 4000 kg milk per lactation from microbial protein (Virtanen 1966; Virtanen et al. 1972). However, by supplying protein for 20% and 40% of the N needed, milk production increased 1000 kg and 1500 kg, respectively. The importance of dietary protein in beef and dairy cattle production was soon recognized. Postruminal administration of AA that were thought to limit milk protein synthesis has shown positive effects on milk protein production (Clark 1975; Schwab et al. 1976; Guinard and Rulquin 1994). The undegradable protein concept was introduced by the NRC (1989) where requirements for rumen degradable (RDP) and rumen undegradable protein (RUP) were separately defined. Use of RUP sources in ruminant diets has become a common practice in diet formulation.

1.1 DIETARY PROTEIN INTAKE AND DIGESTION

The younger the animal or the higher its milk production, the greater its requirement for undegradable protein (Orskov et al. 1980). Quality protein supplements are fed to meet the protein requirements of high producing dairy cows. However, the rumen microflora are highly proteolytic and thus ensure that most protein entering the rumen is degraded to peptides and AA, most of which are subsequently deaminated (Orskov 1992). Consequently, protein supply may still be inadequate to meet the requirements of production. The difficulty arises in part because microbial growth and protein degradation are not coupled directly (NRC 1989). The rate at which energy is generated for microbial growth is not synchronized with the more rapid degradation of protein. Thus, much of the value of protein supplements is lost because dietary protein N is converted to ammonium N.

Ammonium N (NH_4^+ N) promotes microbial growth up to the limit of the microbial N requirement, which is set by the available fermentable carbohydrate, the ATP yield, and the efficiency of conversion to microbial cells (Van Soest 1994). Excess NH_4^+ diffuses from the rumen to the blood where the liver rapidly converts NH_4^+ to urea, which is eventually recycled or excreted as urea in urine (NRC 1989). Increasing excesses of N in the rumen leads to reduced use of the recycled endogenous urea and more excretion in urine and milk (Hof et al. 1997). This results in the inefficient utilization of dietary protein. Large excesses of dietary protein may decrease the energy supply to the cow as the consumption of excess crude protein (CP) increases energy requirements by 13.3 kcal of digestible energy g^{-1} of excess N (NRC 1989).

Urea equilibrates in body water, and kinetic analysis suggests the passive transfer of urea along with water from blood into other body fluids, including saliva, uterine fluid and milk (Baker et al. 1992). High concentrations of urea in body fluids have been associated with negative impacts on health (Carroll et al. 1997), impaired fertility (Ferguson et al. 1993; Butler et al. 1996), reduced metabolic efficiency of milk yield (Tyrrell and Moe 1975), environmental pollution concerns as greater than 95% of endogenous urea is excreted in urine (Baker et al. 1992; Tamminga 1992), and economic loss. In cows fed isonitrogenous diets, blood urea nitrogen (BUN) was elevated with imbalances in RDP and RUP (Baker et al. 1995). BUN concentrations have also been shown to decrease when more optimal levels of ruminally fermentable carbohydrate were supplied to enhance the incorporation of RDP into microbial protein (Roseler et al. 1993). BUN may serve as an indicator of ruminal protein degradability and postruminal protein supply; however, sampling requires invasive techniques, and routine analysis is expensive. Milk urea N has a high correlation with BUN (Roseler et al. 1993; Broderick and Clayton 1997) and has the potential to be utilized as an economical noninvasive measurement to assist in monitoring the protein status of dairy cows fed a particular diet.

1.2 CANOLA MEAL

The economic value of supplemental protein used in rations is largely determined by the amount of RUP that is available for digestion and absorption in the small intestine (Tremblay et al. 1996). Canola meal (CM), a readily available plant protein supplement (36-40% CP, DM basis) rich in calcium, phosphorous and B vitamins, is the principal protein source used in ruminant rations in Canada

(Christensen and McKinnon 1989). Relative to the composition of milk protein, CM has an excellent balance of AA and is a rich source of methionine, cysteine, histidine and threonine (Christensen and McKinnon 1993). However, CM has a relatively low protein efficiency because of extensive rumen degradation relative to by-product feed ingredients such as meat, bone and blood meal. Reported effective rumen degradabilities of CM range from 44.3 (Kendall et al. 1991) to 74.9% (McAllister et al. 1993). The use of CM use as a RUP source is limited because it is a highly degradable protein, leading to surplus NH_4^+ production in the rumen and reduced intestinal AA availability. Several procedures, including chemical and physical treatments, have been studied to alter the rate and extent of rumen microbial protein degradation.

1.3 PROTEIN PROTECTION

The production of insoluble protein through heat (physical) or by complexing (chemical) decreases the rate of proteolytic hydrolysis. This is not only through reduced accessibility of the substrate, but also through the formation of linkages resistant to enzyme attack. Chemical treatments can be divided into methods in which the chemicals actually combine with the proteins, e.g. formaldehyde treatment, and those in which the chemicals denature the proteins, e.g., alcohol, sodium hydroxide and propionic acid. Physical treatments include heat, applied with a wide variation of application methods, or physical encapsulation, in which a protein supplement is coated with a product such as blood that is resistant to rumen degradation (Broderick et al. 1991). Producing resistant linkages with heat or formaldehyde treatment risks permanently reducing the availability of protein, which

then becomes a part of the ultimately indigestible residue. The most successful chemical treatment has been formaldehyde and the most successful and commonly used physical treatment has been heat.

1.3.1 Formaldehyde

Aldehydes, of which formaldehyde is the cheapest and most reactive, form complexes with AA residues rendering proteins less soluble at ruminal pH and more resistant to microbial degradation (Weakley et al. 1983). Formaldehyde treatments are designed upon the presumption that linkages resistant to rumen degradation will be broken by acidic conditions (pH 2-3) in the abosmasum and by enzymatic digestion, making the protein available for digestion and absorption in the lower tract (Van Soest 1994). Protection of this type results in biomanipulation in which digestive processes of supplements fed to ruminants can be modified to resemble those of a monogastric animal in terms of protein composition of the tissue or milk. Overprotection of protein is a major problem in the application of formaldehyde treatment as increasing amounts have resulted in progressively reduced digestibilities (Broderick et al. 1991).

As formaldehyde has been recognized as a carcinogen, there is a concern that formaldehyde used in protein protection may be transferred to milk and therefore present a health hazard. However, Atwal and Mahadevan (1994) reported that feeding formaldehyde treated soybean meal (SBM) did not increase formaldehyde levels above those naturally occurring in milk ranging from 0.02 mg kg^{-1} to 0.2 mg kg^{-1}. Despite other studies that have not indicated any adverse health problems due to

feeding formaldehyde protected proteins, industry and consumer concerns about the potentially adverse health risks limit the use of formaldehyde (Broderick et al. 1991).

1.3.2 Heat Treatment

Heat is the most commonly used treatment because of its efficacy, cost-effectiveness, safety and ease of application (Hussein et al. 1995). The application of heat causes coagulation or denaturation of the protein reducing the solubility and accessibility of the substrate. However, heat treatment primarily decreases protein solubility due to cross-linking between peptide chains and carbohydrates in the Maillard or non-enzymatic browning reaction. Amino acids such as lysine with a free amino group in the peptide form are very reactive (Van Soest 1994). Mild or moderate heating of proteins in the presence of carbonyl compounds, usually reducing sugars, begins with a simple addition to form a Schiff's base, followed by the Amadori rearrangement of the Schiff's base to the 1-amino-1-deoxy-2-ketose (Amadori) compound (Labuza et al. 1977). These intermediate amino-sugar complexes represent the cross-linkage of a sugar aldehyde group to a free epsilon amino group of lysine, while the alpha amino group is bound in the protein structure (Erbersdobler 1977). This complex is more resistant than normal peptides to enzymatic hydrolysis, thus this is the major process by which heating protects protein from rumen degradation. Of critical importance to nutrition is that this reaction is reversible, yielding utilizable lysine from the Amadori compounds following abomasal (pH 2-3) digestion and making it available for intestinal absorption (Erbersdobler 1977).

The advanced stages of the Maillard reaction, precipitated by excessive heat, result in permanent cross-linking of the peptide and carbohydrate chains yielding nutritionally unavailable brown melanoidin polymers (Labuza et al. 1977). These compounds prevent enzyme penetration or mask the sites of enzyme attack (Hurrell and Finot 1985; Ames 1992) which leads to reduced post-rumen availability of sugars and AA. These end products, containing about 11% nitrogen, possess many of the physical properties of lignin and are recoverable in lignin and acid detergent fibre (Van Soest 1994).

The rate and the extent of the Maillard reaction are affected by a number of determinants, the most important being: temperature and length of heat exposure; availability of free amino groups and reducing sugars; moisture content; and pH (Ames 1992). The degree of heating practiced by commercial canola seed processing plants during oil extraction and preparation of CM is insufficient to maximize the escape of protein and AA in CM from the rumen (Moshtaghi Nia and Ingalls 1995). Moshtaghi Nia and Ingalls (1995) found that 15 min of moist heat treatment (autoclaving, 127°C) on CM had no significant effect on in situ total tract disappearance of AA, but 45 min of heat treatment resulted in the classic decrease in digestibility that occurs with extended heating. Moshtaghi Nia and Ingalls (1992) concluded that the beneficial effects of the decreased rate of protein degradation in the rumen appeared to be greater than the decreased protein digestibility in the total GI tract caused by limited heat damage.

The rate of the Maillard reaction is also highly dependent on moisture content, with less heat energy required at higher moisture levels (Lingnert 1990). Maximum reaction rates have been reported at moisture levels of 30%, but the effect of water is

variable (Van Soest 1994). However, high moisture levels are not practical as the energy required to dry a product for extended storage surpasses the energy conserved by heating at high moisture levels.

The most reactive carbohydrates in plants are the hemicelluloses and soluble sugars with cellulose and starch being comparatively more stable (Marounek and Brezina 1993). The order of reactivity of reducing sugars in the Maillard reaction is xylose > arabinose > glucose > lactose > maltose > fructose, with fructose being only one-tenth as reactive as glucose (Ames 1992). Cleale et al. (1987) found that treatment of SBM with xylose was more effective in reducing in vitro degradation of SBM by rumen micro-organisms than was glucose, lactose or fructose.

Calcium-sodium lignosulfonate (LSO$_3$), a non-toxic by-product of the wood pulp industry, is derived from the spent sulphite liquor that is generated during the sodium sulphite digestion of wood. It contains hemicellulose and reducing sugars of which approximately 24% are xylose. Traditionally lignosulfonate products have been used to precipitate protein from packing house waste water, bind to protein in the leather tanning process, and bind feed pellets in the manufacture of animal feeds (Windschitl and Stern 1988). Treatment of SBM and CM with 5% LSO$_3$ in combination with heat has been shown to successfully reduce protein degradation by ruminal microbes (Windschitl and Stern 1988; McAllister et al. 1993). The reductions in CM protein degradation in situ occurred without substantially increasing indigestible protein as assessed by ADIN (McAllister et al. 1993). However, the decreased degradation was less than that observed for SBM by Windschitl and Stern (1988).

Heating SBM at 95°C for 45 min with 15-20% moisture had no effect on protein degradability compared to untreated SBM, but 5% LSO$_3$ and heat resulted in

significantly lower degradation (Windschitl and Stern 1988). Similarly, McAllister et al. (1993) found treatment of CM with 5% LSO_3 followed by heat at 100°C for 60 min with 25% moisture caused a larger reduction in effective rumen degradability of protein than heat treatment without LSO_3. Stanford et al. (1995) concluded that a combination of LSO_3 and heat is necessary to achieve large increases in rumen undegradable protein when CM is heated at temperatures between 95°C and 110°C for 1 to 2 h. Thus, if the Maillard reaction can be controlled in such a manner that the formation of reversible primary products is maximized without the formation of the unavailable terminal products, the value of a supplemental protein could be enhanced.

1.4 EVALUATION OF RUMEN PROTEIN DEGRADABILITY

Accurate determination of the RDP and RUP fractions is of critical importance in the evaluation of the supply of nutrients for absorption and utilization by high yielding cows. The efficacy of treatments designed to alter ruminal protein digestion has been evaluated using in vivo, in vitro and in situ techniques. Conventional in vivo digestibility measurements are believed to accurately reflect the feeding value of total diets (Orskov 1992), however, in addition to being laborious and time consuming, they are limited to a single feedstuff or combination of feedstuffs. A quick, effective and widely accepted method to determine the RDP and RUP fractions of a feedstuff is the dacron polyester or nylon bag technique. This popular technique has been accepted by ARC (1984) and NRC (1989) as the method of choice to determine the protein degradability of a feedstuff (Orskov 1992). The nylon bag technique involves the incubation of a feedstuff in a synthetic nylon bag, which is suspended in the rumen for

varying lengths of time. The pore size (50 um) of the bag allows bacteria to enter and to digest the feed. The nylon bag technique permits a large number of samples to be evaluated at one time and is suitable for the initial evaluation of a feedstuff. The value for the degradation is used to determine both the degradable portion available for micro-organisms and the undegradable protein which may be available for enzymatic digestion in the intestine.

1.4.1 Disadvantages of the Nylon Bag Technique

Care must be exercised in the interpretation of results as the nylon bag technique is used to measure disappearance, and we assume that disappearance is synonymous with degradation (Kennelly and Ha 1983). The strongest criticism of this technique is its low repeatability as suggested by the diversity of values obtained by different researchers for a similar feed sample (Michalet-Doreau and Ould-Bah 1992). Potential explanations for this discrepancy could be: 1) microbial growth within the nylon bag may underestimate N loss (Nocek and Grant 1987); 2) the actual number of microbes within the bag may be an under representation of the level present in the rumen leading to lower loss (Meyer and Mackie 1986); and 3) the rapid removal of soluble materials and very fine particles may overestimate N loss (England et al. 1997).

The greatest error is believed to arise from the net migration of microbes from the rumen into the bag as a result of their attachment to the feedstuff. The challenge is trying to accurately quantify the bacterial component of nylon bag residues following rumen incubation. Bacterial contamination can be a serious problem leading to underestimation of protein degradation for forages (Varvikko and Lindberg 1985;

Nocek and Grant 1987). Correspondingly, others (Mathers and Aitchison 1981; Hof et al. 1990) stated that the problem appears not to be associated with concentrates and that the extent of microbial contamination was nutritionally insignificant. Thus, corrections for bacterial contamination are most appropriate for high fibre, low protein feeds (Waters and Givens 1992).

Several factors identified by Michalet-Doreau and Ould-Bah (1992) and Stern et al. (1994) that need to be controlled if this technique is to be standardized include: 1) the porosity of the nylon bag (must be large enough for the degradation process to take place normally within the bag and sufficiently small to limit the efflux of undegraded feed particles); 2) fineness of the ground sample (must mimic the effect of mastication); 3) the ratio of sample weight to bag surface area; 4) method of bag placement in the rumen; 5) basal diet of the animal; and 6) degree of bacterial attachment to feed residues remaining in the bag. While requiring relatively little equipment, this technique does require surgical preparation of an animal with a ruminal cannula and facilities for its maintenance which may be expensive.

1.4.2 Advantages of the Nylon Bag Technique

Although the in situ nylon bag technique is imperfect in ways that cannot be fully corrected for, it is rapid, fairly reproducible, and requires minimal apparatus (Broderick et al. 1988). The technique has gained popularity due to its versatility and ability to give reliable estimates of in vivo degradation across a wide range of feedstuffs. Most importantly, unlike in vitro procedures, it involves digestive processes that occur in the rumen of a living animal (Nocek 1988). As a relatively

quick and simple procedure, it allows many feedstuffs to be evaluated simultaneously (Stern et al. 1983).

1.5 EVALUATION OF INTESTINAL AND TOTAL TRACT DEGRADABILITY

Low rumen degradability of a feedstuff assessed using the in situ technique does not necessarily mean it is available for digestion and absorption in the lower GI tract. Determination of the proportion of a feed degraded in the intestine can be evaluated using the in situ mobile nylon bag technique. The mobile bag technique developed by Hvelplund (1985) and modified by de Boer et al. (1987) involves a sequence of pre-incubating a feedstuff in a small nylon bag in the rumen for 8 h; incubating the bag in vitro in an acid-pepsin solution; inserting the bag into the duodenum via a cannula; and collecting the bag in the feces. The proportion of protein degraded in the intestines is equal to total tract digestion minus the protein degraded in the rumen.

1.5.1 Disadvantages of the Mobile Nylon Bag Technique

During incubation, microbes attach to the feedstuff and break it down. The degree of contamination of the residue by microbial protein due to sustained attachment has been a subject of controversy (Hvelplund et al. 1992). While Varvikko and Lindberg (1985) reported this to be substantial, others (Hvelplund 1985; Jarosz et al. 1991; Kohn and Allen 1992) have reported limited microbial contamination of feed residues.

This technique has also been criticized because disappearance of the feedstuff in the intestine does not infer that the animal absorbed the material. Estimates of protein degradability using the mobile nylon bag technique are considerably greater

than those observed using conventional in vivo techniques (Broderick 1994). The substantially higher in situ estimates relative to apparent digestibility values reported for conventional in vivo studies, suggests that the endogenous cost of feeding a protein source is significant (Robinson et al. 1992). Kirkpatrick and Kennelly (1984) reported that the discrepancies between conventional and mobile nylon bag digestibility results can be eliminated if endogenous and other contaminating N sources are not removed through the washing of the bags collected in the feces.

Similar to the rumen nylon bag technique, this technique requires animals to be surgically prepared with cannulas both in the rumen and duodenum, which can be time-consuming and expensive.

1.5.2 Advantages of the Mobile Nylon Bag Technique

The mobile nylon bag technique is not as time-consuming or expensive as alternative infusion techniques (Hvelplund et al. 1992). In comparison to infusion studies using sheep, Hvelplund et al. (1992) have shown the mobile bag technique produced comparable results. This technique has also been identified by others (de Boer et al. 1987; Varvikko and Vanhatalo 1991) as a promising technique for evaluating the intestinal degradability of feedstuffs.

1.6 Summary

Diets formulated according to requirements for CP without consideration of RUP and RDP lead to higher urea N concentrations and lowered efficiency of N utilization for milk production and growth. Controlling the rate and extent of degradation of dietary protein to balance the protein supply from microbial synthesis is of great interest to ruminant nutritionists, because inefficient utilization necessitates

the over feeding of protein, the most costly ingredient of a diet. Enhancement of the early stages of the Maillard browning reaction through the use of controlled levels of heat and reducing sugar shows potential to increase the feeding value of CM as a RUP source. Cows supplemented with increased levels of RUP have the potential to increase production and show increased efficiency of N utilization.

1.7 Objectives

The objectives of this study were to ascertain the effects of heat and LSO_3 treatment of CM on 1) in situ ruminal and intestinal disappearance of DM and CP; and 2) in vivo apparent digestibility, milk production and milk composition.

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II. THE EFFECTS OF HYDROTHERMAL COOKING OF CANOLA MEAL ON IN SITU RUMEN AND INTESTINAL DISAPPEARANCE OF DRY MATTER AND CRUDE PROTEIN

2.1 INTRODUCTION

Combinations of both rumen microbial protein and RUP are necessary to provide sufficient protein to support the high milk yields achieved in dairy cattle. Recommendations for RUP levels within diets have been developed to increase the amount of AA presented to the small intestine for utilization (NRC 1989).

The extent to which protein is degraded depends primarily upon microbial proteolytic activity in the rumen, microbial access to the protein, and ruminal retention time of the protein (Stern et al. 1994). Protein structure influences accessibility by proteolytic enzymes, thereby affecting the degradability of protein in the rumen. Some dietary feed ingredients are naturally resistant to ruminal microbial degradation while other ingredients may have greater or lower resistance to microbial degradation because of prior chemical and physical processing (Van Soest 1994).

Canola meal (CM) is used as a supplemental protein in dairy cattle diets because of its high protein quality and its competitive pricing compared to other protein supplements (Christensen and McKinnon 1993). Although, the use of CM is limited by its high rumen degradability, its excellent balance of amino acids relative to milk protein make it a promising candidate for research aimed at increasing its RUP content. Chemical treatments administered to increase RUP content include formaldehyde and acids. Formaldehyde treatment (5% wt wt^{-1}) has been successful in reducing CM's in situ rumen CP degradability by 75% (Bailey and Hironaka 1984). Similarly, treatment of CM with acetic acid (3% vol wt^{-1}) followed by oven drying has

been shown to reduce its in situ rumen protein degradability by 29%, while reducing its total tract digestibility by only 3% (Khorasani et al. 1993). The most successful physical treatment has been heat (Broderick et al. 1991). Heat promotes the Maillard reaction, which decreases the solubility of proteins by creating cross-linkages between sugar aldehyde and free amino groups. These bonds, while resistant to rumen microbial degradation, are cleaved during acid hydrolysis of protein in the abomasum, yielding AA available for absorption in the duodenum. Moshtaghi Nia and Ingalls (1992) used 90 min of moist heat treatment (autoclaving) at 127°C to reduce disappearance of CM protein by 76% following 10 h of rumen incubation. However, total tract digestibility was also reduced by 15%. In subsequent work, Moshtaghi Nia and Ingalls (1995) reported that 45 min of moist heat (127°C) did not affect total tract disappearance of N, with rumen N disappearance still significantly reduced by 68%.

Significant reductions in rumen degradability have resulted from combinations of heat and calcium-sodium lignosulfonate (LSO_3). The LSO_3, containing 24% xylose, is derived from the spent sulphite liquor produced during sodium sulphite digestion of hardwoods. McAllister et al. (1993) decreased the CP degradability of CM treated in the laboratory by 20% with LSO_3 (5% wt wt^{-1}) and heat at 100°C for 60 min. Beauchemin et al. (1995), using similar treatment levels but at a commercial feed manufacturing facility, reduced rumen protein degradability of CM by 52%.

As a means to study the ability of treatments to alter protein degradation, the in situ nylon bag technique has received the most extensive evaluation (Michalet-Doreau and Ould-Bah 1992). It is the most widely used technique for assessing nutrient degradability because it is rapid, fairly reproducible, and generally provides reasonable estimates of protein degradation (Stern et al. 1994).

The objective of this study was to evaluate the effects of the treatment of CM with LSO_3 and graded levels of heat delivered in a hydrothermal cooker on in situ ruminal, intestinal and total tract nutrient degradability.

2.2 MATERIALS AND METHODS

2.2.1 Canola Meal Treatments

Commercially available solvent extracted canola meal (CM) was processed with combinations (Table 2.1) of heat and LSO_3 (LignoTech USA, Inc., Rothschild, WI). Moist heat was applied at 100°C using a hydrothermal cooker (Amandus Kahl Nachf, Hamburg, Germany) installed at Agro Pacific Industries Ltd. (Chilliwack, BC). The hydrothermal cooker is an insulated cylinder, with capacity to cook 2000 kg of CM at once, that uses steam to control and maintain a pre-set temperature. Four probes located at different positions within the cooker measure temperatures, which are monitored by a computer, which in turn controls steam input. CM samples were removed from the cooker after heating for 30, 60, 90 or 120 min (denoted as heat-30, heat-60, heat-90 and heat-120, respectively). Samples were dried with forced air at ambient temperature (20°C) and then stored in plastic bags. Samples of untreated CM, CM before heating and after heating and the final CM product were collected for DM determination. Dry matter content was determined by drying samples in a forced air oven at 105°C for 24 h. To evaluate the effects of LSO_3 on CM compared to SBM, the commercial product Soy Pass® (LignoTech USA, Inc., Rothschild, WI) was included in the in situ trial as a positive control. Soy Pass® is SBM treated with 5% LSO_3 and heated at 100°C for an undisclosed period of time.

2.2.2 Animals and Basal Diet

Two non-lactating Holstein cows, fitted with rumen fistulas and T-shaped duodenal cannulas, were fed a diet consisting of 5.5 kg of grass hay and 3.0 kg of 14% CP, commercially prepared dairy concentrate per day (as fed basis). The cows were fed equal portions of the diet at approximately 0730 h and 1630 h. The animals were cared for according to the standards set by the Canadian Council on Animal Care (1993) and the experimental protocol was approved by The University of British Columbia Animal Care Committee.

2.2.3 Rumen Disappearance

Nylon bags (5 x 10 cm; pore size: 50 ± 15 µm; ANKOM Tech Corp., Fairport, NY) were filled with approximately 3 g of material and sealed using elastic bands. Each sample was incubated in duplicate in the rumen for 24, 16, 12, 8, 4 and 0 h in each of two cows. Nylon bags were suspended in the rumen in a polyester mesh bag (25 x 40 cm; pore size 3 mm) attached to the end of a 50 cm line weighted with a sand filled bottle. Bags were removed at a common time in order to minimize the variation in time that the bags were exposed to air after incubation and to enable simultaneous washing of all bags (Nocek 1985). Following ruminal incubation, the polyester mesh bags containing all the nylon bags (including the zero bags) were rinsed with cold water to remove particulate material. The nylon bags were removed from the mesh bags and placed in a conventional clothes washing machine (von Keyserlingk et al. 1996). The machine was allowed to fill with water and to agitate for 5 min prior to draining, without allowing the bags to be spun. This procedure was repeated until the rinse water remained clear, normally four to five washes. Nylon bags were dried in a

forced air oven at 60°C until constant weight was achieved for the determination of DM disappearance. Nylon bag replicates within cows were pooled and residues were ground through a 0.5 mm screen prior to N analysis.

2.2.4 Intestinal Disappearance

The mobile nylon bag technique, as described by de Boer et al. (1987), was used to measure the DM and CP disappearance during passage through the intestine. Duodenal nylon bags (3.5 x 5 cm; pore size: 50 ± 15 µm; ANKOM, Fairport, NY) were filled with 0.5 g of dry ground (2 mm screen) sample and heat-sealed. Samples were pre-incubated in quadruplicate in the rumen of each cow for 16, 12 and 8 h. Following rumen incubation, the bags were left unwashed and stored at –20°C until the day of insertion into the duodenum. On the day of incubation the bags were thawed at room temperature (20°C) and incubated in a pepsin-HCl solution (1 g of pepsin per L of 0.01 N HCl) for 3 h at 39°C to simulate abomasal digestion (Kirkpatrick and Kennelly 1984). Mobile nylon bags were kept at 4°C until they were randomly inserted into the duodenum every 20-30 min. Duodenal bags were collected from the feces (16 h mean passage time) then hand washed until the rinse water remained clear. Dry matter disappearance was determined for all replicates by drying them in a forced air oven at 60°C until constant weight was achieved. All replicates were ground through a 0.5 mm screen. Due to limited amounts of sample remaining after in situ incubation, replicates within cows were pooled prior to nitrogen analysis. Therefore variation within cows was not measured.

2.2.5 Chemical Analyses

Feeds were analyzed for acid detergent fibre (ADF) and neutral detergent fibre (NDF) using the modified method of Van Soest et al. (1991) called the filter bag technique (ANKOM Co., Fairport, NY; Komarek et al. 1994). Nitrogen determinations, for CP disappearance calculations, were performed using a Leco FP-428 N analyzer (Leco Corp., St. Joseph, MI).

2.2.6 Calculations and Statistical Analyses

Incorporation of microbial protein into the nylon bag contents was not measured in this experiment. This decision was based on the work of Mathers and Aitchison (1981) who stated that in the case of concentrates, the extent of microbial contamination is so minimal that it is nutritionally unimportant. The rumen disappearance of DM and CP at each individual time was calculated as the difference between the feed and the portion remaining after incubation in the rumen. Total tract DM and CP disappearance from each of the nylon bags was calculated as the difference between the feed and the amount remaining (DM basis) after collection of the bag in the feces. The amount of DM and CP that disappeared in the small intestine were calculated for each individual bag by subtracting the amount that disappeared in the rumen from the amount that disappeared in the total tract. Thus, rumen and total tract disappearances were direct measurements while disappearances in the intestines were calculated values. Effective degradabilities of DM and CP have not been included, because the maximum degradation was not reached at 24 h of ruminal incubation (Figures 2.1 and 2.2). Without the establishment of a plateau in degradability values, it was not possible to adequately fit

disappearance data to the effective degradability equations (McDonald 1981). Statistically, the data were analyzed using the General Linear Model procedure of SAS (1990). Least square means between treatments were used to test for significant differences.

2.3 RESULTS

The addition of 5% LSO$_3$ reduced the DM content from 87.49% in untreated CM to 86.00% (Table 2.2). Steam heating in the hydrothermal cooker reduced the DM on average by 2.76 and 3.67 percentage points for heat treated and LSO$_3$ plus heat treated CM, respectively. Thus, the LSO$_3$ plus heat treated products were cooked at higher moisture levels than were the heat treated products. Forced air drying at ambient temperature increased the DM of the feeds on average by 1.97% and 2.20% for the heat and LSO$_3$ plus heat treatments, respectively.

2.3.1 Rumen Disappearance of DM and CP

Results of the in situ rumen DM and CP evaluation of treated CM are shown in Tables 2.3 and 2.4, respectively. For heat treatment, the extents of ruminal DM and CP disappearance at 8 and 16 h of ruminal incubation decreased with heat-120. Values for 30, 60 and 90 min of heat were generally not significantly different from values for the untreated CM and fell between disappearance values of untreated CM and heat-120. Zero, 4, 12 and 24 h DM disappearances of CM were unaffected by heat treatment, except for significant reductions with heat-30 relative to untreated CM at 4 and 24 h.

Application of 5% LSO$_3$ in the absence of heat reduced the extent of DM and CP disappearance at 0, 4 and 8 h compared to untreated, but was not different at 12, 16

and 24 h. DM and CP disappearance of LSO_3-120 was lower than LSO_3 treatment at 0, 4 and 8 h of rumen incubation with disappearances of LSO_3-30, LSO_3-60 and LSO_3-90 in between. Relative to the control, few consistent reductions in 12, 16 and 24 h rumen disappearances of DM and CP were noted for LSO_3 and increasing levels of heat. Disappearance of Soy Pass® DM and CP was significantly lower at all lengths of rumen incubation. The DM and CP degradation characteristics of untreated, heat-120 and LSO_3-120 CM treatments are illustrated in Figures 2.1 and 2.2. They clearly illustrate that treatment differences, in particular with LSO_3-120, were most pronounced following 4 and 8 h of rumen incubation.

2.3.2 Total Tract and Intestinal Disappearance of DM and CP

Neither heat nor LSO_3 plus heat treatment of CM had appreciable effects on total tract disappearance of DM or CP (Table 2.6). The total tract DM disappearance of Soy Pass® was greater than all CM treatments at 12 and 16 h and all but untreated, heat-120 and LSO_3-120 at 8 h rumen incubation. Total tract disappearance of Soy Pass® CP was lower than all CM treatments at all times of rumen incubation.

Few consistent differences in intestinal DM disappearances were found (Table 2.5). The outflow rate of protein supplements from the rumen is about 10% h^{-1} with a high level of feeding of mixed diets to dairy cows (Orskov 1992). Thus, intestinal disappearance results following 8 h rumen incubation were chosen to represent the degradation that would occur in a high producing cows (Figure 2.3). The highest level of heat treatment increased intestinal CP disappearance the most from 15.9 to 23.5% at 8 h and 4.6 to 10.8% at 16 h for untreated and heat-120, respectively. The addition of LSO_3 plus 60, 90 and 120 min of heat increased intestinal disappearance of CM CP

at 8 and 16 h relative to untreated CM. There were no effects of treatments observed at 12 h and significant reductions in degradability of the LSO_3 and LSO_3-30 treated CM were only observed at 8 h of rumen incubation. The greatest increases in intestinal protein disappearance occurred with LSO_3-120 treatment, which increased disappearance from 15.9 to 34.2% at 8 h rumen incubation for untreated and LSO_3-120, respectively. Soy Pass® intestinal DM and CP disappearances were greatest at all levels of incubation, with 8 h intestinal CP disappearance of 73.7% compared to 34.2% for LSO_3-120.

2.4 DISCUSSION

The rumen CP disappearance values at 12 h of incubation for untreated CM were similar to those reported by de Boer et al. (1987), but were 6% lower than those of Bailey and Hironaka (1984). Conversely, the protein disappearance results of untreated CM following 16 h of rumen incubation are 19% greater than those reported by Moshtaghi Nia and Ingalls (1992) and 28% greater than those reported by Moshtaghi Nia and Ingalls (1995). Disappearance values of DM followed similar trends. Discrepancies in reported in situ disappearance values can be attributed to varietal or processing differences in the samples of meal incubated (Kendall et al. 1991) or the in situ technique employed. Differences in the basal diet fed to cows in which the nylon bags are placed (Orskov 1992) or variation in the extent of microbial contamination of the incubated samples (Nocek 1988) might also have contributed to differences in this study compared to published observations. The washing procedure utilized in the present study was similar to that used by de Boer et al. (1987), but longer than that used by others (Bailey and Hironaka 1984; Moshtaghi Nia and Ingalls

1992, 1995), which may have explained the observed differences. Increased agitation may wash away more small particles or be strong enough to stretch pores in the bags allowing for increased escape of undigested substrate through bag pores (Michalet-Doreau and Ould-Bah 1992). Different commercial processing methods may account for some of the discrepancy as Kendall et al. (1991) reported effective degradabilities of CM protein ranged from 44 to 59%, depending on the source. Basal diets with higher roughage or lower CP contents have been associated with increased protein degradation (Orskov 1992). McAllister et al. (1993) reported that the effective degradability of CM ranged from 62% with an 80% concentrate basal diet to 75% with an alfalfa hay basal diet. The concentrate portion of the diet fed to cows was 30% in the present study, 0% in the study of Bailey and Hironaka (1984), and 50% in the studies of Moshtaghi Nia and Ingalls (1992, 1995). Thus, this may have also contributed to higher observed values in this study relative to Moshtaghi Nia and Ingalls (1992, 1995) and lower observed values relative to Bailey and Hironaka (1984). When comparing in situ results between studies from different labs, it is important to consider the in situ method used, in particular the washing technique and basal diet.

Untreated CM was more degradable in the rumen than most treatments. Heat treatment of CM for 2 h at 100°C compared with lower levels of heat decreased rumen CP disappearance. Heat treatment of CM at 125°C or 145°C reduced in situ CP disappearance of CM, but potentially compromised the post ruminal supply of CP (McKinnon et al. 1991). These higher processing temperatures are less desirable because of the formation of indigestible protein (McKinnon et al. 1991). The LSO_3 treatment of CM followed by 2 h of heat at 100°C, compared with lower levels of heat,

reduced rumen CP disappearance. Using similar heat conditions McAllister et al. (1993) treated CM with 10% LSO_3 and 2% xylose, equivalent to the xylose content of 10% LSO_3 and found CP degradability was reduced a further 28 and 20%, respectively, over the reduction observed with 5% LSO_3 treatment. However, because of two and three fold increases in ADIN, which is negatively associated with digestibility, they concluded 10% LSO_3 and 2% xylose levels were too high.

McAllister et al. (1993) reported that heating CM with LSO_3 for 2 h at 100°C also reduced CP degradability more than heating without LSO_3. Windschitl and Stern (1988) found no difference in CP degradability of untreated SBM and heat treated SBM, but lower degradation in LSO_3 plus heat treated SBM. Thus, a combination of LSO_3 and heat is necessary to produce large increases in RUP when CM or SBM are heated at temperatures of 100°C for 1-2 h. The less pronounced response of CM to LSO_3 treatment relative to SBM, supports the results of others (Windschitl and Stern 1988) and our results for Soy Pass®. This may reflect the lower lysine content of CM relative to that of SBM as discussed by McAllister et al. (1993). As lysine is the main reactive site for reducing sugars during the Maillard reaction, lowered CM responses in degradability to LSO_3 may be expected.

Total tract disappearances of untreated CM DM and CP are in agreement with those of others (Moshtaghi Nia and Ingalls 1992, 1995). It was apparent that the levels of moist heat treatments were not excessive as reflected in the unaltered total tract disappearances of treated CM relative to untreated. The higher total tract DM disappearances of Soy Pass® compared to CM, reflect SBM fibre level, which is considerably lower than in CM (NRC 1989). Soy Pass® protein, relative to untreated

SBM protein, may be overprotected as reflected in the consistently lower total tract CP disappearance.

With exposure of CM to moist heat for 2 h, a small depression in total tract digestibility might be expected (McAllister al. 1993). However, this was not observed in the present study and suggests the heat delivered was insufficient. Relatively high rumen degradabilities of CM fractions and the marginal separation between disappearances of heat and LSO_3 treated CM may be attributed to shorter than anticipated exposures to 100°C heat. In the present study, timing of heat exposure began when CM first entered the hydrothermal cooker. However, the period of time for CM within the cooker to reach 100°C varied depending upon the initial temperature of the cooker and the length of time necessary for all of the CM to enter. In future work, to ensure that feeds are heated for specific periods of time, the feed should be added to a preheated cooker in as short a time as possible and timing initiated when 100°C is reached.

In conclusion, the present study indicates that treatment of CM with or without LSO_3 followed by heating at 100°C for 2 h increases the resistance of CM protein to microbial digestion, likely through the formation of primary Maillard products. This shift in degradability from the rumen to the intestines increases the amount of RUP available for digestion and absorption post ruminally. Of greater importance, this resistance to microbial degradation is apparently accomplished without the formation of intestinally indigestible terminal Maillard products. The increased rumen undegradable protein and the availability of this protein for digestion and absorption in the small intestine could benefit rapidly growing ruminants or high producing cows. Further research is recommended to investigate the repeatability of the treatments,

due to the discrepancy in results compared to those of others, and to study the profile

and availability of essential AA in the small intestine.

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Table 2.1. Canola meal processed with graded levels of lignosulfonate (LSO_3) and moist heat at 100°C

Treatment	LSO_3 added (%, wt wt^{-1})	Duration of heat (min)
Untreated	0	0
heat-30	0	30
heat-60	0	60
heat-90	0	90
heat-120	0	120
LSO_3	5	0
LSO_3-30	5	30
LSO_3-60	5	60
LSO_3-90	5	90
LSO_3-120	5	120

Table 2.2. Changes in DM content that occurred during processing of canola meal with graded levels of lignosulfonate (LSO_3) and moist heat at 100°C

DM (%)

Treatment	Untreated	Before cooking	After cooking	Final
Heat Treatments				
heat-30	87.49	87.49	85.24	88.24
heat-60	87.49	87.49	85.25	86.50
heat-90	87.49	87.49	84.07	86.06
heat-120	87.49	87.49	84.34	86.01
Mean	87.49	87.49	84.73	86.70
LSO_3 + Heat Treatments				
LSO_3-30	87.49	86.00	81.86	84.41
LSO_3-60	87.49	86.00	84.17	85.21
LSO_3-90	87.49	86.00	81.88	84.94
LSO_3-120	87.49	86.00	81.42	83.56
Mean	87.49	86.00	82.33	84.53

Table 2.3. DM disappearance (% of initial) of heat and lignosulfonate (LSO₃) treated canola meal and Soy Pass® in the rumen (n=4)

Treatment	Incubation time (h)					
	0	4	8	12	16	24
untreated	36.2a	58.2a	69.7a	74.5ab	79.5a	84.2a
heat-30	35.3ab	51.5bc	65.0abc	67.1e	72.6c	78.2c
heat-60	34.7b	55.4ab	64.9abc	69.2cde	78.1ab	82.6ab
heat-90	35.5ab	56.8a	68.1ab	68.0de	75.1bc	82.5ab
heat-120	35.0b	55.3ab	64.3bc	70.7bcde	74.1bc	82.7ab
LSO₃	33.7c	50.6cd	61.6cd	77.4a	79.4a	83.4ab
LSO₃-30	31.3de	46.6de	57.5de	73.1abc	77.3ab	82.3ab
LSO₃-60	31.6d	45.2e	58.1de	72.7abcd	76.1abc	81.9ab
LSO₃-90	30.9de	44.4e	57.6de	71.7bcde	76.8ab	82.7ab
LSO₃-120	30.5e	45.6e	54.3e	74.0abc	76.6ab	81.8b
Soy Pass®	27.7f	31.2f	34.2f	46.0f	54.8d	67.5e
SE	0.3	1.4	1.7	1.8	1.4	0.8

a-f Means within columns with different letters differ significantly (P<0.05)

Table 2.4. CP disappearance (% of initial) of heat and lignosulfonate (LSO₃) treated canola meal and Soy Pass® in the rumen (n=4)

Treatment	Incubation time (h)					
	0	4	8	12	16	24
untreated	31.3de	61.9a	76.8a	83.4ab	88.5a	92.9a
heat-30	34.8a	57.6b	75.0a	77.1cd	82.9c	88.5c
heat-60	33.5ab	60.6b	73.1ab	78.3bcd	88.0ab	92.2ab
heat-90	33.2bc	60.3b	75.2a	74.9d	83.3bc	91.6abc
heat-120	31.6d	58.5b	68.9bc	78.0bcd	81.8c	91.2abc
LSO₃	33.4bc	56.5bc	68.1c	87.2a	88.9a	92.7ab
LSO₃-30	29.1f	52.6cd	63.6de	81.8abc	86.4abc	92.5ab
LSO₃-60	30.8de	49.6de	65.4cd	81.3abc	84.8abc	90.8abc
LSO₃-90	27.8g	46.7e	63.4def	79.5bcd	84.4abc	91.9ab
LSO₃-120	26.5h	49.4de	58.8f	82.8abc	84.6abc	89.5bc
Soy Pass®	6.1i	7.8f	10.3g	22.8e	32.1d	47.9d
SE	0.5	1.4	1.6	2.1	1.7	1.2

a-i Means within columns with different letters differ significantly (P<0.05)

Table 2.5. The intestinal DM and CP disappearance (% of initial) of heat and lignosulfonate (LSO$_3$) treated canola meal and Soy Pass® (n=8)

Parameters	Dry Matter (%)			Crude Protein (%)		
Incubation time (h)	8	12	16	8	12	16
untreated	12.2ef	6.1ef	3.5cd	15.9hi	9.9de	4.6f
heat-30	11.6f	11.3bc	4.8bcd	15.3i	14.8bc	8.2bcd
heat-60	15.8def	10.7bcd	2.5d	19.9gh	14.1bcd	4.6f
heat-90	11.6f	12.3b	6.3b	17.2hi	17.2b	9.7bc
heat-120	16.0de	9.4bcde	7.2b	23.5efg	14.1bcd	10.8b
LSO$_3$	17.3cd	3.8f	3.5cd	24.2def	5.9e	5.0ef
LSO$_3$-30	21.6c	7.3de	4.8bcd	28.8cd	10.7cd	6.6def
LSO$_3$-60	19.4cd	6.7ef	4.8bcd	26.2cde	10.8cd	7.6cde
LSO$_3$-90	21.9c	8.5cde	4.7bcd	28.9c	12.8bcd	8.3cde
LSO$_3$-120	27.4b	6.6ef	5.2bc	34.2b	9.5de	8.3cde
Soy Pass®	48.5a	43.2a	33.6a	73.7a	66.7a	54.5a
SE	1.6	1.2	0.9	1.5	1.6	1.0

a-i Means within columns with different letters differ significantly (P<0.05)

Table 2.6. The total tract DM and CP disappearance (% of initial) of heat and lignosulfonate (LSO_3) treated canola meal and Soy Pass[®] (n=8)

Parameters	Dry Matter (%)			Crude Protein (%)		
Incubation time (h)	8	12	16	8	12	16
untreated	81.9abc	80.7b	82.9b	92.8a	93.3a	93.0ab
heat-30	76.6f	77.8c	77.4e	90.3b	91.0ab	91.1b
heat-60	79.9cde	79.9b	80.6d	92.3ab	92.3a	92.6ab
heat-90	79.6cde	80.6b	81.3cd	92.4ab	92.6a	93.0ab
heat-120	80.4bcd	80.3b	81.2cd	92.4ab	92.4a	92.8ab
LSO_3	79.1def	81.1b	82.9b	92.6ab	93.1a	94.0a
LSO_3-30	79.1def	80.4b	82.2bc	92.2ab	92.6a	93.3a
LSO_3-60	77.5ef	79.4bc	80.4d	91.6ab	92.1a	92.4ab
LSO_3-90	79.5cde	80.2b	81.5bcd	92.1ab	92.3a	92.7ab
LSO_3-120	81.7abc	80.0b	81.8bcd	93.0a	92.3a	92.9ab
Soy Pass[®]	82.6ab	89.0a	89.2a	84.0c	89.2b	87.8c
SE	1.0	0.7	0.5	0.9	0.8	0.7

a-e Means within columns with different letters differ significantly ($P<0.05$)
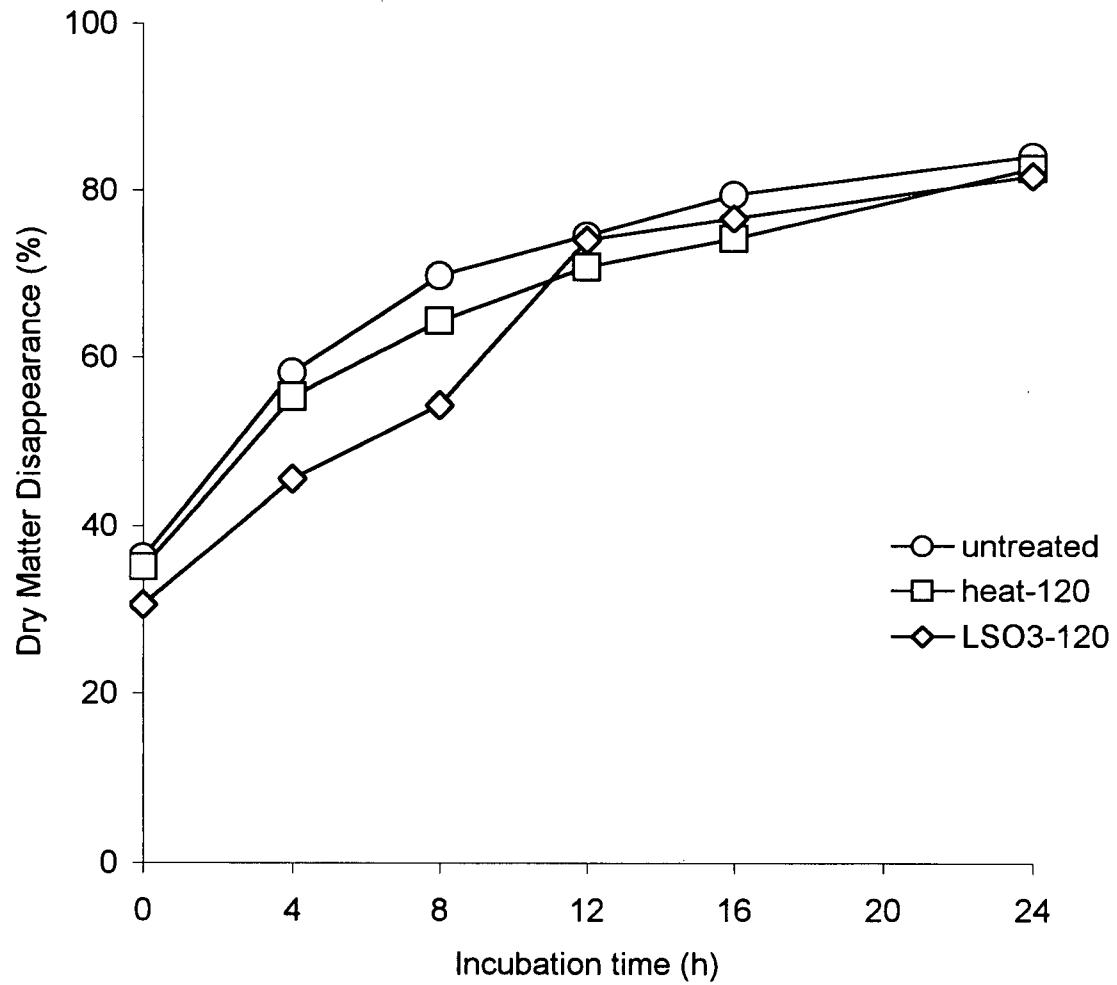
Figure 2.1. The DM disappearance (% of initial) of untreated canola meal and canola meal heated with (LSO$_3$-120) or without 5% LSO$_3$ (heat-120) in the rumen of non-lactating cows
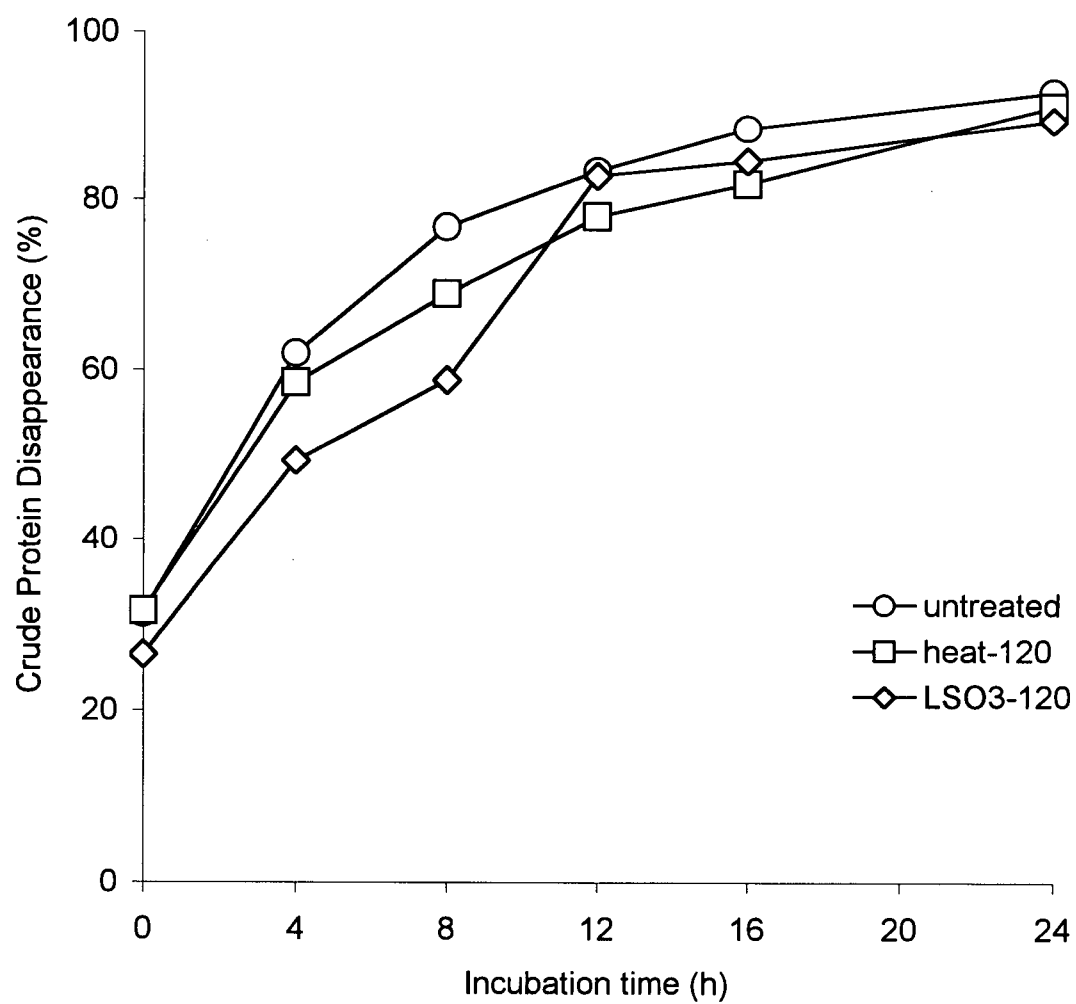
Figure 2.2. The CP disappearance of untreated canola meal and canola meal heated with (LSO$_3$-120) or without 5% LSO$_3$ (heat-120) in the rumen of non-lactating cows
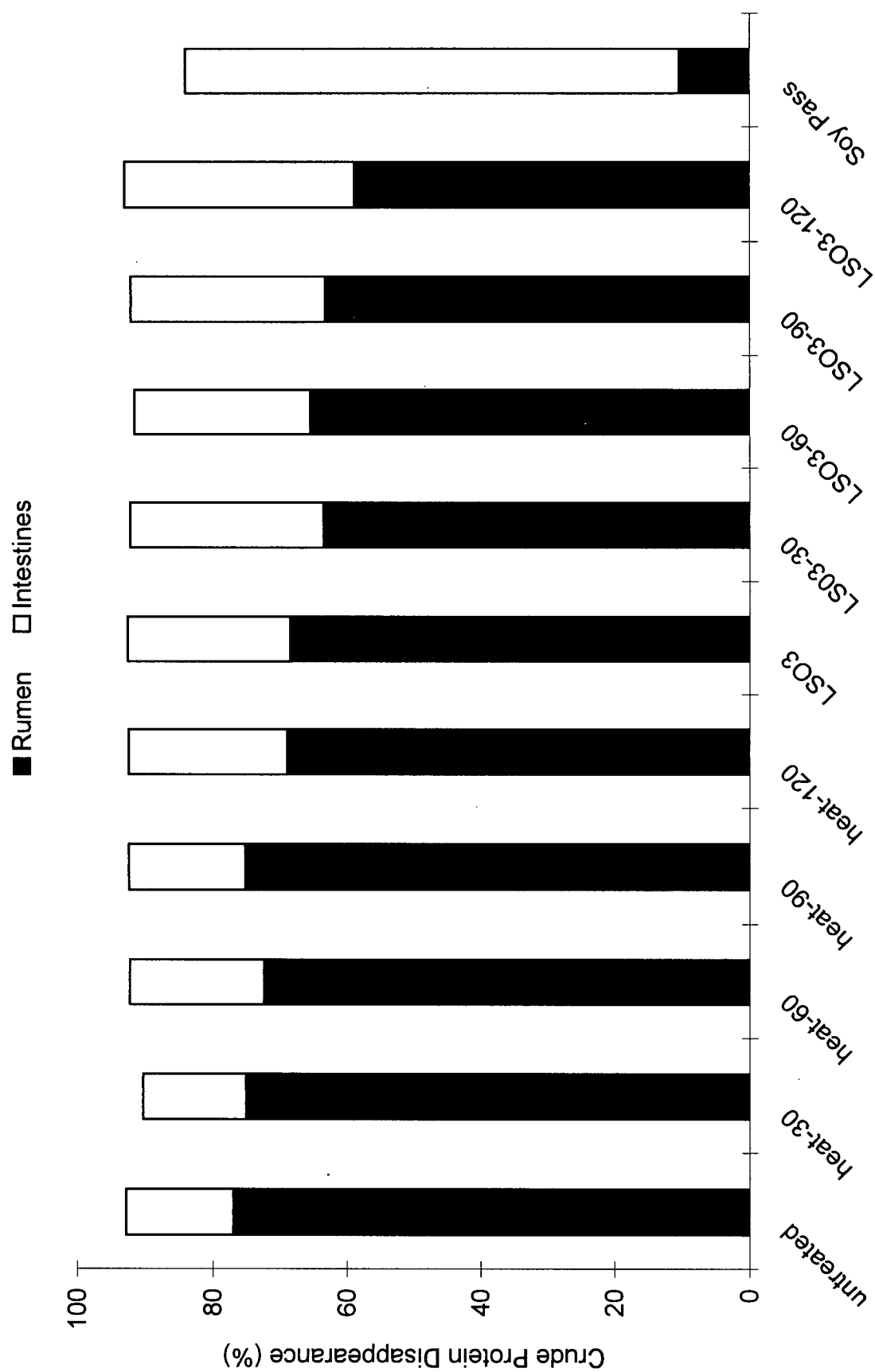
Figure 2.3. Partitioning of total tract CP disappearance of untreated, heat treated and lignosulfonate (LSO_3) plus heat treated canola meal between rumen and intestines following rumen incubation for 8 h.

III. PRODUCTION AND DIGESTION RESPONSES OF LACTATING DAIRY COWS TO THE FEEDING OF HEAT AND LIGNOSULFONATE TREATED CANOLA MEAL

3.1 INTRODUCTION

High producing dairy cows, in addition to their need for sufficient N to optimize microbial growth and function in the rumen, require an appropriate amount of good quality protein that contains the desired amounts of essential amino acids (AA) to be presented to the small intestine to support lactational and metabolic functions. Protein requirements of ruminants are met by microbial protein produced from dietary protein degraded in the rumen (RDP) and undegraded protein (RUP) which escapes microbial degradation in the rumen. Ruminal micro-organisms are a good source of quality protein, however they cannot always supply adequate amounts of metabolizable protein to support production and maintenance (NRC 1989). Dietary RUP can substantially increase the amount of protein for digestion by increasing the outflow and balance of AA to the duodenum for absorption. The potential benefits from feeding increased RUP include: increased level of milk production (Broderick et al. 1990); more efficient use of protein sources (Nakamura et al. 1992); enhanced reproductive performance (Ferguson and Chalupa 1989); and reduced environmental impact (Tamminga 1992). For cows producing 31-37 kg of milk d^{-1}, some studies (Kung and Huber 1983; Broderick et al. 1990) have shown that increasing the amounts of RUP in the diet increased milk production, while other studies (Robinson et al. 1991; Henson 1997) showed no improvement. The lack of response to high RUP may be caused by less than optimum protein protection; decreased microbial protein synthesis from increased RUP leaving insufficient RDP; low postruminal

digestibility of RUP sources; and essential AA profiles of RUP presented for absorption lack sufficient amounts of a limiting AA (Henson et al. 1997).

Treatment of dietary proteins with various agents, including heat, formaldehyde, sodium hydroxide, acids and reducing sugars, has successfully decreased the susceptibility of protein to rumen microbial proteolytic activity. The most successful treatments have incorporated heat and reducing sugars which facilitate the Maillard browning reaction. If the Maillard reaction between the sugar aldehyde group and the free amino groups could be controlled in such a manner that degradation is shifted from the rumen to the small intestine, then animal performance evaluated by either N retention or milk production would be increased (Chalupa 1975). Treatment of soybean meal (SBM) with calcium-sodium lignosulfonate (LSO_3) has successfully decreased the rumen degradability of SBM protein in vitro (Windschitl and Stern 1988b) and in vivo (Windschitl and Stern 1988a). Diets supplemented with LSO_3 treated SBM have supported similar milk production when untreated SBM was replaced with one-half as much protein from LSO_3 treated SBM (Nakamura et al. 1992).

Canola meal (CM), the predominant ruminant protein source in Canada, has a good AA profile relative to milk protein, but its small quantities of RUP limit its usefulness (Christensen and McKinnon 1993). Combinations of heat and LSO_3 have successfully reduced the rumen degradability of CM without affecting its digestibility (McAllister et al. 1993; Stanford et al. 1995). While LSO_3 treated CM has not been fed to lactating cows, its use in feeding trials with lambs and calves has not increased performance (Stanford et al. 1995; Beauchemin 1995).

The objective of this trial was to evaluate the effects of CM protein supplements, varying in ruminal degradability, fed to high producing dairy cows on: 1) dry matter intake, milk yield and composition; 2) in situ and in vivo digestibility; and 3) efficiency of nitrogen utilization.

3.2 MATERIALS AND METHODS

3.2.1 Canola Meal Treatments

Solvent extracted CM was left untreated or was processed with either water or calcium-sodium lignosulfonate (LSO_3) (LignoTech USA, Inc., Rothschild, WI). Either 5% LSO_3 or 2% water was added (wt wt^{-1}) to CM and thoroughly mixed for 10 min prior to heating. The water was added to increase the moisture content of CM to an amount equivalent to the moisture added from LSO_3. The mixture was heated at 100°C for 120 min using moist heat in a hydrothermal cooker (Amandus Kahl Nachf, Hamburg, Germany) located at Agro Pacific Industries Ltd. (Chilliwack, BC. Forced air drying at ambient temperature was used to remove the moisture added prior to heating through water or LSO_3 and the moisture added during heating through steam. The CM was stored in unsealed one tonne bags until it was incorporated in diet concentrate mixtures. Samples for moisture determination were collected prior to water or LSO_3 addition, before heating, following heating and post cooling.

Four batches of each treatment, each about 3 tonnes in quantity, were prepared over the course of the experiment. Each batch was sub-sampled and DM content was determined by drying in a forced air oven at 105°C for 24h. Sub-samples dried at 60°C until constant weight were then ground (1 mm screen) and stored until subsequent chemical analysis. The CM samples were analyzed for acid detergent

fibre (ADF) and neutral detergent fibre (NDF) using the modified method of Van Soest et al. (1991) called the filter bag technique (ANKOM Co., Fairport, NY; Komarek et al. 1994) and for acid detergent insoluble nitrogen (ADIN) and N, using a Leco FP-428 N analyzer (Leco Corp., St. Joseph, MI).

Each batch was also assessed for rumen and intestinal disappearance as described in Chapter II, sections 2.2.2, 2.2.3 and 2.2.4, except intestinal disappearance was only assessed with samples pre-incubated in the rumen for 8 h. The basal diets fed the host cows were different. The non-lactating cows were fed 7.1 kg grass hay (17.1% CP) and 6.9 kg rolled barley (13.0% CP) per day (DM basis).

3.2.2 Experimental Design

Eighteen multiparous, lactating Holstein cows, averaging 60 ± 7 days in milk were used in the production trial. Cows were milked twice daily and housed in a free stall barn located at the Pacific Agri-Food Research Centre (Agassiz, BC). Cows were blocked according to milk production, calving date, body weight and age, and randomly assigned to three treatment groups in a 3 x 3 Latin square design, replicated six times. Cows were randomly assigned to subsequent treatments in second and third periods. Experimental periods were 42 d in length. Individual feed intakes were monitored using Calan feeding doors (American Calan Inc., Northwood, NH). The animals were cared for according to standards set by the Canadian Council on Animal Care (1993) and the experimental protocol was approved by the Research Centre and The University of British Columbia Animal Care Committees.

3.2.3 Diets

The animals were fed isonitrogenous total mixed rations (TMR) consisting of 30% corn silage, 20% grass silage and 50% grain concentrate (DM basis) (Table 3.1). Diets were formulated to contain one of the following CM sources: A) untreated (U-CM); B) heat and water treated (HT-CM); or C) heat and lignosulfonate treated (LSO_3-CM). To provide an isonitrogenous contribution to total CP intake from each protein source, the amounts of CM in each diet were 20.0, 20.6 and 21.5% for cows fed U-CM, HT-CM and LSO_3-CM, respectively (DM basis). Concentrates were prepared in a mash form and were mixed with the silages to minimize sorting. The diets for each treatment group were prepared once daily and cows were fed equal portions at 0830 h and 1530 h. Orts were weighed and removed daily, prior to the 0830 h feeding and the amount of TMR offered was adjusted to maintain about 10% orts (as fed basis). Feed intakes were measured daily for each cow, averaged by week, and corrected for DM content of the TMR in order to calculate dry matter intake (DMI).

3.2.4 Sampling

3.2.4.1 Feed

Total mixed rations were sampled three times per week, composited weekly, and frozen. Weighback samples were taken twice weekly from each cow and composited by week for each diet. Forage and concentrate samples were taken every two weeks. The DM content was determined by drying samples in a forced air oven at 60°C until constant weight was achieved. Samples were then ground (1 mm screen) and stored until subsequent chemical analysis of NDF, ADF and N.

3.2.4.2 Milk

Milk yield was electronically recorded twice daily for all cows. Milk samples were collected from each cow from four consecutive milkings on days 13-14, 20-21 and 34-35 of each treatment period. Milk samples were assayed for fat, protein and lactose content by infrared spectroscopy and analyzed for somatic cell counts (SCC) using a Fossimatic cell counter (BC DHIS Lab, Chilliwack, BC). Milk urea nitrogen (MUN) was also determined after defatting by centrifugation at 1500 x g for 5 min at 4°C before analysis using colorimetric procedures employing a Kodak Ektachem DT 60 Analyzer with Disc Two Module (Clinical Products Division, Eastman Kodak Co., Rochester, NY). Total daily yields of milk components were calculated using the A.M. and P.M. milk yield for the test date. Fat corrected milk (4% FCM) production was calculated using the equation: 4% FCM (kg d^{-1})= (0.4 + (0.15 * % fat)) * kg milk d^{-1} (NRC 1989).

3.2.4.3 Rumen Fluid

Rumen fluid samples (300 ml) were collected by vacuum rumen tube from each cow on days 21 and 35 of each period between 1030 h and 1130 h, approximately 2 h after morning feeding. Rumen fluid pH was determined immediately. A proportion of each sample was frozen at −10°C until ammonium N (NH_4^+ N) analysis (Fawcett and Scott 1960). The remainder of each sample was acidified to pH 2 with 50% sulphuric acid, centrifuged and the supernatant frozen until volatile fatty acids (VFA) were analyzed by gas chromatography. The VFA were determined using a Shimadzu gas chromatograph equipped with a capillary column (30 m x 0.25 mm I.D. Stabilwax-DA). The injection port temperature was set at 170°C.

The column temperature was set at 120 to 180°C at 10°C min^{-1}, with an initial time of 1 min and a final time of 2 min. The internal standard used was isocaproic acid (0.70 g in 200 ml water).

3.2.4.4 Blood

Samples of blood (20 ml) were taken in heparinized Vacutainers by jugular venipuncture on day 21 and 35 of each period between 1030 h and 1130 h. Hematocrit was determined and then blood was centrifuged and plasma was frozen for later analysis for urea nitrogen (BUN) and glucose by colorimetric procedures using a Kodak Ektachem DT 60 Analyzer with Disc Two Module (Clinical Products Division, Eastman Kodak Co., Rochester, NY).

3.2.5 Total Collections

For the last 5 d of each period, half of the cows on each treatment were confined to metabolism stalls for the total collection of urine and feces, and the measurement of individual water intakes. Foley bladder catheters (French 75, Rusch of Canada, Scarborough, ON) were used to collect urine. Flexible rubber tubing connected the catheter to a stainless steel container holding sufficient sulphuric acid (50%) to acidify the urine. Urine was sampled daily from each cow (250 ml), composited for each 5 d collection period and frozen at -10°C until further analysis. Kjeldahl N contents of the urine samples were determined after digestion on a block digester (Parkinson and Allen 1975; Fukumoto and Chang 1982). Feces were collected in large pans placed in the gutter behind the cows. Fecal material for each cow was removed from the pans every 4 h and placed in a covered garbage can. Daily fecal composites were mixed using a Hobart mixer, sampled (700 g) and frozen

at -10°C. Fecal samples were later dried in a forced air oven at 60°C until constant weight was achieved for the determination of DM. Dried fecal samples were then ground (1 mm screen), composited for each cow-treatment period, and stored. Samples were later analyzed for ADF, NDF and N.

Body weights for each cow were recorded on three consecutive days at the beginning of each period and at the end of the experiment, and means for individual cows were used to provide an estimate of change in body weight.

3.2.6 Efficiency Calculations

Energy expenditure, expressed in Mcal of net energy for lactation (NEL), was calculated as the sum of the energy output for milk, maintenance requirements, and body weight change. The energy value of milk was calculated using the following equation: energy (kcal NEL kg^{-1}) = 2.2[41.84(% milk fat) + 22.29(% milk solids not fat) − 25.28] (Tyrrell and Reid 1965). Maintenance requirement was assumed to be 73 kcal NEL kg^{-1} BW$^{0.75}$ with a 10% allowance for activity added (NRC 1989). The amount of energy spared or required respectively, was assumed to be 4.92 Mcal NEL kg^{-1} for BW loss and 5.12 Mcal NEL kg^{-1} of BW gain (NRC 1989). Efficiency of DM utilization was expressed in terms of the daily energy expenditure divided by DMI.

3.2.7 Statistical Analysis

Statistical analysis was via least squares ANOVA, following the GLM procedure of SAS (1990). Effects were considered to be significant at P<0.05 unless otherwise specified. The model used for this experiment was:

$Y_{ijkl} = \mu + \alpha_k + \tau_j + \beta_l + \gamma_{i(k)} + \varepsilon_{ijl(k)}$

μ = overall mean,

α_k = effect of square (k = 1,...,6),

τ_j = effect of treatment (j = 1,...,3),

β_l = effect of period, (l = 1,...,3)

$\gamma_{i(k)}$ = effect of cow within square, (i = 1,...,3)

$\varepsilon_{ijl(k)}$ = experimental error

3.3 RESULTS

3.3.1 Feed Composition

The DM content of U-CM from different batches, derived from different rail car shipments, was consistent at about 89% (Table 3.2). The addition of 2% water in HT-CM processing reduced the DM content on average by 2.98 percentage points, whereas the addition of LSO_3 in LSO_3-CM processing reduced the DM content on average by 1.88 percentage points. The use of steam to heat and maintain 100°C cooking temperature in the hydrothermal cooker further reduced the DM content on average by 1.73 and 3.24 percentage points for HT-CM and LSO_3-CM, respectively. The cooling process increased the DM content on average by 2.17 and 2.68 percentage points to yield the HT-CM and LSO_3-CM final products, respectively. However, the final DM content for both HT-CM and LSO_3-CM was less than that of the original untreated CM. The DM content of batch 3 of the HT-CM and to a lesser degree LSO_3-CM were too low, which resulted in the growth of mold. As a consequence none of the HT-CM batch 3 and only half of the LSO_3-CM batch 3 were fed during the lactation trial.

Heat in combination with LSO_3 (LSO_3-CM) increased NDF content to 33.4%, whereas heat alone (HT-CM) marginally increased the NDF to 25% from 24% in U-

CM (Table 3.3). The ADF levels were increased in LSO$_3$-CM and to a lesser degree in HT-CM over that in U-CM. ADIN levels were increased from 1.8% in U-CM to 2.0% in HT-CM and markedly increased to 4.1% in LSO$_3$-CM.

The 8 h rumen, intestinal and total tract disappearances of U-CM were similar to values reported by Moshtaghi Nia and Ingalls (1995). The rumen disappearance of HT-CM DM was not different than U-CM except at 8 h incubation where there was greater disappearance of HT-CM (50 and 59%, for U-CM and HT-CM, respectively) (Table 3.4). Other than no difference at 0 h, the disappearance of DM from LSO$_3$-CM was consistently lower than that of U-CM and HT-CM. While the rumen disappearance of CP followed the same trend as DM, the differences between LSO$_3$-CM and that of U-CM and HT-CM were markedly pronounced (Table 3.5). At all incubation times the rumen disappearance of LSO$_3$-CM CP was at least 45% below that of U-CM and HT-CM.

The total tract disappearances of both DM and CP following 8 h rumen incubation were not different across treatments (Table 3.6). LSO$_3$-CM had the greatest 8 h intestinal DM disappearance at 45% compared to 26% for U-CM and 18% for HT-CM (Table 3.6). As CP accounts for 35% of the DM content of CM, the 8 h disappearance value for LSO$_3$-CM was also greatest at 72% relative to the disappearance values of 36 and 26% for U-CM and HT-CM, respectively.

The CP levels of corn silage, grass silage and barley were 8.5, 11.7 and 10.5%, respectively (Table 3.7). Throughout the experiment the composition of each diet remained fairly constant, as reflected in the low standard errors for nutrient analysis (Table 3.8). The CP level in LSO$_3$-CM diet was 17.5%, which was greater

than HT-CM diet (17.2%), but not different than U-CM diet at 17.3%. The LSO$_3$-CM diet contained higher NDF at 34.4% compared to 32.5 and 33.2% for U-CM and HT-CM diets. ADF was greater in LSO$_3$-CM diet than in U-CM diet but not different from HT-CM diet (19.8, 20.3 and 20.8% for U-CM, HT-CM and LSO$_3$-CM diets, respectively). The higher fibre levels in LSO$_3$-CM diet reflect the higher fibre levels in the LSO$_3$-CM supplement.

3.3.2 Dietary Intakes and Milk Production and Composition

Reported DMI, milk production and milk composition results represent data collected during day 15-35 of each period. No significant differences were apparent within the first 14 d. The inclusion of LSO$_3$-CM treated CM in the diet increased DMI over cows fed diets supplemented with U-CM or HT-CM (Table 3.9). Milk production in cows fed LSO$_3$-CM diet was greater than those fed the U-CM diet, but not different from those fed the HT-CM diet (Table 3.9). The FCM production was higher in cows fed LSO$_3$-CM diet compared to HT-CM diet, but not different from the U-CM diet. The MUN level was lowered in cows fed the LSO$_3$-CM diet relative to those fed the U-CM and HT-CM diets. Dietary treatment had no impact on body weight, body weight change, milk composition and component yield or SCC.

3.3.3 Blood Composition

Hematocrit and blood glucose values were not influenced by treatment (Table 3.10). The values for BUN were lower at 16.7 mg dl^{-1} in cows fed LSO$_3$-CM diet compared to 18.6 and 18.2 mg dl^{-1} for cows fed U-CM and HT-CM supplemented diets.

3.3.4 Rumen Fluid

The pH of the rumen fluid was not affected by dietary treatment (Table 3.11). The rumen NH_4^+ N levels were lower for cows fed LSO_3-CM diet than those fed the U-CM diet, but were not different for those fed HT-CM diet. The molar proportion of acetate was higher in cows fed LSO_3-CM diet than those fed U-CM and HT-CM diet. Cows fed LSO_3-CM diet produced a lower proportion of propionate than cows fed HT-CM diet, but were not different from the U-CM diet. Molar proportions of branched chain fatty acids, isobutyrate and isovalerate, were lower in cows fed the LSO_3-CM diet. There was no apparent influence of dietary treatment on rumen butyrate or valerate concentrations. The ratios of acetate to propionate and acetate plus butyrate to propionate were higher in cows fed LSO_3-CM diet due to the higher acetate and lower propionate proportions.

3.3.5 Water Intake and Waste Excretion

Water intake and urine output were not affected by dietary treatment (Table 3.12). Cows fed LSO_3-CM diet had a 6% greater output of fecal DM than those fed U-CM diet, but were not different than HT-CM diet. This reflects the increased DMI of 7% in cows fed LSO_3-CM diet over that of U-CM diet.

3.3.6 Apparent Digestibility of Nutrients

The effect of diet on apparent digestibility of nutrients is given in Table 3.13. The apparent digestibility of CP was decreased in cows fed LSO_3-CM diet compared to cows fed U-CM or HT-CM diets (74, 73 and 71% for U-CM, HT-CM and LSO_3-CM, respectively). However, the apparent digestibility of NDF and ADF was increased in

cows fed LSO_3-CM diet over that of the U-CM and HT-CM diets. No differences were observed among treatment groups in the apparent digestibility of DM.

3.3.7 Nitrogen Balance

Nitrogen intakes were higher for cows fed the LSO_3-CM diet than for cows fed U-CM diet, but not different than those fed HT-CM diet (Table 3.14). This reflects the higher CP content of the LSO_3-CM diet relative to HT-CM (Table 3.8) and the increased DMI in cows fed LSO_3-CM over that of cows fed U-CM and HT-CM diets (Table 3.9). Fecal N excretion was greater in cows fed LSO_3-CM diet than U-CM and HT-CM diet. Conversely, urinary N excretion was lower for cows fed LSO_3-CM diet (0.227 kg d^{-1}) when compared to cows fed U-CM and HT-CM diets (0.258 and 0.258 kg d^{-1}, respectively). The excretion of N in milk was unaffected by dietary treatment. Cows fed LSO_3-CM diet retained more N than cows fed the U-CM diet, but was not different than those fed HT-CM diet.

3.3.8 Nitrogen Efficiency

The percentage of N intake excreted in feces (Table 3.15) was significantly greater for cows fed LSO_3-CM diet than those fed U-CM and HT-CM (26, 27 and 29%, for U-CM, HT-CM and LSO_3-CM diets, respectively). Conversely, the urinary excretion of N, as a percentage of N intake, was significantly reduced at 32% in the LSO_3-CM diet compared to 38 and 37% for U-CM and HT-CM diets, respectively. Cows fed LSO_3-CM diet tended ($P=0.06$) to retain greater amounts of N, as a percentage of N intake (10, 10 and 14% for U-CM, HT-CM and LSO_3-CM, respectively). Milk N excretion, as a percentage of dietary N, was not affected by treatment.

3.3.9 Energy Efficiency

Cows on LSO_3-CM diet tended (P=0.10) to gain weight faster at 0.337 kg d^{-1} than those fed U-CM and HT-CM at gains of 0.158 and 0.125 kg d^{-1}, respectively (Table 3.9). The output of energy for maintenance, milk production and body weight gain or loss was greater at 39.5 Mcal of NEL in cows fed the LSO_3-CM diet than those fed U-CM and HT-CM diets (37.1 and 36.9 Mcal of NEL, respectively) (Table 3.16). The energy efficiency of DM utilization by cows was unaffected by treatment.

3.4 DISCUSSION

This study was designed to evaluate the influence of the amount of supplementary RUP from untreated and treated CM on DMI, milk production and digestibility. Heat and LSO_3 treatments were designed to shift the digestion of the CM protein from the rumen to the small intestine through the enhancement of the Maillard browning reaction. During the processing of CM, the formation of primary Maillard reaction products resistant to rumen proteolysis was reflected in the elevated (39%) NDF levels and reduced rumen CP degradability in LSO_3-CM. Heat treatment alone produced few differences in levels of NDF, ADF and ADIN or in in situ degradability between HT-CM and U-CM. This suggests that the extent and rate of the Maillard reaction was not sufficient to alter digestion and thus marginal lactation responses to heat treatment would be anticipated. The significantly greater responses of CM to treatment in the present study relative to the initial in situ study (Chapter II) suggests the heat applied in the present study was longer than that in the first. In the present study, 120 min of heat treatment represented the length of time the CM was exposed

to 100°C. It did not include the time required for the temperature to reach 100°C which was included in the 120 min of heat treatment in the initial study.

The elevated ADF (22%) and associated ADIN in LSO₃-CM suggest that indigestible terminal Maillard reaction products were formed. This was not apparent in the in situ analysis of LSO₃-CM, as total tract disappearances of DM and CP were not significantly different in LSO₃-CM compared to that of U-CM. Similarly in the evaluation of heat treated soybean proteins Faldet et al. (1991) failed to observe any reductions in total tract N disappearance despite small increases in ADIN with extended heating. They concluded that the mobile nylon bag technique may be insensitive to small differences in intestinal digestibility. A small reduction in treated CM in vivo apparent CP digestibility and a corresponding increase in fecal N output as a percentage of N intake also suggest the formation of indigestible Maillard reaction products. Commercial application of this technology would require routine quality control analysis to ensure a consistent product of high quality. Replacement of the cooler used in the current study with a more efficient one would dry the final product more effectively and prevent the spoilage of the feed that was observed with the third processing batch.

The diets were formulated to be isonitrogenous such that the only differences between diets reflected the inclusion of either U-CM, HT-CM or LSO₃-CM. Analysis of the total mixed rations showed significant differences in CP content (range 17.2-17.5%); however the biological significance of these relatively small differences might be questionable. Supplementation with LSO₃-CM increased milk yield by 1.8 kg d⁻¹ which may have resulted from the increased amount of protein passing to the intestine or from a direct effect of the LSO₃-CM protein on microbial growth and rumen

digestion. Stabilizing ruminal fermentation, through improvement in the synchrony of protein and organic matter degradation in the rumen, can stimulate microbial growth and protein synthesis (Khorasani et al. 1994). High producing cows supplemented with RUP sources have shown increased flows of essential AA critical for milk protein synthesis to the small intestine resulting in increased milk and milk component production (Baker et al. 1996; Wright et al. 1998). However, other researchers (Lundquist et al. 1986; Keery and Amos 1993) reported no improvement in milk yield from cows fed diets containing SBM with low ruminal degradability compared with those fed untreated SBM. Numerical increases in milk composition and component yields of fat, protein, lactose and total solids for cows fed LSO_3-CM diet were not significant in the present study. This is in agreement with results of Oldham et al. (1985) and Khorasani et al. (1996) but differ from those of Erfle et al. (1983) and Sloan et al. (1988), who reported that increased dietary RUP reduced milk fat percentage and milk fat yield. An absence of RUP influence on milk protein and lactose concentrations was also observed by others (Erfle et al. 1983; Khorasani et al. 1996). In a study by Baker et al. (1995) in which diets were fed containing different levels of RDP and RUP no differences in CP content were observed. However, the milk from cows fed the higher levels of RUP had lower proportions of non-protein nitrogen and higher proportions of true protein.

While cows fed the LSO_3-CM diet produced more milk, they also had increased DMI of 1.8 kg d^{-1} over that of the control. There may be confounding effects of increased RUP and DMI on energy status that contributed to increased milk production. Cunningham et al. (1996) observed tendencies (P = 0.08) for increased milk and milk component yields for high RUP diets, however, the flow of essential AA

to the duodenum was unchanged relative to low RUP diets. The researchers attributed the increased yields to improved energy status of cows due to an 8.5% (not significant) increase in organic matter intake for cows on high RUP diets relative to low RUP diets. In the present study, higher DMI with LSO_3-CM supplementation may have improved the supply of metabolizable energy and accounted for the increase in milk yield.

Increased RUP in the diet results in less AA deamination and lowered ruminal NH_4^+ N concentrations. The 29% reduction in rumen NH_4^+ N concentrations in cows fed LSO_3-CM diet compared to U-CM diet is in agreement with the in situ results showing reduced ruminal degradation. The degradation of CM protein was shifted to the intestine as the intestinal disappearance of LSO_3-CM CP was 72%, a 100% increase over that of U-CM (36%). The reduction in BUN levels by 10%, to 16.7 mg dl^{-1} and MUN levels by 12%, to 13.9 mg dl^{-1} in cows fed LSO_3-CM diets relative to cows fed control diets is in agreement with results of Roseler et al. (1993) who fed increasing levels of RUP. The BUN and MUN levels for cows fed HT-CM while numerically lower, were not significantly different, confirming minimal rumen protection of the protein. To maximize organic matter digestion in the rumen Journet et al. (1983) stated rumen NH_4^+ N concentrations of greater than 8 to 15 mg dl^{-1} or corresponding BUN concentrations of greater than 8 to 10 mg dl^{-1} are required. Correspondingly, Wohlt et al. (1978) observed higher DM digestibility when NH_4^+ N levels were greater than 5 mg dl^{-1} compared with concentrations of less than 5 mg dl^{-1}. As urea concentration is a metabolic indicator of N wastage (Baker et al. 1992), the lowered BUN and MUN levels for cows fed the low RUP treatment supports a theory for higher efficiency of N utilization in cows fed LSO_3-CM.

Due to the solubility of urea, it appears that MUN behaves very similarly to BUN (Baker et al. 1992). However, urea levels go through peaks and valleys, depending on time of the last feeding and number of feedings, and whether or not forages and concentrates were fed separately or together as a TMR. Rumen NH_4^+ N levels have been reported to peak at 2 h (Rodriguez et al. 1997) or 1 h after feeding followed by a decline to a baseline value by 6 h after feeding (Gustafsson and Palmquist 1993). The BUN concentrations of high producing cows have been reported to peak 1.5-2.0 h after the rumen NH_4^+ N peak, at levels 70-85% higher than the lowest concentrations of BUN (Gustafsson and Palmquist 1993). Levels of MUN equilibrated with BUN after a lag of 1-2 h when the rate of BUN change was 3-6 mg dl^{-1} h^{-1} (Gustafsson and Palmquist 1993). In the present study, higher BUN values than MUN values were expected as BUN values reflect blood samples taken 2 h following feeding whereas MUN values reflect a composite of milk samples taken 2 h prior to the morning feeding and 0.5 h prior to the afternoon feeding. Despite differences in magnitude, blood and milk urea nitrogen levels showed the same trends across treatments and were both useful indicators of NH_4^+ loss from the rumen and reflected the efficiency of N utilization. Others (Roseler et al. 1993; Hof et al. 1997) have also suggested that MUN is closely related to the ratio of dietary protein to energy intake and a correct balance between RDP and energy intake is essential for the efficient synthesis of milk protein. Thus, high urea levels can either be absolute (just too much protein) or indirect (not enough available rumen energy to match the available RDP). As percentages of CP in milk are not adequate to assess the efficiency of diet formulation and BUN analysis is invasive and expensive, routine

analysis of MUN content could be useful to assess efficiency of protein feeding on dairy farms.

Molar proportions of acetate were higher and propionate were lower for cows fed LSO_3-CM compared to other treatments. In contrast, Cunningham et al. (1996) found the molar percentages of acetate, propionate, isobutyrate, isovalerate, and valerate were unaffected by increasing dietary RUP. In agreement with the present study, Veen (1986), Windschitl and Stern (1988a), and Arieli et al. (1996) reported increases in acetate and decreases in propionate concentrations when cows were fed diets of relatively low degradability. To explain the lowered propionate concentrations Veen (1986) suggested that under the influence of bacterial fermentation, proteins give rise to the formation of relatively more propionate, and therefore, when proteins are less degradable or fermentable, propionate concentrations are decreased.

With enhanced fibre digestion and increased proportions of acetate in cows fed the LSO_3-CM diet, a corresponding increase in butyrate was not observed. Other researchers (Veen 1986; Cunningham et al. 1996) have reported increased molar percentages of butyrate as dietary RUP increased, while Folman et al. (1981) reported a decrease. Valerate concentration was unaffected by dietary treatment in the present study, however in contrast, Windschitl and Stern (1988a) found valerate was reduced with the feeding of xylose or LSO_3 treated SBM.

A depression in branched chain VFA concentrations were observed in cows supplemented with LSO_3-CM compared to U-CM and HT-CM. In contrast, others (Windschitl and Stern 1987; Windschitl and Stern 1988a) found branched chain VFA concentrations were not affected when xylose or LSO_3 treated SBM were fed. Similar

to the present study, Veen (1986) and Windschitl and Stern (1988b) observed decreases in branch chained VFA when low degradable proteins were fed. The branched chain VFA depression may be attributed to the lower protein degradation found with LSO_3-CM, since isobutyrate and isovalerate are derived from the branched-chain AA, valine and leucine, respectively (Harwood and Canale-Parola 1981).

While numerically lower in animals fed the HT-CM and LSO_3-CM diets, the total VFA levels were not significantly different from cows fed the U-CM diet. Others (Folman et al. 1981; Veen 1986) have observed in vivo decreases in total VFA concentrations when low degradable proteins were fed. Stern (1984) and Windschitl and Stern (1988b) reported decreased cellulose and ADF digestion and lower total VFA flows from continuous culture fermenters when LSO_3 treated SBM was used in the diet. With high amounts of RUP, a shortage of RDP may limit the degradative activity of the rumen microbes, resulting in a combination of reduced intake, energy supply and protein supply (Tamminga 1992). Thus, it is important to include a source of RDP when high RUP supplements are fed, to ensure sufficient ruminally available N to meet the requirement for optimum growth of cellulolytic bacteria (Waltz et al. 1989; Wohlt et al. 1991). By infusing urea into culture fermenters, Windschitl and Stern (1988b) demonstrated that in vitro organic matter digestion could be enhanced for corn based diets containing protein of relatively low rumen degradability. In the lactation study by Cunningham et al. (1996), diets containing protected SBM in combination with urea supplementation appeared to provide sufficient RDP to meet the N needs of ruminal micro-organisms for protein synthesis. Other researchers (McCarthy et al. 1989; King et al. 1990; Keery and Amos 1993) have reported that the

source and amount of RUP in diets fed to lactating dairy cows appears to have only small effects on the digestion of fibre in the rumen and total tract.

The increased flow of digesta through the gastrointestinal tract resulting from the higher DMI of cows supplemented with LSO_3-CM would tend to decrease digestibility. However, cows fed LSO_3-CM had increased apparent digestibilities of NDF and ADF over that of the control and HT-CM diets. This suggests that the rapid rate and high degradability of protein in U-CM and HT-CM diets was not synchronized with the rate at which energy was generated for microbial growth. Hence, this resulted in uncoupled fermentation and inefficient protein utilization (Broderick et al. 1991). Additionally, the increased apparent fibre digestibilities of the LSO_3-CM diet suggests that NH_4^+ N levels were not limiting with regard to supporting optimal fibre digestion as they were in other studies (Stern 1984; Windschitl and Stern 1988b). This apparent inconsistency, between our results and those of others, on the influence of high RUP on VFA concentrations and fibre digestion may have been due to our use of barley versus corn. Barley is more rapidly and efficiently colonized by rumen microbes than is corn (McAllister et al. 1990), resulting in a more rapid digestion of barley protein and higher production of NH_4^+ with barley based than corn based diets. Additionally, the greater intake of NDF and ADF with the LSO_3-CM diet might have improved ruminal conditions for fibre fermentation, as reflected in the increased rumen concentrations of acetate.

Based on studies with fish meal, SBM and urea-casein, McAllan and Griffith (1987) suggested that ruminal fibre digestibility might be enhanced with proteins that are more slowly degraded in the rumen. Slowly fermentable proteins may result in a more gradual release of NH_4^+ N, peptides, and branched chain fatty acids; therefore,

these essential growth factors are available to the cellulolytic bacteria for an extended period of time after feeding (Veen 1986). While cows in this study had access to feed the majority of the time, lower fibre digestion with U-CM and HT-CM diets suggests uncoupled ruminal fermentation of carbohydrate and protein due to high RDP (McAllan and Griffith 1987). Treatment with LSO_3 may have ensured that the necessary N growth factors were available to the cellulolytic bacteria on a relatively constant basis. As NH_4^+ N, BUN and MUN levels of all three dietary groups were sufficiently high to indicate that all diets contained ample amounts of RDP and as milk protein concentrations were normal ($\geq$ 3.2%), cows in the present study probably consumed an adequate supply of ruminally fermentable energy. Had milk protein concentrations been lower ($\leq$ 3.2%), this would have indicated reduced bacterial crude protein supply caused by an inadequate supply of fermentable energy (Kaufmann 1982).

Fecal N losses result from the excretion of undigested feed N, undigested microbial N and endogenous N. The 17% increase in fecal N excretion for cows fed LSO_3-CM is unlikely to be due to differences in microbial N digestibility or endogenous N, but more likely due to feed N factors (Tamminga 1992). An increase in N intake of 6% and a reduced apparent N digestibility were probably the contributing factors. The reduction in apparent CP digestibility from 74 and 73% for U-CM and HT-CM to 71% with LSO_3-CM diet supports the fibre and ADIN levels observed for the respective diets and suggests that LSO_3-CM protein was overprotected to a small degree. The study of Windschitl and Stern (1988a) showed increased fecal N output due to a large reduction (77 to 71%) in total tract apparent N digestibility with the inclusion of LSO_3 treated SBM compared to untreated SBM. Cunningham et al.

(1996) reported no differences in apparent total tract N digestion between SBM based diets of low and high RUP and concluded that the treated SBM was not overprotected. It has been suggested by Owens and Bergen (1983) that a slight depression in total tract N digestion may be necessary in order 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 value of treated proteins. In agreement with Wright et al. (1998), efficiency for retained N, expressed as a percentage of N intake, tended (P=0.06) to increase with increased RUP levels.

Losses of N in urine originate from various sources including: rumen loss; the replacement of metabolic losses in the gut; the incorporation of dietary protein into microbial nucleic acids which are not available for metabolism by the cow; loss in maintenance; and loss caused by the inefficient conversion of absorbed AA into milk and body proteins (Tamminga 1992). Rumen loss can be decreased by reducing dietary CP, by reducing protein degradability, and by enhancing microbial protein synthesis by increasing the capture of rumen degraded protein. The greater the rumen degradability, the greater will be the rumen AA deamination yielding the highest N excesses, thus the observed higher urinary N losses with the control and HT-CM. The higher urinary N excretion may also be indicative of insufficient energy substrates available in the rumen for productive use of the N (Tamminga 1996; Wright et al. 1998). An improved balance between the quantity of carbohydrate and protein and improved synchrony between the rate at which they were degraded may have contributed to lower N losses from the rumen in cows fed the LSO_3-CM diet (Stern et al. 1994; Tamminga 1996). Similar to the present study, Wohlt et al. (1991) reported a decrease in urinary N, as a percentage of absorbed nitrogen, from 42% for

cows fed SBM to 38% for cows fed fish meal which is a less rumen degradable protein.

The improvement in N efficiency has important implications for dairy producers and the environment. Not only is excess dietary N expensive, there is the energy cost associated with conversion of ammonium to urea.

A considerable part of the excreted N in feces and urine, is lost by NH_3 volatilization, nitrate leaching or nitrous oxide emissions (Hof et al. 1997). These losses contribute to environmental deterioration through eutrophication of water, acidification of soil and depletion of the ozone layer (Tamminga 1996). Decreased N concentrations in the manure (feces and urine) from dairy cows fed diets lower in CP has been shown to result in decreased NH_3 emission from the manure and decreased N volatilization as a fraction of the excreted N (Paul et al. 1998). The primary source of NH_3 emissions has been shown to be the urine, with the feces contributing very low emissions (Paul et al. 1998). In the present study lower NH_3 emissions from the waste of cows fed LSO_3-CM diet would be expected, as the urinary N excretion was reduced 17%, even though fecal N excretion was increased 10%.

The increased energy expenditure of cows supplemented with LSO_3-CM reflects their increased milk production and their numerically higher BW gain and retention of N relative to cows fed control and HT-CM diets. In agreement with the study of Keery and Amos (1993) the energy efficiency was not affected by the level of UIP supplied by the different CM supplements.

Results of this study indicate that the degradation of CM, treated with 5% LSO_3 and heated at 100°C for 2 h in a hydrothermal cooker, was shifted from the rumen to the small intestine. This increased supply of RUP resulted in increased DMI, milk

production and efficiency of N utilization in cows supplemented with LSO₃-CM. The

lower rumen degradation of protein may have resulted in a better balance of protein

and carbohydrate breakdown that led to greater synchrony in the release of ruminally

degraded N and microbial N capture. The level of undegradable protein makes LSO₃-

CM an effective source of bypass protein for high producing cows.

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Table 3.1. Ingredient composition of the total mixed rations

Ingredient	Diet[1]		
	U-CM	HT-CM	LSO$_3$-CM
	(%, DM basis)		
corn silage	30.0	30.0	30.0
grass silage	20.0	20.0	20.0
barley	25.0	24.4	23.5
untreated canola meal	20.0	0	0
HT canola meal[2]	0	20.6	0
LSO$_3$ canola meal[3]	0	0	21.5
soybean meal	3.67	3.68	3.68
limestone	0.96	0.95	0.94
salt	0.25	0.25	0.25
mineral-vitamin premix[4]	0.10	0.10	0.10

[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal
[2]Canola meal with 2% added water hydrothermally cooked at 100°C for 120 min.
[3]Canola meal treated with 5% lignosulfonate and hydrothermally cooked at 100°C for 120 min
[4]Premix contained 40,000 mg kg^{-1} of Mn, 40,000 mg kg^{-1} of Zn, 16,000 mg kg^{-1} of Fe, 12,000 mg kg^{-1} of Cu, 640 mg kg^{-1} of I, 240 mg kg^{-1} of Se, 160 mg kg^{-1} of Co, 4,000 KIU kg^{-1} of vitamin A, 800 KIU kg^{-1} of vitamin D and 10 KIU kg^{-1} of vitamin E on a DM basis

Table 3.2. Changes in moisture content that occurred during processing of individual batches of treated canola meal cooked in a hydrothermal cooker for 120 min at 100°C

	DM (%)			
Batch	Untreated	Before cooking	After cooking	Final
	HT-CM[1]			
1	88.66	87.53	85.35	88.75
2	88.99	---[3]	85.31	87.13
3	89.02	84.08	81.69	82.84
4	88.48	85.83	83.95	86.27
Mean	88.79	85.81	84.08	86.25
	LSO$_3$-CM[2]			
1	88.66	87.01	84.46	86.72
2	88.99	87.09	83.41	85.99
3	89.02	86.69	82.56	85.70
4	88.48	86.85	84.26	87.00
Mean	88.79	86.91	83.67	86.35

[1]HT-CM = heat (100°C, 120 min) and water (2%) treated canola meal
[2]LSO$_3$-CM = heat (100°C, 120 min) and lignosulfonate (5%) treated canola meal
[3]missing data

Table 3.3. Chemical composition of individual batches of untreated canola meal and canola meal cooked in a hydrothermal cooker for 120 min at 100°C, with or without 5% LSO₃

Batch	DM (%)	CP (%)	NDF (%)	ADF (%)	ADIN (%)
			U-CM[1]		
1	88.66	34.8	24.7	19.0	1.8
2	88.99	37.2	22.8	17.0	1.8
3	89.02	36.2	22.9	17.4	1.9
4	88.48	35.0	25.6	19.0	1.8
Mean	88.79	35.8	24.0	18.1	1.8
			HT-CM[2]		
1	88.75	35.5	24.6	18.0	1.9
2	87.13	35.7	24.2	17.4	2.2
3	82.84	34.6	26.9	21.5	1.9
4	86.27	35.1	24.2	18.1	1.9
Mean	86.25	35.2	25.0	18.8	2.0
			LSO₃-CM[3]		
1	86.72	34.7	33.6	21.3	4.2
2	85.99	36.3	34.2	21.9	4.6
3	85.70	35.3	32.4	20.7	2.8
4	87.00	34.0	33.5	24.6	4.9
Mean	86.35	35.1	33.4	22.1	4.1

[1]U-CM = untreated canola meal
[2]HT-CM = heat (100°C, 120 min) and water (2%) treated canola meal
[3]LSO₃-CM = heat (100°C, 120 min) and lignosulfonate (5%) treated canola meal

Table 3.4. The influence of processing batch on the rumen DM disappearance (% of initial) of untreated and treated canola meal fed during the lactation trial (n=4)

Treatment Batch	Incubation time (h)					
	0	4	8	12	16	24
U-CM						
1	21.5	40.9	49.9	64.6	68.3	76.8
2	20.4	40.3	51.2	63.6	71.9	80.2
3	19.9	45.2	48.7	60.7	69.5	79.1
4	17.7	39.9	49.4	65.1	66.9	74.6
Mean	19.9	41.6a	49.8b	63.5a	69.2a	77.7a
HT-CM						
1	19.8	45.5	60.3	64.1	70.6	76.7
2	16.9	50.6	59.8	67.2	71.5	78.9
3	11.6	37.4	53.6	60.2	64.3	72.2
4	16.9	42.9	61.0	65.3	70.8	77.8
Mean	16.3	44.1a	58.6a	64.2a	69.3a	76.4a
LSO₃-CM						
1	15.2	24.8	30.6	39.8	49.5	55.5
2	14.3	23.2	30.2	42.3	44.1	54.9
3	16.8	28.1	34.6	47.6	53.8	59.4
4	15.7	24.7	27.9	40.7	45.8	55.5
Mean	15.5	25.2b	30.8c	42.6b	48.3b	56.3b
SE	1.1	1.8	1.3	1.4	1.7	1.3

a-c Means within columns with different letters differ significantly (P<0.05)

Table 3.5. The influence of processing batch on the rumen CP disappearance (% of initial) of untreated and treated canola meal fed during the lactation trial (n=4)

Treatment Batch	Incubation time (h)					
	0	4	8	12	16	24
	U-CM					
1	15.7	45.5	55.8	73.7	77.4	88.2
2	20.6	44.3	56.1	69.8	80.1	90.2
3	15.8	50.5	53.7	66.5	77.0	88.9
4	10.0	42.6	54.6	75.4	78.2	87.0
Mean	15.5a	45.7a	55.0b	71.3a	78.2a	88.6a
	HT-CM					
1	12.6	47.7	66.6	70.9	79.5	86.5
2	10.3	53.4	65.3	74.2	80.1	88.9
3	7.7	43.4	62.9	71.0	75.4	83.0
4	7.6	45.4	66.5	70.6	77.6	84.7
Mean	9.6b	47.5a	65.3a	71.7a	78.3a	85.8a
	LSO$_3$-CM					
1	0	8.8	13.3	22.5	33.1	41.5
2	1.1	10.2	18.1	27.1	30.2	42.3
3	2.7	23.7	29.0	44.7	50.4	54.6
4	1.2	7.0	11.5	25.1	31.5	42.1
Mean	1.3c	12.4b	18.0c	29.9b	36.3b	45.1b
SE	1.5	2.7	2.3	3.2	2.8	2.0

a-c Means within columns with different letters differ significantly (P<0.05)

Table 3.6. The intestinal and total tract DM and CP disappearance (% of initial) following 8 h rumen incubation of different processing batches of untreated and treated canola meal fed during the lactation trial (n=8)

Treatment Batch	DM disappearance (%)		CP disappearance (%)	
	Intestinal	Total tract	Intestinal	Total Tract
U-CM				
1	26.6	76.5	35.4	91.2
2	27.3	79.2	35.8	92.6
3	27.9	77.1	37.6	91.8
4	23.4	72.7	35.5	90.1
Mean	26.3b	76.4	36.1b	91.4
HT-CM				
1	16.5	76.2	25.7	90.9
2	19.5	79.5	28.2	92.4
3	17.4	73.4	25.8	90.6
4	17.5	79.0	25.5	92.0
Mean	17.7c	77.0	26.1c	91.5
LSO$_3$-CM				
1	47.1	77.7	77.4	90.7
2	46.0	76.2	71.4	89.5
3	39.4	74.0	61.4	90.3
4	45.7	73.6	77.7	89.1
Mean	44.6a	75.4	72.0a	89.9
SE	1.2	1.3	2.2	0.4

a-c Means within columns with different letters differ significantly (P<0.05)

Table 3.7. Chemical composition of major diet components

Nutrient	corn silage	grass silage	barley
	(%, DM basis)		
DM	36.88	30.30	90.25
CP	8.49	11.73	10.46
NDF	39.19	61.73	16.01
ADF	22.61	37.54	5.76

Table 3.8. Chemical composition of the total mixed rations

| Nutrient | n | Diet[1] | | | SE |
		U-CM	HT-CM	LSO$_3$-CM	
		(%, DM basis)			
CP	19	17.3[ab]	17.2[a]	17.5[b]	0.1
NDF	19	32.5[a]	33.2[a]	34.4[b]	0.4
ADF	19	19.8[a]	20.3[ab]	20.8[b]	0.2

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat (100°C, 120 min) and water (2%) treated canola meal, and LSO$_3$-CM = heat (100°C, 120 min) and lignosulfonate (5%) treated canola meal

Table 3.9. The effects of diets supplemented with different canola meal treatments on DMI, body weight, milk yield and milk composition

		Diet[1]			
	n	U-CM	HT-CM	LSO$_3$-CM	SE
DMI					
kg d^{-1}	53	24.6^b	25.1^b	26.4^a	0.2
% of BW	52	3.8	3.8	3.9	<0.1
Body wt					
kg	52	665.2	666.4	672.9	3.4
gain, kg d^{-1}	51	0.158	0.125	0.337	0.093
Milk, kg d^{-1}	52	34.8^b	35.3^{ab}	36.6^a	0.6
4% FCM, kg d^{-1}	52	33.8^{ab}	33.4^a	35.2^b	0.6
Milk compositon, %					
Fat	53	3.80	3.68	3.82	0.06
Protein	53	3.25	3.28	3.31	0.04
Lactose	53	4.41	4.50	4.54	0.05
Total solids	53	12.70	12.69	12.92	0.12
Component Yield, kg d^{-1}					
Fat	53	1.33	1.27	1.36	0.03
Protein	53	1.14	1.14	1.18	0.02
Lactose	53	1.56	1.57	1.63	0.03
Total solids	53	4.47	4.42	4.62	0.09
Milk SCC, x 10^3 ml^{-1}	53	223	229	372	105
MUN, mg dl^{-1}	53	15.68^a	15.34^a	13.86^b	0.27

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal

Table 3.10. The effects of diets supplemented with different canola meal treatments on blood composition

| | n | Diet[1] | | | SE |
		U-CM	HT-CM	LSO$_3$-CM	
Hematocrit, %	53	31.1	30.6	31.1	0.3
Blood glucose, mg dl^{-1}	53	68.2	70.2	69.1	0.9
BUN, mg dl^{-1}	53	18.6[a]	18.2[a]	16.7[b]	0.3

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal

Table 3.11. The effects of diets supplemented with different canola meal treatments on ruminal pH, ammonium nitrogen (NH_4^+ N) and VFA concentrations

| | n | Diet[1] | | | SE |
		U-CM	HT-CM	LSO_3-CM	
pH	53	6.79	6.83	6.84	0.03
NH_4^+ N, mg dl^{-1}	53	11.24^a	9.45^{ab}	8.00^b	0.73
Total VFA, mM	53	100.6	95.7	95.1	2.6
VFA, mol 100mol^{-1}					
Acetate (A)	53	59.5^b	59.4^b	60.4^a	0.2
Propionate (P)	53	22.1^a	22.2^a	21.3^b	0.3
Isobutyrate	53	1.07^a	1.06^a	0.95^b	0.01
Butyrate (B)	53	12.7	12.6	12.8	0.1
Isovalerate	53	2.32^a	2.30^a	2.10^b	0.04
Valerate	53	1.85	1.87	1.79	0.03
Caproate	53	0.50^b	0.53^b	0.64^a	0.03
A:P	53	2.73^b	2.70^b	2.88^a	0.04
A+B:P	53	3.32^b	3.28^b	3.49^a	0.05

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO_3-CM = heat and lignosulfonate treated canola meal

Table 3.12. The effects of diets supplemented with different canola meal treatments on the water intake and urine and fecal outputs by cows

Measurement	n	Diet[1] U-CM	HT-CM	LSO$_3$-CM	SE
Water intake, l d^{-1}	15	65.62	68.87	66.55	5.26
Urine output, kg d^{-1}	27	18.84	19.63	18.92	0.47
Fecal output, kg of DM d^{-1}	27	7.38[a]	7.78[ab]	7.89[b]	0.14

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal

Table 3.13. The apparent digestibilities of diets supplemented with different canola meal sources

| Apparent digestibility | n | Diet[1] | | | SE |
		U-CM	HT-CM	LSO$_3$-CM	
		(%, DM basis)			
DM	27	69.6	69.1	69.3	0.4
CP	27	73.6[a]	73.0[a]	70.9[b]	0.4
NDF	27	50.1[b]	50.9[b]	54.0[a]	0.6
ADF	27	45.2[b]	45.0[b]	48.3[a]	0.9

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal

Table 3.14. Nitrogen balance measurements for cows fed three different canola meal treatments

| Measurement | n | Diet[1] | | | SE |
		U-CM	HT-CM	LSO$_3$-CM	
		(kg d^{-1})			
N intake	27	0.68^a	0.70^{ab}	0.72^b	0.01
Fecal N	27	0.179^b	0.189^b	0.209^a	0.004
Urinary N	27	0.258^a	0.258^a	0.227^b	0.008
Milk N	27	0.174	0.181	0.182	0.004
Retained N[2]	27	0.065^a	0.071^{ab}	0.102^b	0.011

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal
[2]Retained N = Intake N – (fecal N + urinary N + milk N)

Table 3.15. The utilization of N, as a percentage of N intake, in cows fed different canola meal sources

Measurement	n	Diet[1]			SE
		U-CM	HT-CM	LSO$_3$-CM	
		(% of N intake, DM basis)			
Fecal N	27	26.4[b]	27.0[b]	29.1[a]	0.4
Urinary N	27	38.3[a]	36.9[a]	31.6[b]	1.1
Milk N	27	25.8	25.9	25.4	0.9
Retained N[2]	27	9.6	10.1	13.8	1.5

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal
[2]Retained N = 100((Intake N − (fecal N + urinary N + milk N)) / Intake N)

Table 3.16. The effect of canola meal treatment on energy expenditure and efficiency

		Diet[1]			
	n	U-CM	HT-CM	LSO$_3$-CM	SE
Energy output[2], Mcal of NEL	50	37.1b	36.9b	39.5a	0.7
Efficiency, Mcal NEL kg^{-1} DMI	50	1.51	1.47	1.49	0.03

[a,b] Means within rows with different letters differ significantly (P<0.05)
[1]U-CM = untreated canola meal, HT-CM = heat and water treated canola meal, and LSO$_3$-CM = heat and lignosulfonate treated canola meal
[2]Energy output = NEL for maintenance, milk yield, and BW change

IV. GENERAL CONCLUSIONS

In the first experiment the rumen protein degradability of untreated CM was moderately decreased by heating and substantially decreased by LSO_3 plus heat treatment. Treatment of CM with 5% LSO_3 followed by heating at 100°C for 2 h was the most effective at decreasing rumen microbial degradation and increasing the amount of CP and DM delivered to the intestines. In the second experiment cows supplemented with 5% LSO_3 plus heat (100°C, 120 min) treated CM had increased milk production and showed increased efficiencies of N utilization which were reflected in lowered rumen NH_4^+ N levels, circulating urea N levels and urinary N excretion, as a percentage of intake. Dietary N supplied to the rumen in excess of microbial needs is wasted as urea and reduces the efficiency of N utilization for product formation. Thus, the formulation of rations with slowly degraded proteins will improve utilization of dietary N by balancing carbohydrate breakdown with the rate of crude protein breakdown (Tamminga 1992). With modest changes in processing methods the hydrothermal cooking of LSO_3 treated CM has the potential to produce a commercially viable RUP source for lactating cows. In addition to the direct benefits measured, the formulation of diets with LSO_3-CM also has positive implications for health, fertility and reduced environmental impact.