THE EFFECTS OF MOISTURE, LIGNOSULFONATE, ALCOHOL AND HEAT TREATMENTS ON CANOLA MEAL PROTEIN DEGRADATION AND DIGESTION IN RUMINANTS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Yuqun Wu

In Partial Fulfilment of the

Requirements for the Degree

of

Doctor of Philosophy

Department of Animal Science

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

THE EFFECTS OF MOISTURE, LIGNOSULFONATE, ALCOHOL AND HEAT TREATMENTS ON CANOLA MEAL PROTEIN DEGRADATION AND DIGESTION IN RUMINANTS

BY

YUQUN WU

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree

of

DOCTOR OF PHILOSOPHY

ABSTRACT

Wu, Yuqun. Ph.D., The University of Manitoba, May, 1998. The Effects of Moisture, Lignosulfonate, Alcohol and Heat Treatments on Canola Meal Protein Degradation and Digestion in Ruminants. Major Professor: J. R. Ingalls.

Four experiments were conducted to investigate the methods for protecting canola meal (CM) protein from ruminal degradation. Canola meal was moist heat treated at 110°C for 60 min. The treated and untreated CMs were used to formulate a treatment and a control diet which were fed to two groups of lambs. The plasma total amino acid (AA) concentration was increased and weight gain efficiency was improved in lambs fed treatment diet compared to control. The CM lignosulfonate (LS), water, alcohol and heat treatments were tested in a 4x3x2x4 factorial design. The increasing LS level increased digestible undegradable intake protein (DUIP) of CM protein by 41% with 6.5 % LS added. Three temperature and time levels were not different in improving the DUIP in CM in the presence of 4.0 and 6.5% of LS. Generally addition of water and alcohol did not improve the DUIP in CM. Canola meal was heat treated with or without LS on an industrial scale resulting in four CM treatments, i.e. untreated, or heated to 129°C with 6.5% LS, 154°C with or without 6.5% LS, and held for 32 min. These four CM treatments were tested with four cannulated heifers in a 4 x 4 Latin square design. Canola meal heated to 154°C with LS had a lower in situ effective degradability of protein compared to the control. These four CM treatments were further studied in the same four heifers during their first lactation over four periods in a 4 x 4 Latin

square design. The in vivo undegradable intake nitrogen was increased in the diet containing CM heated to 154°C with LS compared to those obtained with the control diet and the diet containing CM treated to 129°C with LS. The in vivo apparent digestibility of dietary N and DM in the lower and total GI tract was unaltered by the inclusion of treated CM in the diet. Heating to 154°C with LS and held for 32 min thus was sufficient to reduce the ruminal degradability without damaging the apparent digestibility of CM protein, and improve the nutritive value of CM to the dairy cows.

Key words: Canola meal, Protein protection, Heat, Moist, Lignosulfonate, Alcohol, Milk, Lamb, Cow.

DEDICATION

To my parents

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor Dr. J. R. Ingalls for his professional guidance, kind support and great patience throughout my Ph.D. program. I also wish to thank my committee members, Drs. R. R. Marquardt, Dr. M. Scanlon (Department of Food Science) S. Venkataram and K. M. Wittenberg for their suggestions and encouragements during the program. My sincere gratitude also goes to the external examiners Dr. G. W. Mathison (University of Alberta) for examining the thesis.

I am very grateful to Dr. G. H. Crow for his kind advice in statistical analysis, Dr. L Onischuk for her technical support in statistics and computer usage. Dr. N. E. Stanger for the surgical implantation of cannulas and Terri Garner for sample and data collection. Thanks also go to P. Mills for the AA and VFA analysis, P. Charles and A. H. Tarr for sample analysis.

I wish to thank the staff at the Animal Science Research Unit and Glenlea Dairy Research Unit for their support and cooperation during the trial, LignoTech USA, Inc., WI, USA for the sponsoring of lignosulfonate, and Sureleen-Albion Agra Inc. West, Airdrie, AB for the sponsoring of canola meal and the processing. My appreciation also goes to the academic and administrative staff in the Department of Animal Science for their kind support and understanding, and to my fellow graduate students in the department for their kind help and warm friendship.

FOREWORD

The studies comprised in this thesis have resulted the following publication:

Wu, Y. and Ingalls, J. R. 1994. The effects of lignosulfonate and heat treatments on the rumen degradation and post-rumen digestion of canola meal. J. Anim. Sci. 74 (suppl.):274 (Abstr.).

The manuscripts from these studies will be submitted for publications as follows:

1. Wu, Y. and Ingalls, J. R. 1998. The effect of moist heat treated canola meal on lamb growth. Animal Feed Science and Technology.

2. Wu, Y. and Ingalls, J. R. 1998. The effects of lignosulfonate, water, alcohol and heat treatment on the rumen degradation and post-rumen enzyme digestion of canola meal protein. Canadian Journal of Animal Science.

3. Wu, Y. and Ingalls, J. R. 1998. Effect of lignosulfonate and heat treatment on canola meal protein degradation in the rumen, post-rumen digestion and production. Journal of Dairy Science.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AA	-Amino acid
ADF	-Acid detergent fiber
ADIN	-Acid detergent insoluble nitrogen
ALC	-Alcohol
AO	-*Aspergillius oryzae*
C	-Control
CM	-Canola meal
CP	-Crude protein
DE	-Digestible energy
DIP	-Degradable intake protein
DM	-Dry matter
DMI	-Dry matter intake
DUIP	-Digestible undegradable intake protein
EAA	-Essential amino acid
ED	-Effective degradability
FAA	-Free amino acid
GI	-Gastrointestinal
LS	-Lignosulfonate
MNB	-Mobile nylon bag
MUN	-Milk urea nitrogen
n	-Number of observation
N	-Nitrogen
NAN	-Non-ammonia nitrogen
NBD	-Nylon bag disappearance
NDF	-Neutral detergent fiber
NDIN	-Neutral detergent insoluble nitrogen
NE(L)	-Net energy (for lactation)
NEAA	-Non-essential amino acid
NH_3	-Ammonia
NPN	-Non-protein nitrogen
p	-probability
PPD	-Pepsin and pancreatin digestibility
PUN	-Plasma urea nitrogen
r	-Correlation coefficient
SAA	-Short-peptide form amino acid
SBM	-Soybean meal
SC	-*Saccharomyces cerevisiae*
SEM	-Standard error of means
SNF	-Solid non-fat
T	-Treatment

TAA	-Total amino acid
TCA	-Trichloroacetic acid
TDN	-Total digestible nutrient
TP	-True protein
TT	-Temperature and time
UIAA	-Undegradable intake amino acid
UIN	-Undegradable intake nitrogen
UIP	-Undergraded (undegradable) intake protein
VFA	-Volatile fatty acid
W	-Water
WG	-Weight gain
WoG	-Wool growth
YC	-Yeast culture

INTRODUCTION

Diets consumed by ruminants are exposed to the fermentation activities of microbes in the rumen prior to gastric and intestinal digestion. The microbial biomass in the rumen is made up largely of bacteria, protozoa and fungi. Bacteria are usually predominant in the rumen. Ciliate protozoa may represent up to 40% of the rumen microbial nitrogen (N) when conditions are ideal for them, and fungi represent up to 8% of the total biomass (Nolan 1989). Many species of bacteria and protozoa display proteolytic activities, in addition to their cellulolytic activities. Bacteria in the rumen locate in three phases, i.e. they attach to the rumen wall or attach to the solids in the rumen or stay free in rumen fluid. Those bacteria attached to the rumen solids and in fluid play a more important role in proteolysis than those attached to the rumen wall (Broderick et al. 1991). Protozoa are predators to the rumen bacteria and are probably of minor importance to dietary proteolysis (Wallace 1988), although they do engulf small dietary particles of appropriate size. Fungi also show proteolytic activities, but their ecological role is less known (Wallace 1988).

Dietary nitrogenous compounds and polysaccharides are degraded extensively by ruminal micro-organisms to produce ammonia (NH_3) and energy for growth. The host animal in turn uses the degradation end products, the undegraded dietary nutrients and the microbes which leave the rumen as energy and protein or amino acid (AA) sources. The NH_3 produced in the rumen can be reused by microbes either directly (Bryan 1974) or recycled via the liver (Huntington et al. 1995) or lost through lactation (Clark et al. 1978) and/or urination (Nolan

and Leng 1972).

To improve the efficiency of the protein utilization in ruminants, high quality dietary protein sources such as soybean meal (SBM) and canola meal (CM) have been treated in many ways to limit protein loss from ruminal degradation and thus increase the amount of dietary protein available for post-rumen digestion (Broderick et al. 1991). The objectives of this study were to investigate the effects of the moisture, lignosulfonate (LS), alcohol and heat treatment on the rumen degradation and the post-rumen digestion of CM protein when fed to ruminants.

LITERATURE REVIEW

RUMEN METABOLISM OF NITROGENOUS DIETARY COMPOUNDS

Protein Degradation

Most of the ingested protein entering the rumen is degraded to NH_3 and volatile fatty acids (VFAs, such as acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and 2-methylbutyric acid). Microbial activities include protein hydrolysis, peptide degradation, AA deamination, and fermentation of the resultant carbon skeletons.

Hydrolysis of dietary protein by microbial protease into oligopeptides is the initial step. Rumen microbe proteolytic activity has an optimum pH in a broad neutral range. Cysteine proteases are major contribution to proteolytic activity with large contributions from serine- and metallo- protease (Mackie and White 1990). Bacteria producing these proteases comprise about 38% of the total bacterial population in the rumen (Fulghum and Moore 1963).

Proteolytic enzymes of ruminal bacteria appear to be predominantly cell associated, as little proteolytic activity can be detected in cell-free rumen fluid (Brock et al. 1982). Bacteria have both cell surface-bonded and intracellular peptidase activity. The first step in the breakdown of dietary protein by bacteria is adsorption, either soluble protein is adsorbed to the bacterial

surface (Wallace 1988) or bacteria are adsorbed to the insoluble protein particle. Susceptibility of different proteins to hydrolysis may correspond to their relative adsorption affinities. Protozoa engulf bacteria and dietary particles of small size and carry on further intra-cellular proteolysis.

Protozoal protease is mainly of the cysteine type but the aspartic activity is also significant (Forsberg et al. 1984). Proteolytic, peptidolytic and deamination activities of ciliate protozoa and, probably to a lesser extent, anaerobic fungi are considered less important than bacteria, however, the removal of ciliate protozoa can still cause a significant decline in proteolysis of dietary protein in the rumen (Broderick et al. 1991).

The resulting oligopeptides can be absorbed directly into the cell or be hydrolysed into small peptides by peptidase on the cell surface and then be absorbed (Cotta and Hespell 1986). The absorbed peptides will be hydrolyzed into AAs by intra-cellular peptidase. The resulting AAs are deaminated to produce NH_3 and VFAs (Cotta and Hespell 1986). Bacteria can use short chain peptides, AAs and NH_3 for their own protein synthesis, but peptides are more effectively incorporated into bacterial protein (Mackie and White 1990) and a greater proportion of AAs undergo rapid deamination (Wright 1967). The importance of deamination of AAs may exist in the fact that the branched-chain fatty acids resulting from the deamination and degradation of specific AAs such as leucine, isoleucine and valine are either required or highly stipulatory to the growth of many ruminal bacteria, particularly the fibrolytic species (Bryant and Robinson 1963; Mackie and White 1990). Ciliate protozoa

seem to have a significant role in deamination. Most species of protozoa produce NH_3 from the intra-cellular hydrolysis of protein or AA. Specific deamination activities of mixed protozoa were found to be about three times those of bacteria (Hino and Russell 1985). Excess NH_3 and VFAs will be diffused into the rumen from microbial cells.

Reticulum function is similar to that of the rumen and thus is considered a part of the rumen in this discussion. The omasum's function in metabolism of nitrogenous products is less known (Asplund 1994), although its environment is favourable for microbial growth (Smith 1989). Some evidence suggested that the rumen and omasum may be an important site for peptide absorption throughout the length of the ruminant gastrointestinal tract (Webb et al. 1992).

The undegradable dietary protein, synthesised microbial protein and non-protein N (NPN) or the residue of degraded protein will be turned over to the abomasum for host animal digestion. Quantity of N flowing out of the rumen will depend on the dietary protein or N intake, the degradability of dietary protein and the production of microbial protein in rumen.

Utilization of NPN Compounds

Non-protein N can appear as a form of nucleic acids, nitrate, ethanolamine or choline from natural feedstuffs (Wallace 1988) or as NH_3, urea or slow NH_3-release compounds such as biuret, isobutylidene diurea and lactosy urea from supplements (Smith 1989). The nucleic

acids comprise about 5.2 - 9.5% of total N in grasses and hay (Smith and McAllan 1970) and about 20% of rumen bacterial N (NRC 1985b). The nucleic acids are rapidly hydrolysed in the rumen and degradation products of ribonucleic acid and deoxyribonucleic acid introduced into the rumen were readily identifiable as pyrimidine nucleosides, uracil, hyproxanthine, xanthine, guanosine and thymine (McAllan and Smith 1973). These products disappear completely after 4 h in the rumen (McAllan and Smith 1973) and are presumably degraded and/or incorporated by microorganisms (Wallace and Cotta 1988). Nitrate is quite abundant in some plant materials (Wallace and Cotta 1988). Nitrates are reduced in the rumen to nitrites, and then can be reduced to hydroxylamine and to NH_3 (Shirley 1986). Choline is essential for the growth of the protozoan *Entodinium caudatum* and is rapidly incorporated into phospholipid (Broad and Dawson 1975). However the main fate of choline in the mixed microbial population is to be converted into trimethylamine which in turn is converted into methane by methanogen bacteria (Wallace and Cotta 1988).

Urea is broken down extremely rapidly in the rumen to release NH_3. Urease activity is greatest within the stratified layers of rumen epithelium and has been shown to be associated with the adherent, facultatively anaerobic and strongly proteolytic bacterial population which colonizes this tissue (Whitelaw et al. 1991). The urease activity in normal rumen contents is thought to result mainly from the sloughing of heavily-colonized epithelial cells (Wallace and Cotta 1988). Numerous populations of strict anaerobes in the rumen contents in general have low urease activity, whereas a much smaller population of atypical, facultative anaerobes are characterized with high specific urease activity (Hobson and Wallace 1982). The organisms

residing on the rumen wall may be most important in the hydrolysis of urea transferred across the rumen wall, while the organisms in the fluid may be important in the hydrolysis of dietary and salivary urea (Wallace and Cotta 1988). The degradation of biuret, isobutylidene diurea and lactosy urea are slower than that of urea in the rumen (Smith 1989). The NPN substances that have ionic bonds, such as ammonium sulfate, ammonium phosphate and ammonium chloride, will dissociate in water to release ammonium ions without enzymatic activity (Shirley 1986).

Most species of bacteria can use NH_3 as their source of N for growth and in fact, NH_3 is essential for the growth of many species (Bryant 1974). Efforts have been made to determine the optimal rumen NH_3 level for maximum microbial growth or rumen fermentation. Allen and Miller (1976) reported that abomasum non-ammonia N (NAN) flow was maximized when NH_3 concentration reached 16 mg/dl for wethers fed a low crude protein diet supplemented with urea. Mehrez et al. (1977) found that the minimal NH_3 N concentration for maximal rate of barley fermentation (nylon bag DM disappearance) was 19 mg/dl for sheep. Roffler et al. (1975) reported that the total uptake of NH_3 by rumen microorganisms remained static and was unaffected by the change in concentration of NH_3 N over 5 mg/dl. Russell and Strobel (1987) also showed that 4 mg/dl of NH_3 N was required for maximal microbial protein synthesis when mixed ruminal bacteria were provided with growth rate limiting amounts of mixed carbohydrates. Russell and Strobel (1987), however, suggested that the bacterial growth rate in their experiment was possibly far too low (0.09/h) compared to rate in the experiment of Mehrez et al. (1977). Odle and Schaefer (1987) point out that the optimal NH_3

N concentration required to maximize the rate of grain digestion in the rumen might be influenced by the chemical or structural characteristics of the grain. Carbohydrates and proteins are the major nutrients supporting microbial growth, and the diet of a lactating cow can vary considerably in the quantity and composition of both nutrient sources (Hoover and Stokes 1991). Based on data from in vitro and in vivo studies, Hoover and Stokes (1991) concluded that the rate of digestion of carbohydrates is the major factor controlling the energy available for microbial growth. Protein affects both total fermentation and production of microbial DM per unit of carbohydrate fermented. Maximum DM digestion, microbial protein synthesis efficiency and microbial protein yield were achieved when the diets contained 10 to 14% degradable intake protein and 56% of total carbohydrate as non-structural carbohydrate.

The rumen NH_3 which resulted from the degradation of dietary or microbial true protein or of NPN can be absorbed into the rumen wall and enter the bloodstream (Nolan and Leng 1972). The NH_3 concentrations in portal blood tend to parallel rumen NH_3 concentrations (Bartley et al. 1981). The NH_3 in blood may also be derived from the deamination of AAs in the tissues (Nolan and Leng 1972). Liver synthesises urea from blood NH_3 and released the urea into the blood stream (Krebs and Henseleit 1932). The kidney might also produce small amounts of urea (Huntington et al. 1996). Nolan and Leng (1972) reported that urea was synthesized in the body (sheep) at a rate of 18.4 g N/d from 2 g N/d of NH_3 absorbed through the rumen wall and 16.4 g N/d apparently arising from other NH_3 sources. It is noteworthy that many factors may affect the urea concentration in blood. Huntington et al. (1996) showed that total urea removed from blood increased as the level of dietary

concentrate increased for beef steers. Blood urea N was positively associated with intakes of ruminally degradable and undegradable protein and negatively associated with the intake of net energy (DePeters and Ferguson 1992; Roseler et al. 1993). A large part of blood NH_3 can be derived from the deamination of AAs in body tissue (Bruckental et al. 1980). The urea N level in blood can be affected by the quality and quantity of protein and AAs absorbed from the intestine (DePeters and Ferguson 1992) and the nutritional and physiological status of animals (James and Olson 1995).

About 46 to 91% of endogenous production of urea can be recycled to the lumen of the gut (Norton et al. 1979; Kennedy 1980) and the principal sites of recycling of urea are saliva, ruminal and post-ruminal (Nolan and Leng 1972). Urea transfer from blood to rumen occurred rapidly by simple diffusion and the urease activity in the rumen is able to hydrolyse all urea presented (Whitelaw 1991). Presumably, this urea was also recycled to saliva and to the lower gut through diffusion. The amount and proportion of urea recycling respond to a variety of dietary factors which include N intake, degradability of dietary N in the rumen, forage type, hay : grain ratio and fermentability of carbohydrate in the rumen (Huntington et al. 1996).

Urea can be excreted out of the body in many ways. A substantial amount of urea is sequestered by the kidney for excretion in urine (Nolan and Leng 1972). Some urea recycling to the lower gut from blood can be lost in a form of microbial protein through the feces excretion after the urea is incorporated into microorganisms (Nolan and Leng 1972; Nolan

et al. 1976). Urea is also secreted into the milk through passive transfer from blood (Clark et al. 1978). The concentration of milk urea N is highly correlated (r = 0.89) to the concentration of blood urea N (Roseler et al. 1993). Factors affecting the concentration and flux of blood urea N will also affect the milk urea N (DePeters and Ferguson 1992). Milk urea N data should be interpreted with caution in relating to the dietary nutrient supplies and the body conditions but can give some clues as to nutrient balance in dairy cow diets.

DETERMINATION OF RUMEN DEGRADABILITY OF DIETARY PROTEIN

A great deal of effort has been directed towards developing methods for determination of the protein degradability of feed ingredients. The objectives of developing these methods are to provide accurate data for ruminant diet formulation and to compare different processing treatments for ruminal protein protection. Three types of methods, i. e. in vivo, in situ and in vitro measurements have been used in ruminal protein degradability determinations.

In Vivo Measurement

In vivo measurement serves as a standard for evaluating the efficacy of other methods in estimation of protein degradabilities (NRC 1985b). Animals cannulated in the rumen and abomasum or proximal duodenum are used to make measurements. The amount and composition of digesta flow are measured. Two types of duodenal cannulas have been used, i.e. re-entrant and simple "T" types. Re-entrant cannulas offer the advantage of the

conduction of total digesta collections, thus eliminating the need for digesta phase markers (Nocek 1988) and providing the most representative digesta sample. "T" cannulas are more physiological and give less disturbance to the digestive tract (Nocek 1988) and require less labour and effort to care for animals. Thus at present "T" cannulas are used most often in digestive studies for in vivo measurements.

Markers used in measuring digesta flow from the rumen to small intestine were reviewed extensively (Owens and Hanson 1992). There are two types of markers, i. e. the internal markers which are the components of feedstuffs and the external markers which are inert compounds added to diets. Markers can be administered either as a single pulse-dose, or can be provided constantly (or frequently) for a period of days (Owen and Hanson 1992). Pulse-dosing typically is used to estimate digesta volume and retention time, while continuous dosing is used primarily to measure instantaneous flow at a specific point in the digestive tract. Chromic oxide powder has been used frequently and successfully in several recent studies (Zinn 1993; Demjanec et al. 1995; Overton et al. 1995; Streeter and Marthis 1995). Both curve-peeling (linear regression) and nonlinear regression methods can be equally useful for evaluating passage kinetics of grain in dairy cows (Poore et al. 1991). The sampling site, i. e. duodenum or rectum, and the dosing time of markers have little effect on passage parameter estimates (Poore et al. 1991).

Microbial flow can be determined by employing microbial markers. Broderick and Merchen (1992) reviewed the possible markers for quantification of microbial protein synthesis in the

rumen. They recommended the use of the total purine and/or ^{15}N techniques with either continuous intraruminal infusion or discontinuous dosing via feed. The total purine isolation-quantification procedure described by Zinn and Owens (1986) has been widely accepted for microbial protein quantification in duodenal digesta (Zinn 1993; Murphy et al. 1994; Demjanec et al. 1995; Overton et al. 1995; Streeter and Marthis 1995). It is noteworthy, however, that the contribution of protozoal protein would be underestimated if total microbial crude protein flow were computed based on the bacterial purine : N ratio. That is because the protozoal purine : N ratio is about half of bacterial ratio (0.44 : 1 vs. 0.90 : 1, Broderick and Merchen 1992).

There are two common methods available for determining rumen protein degradability in vivo, i.e. incremental and difference methods (Nocek 1988). In the incremental method, degradability of a given protein source is measured based on the increase in flow of protein to the small intestine in response to incremental additions of the protein source to the basal diet. For the difference method, the quantity of undegraded intake protein (UIP) or AAs in the diet are calculated from the difference between NAN flow and the sum of endogenous and microbial protein flows in abomasum or proximate duodenum digesta.

The incremental method (Stern and Satter 1980) can measure the protein degradability of individual feedstuffs and does not require the measurement of endogenous protein contribution separately. The bias of this method may exist in the usage of a relatively high protein level with the basal diet and the assumption that protein contents in the rations do not

influence the measurement (NRC 1985b). The difference method measures protein degradability of the whole diet only. A basal diet also can be used as a reference for the extent of its contribution to the duodenal protein flow. The degradability of an individual feedstuff can be evaluated by subtracting the effect of basal diet from the experimental diet (Siddons et al. 1985; Cleale et al. 1987b). Endogenous protein has to be estimated in this method to increase the accuracy (Nocek 1988). Estimations of the endogenous contribution to the duodenal protein flow are very variable, difficult to obtain, and thus often ignored which may lead to an under-estimation of protein degradability (NRC 1985b).

In Situ Measurement

Protein degradability estimated by artificial fibre (i.e. Dacron or nylon) bag incubation in the rumen (in situ) has gained widespread application since it was suggested as a routine method for testing by Mehrez and Ørskov (1977). In this method a feed sample is sealed in the bag and several of these bags suspended in the rumen via a rumen cannula for a serial period of times. The bags are withdrawn and washed after suspension in the rumen. The protein disappearance from the bag is cited as the protein's degradability in a certain time period. Mathematical models are developed to describe protein disappearance from bags in the rumen over a series of time periods. These models are used to calculate effective degradability of crude protein (CP) of a feedstuff by taking into consideration the outflow rate of protein from the rumen (Ørskov and McDonald 1979; Michalet-Doreau and Ould-Bah 1992; Ørskov 1992).

Factors affecting in situ results have been reviewed and recommended procedures were given by Nocek (1988) and Ørskov (1992). An appropriate bag porosity is very important in reaching a compromise among limiting influx of rumen contents not associated with the test sample, allowing influx of microbial populations to degrade the test sample, and at the same time limiting the efflux of undegradable particles of test sample during the rumen incubation (Nocek 1988). With a preruminal incubation (at 39°C for 15 min) of SBM in water, Nocek (1985) showed that the N washout of SBM from the bags of porosity from 6 to 20 μm was lower than that from 80 to 102 μm, with the 40 and 59 μm not being different from all others. The author further demonstrated that bags of low porosity (6 to 20 μm) underestimated, whereas bags of porosity larger than 40 μm compared favourably with in vivo protein ruminal degradability. Bag porosity thus was suggested to be 40 to 60 μm for in situ measurements (Nocek 1988; Michalet-Doreau and Ould-Bah 1992; Ørskov 1992). Onyango (1995) further compared two types of nylon bag, i. e. monofilament (which has a greater percentage open area) and multifilament bag types, in the rumen incubation. The monofilament type was found to give a relative higher N disappearance from the bag compared to multifilament type. This research implies that not only the pore size, but also the total pore area will affect the extent of protein disappearance from the bags. Currently no recommendation is available on the total pore area of nylon bag.

The ratio of sample weight to bag surface area is also important for in situ measurement, as this ratio may restrict the circulation of bacteria and protozoa responsible for rumen degradation (Michalet-Doreau and Ould-Bah 1992). Increasing the sample weight to bag

surface area will decrease the rumen DM (Udén et al. 1974) and protein disappearance (Nocek 1985). A ratio of 12.6 mg/cm^2 was found to result in a ruminal available protein of SBM that most closely approximated in vivo results (Nocek 1985). Nocek (1988) further suggested that the range in sample size to bag surface area should be 10 to 20 mg/cm^2 for most forage and concentrate type ingredients. Michalet-Doreau and Ould-Bah (1992) suggested 15 mg/cm^2 should be used.

Data from Michalet-Doreau and Cerneau (1991) showed that concentrate N degradability in the rumen decreased as the screen size of grinding changed from 0.8 to 6.0 mm, but forage N degradability did not. This increase in N degradability may be partially attributed to the increase of N losses through the bag pores (Michalet-Doreau and Cerneau 1991). The fine grinding of feed can also correspond to an increase in microbial population (Legay-Carmier and Bauchart 1989) and thus could over-estimate degradability. Michalet-Doreau and Cerneau (1991) further reported the considerable differences in particle size between feeds ground with the same screen. To reach some degree of uniformity in size within major categories of feedstuffs, Nocek (1988) proposed that protein supplements and by-product type ingredients should be ground to pass a 2 mm sieve prior to in situ digestion with a 5 mm sieve for forage.

The extent of N disappearance from nylon bags was different between high forage and high concentrate diets fed to cows (Weakley et al. 1983), with the highest concentrate diet having the lowest N disappearance. Nocek (1988) indicated that diet was the major factor

determining the quantity and types of microbes and, therefore, the rate and extent of digestion of dietary nutrients in the rumen. Ørskov (1992) suggested that the diet given to animals fitted with nylon bags be similar to the diets for which the results were to be applied. Onyango (1995) demonstrated that the in situ DM and N disappearances (12 to 24 h ruminal incubation) of test feedstuffs such as untreated CM, heat-treated (at 110°C for 60 min) CM or a formulated animal-vegetable protein supplement could behave differently if these feedstuffs were tested in cows fed diets containing the different test protein supplements.

The incubation times of bags in the rumen may be different for different kinds of feed samples (Ørskov 1992). For an adequate and reliable mathematical description, the most sensitive part of the curve should be well supported by observation, and the asymptote must be well described. Bag incubation sequence can influence dietary digestion rates in the bag. The procedure of bag introduction at different times with removal all at once gives a higher rate constant for DM and N disappearance compared to the reversed procedure (Nocek 1985). Standard ingredient(s) were suggested to be used in each incubation time suspended with test samples (Nocek 1988) to give confidence for results from different runs.

Madsen and Hvelplund (1994) investigated the predictions of in situ protein ruminal degradability in 23 European laboratories. They reported that the differences found in protein degradabilities between laboratories were too large to be acceptable. The variation was mainly associated with differences between laboratories in the methods used for sample preparation and processing. Thus standardization of the procedure for in situ measurement

is necessary to allow data comparison between laboratories.

The assumption that all protein disappearing from the bag is readily degraded may result in serious bias. By incubating many protein sources in vitro at 37°C with protease from *Bacteroides amylophilus*, Mahadevan et al. (1980) demonstrated that soluble and insoluble proteins of soybean were hydrolysed at almost identical rates though soluble, serum albumin and ribonuclease A were resistant to hydrolysis. Furthermore, undegraded particles of the test sample may be lost by passing through the bag pores during rumen incubation and bag washing (Michalet-Doreau and Cerneau 1991). In spite of these possible biases, in situ measurements were shown to have a close relation to the in vivo results (Madsen and Hvelplund 1985).

In Vitro Measurement

In vitro methods were developed to give rapid, low cost and consistent laboratory means for protein degradability measurements.

Solubility had long been used to estimate protein degradability by incubating samples in a solvent such as distilled water, saline solution or autoclaved rumen fluid (Little et al. 1963; Waldo and Goering 1979; Crooker et al. 1978). Currently this method is seldom used, because it does not reflect the protein degradation dynamics in the rumen (NRC 1985b).

Enzymatic techniques have been employed in protein degradability determination. Enzymes can come from two sources, i.e. rumen microbial origin or commercial. Poos et al. (1980) used five commercial proteolytic enzymes: a bacterial protease (*Streptomyces griseus*); three plant proteases, papain (*Ananas comosus*), ficin (*Ficus glabrata*) and bromelain (*Ananas comosus*); and a neutral fungal protease (*Aspergillus oryzae*) to determine the ruminal degradabilities of nine concentrate samples. They found that in vitro enzymatic degradability was highly correlated to protein bypass determined by animal performance. Fungal protease gave the highest correlation across all incubation times (average $r = 0.86$, $n = 4$). These results were re-confirmed by Poos et al. (1985). Pepsin, pancreatin and a protease (Type XIV) were also used in the estimation of N degradability in the rumen (Siddon et al. 1985). None of these three enzymes, however, ranked the feedstuffs (SBM, groundnut meal and fish meal) in the same order for degradability as in vivo procedures (Siddon et al. 1985).

Krishnamoorthy et al. (1983) fractionated total feed N into pool A (N soluble in borate-phosphate buffer), pool B (total N - (pool A + pool C)) and pool C (acid-detergent-insoluble N or residual N after 24 - 48 h incubation in a protease enzyme (Streptomyces griseus, type XIV) solution. Pool B was further divided into subfractions B1, B2 and B3 by means of curve peeling the natural logarithm of pool B vs. time of the protease incubation of substrate in vitro. The ruminally undegraded dietary N of different N sources in the mixed diets was predicted by using a mathematical model with data inputs of rate-constants and the pool size of N fractions obtained from in vitro protease incubation. A good agreement ($r = 0.78$, $n = 12$) was obtained between the ruminally undegraded dietary N values estimated from

simulated rumen proteolysis and those determined in vivo for the mixed diets. Roe et al. (1991) investigated the accuracy of in vitro methods using *Streptomyces griseus*, ficin and neutral protease with amylase in predicting in situ protein degradation of concentrate feeds. The authors found that the in vitro methods generated degradability estimates in conflict with those obtained from the in situ method, although high correlation were found between in situ and in vitro measurements across a series of time periods (0 to 48 h) for the individual feeds. The method of using neutral protease with amylase ranked the test feeds according to the extent of CP degradation at 24 h most similar to that of in situ. At least 67% of the variation in the in situ estimates (18 h ruminal incubation) could be explained by the in vitro estimates (neutral protease and amylase incubation for 1 to 12 h) (Roe et al. 1991). The advantage of using amylase in addition to the protease in an in vitro incubation may exist in increasing the access of protease to the dietary protein exposed by amylase which is more similar to the feed degradation process in the rumen.

Luchini et al. (1996) used mixtures of commercial proteases to mimic the rate and extent of protein degradation obtained by using strained ruminal fluid. The following mixtures of proteolytic enzymes were tested: a mixture of *Streptomyces griseus* protease, chymotrypsin and proteinase K at 0.042, 2.5 and 0.5 enzyme units/ml, respectively, and a mixture of trypsin, carboxypeptidase A & B and chymotrypsin at 116.5, 0.5, 0.5 and 2.5 enzyme units/ml respectively. The protein degradation rates obtained with strained ruminal fluid were two to six times faster than those obtained with enzyme mixtures. Thus they concluded that the commercial enzymes tested did not mimic the protein degradation activity of strained ruminal

fluid. One limitation which may be associated with this research is that the proteolytic activity was characterized by using 13 L-amino acid p-nitroanilides as the artificial substrate. The protein degradation activity in the incubations where feed protein was the substrate was different from that where artificial protein was used (Luchini et al. 1996).

A partially purified protease originating from *Bacteroides Amylophilus* was used to study the degradation of soluble and insoluble proteins in vitro (Mahadevan et al. 1980). Results indicated that the rankings of degradabilities of soluble and insoluble proteins from SBM were similar between the data obtained from the protease incubations and crude microbial fraction in vitro incubations. Mahadevan et al. (1987) further prepared the protease from mixed microorganisms originating in rumen fluid. The majority of the protease extracted had a molecular weight greater than 300,000. The relative degradation rates of the true proteins from different feedstuffs incubated in the rumen protease were: SBM > fish meal > linseed meal and blood meal > rapeseed meal > corn gluten meal. They also found that substituting *Streptomyces griseus* protease for the rumen protease gave results which were very different from those obtained with the ruminal protease. The authors concluded that protease other than rumen protease may not have the same action on feed proteins as rumen protease and thus might give misleading results. The preparation of protease from mixed ruminal microorganism does not solve the problem of variable proteolytic activity of protease from different sources.

Rumen micro-organism inoculations would seem to provide a more physiologically sound

procedure to simulate rumen protein degradation (Tilley and Terry 1963; Slyter et al. 1964; Hoover et al. 1976; Czerkawski and Brekenridge 1977; Broderick 1978; Raab et al. 1983; Luchini et al. 1996). These methods also have problems such as accumulation of degradation end products (Raab et al. 1983), presence of inhibitors and low microbial activities (Broderick 1978; Broderick and Balthrop 1979; Luchini et al. 1996), decreased microbial numbers and change in the proportion of microbial species (Hoover et al. 1976; Slyter and Putnam 1967) in the in vitro incubations which may limit the usefulness of these methods.

For future studies, in order to obtain a representative result, some purified rumen microbial enzymes such as protease, cellulase and amylase need to be developed for the use of in vitro enzymatic methods in determining dietary protein ruminal degradability. Including enzymes in addition to protease, such as amylase for high concentrate feeds or cellulase for high fibre feeds in an in vitro incubation system and using a natural protein as substrate in characterizing the proteolytic activity will increase the accuracy in measuring.

POST-RUMEN DIGESTION, ABSORPTION AND METABOLISM OF NITROGENOUS COMPOUNDS

Abomasum Protein Digestion

Omasal digesta entering the abomasum for further digestion consists of various nitrogenous compounds which include microbial protein, undegraded dietary protein, the endogenous

protein and the intermediate or end products of degraded protein such as peptides, AA and NH_3. Protein is hydrolysed into large or medium peptide segments by pepsin which was secreted into the acidic abomasum environment by the cells on the abomasum wall. The absorption of N compounds from the abomasum is probably quantitatively unimportant (Smith 1989).

Small Intestinal Protein Digestion and Absorption

Peptides and undigested proteins from the abomasum are subjected to extensive digestion by pancreatin and intestinal proteases in the small intestine to produce small peptides and AAs which can be absorbed into intestinal mucosa (Friedrich 1989; Webb and Matthews 1994). The absorption of small peptides are more rapid than that of AAs and an independent transportation system for small peptides is possible (Webb and Bergman 1991). While large peptides may be absorbed from the small intestine, the absorption of quantitative importance is limited to di- and tripeptides. The absorption of these peptides can be affected by many factors, for instance, the peptide AA composition, the location of a specific AA in a peptide and the competition among peptides (Webb and Bergman 1991).

The quantity and the proportion of AAs in the small intestine are mainly determined by the influxes of microbial protein, dietary protein and endogenous protein (Clark et al. 1992). The peptides and AAs which escape rumen degradation may affect the AA composition of small intestine digesta (Webb et al. 1993). After summarizing 152 dietary treatments from the

literature, Clark et al. (1992) reported that microbial N supplied an average of 59% of NAN that passed to the small intestine of dairy cows with a range of 34 to 89%. The apparent digestibility of rumen bacterial preparations measured between the abomasum and feces in sheep is 77.5% on average and the true digestibility of microbial AAs is 84.7% (Ørskov 1992).

Most of the absorbed peptides from the small intestine will be hydrolysed into AAs in the cytosol of the intestinal wall cell. A small part of absorbed peptides can be released into the blood stream together with some AAs which are absorbed from the intestine or derived from the hydrolyzation of peptides in the cell (Friedrich 1989; Webb and Bergman 1991). A substantial part of absorbed AAs are metabolized in the intestinal wall (NRC 1985b). It was shown that 10 - 35 % the amino N entering the blood during the absorption of the partial hydrolysate of casein and soybean might well have been in the form of small peptides (Matthews 1991). The author further suggested that red blood cells might carry more peptides than plasma.

The AAs are transported in blood for utilization by body tissues (Armentano 1994). The hepatic uptake of amino N is considered to be around 23% of duodenal entry while the essential AAs utilized by liver can account for approximately half of the non-mammary utilization of duodenal entry (Armentano 1994). The author also suggested that hind limbs of growing calves accounted for roughly one-fourth to one-third the rate of hepatic AA uptake. The mammary gland utilized substantial quantities of AAs from blood (DePeters and

Cant 1992; Armentano 1994) in the lactating cow. Based on the composition in milk, individual AAs can be characterized into three groups (DePeters and Cant 1992). Group I AAs, i.e. phenylalanine, tyrosine, methionine and tryptophan, are transferred from blood to milk without alteration. Group II AAs, which include the remainder of essential AAs, are taken up from blood in excess of the milk output. Group III AAs, which include the reminder of non-essential AAs which are taken up from blood in insufficient quantities to account for their secretion in milk. The authors suggested that the group I AAs are likely to be the most limiting AAs in milk protein synthesis. After examining many experiments conducted to define the AAs that limited milk yield, the authors concluded that, in general, methionine and lysine are considered to be the limiting, often termed co-limiting AAs for milk yield with normal dairy cow feeding practices.

Rulquin (1994) and Rulquin et al. (1995) studied the responses of milk protein content to the addition of rumen-protected methionine and lysine in diets. The results suggested that the milk protein responses were dependent on the methionine and lysine content of the protein concentrate and the physiological state of the cow. The author showed that the response to the addition of protected methionine and lysine was low with fishmeal which has a high lysine and methionine content, high with groundnut meal which has a low lysine and methionine, while intermediate with corn gluten meal (low in lysine and high in methionine) and blood meal (low in methionine and high in lysine). The allowances for lysine and methionine as a percentage of total intestine digestible protein in diets were recommended to be 7.3 and 2.5%, respectively (Rulquin 1994; Rulquin et al. 1995). Schwab (1995) further suggested that lysine

should contribute at least 15.0% of total essential AA in duodenal digesta for maximum content and yield of milk protein, and methionine should contribute about 5.3% of total essential AA in duodenal digesta when and only when, lysine made up approximate 15.0% of total essential AA.

Erythrocytes were demonstrated to transport free AA to tissues such as the mammary gland (Hanigan et al. 1991). The authors suggest that uptake data derived from plasma is not adequately representing AA uptake by whole blood. Moreover, data from Pocius et al. (1981) further suggested that small peptides, such as glutathione might be absorbed by the mammary gland and thus contribute to the AA pool available for utilization by the mammary gland. Thus the AA utilization in the mammary gland needs to be examined more carefully. Small peptides, especially limited to di- or tripeptides, also can be absorbed and utilized by other tissues such as liver, kidney and muscle (Webb et al. 1993). The contribution of small peptides to the AA nutrition of animal body tissues needs to be investigated in more detail.

Other nitrogenous compounds present in small intestine digesta include amides, NH_3 and nucleic acids (Oldham and Tamminga 1980). Amides and NH_3 can be absorbed from the small intestine (Church 1976). Nucleic acids are broken down into nucleotides , nucleosides and bases by deoxyribonuleases, ribonuleases, phosphodiesterases and phosphomonesterases and absorbed in the small intestine (NRC 1985b).

Endogenous protein in the small intestine includes enzymes, bile, mucus, serum albumin,

lymph, epithelial cells and other degradation products from the gastrointestinal lining (Swanson 1982). Swanson (1982) reported that a large influx of N, presumably endogenous, in the first part of the small intestine raised the protein percentage almost 45% above that of abomasal content in steers. The amount of endogenous N can be equivalent to the sum of N of undegraded intake protein and microbial protein present in sheep small intestine (NRC 1985b). Thus, endogenous protein may play an important role in protein nutrition of ruminants.

Large Intestine N Metabolism

Protein and NPN passing from the ileum can be degraded by microbes present in the large intestine to produce NH_3 which can be used for microbial growth or can be absorbed into blood through the intestine wall (NRC 1985b). The undegraded protein and unabsorbed NPN will be excreted in feces together with endogenous N and the microbial protein synthesised in the large intestine.

DETERMINATION OF POST-RUMEN PROTEIN DIGESTIBILITY AND ABSORPTION

In Vivo Measurement

Apparent protein or AA absorption (g/d) in the small intestine can be estimated by measuring

the disappearance of nutrient (g/d) between the proximal duodenum and the terminal ileum (Zinn and Owens 1982; Santos et al. 1984). True absorption is the sum of apparent absorption and endogenous loss, i.e. the endogenous input that is not reabsorbed. Through the regression of apparent disappearance in the small intestine (Y, g/d) against the supply to the proximal duodenum (X, g/d), using the equation $Y = a + b*X$, the true absorption (b, % of the supply to the proximate duodenum) and the negative value of endogenous losses (a, g/d) can be derived (Zinn and Owen 1982).

Apparent digestible intake protein or AA (g/d) in the whole digestive tract is equal to the disappearance of intake protein or AA (g/d) as measured by difference between dietary intake (g/d) and fecal loss (g/d). True digestible protein or AA (g/d) equals the sum of the apparent digestible protein or AA (g/d) and the fecal endogenous loss (g/d). A linear regression of the apparent digestible protein or AA content (% of dietary DM) against the dietary protein or AA content (% of dietary DM) can be plotted and the slope will represent the true digestibility (% of dietary protein or AA) (Harris et al. 1972).

Mobile Nylon Bag Technique

Sauer et al. (1983) introduced a mobile nylon bag (MNB) technique to determine the apparent digestibilities for pigs. This method was adapted to study the post-rumen digestion of dietary protein and AAs in ruminants (de Boer et al. 1987; Kendall 1988).

Presently, the MNB technique is used routinely in the University of Manitoba with 1 g samples that are sealed in a 3.5 X 5.5 cm nylon bags of 50 μm pore size (Sauer et al. 1983; Kendall et al. 1991; Moshtaghi Nia and Ingalls 1992). The nylon bags are incubated in the rumen for 16 hours. Some incubated bags are washed and dried and the residues in the bags are measured for disappearance in rumen. The remaining bags are incubated in pepsin-HCl solution for three hours (Moshtaghi Nia and Ingalls 1992) to simulate the abomasum digestion and then the bags are inserted into duodenum and collected from feces. The true digestibility of dietary protein or AAs can be measured through their disappearance from the bags.

De Boer et al. (1987) tested six feedstuffs using a simular MNB technique as the one used in the University of Manitoba excepting that they used nylon bags of 48 μm pore size and omitted the pepsin digestion step. The measured intestinal availabilities of undegradable protein (based on 8 h rumen incubation) are 73 and 79% for the meat and bone meal and the CM, respectively. These values are higher than the values of true N intestinal digestibility of ruminal residues (based on 8 or 24 h rumen incubation) of meat and bone meal (55%) and rapeseed meal (67%) in rats (Rooke 1985). However the values obtained for fish meal are quite close (83% in MNB vs. 86% in rats digestibility) between these two methods.

Hvelplund et al. (1992) measured the true digestibility of undegraded dietary protein from 12 different feeds using the MNB technique. Samples were put into nylon bags (9 um pore size) and incubated in the rumen for 0 to 96 h. After incubation the sample residues were

preincubated in a pepsin-HCl solution (100 mg/l pepsin, pH 2.4) prior to insertion into the small intestine. The results suggested that the effect of preincubation in rumen was numerically very small for the four concentrates, but statistically significant excepting one. The values of true digestibility of the intact feed protein estimated by the MNB technique (Hvelplund et al. 1992) are much higher than those in vivo (Titgemeyer 1988) and in rat digestion trials (Rooke 1985). The small intestinal digestibility of undegradable CM protein (MNB technique) calculated from the data presented by Moshtaghi Nia and Ingalls (1995b) is 82.8% with a rumen degradability of 69.1% for the original CM. Moshtaghi Nia and Ingalls (1992), however, reported a lower value (63%) of small intestinal digestibility of undegradable CM protein (MNB technique) together with a higher ruminal protein degradability (74.4%) of CM. Thus the extent in ruminal protein degradability among different sources of the same feedstuffs may influence the digestibility of the undegradable dietary protein in small intestine as measured by the MNB technique.

Hvelplund et al. (1992) indicated that the results obtained with MBN technique showed good agreement with the true intestinal digestibility results obtained in infusion experiments with sheep. More data regarding the direct comparison between the in vivo and the MNB technique in measuring intestinal digestibility of dietary protein are required before a conclusion can be drawn on the accuracy and reliability of the MNB technique.

In Vitro Measurement

In vitro methods such as enzymatic digestion, chemical methods and dye binding were developed to estimate the digestibility or availability of protein or AAs of foods or feedstuffs in poultry, swine or human nutrition (Akeson and Stahmann 1964; Hsu et al. 1977; Sibbald 1987).

Enzymatic methods were used very successfully in predicting protein biological values (Akeson and Stahmann 1964) and estimating protein digestibility (Hsu et al. 1977). Akeson and Stahmann (1964) incubated 100 mg protein with 1.5 mg pepsin in 15 ml of 0.1 N hydrochloric acid at 37°C for 3 h. After the pepsin incubation, the digestion mixture was neutralized with 7.5 ml of 0.2 N NaOH and 4 mg pancreatin in 7.5 ml of pH 8.0 phosphate buffer, and was incubated further for an additional 24 h at 37°C. Merthiolate (50 ppm) was added to the digestion mixture to prevent the growth of micro-organisms. The amount of AA released from the incubation was used to calculate the pepsin pancreatin index of the dietary protein. The authors reported that the values of pepsin pancreatin index for 12 proteins were highly correlated ($r = 0.99$) to the biological values of these proteins in growing rats. Hsu et al. (1977) used a multienzyme system, which consists of trypsin, chymotrypsin and peptidase, to evaluate the protein digestibility in vitro. An aqueous protein suspension was incubated in the multienzyme solution at 37°C. A rapid decline in pH of the incubation solution occurred, caused by the freeing of AA carboxyl groups from the protein by the proteolytic enzymes. The authors found that the pH of the protein suspension after 10 min

of digestion was highly correlated ($r = 0.9$, $n = 23$) with the in vivo apparent digestibility by rats. The advantage of this multienzyme digestion method is that it is rapid (completed within 1 h) and simple, with a high degree of sensitivity (Hsu et al. 1977).

Recently, Calsamiglia and Stern (1995) developed a three-step in vitro procedure for the measurement of intestinal digestibility of protein in ruminants. The pancreatin digestion step in this procedure was modified from the method used by Akeson and Stahmann (1964). Feed samples (1.5 g) were ground through a 2 mm screen, heat-sealed in 6 x 10 cm Dacron polyester bags and suspended in the rumen for 16 h. Sample residue (15 mg N) was incubated in 10 ml of pH 1.9, 0.1 N HCl solution containing 1 g/l of pepsin for 1 h in 38°C. After pepsin incubation, 0.5 ml of 1 N NaOH solution and 13.5 ml of pancreatin solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 containing 50 ppm of thymol and 3 g/l of pancreatin) was added into the digestion solution. The sample was incubated at 38°C for 24 h with votexing at 8 h intervals. Three ml of 100% (w/v) trichloroacetic acid (TCA) solution was added to the digestion solution to stop enzymatic action and precipitate undigested proteins after the enzymatic incubation. The digestion solution was centrifuged at 10,000 x g for 15 min and the supernatant was measured for soluble N content. Thus the pepsin pancreatin digestibility (PPD) of protein is calculated as TCA-soluble N divided by the amount of N in the Dacron bag residue. The pancreatin assay was validated by using duodenal samples obtained from animals fed diets containing a wide variety of protein supplements. Thirty-four duodenal samples, from which small intestine CP digestibility was determined in vivo, were measured for the pancreatin digestibility. A high correlation ($r = 0.91$) was found between

in vivo estimates and the pancreatin digestion.

The intestinal absorbable dietary protein (% of CP), the product of rumen UIP (% of CP) measured by the in situ Dacron bag technique and the PPD (% of UIP), can be used further to evaluate the intestinal availability of dietary protein and AAs for ruminants (Stern et al. 1995). The three-step in vitro procedure provided an alternative to the use of intestinally cannulated animals for estimating intestinal digestion of protein supplements (Calsamiglia and Stern 1995).

MANIPULATING PROTEIN FLOW FROM THE RUMEN

Dietary protein is degraded extensively in the rumen by microbes to produce NH_3 which can diffuse into blood and be lost largely through urine in ruminants. To use high quality dietary protein sources more efficiently, many techniques have been tried to manipulate the protein flow from the rumen and protect dietary protein or AA from ruminal degradation.

Ionophores

Some efforts have been made to manipulate the rumen fermentation environment so as to reduce the rumen protein degradation. Carboxylic polyether ionophores such as monensin, tetronasin, lasaloid, lysocellin, marasin and laidlomycin are antibiotics produced by various strains of *Streptomyces* (Bergen and Bates 1984; Russell and Strobell 1989). Various

ionophores have been used to improve the production efficiency of growing ruminants since the 1970s, with monensin being the most extensively studied (Goodrich et al. 1984; Shirley 1986). Hanson and Klopfenstein (1979) investigated the effects of monensin on growing steers fed different protein sources and levels. Supplementation of monensin at 200 mg/head/day to 10.5 and 12.5% CP diets resulted in 16.3 and 8.7% improvements in feed/gain efficiency, respectively. Monensin effectively altered volatile fatty acid concentrations in favour of propionate. Rumen NH_3 N concentration was reduced by monensin. Lysocellin (80 mg/head/day) and tetronasin (60 mg/head/day) were shown to increase average daily gain of steers grazing ryegrass by 9.6 and 7.0% respectively, compared to the control (Sticker et al. 1991). However, lysocellin and tetronasin did not affect the rumen NH_3 concentration (Sticker et al. 1991).

Ionophores are regarded as having "protein-sparing" effect on dietary protein utilization in ruminants (Russell and Strobel 1989), as they generally improve weight gain while decreasing feed intake (Goodrich et al. 1984). Ionophores were shown to decrease NH_3 production in vivo (Hanson and Klopfenstein 1979) and in vitro (Chen and Russell 1990; Newbold et al. 1990). Ruminal bacteria with a high specific activity of NH_3 production were found to be sensitive to monensin (Russell et al. 1988; Chen and Russell 1989). Chen and Russell (1989) further found that monensin had little effect on protein degradation, but it caused a large decrease in NH_3 production and an increase in non-NH_3-protein-N in vitro. Thus they suggested that monensin could increase peptide N flow from the rumen. Newbold et al. (1990) also investigated the effects of an ionophore, tetronasin, on N metabolism by ruminal

microorganisms in vitro. Proteolytic activity (14C-labelled casein hydrolysis) was unaffected, but the NH_3 release rate from AAs was decreased by 87%. The addition of tetronasin to the cultures of rumen bacteria *Bacteroides ruminicola* and *Ruminobacter amylophilas* did not decrease bacteria proteolytic and deaminase activities. However, after they adapted to and were further incubated in the presence of tetronasin, their deaminase activities were completely inhibited, although the proteolytic activities were not decreased. The authors speculated that the mechanism employed by these species to grow in the presence of tetronasin interfered with the uptake or metabolism of AAs, perhaps by altering permeability characteristics of the bacterial cell envelope. The authors suggested that the effects of tetronasin, and probably of other ionophores, on AA deamination could be the elimination of gram-positive deaminating bacteria and an interference in AA breakdown in surviving species. It was reported that the driving force for the active transport of AA into the cell is a Na^+ gradient, while the active transport of peptides is driven by a H^+ gradient and possibly by a Ca^{2+} gradient (Webb and Bergman 1991; Webb et al. 1993). Ionophores which are able to dissipate the proton and Na^+ gradients across the cell membrane (Bergen and Bates 1984; Russell and Strobel 1989) thus may interfere with transport of AAs and peptides across membranes for further metabolism. An in vivo study demonstrated that monensin was able to increase rumen protein bypass from 22 to 55%, while decreasing microbial NAN flow and microbial growth efficiency or protein synthesis (g microbial CP/ 100 organic matter digested ruminally) on average of 15 and 21.5%, respectively (Bergen and Bates 1984). Protozoal inhibition by monensin and lasalocid was reported to be transient because prolonged antibiotic feeding resulted in the selection of a resistant population in the rumen of cattle (Dennis et al.

1986). Fungi are also inhibited by ionophores (Wallace 1994).

Microbial Cultures

Microbial feed additives, i.e. live microbial cultures (including their extracts) such as *Aspergillus oryzae* (AO) and *Saccharomyces cerevisiae* (SC) have been used as ruminal fermentation manipulating agents in ruminant diets (Wallace and Newbold 1993). The use of small amount of yeast culture (YC, *Saccharomyces cerevisiae* plus growth medium), typically 4 - 100 g/d (Wallace 1996), has been associated with improved feed intake ($P < 0.10$, Erasmus et al. 1992) and milk yield ($P < 0.05$, Williams et al. 1991) in dairy cows, and alternations in milk composition and improved weight gains and feed efficiencies in meat producing animals (reviewed by Dawson 1990). The most reproducible effect of microbial feed additives is that they increase the viable count of total anaerobic, especially cellulolytic, bacteria recovered from ruminal fluid (Wallace and Newbold 1993). Harrison et al. (1988) supplemented 114 g/d of YC to a 40% corn silage and 60 % concentrate (DM basis) diet to cows. They observed 58 and 82% increases in the concentration of anaerobic and cellulolytic bacteria, respectively, in rumen fluid, while the ruminal pH, NH_3 concentration and the acetate:propionate ratio were lower when YC was included in diets. Dawson et al. (1990) recorded 10 fold increase in total culturable bacteria and about 8 fold increase in cellulolytic bacteria in vivo using cannulated steers.

Furthermore, Erasmus et al. (1992) found that the AA profile of bacterial protein was altered

such that the proportion of some AAs , i.e. cystine, glutamine, serine and threonine ($P < 0.05$) and methionine ($P < 0.10$) was increased and the duodenal flow of methionine was increased ($P < 0.05$, treatment 58 vs. control 41 g/d) when YC was supplemented in the diets for the lactating cows.

El Hassan et al. (1993) suggested that yeast cells do not grow in the rumen and YC need to be both viable and metabolically active for the full effects of YC on the rumen fermentation to be realized. The authors demonstrated that after 4 g YC/d was fed to sheep the viable count of yeast cells in the rumen declined at a similar rate as to the outflow (0.086 vs. 0.095) of chromium mordanted yeast added to the rumen. Wallace (1996) pointed out that the enormous changes in the viability of the bacterial cells that are presented when YC is used do not necessarily mean that the total number of bacterial particles is different, only that a higher proportion is alive or at least vigorous enough to survive in viable counting. The author suggested that YC must in some way improve conditions for the cultivation of rumen bacteria.

The available experimental evidence thus suggests that the microbial feed additives increase the rate of fibre breakdown through stimulating bacterial growth, which in turn will enhance feed intake and increase the flow of nutrients such as protein and energy to the small intestine for animal digestion and absorption, and further improve the animal production.

Defaunation

Defaunation was found to reduce NH_3 concentration, dietary protein degradation and increase bacteria production in the rumen (Ushida et al. 1986; Punia et al. 1987) and thus improve N or NAN flow to the lower gut (Veira 1984; Ushida et al. 1986; Punia et al. 1987). Protease, peptidase and deaminase activities were found in protozoa (Williams 1989). Entodiniomorphid (oligotrich) protozoa use only a particulate protein source, whereas the isotrichid (holotrich) protozoa are able to utilize both particulate and soluble proteins (Hino and Russell 1987). The elimination of protozoa, the predator of rumen bacteria, generally increase rumen bacterial counts fourfold (Nagaraja et al. 1992), and increase microbial N net synthesis in the rumen (Demeyer and Van Nevel 1979), and thus increase the bacterial N flow to the duodenum (Ushida et al. 1986). In vitro ruminal bacterial and protozoa incubations demonstrated that isolated bacteria always produce more NH_3 than isolated protozoa, but the difference was less with heat-treated, particulate proteins (Hino and Russell 1987). These results suggest that the presence of protozoa may increase the availability of insoluble proteins for microbial utilization and thus increase dietary protein degradability in the rumen. Protozoa also were shown to be limited in their capacity to take up peptides and AAs, as non-NH_3-NPN was higher in incubations containing protozoa than bacteria, while bacteria on the other hand have a high capacity to take up AA sources and deaminate (Hino and Russell 1987). Thus the synergism was observed between the protozoa and bacteria in NH_3 production (Hino and Russell 1987). Wallace et al. (1987) further found that the leucine aminopeptidase, deaminase and trypsin-like protease activities were 58, 64 and 55% higher

when natural mixed protozoa were present in sheep compared to ciliate-free.

Protozoa contribute significantly to cellulolytic and amylolytic activities in the rumen (Williams 1989). Defaunation can cause general depression of fibre digestion (Williams and Coleman 1988). Coleman (1986) found that the carboxymethylcellulase activity in rumen was decreased 50% due to defaunation in sheep. Veira (1984) also indicated that, on average, the extent of ruminal organic matter disappearance from the rumen in ciliate-free sheep was only 85% of that observed in faunated sheep. Thus the benefits of defaunation to ruminant production have to be examined carefully.

PROTECTING DIETARY PROTEIN FROM RUMEN DEGRADATION

Emphasis has been placed on the development of physical or chemical methods in treating dietary protein directly to protect it from microbial degradation in rumen. Broderick et al. (1991) and Atwal (1995) have written extensive reviews on this subject.

Heat Treatment

Heating has long been used as a method to reduce the protein degradability in the rumen (Sherrod and Tillman 1964; Stern 1984; Faldet et al.1991; Satter et al. 1994; Moshtaghi Nia and Ingalls 1992, 1995a,b; Onyango and Ingalls, 1994; Onyango 1995). The mechanism of protection in heat treatment involves principally the Maillard reaction (or non-enzymatic

browning) between the free amino group of lysine residues in protein and the aldehyde groups of reducing sugar such as glucose, fructose and xylose which are present in or are added to diets (Hurrell and Finot 1985). In the initial stages of the Maillard reaction (Finot et al. 1977), a Schiff's base is formed and a molecule of water is released through the addition reaction between the amino and aldehyde groups. The Schiff's base is reversible and can be transformed to other reversible intermediates before the formation of a stable and irreversible Amadori product (ketose form). The Schiff's base and its reversible intermediates are fully bio-available to animals (Finot et al. 1977). Heat can enhance the formation of Schiff's base and its reversible intermediates. Schiff's base is more stable at a neutral pH while an acidic environment can induce the reversal hydrolysis process to produce the original sugar and lysine residue. Thus, it can be assumed that the Schiff's base formed through heat treatment stabilizes the protein and renders it less degradable in the rumen (pH 5.5 to 7.5), whereas it remains digestible in the acidic condition of the abomasum (pH < 3.0).

However, Hurrell and Carpenter (1977) showed that excessive heat may induce the formation of Amadori products, its further derived products, and the stable final products premelanoidins and melanoidins (poly-polymeric nitrogenous compounds). These reactions are irreversible and thus the products are indigestible to the host animal because of the 'advanced' Maillard reaction. The modified lysine involved in the reaction also becomes nutritionally unavailable (Hurrell and Carpenter 1977). The authors further indicated that severe or prolonged heating can cause the formation of new isopeptide bonds between lysine and aspartic or glutamic acids, and also cause the formation of cross-linkage products such

as lanthionine and lysinoalanine within the protein. Thus the digestibilities or biological availabilities of proteins and lysine especially are decreased during severe heat treatments (Hurrell and Carpenter 1977). Prolonged heating can also destroy other AAs such as methionine, cystine, leucine, arginine or histidine and the damage to AAs becomes more severe in the presence of sugar (Evans and Butts 1949; Miller et al. 1965).

Thus, it is important to control the reaction conditions in heat treatment so as to limit the Maillard reaction to its initial reversible step. Two major variables having the most effect on the formation of Maillard products are temperature and duration of heating (Satter et al. 1994). Most studies on heat treatments have involved defining the range of heating temperature and time for optimum treatment which will supply maximum available dietary protein for small intestine digestion and absorption through maximizing the protection while minimizing the damage effect to the protein through heating.

Extrusion was used as a method to apply heat to diets to increase protein bypass rate. The temperature of soybeans exiting the extruder can be in the range of 132 to 149°C (Satter et al. 1994). A review on the practical usage of extrusion treatments (Atwal 1995), indicated improvements in milk production in several studies but not all. The lack of response to extrusion may be the result of too short heating time and/or the temperature is too low for effective treatment.

Drum roasters (e.g. Roast A - Matic or Gem) and high temperature air dryers (e.g. Jet-Pro

or Thermo-Pro) were used most often in applying heat to proteins (Satter et al. 1994). Protein supplements were exposed to the hot air environment of 204°C to 316°C or even higher for a short period before exiting the heating apparatus. These treatments became more effective with steeping or conditioning for an additional period. That is the heated protein products have to be held for a certain time period in a container with slow cooling. The air dryer was considered to be more efficient and caused less scorching of treated materials in comparison to drum roasters (Satter et al. 1994).

Plegge et al. (1985) found that roasting at an exit temperature from 115 to 145°C had a linear effect on the amount of SBM N escaping ruminal degradation, which was increased from 14.7 to 47.3%. However, acid-detergent-insoluble-N and acid pepsin insoluble N in SBM were increased three fold when SBM was roasted to 145°C, compared to control. Faldet et al. (1991) observed a significant increase in estimated postruminally available lysine by roasting and holding SBM at a temperature of 110 - 120°C for 0.5 to 3 h. McKinnon et al. (1990) treated CM at 125 and 145°C for 10, 20 and 30 min in a vacuum tumble dryer. The authors observed the decreases of in situ DM and CP disappearance and an increase in post ruminal supply of DM and CP due to the treatment effects. Satter et al. (1994) reported that soybeans should be heated to approximately 146°C exit temperature and then held without cooling for approximately 30 min. Higher undegradable protein, post-ruminal available lysine, plasma branched-chain AAs concentration and milk production were achieved under this treating condition.

Recently, moist heat treatment was studied extensively at the University of Manitoba (Moshtaghi Nia and Ingalls 1992, 1995a,b; Onyango and Ingalls 1994; Onyango 1995;). The CM was treated at 127°C with a steam pressure of 117 kPa for 15 to 90 min and the treated CM showed a decrease in N disappearance in rumen from 74.4 to 18.9%, but an increase in N availability in the lower GI tract from 16.2 (control) to 64.2% (45 min) (Moshtaghi Nia and Ingalls 1992). However, the concentrations and apparent digestibilities of lysine and arginine were reduced in 45 min moist heat treatment (Moshtaghi Nia and Ingalls 1995a). Moist heat treated CM at 110°C for 60 min increased milk yield about 2 kg/day over control in primiparous cows during early lactation (Onyango and Ingalls 1994). In an earlier study with autoclaving, which is similar to moist heat treatment, Broderick and Craig (1980) also observed that the protein degradation rate of cotton seed meal decreased along with the increase of autoclaving time from 0 to 240 min at 121°C and the intestinal protein digestibility increased to a maximum at 60 min and then declined.

Heat treatment in the presence of reducing sugar has received more and more research interest (Stern 1984; Cleale et al. 1987a,b,c; Nakamura et al. 1992; Wallace and Falconer 1992; McAllister et al. 1993). Cereals and oilseeds contain little reducing sugar (Hurrell and Carpenter 1977). The addition of reducing sugar to heat treatment will help to establish the Maillard reaction and reach the potential of maximum protein protection by supplying adequate reducing agents.

Cleale et al. (1987a,b,c) investigated the effects of various reducing sugar sources and

treatment conditions on SBM protein protection. Cleale et al. (1987a) found that xylose as a reducing sugar was more effective than glucose, fructose or lactose. Increasing the sugar levels (from 1 to 5 mol/mol SBM lysine) resulted in a linear decrease in NH_3 release in vitro. Treatment with xylose and heat at pH 10 gave the lowest NH_3 release. The increase of treating time (from 30 to 60 min) decreased NH_3 release in vitro (Cleale et al. 1987a). The treated SBM (heated at 150°C for 30 min at pH 8.3 and 83% DM) showed an increase of 157% (33.7% vs. 13.1%) of in vivo ruminal protein escape over control (Cleale et al. 1987b). Xylose, a more expensive reducing sugar source, could be substituted by glucose when heating time was increased to 60 min at 150°C (Cleale et al. 1987c).

Wallace and Falconer (1992) treated carbon-14 labelled casein for various time durations (5 min to 6 h) with a range of temperature (60 to 180°C) in the presence of 8 % glucose, sucrose or xylose or 12.4 % cane molasses. These researchers reported that as the time and temperature of heating increased the in vitro protein degradability was affected before digestibility, such that an efficacy window for producing protected protein became apparent, in which degradability was depressed without significantly affecting digestibility. Their results, in agreement with Cleale et al. (1987a), showed that xylose was the most active agent in inducing the optimum protection of protein. The temperature needed to be increased to 140°C for sucrose and molasses and to 120°C for glucose to achieve a similar protein protection effect (protein degradability 18%) of xylose at 100°C with a 30 min heating period (Wallace and Falconer 1992).

Stern (1984) showed that calcium lignosulfonate (CL), a by-product of the wood industry which consisted mainly of reducing sugar such as xylose, decreased the protein degradability of SBM incubated in a continuous culture apparatus after CL was pelleted with SBM. The partitioning of effluent N into NH_3 N and bacteria N was decreased, while the dietary N portion was increased more than 70% when the untreated SBM was replaced by CL treated SBM. In another experiment, Windschitl and Stern (1988) reported that a diet (16% CP, DM basis) made up of 17% SBM which was treated with 5% CL at 95 - 100°C for 30 min in a steam-jacketed processor and held for 45 min at 90 - 95°C, decreased the protein degradability and NH_3 concentration in the continuous culture apparatus compared to control.

Nakamura et al. (1992) demonstrated that similar milk production could be obtained when untreated SBM was replaced with one-half as much protein from SBM treated with 5% sulfite liquor (containing 20% xylose) at 93.5°C for 2 h in dairy diet (16% CP, with 13.6% of SBM). McAllister et al. (1993) treated CM with 5 or 10% LS (containing 20% reducing sugar) or 2% xylose for 1 and 2 h at 100°C. The results indicated that heating reduced in situ protein ruminal degradability and the reduction was more pronounced with LS or xylose addition. The treatments with 10% LS and 2% xylose were not different, while both of them were more effective than 5% LS treatment.

Chemical Treatment

Formaldehyde treatment

Formaldehyde has been used to treat casein, oil seed meals, forage and silage to reduce ruminal degradation of protein and thus enhance the passage of dietary protein to the lower gut for digestion (Broderick et al. 1991). Formaldehyde treatments were found to increase wool growth, N retention and body weight gain in sheep production trials (Clark et. al. 1974). Broderick et al. (1991) reviewed the literature and summarized that: formaldehyde can form a Schiff's base with the lysine residue present in protein; formaldehyde also can react with other AAs (such as arginine, histidine, methionine, tryptophan and tyrosine); the amide groups of asparagine and glutamine and the peptide bond also react with formaldehyde and these reactions lead to the formation of methylene bridges between polypeptide chains. The protein thus was stabilized by formaldehyde (Walker 1964).

However, many studies conducted recently failed to show any benefits of formaldehyde treatments of proteins on dairy cow production (Rae et al. 1983; Bailey and Hironaka 1984; Crawford and Hoover 1984; Erfle 1986), even though in situ protein degradability and ruminal NH_3 release were decreased and total essential AA (EAA) absorption was increased (Rae et al. 1983; Bailey and Hironaka 1984). The problem of protein over-protection (Finlayson and Armstrong 1986), the carcinogenic potential of formaldehyde and the concern of environmental pollution associated with the treatment limit its application (Atwal 1995).

Tannin treatment

Feeding tannin-containing forage together with white clover, red clover or lucerne was found to prevent cows from the bloating by formation of stable foams in the rumen (Jones et al. 1973). The latter forage contains a large quantity of soluble leaf protein. Condensed tannin present in the forage appeared to prevent bloating by precipitating bloat causing proteins (Jones et al. 1973). The complexes formed between condensed tannins and proteins were stable at pH 3.5 - 7.0, but readily disassociated below pH 3 (Jones and Mangan 1977). Plant leaves with high condensed tannin content were fed to ruminants to reduce dietary protein ruminal degradation (Barry et al. 1986; Waghorn et al. 1987; Makkar et al. 1988). Barry et al. (1986) showed that rumen NH_3 concentration was reduced and, the flow of non-NH_3-N (NAN) to the small intestine and N retention were increased due to the increase of dietary reactive condensed tannin when *Lotus pedunculatus* (containing 95 g condensed tannin/kg DM) was fed to sheep. Waghorn et al. (1987) also reported a decrease in NH_3 concentration and protein degradation in rumen and an increase in NAN and EAA flow to small intestine when a low condensed tannin lotus (*Lotus corniculatus L.*, 22 g condensed tannin/kg DM) was fed to sheep.

Driedger and Hatfield (1972) treated SBM with Allepo tannin (tannic acid). They observed that the optimum protein protection was reached at 10% tannin addition which decreased in vitro deamination 92%. The authors further reported that the SBM treated with 10% Tara tannin increased lambs' daily body weight gains and daily N retention. The pelleting of treated

SBM resulted in superior N retention over an unpelleted treated SBM (Driedger and Hatfield 1972).

Tannin treatments thus presented an alternative method in protecting dietary protein from extensive ruminal degradation. However, tannin also has the potential for toxicity to animals and consistently had negative effects on nutrient digestion and growth in non-ruminant animals (Marquardt 1989; Salunkhe et al. 1990). Tannin was reported to reduce the small intestinal digestion (Driedger and Hatfield 1972) and the energy and N metabolism (Barry et al. 1986, Waghorn et al. 1987) in ruminants. The application of tannin to reduce rumen degradation of dietary protein requires further examination.

A number of other chemical treatments to reduce protein ruminal degradation have been studied. Lynch et al. (1987) treated SBM (800 g) with 70% ethanol (2000 ml) at 23 and 78°C for 1 h. They found that the SBM N disappearance from nylon bag (3 to 12 h ruminal incubation) was significantly decreased ($P < 0.05$) due to the treatment effects. The N retained by lambs was higher ($P< 0.05$) for lambs fed SBM treated at 78°C than that of control group. Mir et al. (1984) treated SBM and CM with 50% solution of NaOH at levels of 1, 2, 3 and 4 g NaOH/100 g DM for 24 h and then dried at room temperature. The authors reported that the effective degradability of CM and SBM were decreased ($P < 0.05$) when they were treated with 2 to 4 g NaOH/100 g DM. Atwal et al. (1974) pelleted SBM with volatile fatty acid (5 to 20% of protein). They found that the NH_3 accumulation rate (mM/h) in the rumen was inhibited by 57 to 84% following the provision of the treated SBM protein

(300g) either directly through the rumen via fistula or through the feeding to sheep compared to the control. Data from Cecava et al. (1993) showed that the measured ruminal escape N content of SBM and zinc-treated SBM (prepared by Central Soya, Decatur, IN) were 30 and 57% (n = 2), based on a 12 h Dacron bag ruminal incubation.

Physical Treatment

Physical application of coating materials onto the surface of dietary protein particles could be a useful alternative to chemical treatments. Coating materials should be designed to sustain the attacks from microbial degradation activities, the existing abrasive forces in the rumen, and remain ruminally insoluble but become soluble or digestible post-rumenly so as to release the coated protein for animal digestion. The key compounds of coating materials can be modified or made from natural polymeric products or from synthesized polymers.

Fresh blood (Ørskov et al. 1980; Mosimanyana and Mowat 1992) and fish hydrolysate (Mir et al. 1984) were used in coating SBM and/or CM and were found to be effective in reducing rumen microbial degradation of dietary protein. The effectiveness of these products as coating materials is limited because they are hydrolysable in the rumen, although their degradabilities are much lower than the one to be protected (NRC 1989). Mosimanyana and Mowat (1992) included xylose in blood and used the resulting mixture to coat SBM. Xylose can reduce the degradability of the protein in blood through the mechanism discussed for heat treatment. Wallace (1989) also used xylose to treat casein and hydrolysed gelatin so as to

form a sugar-protein mix. The SBM was encapsulated with this mixture et 140°C for 1 h. This treatment resulted in a decrease of about 50% in ruminal degradability of SBM.

Lipid has also been used to coat the particles of protein supplements to reduce their degradabilities in the rumen (Glenn et al. 1977; Lynch et al. 1987). Rumen microbes have very limited abilities in utilization of long-chain fatty acids (Wood et al. 1963), although they are able to hydrolyse lipid to produce fatty acids (Hawke and Silcock 1970; Hespell and O'Bryant-Shah 1988). A limitation of this treatment is that lipid used in coating could also depress digestibility of nutrients other than lipid in diets (Shirley 1986). Coconut oil-coated linseed meal reduced the NH_3 concentration in rumen, but also decreased dry matter and cellulose digestibilities (Glenn et al. 1977). Smith and Boling (1984) used a lipid-protein matrix (50% finely ground corn, 37.5% coconut oil and 12.5% zein) as a coating for DL-methionine and thus increased its post-rumen availability and absorption. Zein, an alcohol-soluble fraction of corn protein, is insoluble in water and also is low in rumen degradability (NRC 1989).

Calcium soaps of long chain fatty acids, a by-pass fat, were used successfully as a coating material to protect protein from rumen degradation (Sklan 1989; Sklan and Tinsky 1993). The calcium soaps of long chain fatty acids are insoluble in water and can not be utilized by microbes. And most important, they will not cause the side effects of lipid treatments mentioned before. One limitation associated with this method was a large quantity of calcium soaps (about 75 - 85% of finished coated product) had to be used for adequate protection.

Recently, Agriculture Canada developed a process for protecting SBM (Mahadevan 1990). In this process chemically modified zein was used as a coating material for SBM. The protected SBM was reported to have up to 90% ruminal resistance (Ivan et al. 1996). The authors further showed that dietary N flow to the small intestine was increased by 231% (9.08 vs. 2.74 g/kg of OM intake) when the treated SBM was fed to sheep compared to control. However, the treatment decreased the bacteria N flow by 18% (15.78 vs. 19.13 g/kg of OM intake) and thus had no effect on the total NAN flow (25.77 vs. 23.05 g/kg of OM intake) to the small intestine (Ivan et al. 1996). Milk production was significantly increased during week 7 to 16 of lactation for cows fed treated SBM diets (Atwal et al. 1995). The drawbacks of this method (Atwal et al. 1995) were that the treatment preparation procedure was very time consuming (about a month) and included a costly coating material (zein) and solvent.

Methods developed for AA (i. e. methionine and lysine) protection from rumen degradation also may be useful for dietary protein protection. Some coating materials of pH-sensitive polymer such as vinypyridine-styrene copolymer (Dannelly 1980; Wu and Sandhu 1984), vinylpyridine-styrene-lauryl acrylate polymer (Wu et al. 1989), Eudragit E 100 (Morita et al. 1986) or NN-dimethylaminoethyl methacrylate - Et methacrylate copolymer (Ueda et al. 1990) were used and patented for protecting AAs from ruminal degradation with release for post-rumen absorption. These coating materials were suggested to be stable above pH 5.5 in the rumen but labile below pH 3.5 in abomasum. These polymers are sensitive to pH change and have excellent coating properties. The difficulties in using them are that they have high product costs, commercial availabilities are limited for some and the coating procedures

are complicated and/or expensive. Less expensive polymers and simpler coating procedures need to be developed before they can be used for dietary protein protection.

CANOLA MEAL

Canola is the registered name for rapeseed containing less than two percent of the total fatty acids in the oil as erucic acid and less than 30 umol of alkenyl glucosinolates per gram of oil-free dry matter of the seed (Bell 1993). The CM is the by-product of canola oil industry, resulting from the crushing and the oil extraction of canola seed. After surveying the chemical composition of commercial CM produced in seven western Canadian crushing plants, Bell and Keith (1991) reported that the mean of aliphatic glucosinolates concentration in CM was 16 μmol/g which was much lower that the 110 - 150 μmol/g in the earlier varieties of rapeseed (Bell 1993). The CP percentage of CM was 41.85% (DM bases, Bell 1993) on average which was lower than the 52.7%, dry matter bases of SBM (Zinn 1993). Compared to SBM, CM had lower lysine but higher methionine and cystine concentrations (Table 1). The lysine/methionine ratio of CM is closer to those of milk and beef compared to that of SBM (Table 1). Thus CM is considered to have a better AA profile and protein quality compared to SBM. Presently CM is widely used as a protein source for ruminants in western Canada.

The CM has a relatively high rumen degradation rate. The degradabilities of CM protein were reported to be 75% (Rook et al 1983) and 72% (NRC 1989), higher than or close to

those of SBM protein, i.e. 73% (Satter 1983) and 65% (NRC 1989). Many dairy diets bassed on forage, cereals and SBM and/or CM do not meet requirements of about 37% undegradable intake protein (NRC 1989). Reducing the rumen degradability of CM protein will thus greatly improve the efficiency of CM protein utilization.

Table 1. The composition of essential amino acid (% dry matter) in canola meal (CM), soybean meal (SBM), milk and beef.

Amino acid	CM[a]	SBM[b]	Milk[c]	Beef[c]
Arginine	6.0	7.3	4.0	6.7
Histidine	3.4	2.7	3.0	3.7
Isoleucine	4.1	4.6	5.6	5.1
Leucine	7.0	7.8	10.2	8.0
Lysine	5.7	6.1	8.2	9.1
Methionine	2.0	1.2	2.9	2.7
Phenylalanine	3.9	5.1	5.4	4.5
Threonine	4.4	3.9	5.0	4.6
Valine	5.3	4.8	7.4	5.3
Lysine/Methionine	2.9	5.2	2.8	3.4

[a]Value calculated from Bell and Keith (1991).
[b]Value calculated from Zinn (1993).
[c]Value adapted from Ørskov (1992).

MANUSCRIPT I

THE EFFECT OF MOIST HEAT TREATED CANOLA MEAL ON LAMB GROWTH

ABSTRACT

Canola meal (CM) was moist heat treated at 110°C for 60 min to reduce protein ruminal degradability and increase protein post-ruminal flow, and thus to improve the lamb growth. The treated and untreated CM were used to formulate an iso-caloric and iso-nitrogenous treatment and control diet. Twenty-four Suffolk lambs, averaging 100 days old and 20.5 kg of body weight, were split into two groups (12 lambs including 6 male and 6 female per group) and two diets were assigned to these two groups of lambs randomly. The feeding period was 8 weeks. Daily dry matter (DM) intake, weight gain, wool growth, and wool growth efficiency (wool growth/DM intake) were not different between lambs fed the control and treatment diet. However, feed efficiency (weight gain/DM intake) was increased in lambs fed the treatment diet relative to lambs fed the control diet. Concentration of total amino acid, which equals the sum of free and short-peptide form amino acids, was increased in the deproteinized plasma in lambs fed the treatment diet relative to the lambs fed control diet. The moist heat treatment (at 110°C for 60 min) thus increased the nutritive value of CM for the growing lambs.

Keyword: Moist heat processing, protein protection, canola meal, lambs, plasma amino acid, weight gain, wool growth.

INTRODUCTION

Heat treatment has been used extensively as a method to reduce the dietary protein degradability in the rumen (Sherrod and Tillman 1964; Stern 1984; Faldet et al.1991; Moshtaghi Nia and Ingalls 1992, 1995a,b; Onyango and Ingalls 1994;Satter et al. 1994; Onyango 1995). The mechanism of protection in heat treatment involves principally the initial stage of Maillard reaction. The reaction is between the free amino group of lysine in protein and the aldehyde group of reducing sugars such as glucose and fructose which was present in the feedsuff (Hurrell and Finot 1985). The products (the Schiff's base and its intermediates) of this initial stage of the Maillard reaction are reversible and thus are bio-available to animals (Finot et al. 1977). Heating may enhance the formation of these reversible products which are more stable at a neutral pH and are liable in an acidic environment. Heat treatment thus is assumed to stabilize the dietary protein and render it less degradable in the rumen (pH 5.5 to 7.5) and thus increase the flow of dietary protein to the abomasum (pH< 3.0) for further digestion by the animal.

Canola meal (CM) has a high protein content (up to 42% crude protein (CP) on dry matter (DM) basis, Zinn 1993) and also is a good source of sulfur amino acid (AA) (methionine plus cystine). The sulfur AA content of CM is 4.7% on a DM basis (Bell and Keith 1991) which is higher than that of soybean meal (3.1% on a DM basis, Zinn 1993). Sulfur AAs have been shown to have a positive influence on wool growth in wethers (Radcliffe et al. 1985; Stephenson et al. 1991). Canola meal protein however has a relatively high rumen

degradation rate (72%, NRC 1989). Some moist heat is applied to CM during the oil extraction process at the crushing plants. Additional moist heat treatment to CM may be a convenient method for reducing the rumen degradability of CM protein and AA and thus increasing their amounts post-ruminly. Onyango (1995) reported that the effective degradability of CM protein was decreased ($P < 0.05$) when CM was moist heat treated at 100°C for 60 min utilizing pilot plant equipment compared to control. Milk yield was increased ($P < 0.05$) about 2 kg/d in cows fed treated CM vs. control in primparous cows (Onyango and Ingalls 1994). The objectives of the present research were to study the effect of this same moist heat treated CM (110°C for 60 min) on the rumen condition, plasma AA level and the growth of lambs.

MATERIALS AND METHODS

Heat Treatment of CM

The CM was heat treated at the Protein Oil Starch pilot plant (Saskatoon, SK). Heat treatment involved passing CM through a steam jacketed conveyor set at the lowest speed (90 kg CM/h) with 5% steam addition and at the maximum steam pressure (130 psi). The water was added at 45 kg/h to generate steam. The CM was then held on hot trays and steeped for 60 min at 110°C on average (Onyango 1995).

Animals, Diets and Experimental Design

Twenty-four Suffolk lambs (made up of 12 male and 12 female, averaging about 100 days old and 20.5 kg body weight) were randomly divided into two groups which were balanced for sex. Lambs were housed in pens (two lambs of the same sex per pen) in the Animal Science Research Unit (University of Manitoba, Winnipeg, MB) such that there were 6 pens per dietary treatment. Lambs were fed one of the two pelleted diets, the control and treatment (Table 1.1), on an ad lib basis for 8 weeks. Feed was offered once daily at 9:00 pm. The diets were formulated to meet the maintenance and growth requirements of lambs (NRC 1985a) and to be iso-caloric and iso-nitrogenous. Canola meal protein made up 38% of total diet crude protein. The lambs were adapted to a concentrate diet over a period of 10 days and then adapted to the experimental diets over a four day period. Feed offered was recorded daily and the weight backs were recorded weekly. Feed samples were colleted from each 500 kg bulk bag of pelleted feed and composited over the experimental period. The composited feed samples were analyzed for DM (934.01, AOAC 1990), CP (796.06, AOAC 1990), acid detergent fiber (ADF, 973.18, AOAC 1990) and neutral detergent fiber (NDF, Goering and Van Soest 1970).

Lambs were weighed on two consecutive days prior to and immediately after the finish of the trial. They also were weighed every two weeks during the trial. The lambs were shaved on both flanks in the beginning of trial. A 10 x 10 cm patch of wool on both flanks was clipped and weighed upon completion of the trial. The clipped wool was washed by immersing in 400

ml of commercial petroleum ether (Fisher Scientific, Nepeon, ON) for 10 min with manual agitation and repeated squeezing (Radcliffe et al. 1985). The wool was washed twice in petroleum ether followed by a final rinse in distilled water (50°C), and was dried at 70°C for 24 h and weighed to calculate daily wool growth (Radcliffe et al. 1985).

Rumen Fluid, Blood Sampling and Analysis

Rumen fluid and blood samples were collected over three alternate days during the 5th week of the experimental period. Lambs within control and treatment groups were each randomly divided into three groups. These three groups of lambs (balanced in control and treatment) were assigned to three sampling patterns (i.e. 1st, sampled at 0 and 10 h post-feeding; 2nd, sampled at 2 and 14 h post-feed; 3rd, sampled at 6 and 22 h post-feeding) over the three alternate sampling days via a Latin square design. Samples of rumen fluid and blood were obtained to represent the samples of six time points, i.e. 0 (right before feeding) , 2, 6, 10, 14 and 22 h after morning feeding. The rumen fluid samples at the 2 and 14 h (after morning feeding) time points were not available due to the difficulty in obtaining rumen fluid at these times.

Rumen fluid samples (30 ml) were obtained from the rumen by using an oesophageal tube (Ingalls et al. 1980). The samples were measured for pH and were then stored frozen (-20°C). They were then thawed at room temperature and centrifuged at 25,000 x g for 20 min to obtain a clear supernatant which was stored frozen (-20°C). The ammonia (NH_3)

concentration in the clear rumen fluid (thawed at room temperature) was measured with an NH_3 electrode (model 95-10, Orion Research Inc., Cambridge, MA).

Blood samples (10 ml) were collected into two 10 ml vacuumed and heparinized tubes through cervical venipuncture. The blood sample was centrifuged immediately at 2000 x g for 10 min to obtain plasma which was then stored frozen (-20°C). The plasma samples were chosen randomly from one of the lambs in each pen to be analyzed for AA composition. Three ml of plasma (thawed at room temperature) was deproteinized by the addition of a 75 mg 5-sulphosalicylic acid and the resulting mixture was centrifuged at 27,750 x g for 10 min to obtain a deproteinized plasma. One ml of deproteinized plasma was lyophilized at 30°C for 72 h (Model Genesis LE 25, The Virtis Company, Gardiner, NY) in a hydrolysis tube, and 1 ml of 3 N HCl and 2 drops 2-octanol was added into the tube after the lyophilizing. The tube was evacuated and stoppered. The mixture was then hydrolyzed at 110°C for 24 hour on a heat block. The cooled hydrolysate was neutralized with 0.5 ml of 25% w/v NaOH and cooled to room temperature. The resulting mixture was made up to 10 ml in a volumetric flask and filtered through #40 filter paper and stored frozen (-20°C) for the analysis of AAs composition. Another 1 ml of deproteinized plasma was lyophilized in a hydrolysis tube and oxidized with 1 ml performic acid (88% formic acid and 35% H_2O_2) and 2 drops of 2-octanol for 20 h at 4°C. A 6 N HCl (0.25 ml) solution was added to the mixture to react with the remaining performic acid for 6 h in a fume hood. Another 1 ml of 6 N HCl was added to the tube to hydrolyze the mixture at 110°C for 16 h. The tubes were covered with marble balls during the oxidization and hydrolysis processes. The cooled hydrolysate was neutralized with

0.625 ml 25% w/v NaOH and cooled to room temperature. The mixture was made up to 10 ml in a volumetric flask and filtered through #40 filter paper and stored frozen (-20°C) for further analysis of cysteine and methionine. All the hydrolyzed samples were analyzed for AA contents by high pressure liquid chromatography (Model Alpha Plus 4151, LKB/BIOCHROM, Cambridge, England). About 0.3 ml of deproteinized plasma was prepared and analyzed for free AA content by ion-exchange chromatography (Model Biochrom 20 AA Analyzer, Pharmacia Biotech (Biochrom) Ltd., Cambridge, England). The AA content of short peptides in deproteinized plasma were calculated by the difference of AA content of hydrolyzed deproteinized plasma and the free AA content in deproteinized plasma (McCormick and Webb 1982).

Statistical Analysis

The experiment was a completely random design. All the data were analyzed statistically by using GLM (general linear models) procedures of SAS System (SAS 1989). Statistical differences between treatment means were tested by using Tukey's studentized range test (SAS 1989).

The treatment effect on DM intake, weight gain, wool growth, feed efficiency and wool growth efficiency was evaluated by using the following model:

$$Y_i = \mu + T_i + e_i$$

where

Y_i = observation,

μ = overall mean,

T_i = treatment effect,

e_i = residue,

i = 1 to 12 animals for weight gain and wool growth, 1 to 6 pens for the remainder.

The treatment effect on the pH and NH_3 concentration of the rumen fluid, and the plasma AA concentration were evaluated by using the following model:

$$Y_{ijklm} = \mu + T_i + G_{j(i)} + D_k + C_l + T_i*G_j*D_k*C_l + S_m + C_l*S_m + T_i*S_m + S_m*D_k + T_i*C_l*S_m + T_i*S_m*G_j*D_k*C_l + e_{ijklm}$$

where

Y_{ijklm} = observation,

μ = overall mean,

T_i = treatment effect (i = 1,2),

$G_{j(i)}$ = animal group effect (j = 1,2,3),

D_k = sample collection day effect (k = 1,2,3),

C_l = sample collection pattern effect (l = 1,2, for pH and NH_3; 1, 2, 3 for plasma AA),

S_m = sample collection sequence effect (m = 1,2),

$T_i*G_j*D_k*C_l$, C_l*S_m, T_i*S_m, S_m*D_k, $T_i*C_l*S_m$, $T_i*S_m*G_j*D_k*C_l$ = interaction effects,

e_{ijklm} = residue.

There were 6 and 10 observations missing for rumen fluid pH and NH_3 concentration, respectively. Only the treatment effect (i.e. T_i) and the interaction effect (i.e. $T_i*C_j*S_m$) between the treatment and the sample collection time were of interest and investigated in the present study.

RESULTS AND DISCUSSION

All of the 24 lambs completed the 8 weeks trial. The concentration of digestive energy in the diets (Table 1.1) estimated from NRC (1985a) were adequate for lambs ranging from 20 - 60 kg body weight to have weight gain ranging from 300 - 400 g/d. The actual CP level in the diets (Table 1.1) was close to the NRC (1985a) level (16.9 %, DM basis) for lambs averaging 20 kg body weight with 300 g weight gain/d, and would be adequate for lambs ranging from 30 - 60 body weight with weight gain ranging from 325 to 400 g/d (NRC 1985a). The DM intake of lambs as kg/d or percentage of body weight (Table 1.1) was within the range of predicted value for lambs ranging from 20 to 60 kg body weight with weight gain ranging from 300 - 425 g/d (NRC 1985a). Thus the diets formulated in the present study were able to meet the nutrient requirements of the growing lambs.

Differences were shown between the control and treatment diets in the measured CP, NDF and ADF contents (Table 1.1). These differences might be caused by the variations of feed sampling. Data from Onyango (1995) showed that the same moist heat treatment (110°C, 60 min) did not alter ($P > 0.05$) the CP content (39.6 vs. 40.4%, SEM = 0.1%) and the ADF

content (18.1 vs. 16.1%, SEM = 0.2%), but increased ($P < 0.05$) the NDF content (25.1 vs. 21.1%, SEM = 0.6%) in CM compared to the control. A higher ($P < 0.05$) NDF and ADF content in the heat treated protein meal was observed by Moshtaghi Nia and Ingalls (1992) and Hussen et al. (1995). This increase in NDF and ADF content may be attributed to the increase in the content of protein which is less soluble in the neutral and acid detergent buffers due to the heat denaturing effect to the protein during the heating process (Moshtaghi Nia and Ingalls 1992; Van Soest 1994).

The daily dry matter intake, weight gain, wool growth and the wool growth efficiency were not different between lambs fed the control or treatment diets (Table 1.2). However, feed efficiency was increased when lambs were fed moist heat treated CM (at 110°C for 60 min) diet compared to the control (Table 1.2). These results were consistent with the finding of Onyango and Ingalls (1994) whose data also showed an increase in milk yield efficiency (milk yield/DM intake, 1.62 vs. 1.49 as calculated) in cows fed the diet containing moist heat treated CM relative to the cows fed the control diet. They observed a 7.3% increase ($P< 0.05$) in milk yield (29 vs. 27 kg/d) in primparous cows due to the treatment effect.

Onyango (1995) showed that the ruminal effective degradability of CM treated with moist heat at 110°C for 60 min was lower ($P < 0.05$) than that of untreated CM (44.35 vs. 56.15%,). The decrease in the protein degradability of moist heat treated CM thus might be expected to cause a decrease in NH_3 production in the rumen when the treated CM was fed to lambs in replacing the untreated. However the rumen NH_3 concentration and rumen pH

which might serve as an index for the extent of ruminal degradation of dietary nutrient were not affected by the inclusion of treated CM in the diet (Table 1.2) in the present study. The interaction effect between treatment and rumen fluid sampling time was not significant on the rumen pH and the rumen NH_3 concentration.

The concentration of total AA (i.e. the sum of free and short-peptide form AA) in deproteinised plasma were increased ($P< 0.05$) in total AA, in essential and non-essential AA, and in individual AA, i.e. alanine, arginine, aspartic acid, glutamine, isoleucine, leucine, phenylalanine, proline, threonine and serine for lambs fed moist heat treated CM compared to the lambs fed the control diet (Table 1.3). This increase in AA level in the plasma may indicate that the amount of AA absorbed from the small intestine was increased due to the heat treatment effect which may cause the decrease of the protein degradability in the rumen and thus increase the flow of CM protein or AA to the small intestine for animal digestion. This result is consistent with the observation by Onyango (1995). He showed that the availability of AA in lower GI tract from CM was increased by 146% as measured by mobile nylon bag when the CM was moist heat treated at 110°C for 60 min compared to that of untreated CM (67.16 vs. 27.28%). The moist heat treatment (at 110°C for 60 min) thus increased the by-pass protein in CM and increased the protein supply to the lower GI tract for animal digestion and absorption. No measure of bacterial protein was made. The interaction between treatment and blood sampling time was not significant for the concentration of total AA with the exception of threonine in the deproteinized plasma.

Wool is especially rich in the sulfur-containing AA, i.e. cystine and methionine (Bradbury 1973) relative to beef (Ørskov 1992), CM (Bell and Keith 1991) and SBM (Zinn 1993). Methionine is usually the most limiting AA for wool production (Ensminger and Parker 1986). The total sulfur-containing AA levels in deproteinized plasma were not improved by the inclusion of treated CM in the diet compared to control (Table 1.3), even though in situ data suggested a 132% increase (57.9 on average vs. 25%) in the percentage of ruminal escape of these AAs following a 16 h nylon bag ruminal incubation (Onyango 1995) using the same moist heat treated CM related to untreated CM.

The total AA is made up of the free and short-peptide form AAs in deproteinized plasma (McCormick and Webb 1982). The concentration of free and short-peptide AAs in deproteinized plasma were not different ($P > 0.05$) in total and in individual (excepting free alanine and cystine, and short-peptide form aspartic acid, serine, isoleucine, leucine and phenylalanine) between lambs fed treatment and control diets (Table 1.3). The interaction effect between treatment and blood sampling time (i.e. the 6 time points of collection pattern x collection sequence) was not significant for free AA and short-peptide form AA concentrations in deproteinized plasma in lambs fed the treatment and control diet. The total (i.e. free plus short-peptide form) AA level in the deproteinized plasma reflected more changes in AA level than the free or short-peptide AA did individually (Table 1.3). Therefore, the concentrations of the total AA in the deproteinized plasma is more representative in reflecting the AA nutritive status in the lower gastrointestinal tract in animal than the concentration of free or short peptide AA.

In conclusion, moist heat treatment of canola meal at 110°C for 60 min improved the absorption of dietary AA in the lower gastrointestinal tract and the feed efficiency of a barley based diet when treated CM was fed to lambs in place of commercial CM. Moist heat treatment thus improved the nutritive value of canola meal for growing lambs.

Table 1.1. The ingredient and nutrient composition of the diets containing control and moist heat treated canola meal for lambs.

ITEM	Diet	
	Control	Treatment
Ingredient (%, as fed)		
Barley grain (rolled)	69.2	69.2
Canola meal (untreated)	13	--
Canola meal (treated)	--	13
Sunflower seed hulls	12	12
Beet molasses	1	1
Calcium carbonate	1.2	1.2
Megalac	2	2
Mineral premix [1]	1	1
Vitamin premix [2]	0.4	0.4
Bovatec[3]	0.02	0.02
Nutrient[4] (DM basis)		
TDN (%)	76.1	76.1
DE (Mcal/kg)	3.4	3.4
CP (%)	15.2	13.9
NDF (%)	29	23.8
ADF (%)	16.1	13.4

[1]The mineral premix includes: 93.10% cobalt iodized salt, 5% dynamate, 0.93% zinc sulphate, 0.84% manganese sulphate, 0.13% copper sulphate (25% Cu) and 0.003% Sodium selenite (30% Se).

[2]The vitamin premix includes: 967 IU vitamin A/mg, 76 IU vitamin D/mg and 1 IU vitamin E/mg.

[3]Bovatec, a lasalocid premix, consists of 15% of lasalocid, from Hoffmann - La Roche, Nutley, NJ.

[4]The TDN (total digestible nutrients) and DE (digestible energy) values were estimated from NRC (1985), and the DM, CP, NDF (neutral detergent fiber) and ADF (acid detergent fiber) were determined.

Table 1.2. The effect of moist heat treated canola meal on daily dry matter intake (DMI), daily weight gain (WG), wool growth (WoG), feed efficiency, wool growth efficiency, rumen fluid pH and rumen fluid ammonia (NH_3) concentration of lambs during an eight-week growth trial.

Item	n[1]	Diet		SEM[2]
		Control	Treatment	
Initial body weight	12	20.4	20.5	0.6
Dry matter intake (DMI)				
kg/d	6	1.29	1.26	0.05
% of body weight	6	3.79	3.65	0.08
Weight gain (kg/d, WG)	12	0.4	0.43	0.02
Wool growth ($mg/cm^2/d$, WoG)	12	0.97	0.94	0.06
Feed efficiency (WG/DMI)	6	0.31b	0.34a	0.01
Wool growth efficiency (WoG/DMI)	6	0.76	0.75	0.06
Rumen fluid[3]				
pH	45[4]	6.04	5.95	0.04
NH_3 (mg/dl)	43[5]	10.6	8.6	0.8

a,b: means in rows with different letters were different ($P < 0.05$).
[1]Observation number.
[2]Standard error of means.
[3]Least squares means.
[4]Average of observation numbers of two treatments with n = 46 for control and n = 44 for treatment.
[5]Average of observation numbers of two treatments with n = 45 for control and n = 41 for treatment.

Table 1.3. The effect of moist heat treated canola meal on the composition of total AA (TAA), free AA (FAA) and short-peptide form AA (SAA) in deproteinized plasma of lambs ($n^1 = 36$).

AA (mg/dl)	TAA			FAA			SAA[5]		
	C[2]	T[3]	SEM[4]	C	T	SEM	C	T	SEM
Individual									
Aspartic acid	4.18b	5.01a	0.11	0.96	0.91	0.06	3.22b	4.09a	0.16
Threonine	4.10b	4.38a	0.07	3.31	3.34	0.24	0.79	1.04	0.18
Serine	3.18b	3.66a	0.09	1.59	1.56	0.11	1.60b	2.10a	0.13
Glutamine	12.07b	13.65a	0.15	5.6	6.15	0.36	4.47	7.5	0.38
Proline	4.75b	5.33a	0.11	2.21	2.47	0.17	2.53	2.86	0.11
Glycine	6.90a	6.07b	0.13	4.96	4.43	0.22	1.93	1.64	0.17
Alanine	3.54b	4.02a	0.1	2.27b	2.71a	0.11	1.27	1.49	0.09
Cystine	1.17	1.19	0.03	0.26b	0.29a	0.01	0.91	0.9	0.03
Valine	5.83	6.23	0.14	4.15	4.25	0.18	1.68	1.98	0.13
Methionine	1.88	1.84	0.06	0.65	0.65	0.03	1.23	1.19	0.05
Isoleucine	2.28b	2.65a	0.09	1.71	1.82	0.1	0.56b	0.83a	0.03
Leucine	5.34b	6.52a	0.06	2.6	2.94	0.13	2.74b	3.58a	0.13
Tyrosine	1.18	1.63	0.13	3.2	3.32	0.17	--	--	--
Phenylalanine	6.32b	7.18a	0.14	2.12	2.25	0.1	4.20b	4.92a	0.16
Histidine	2.89	3.19	0.13	2.76	3.09	0.15	0.13	0.1	0.19
Lysine	8.24	8.41	0.2	6.59	6.65	0.36	1.65	1.76	0.26
Arginine	4.85b	5.63a	0.18	3.19	3.52	0.19	1.66	2.1	0.18
Total	78.70b	86.78a	0.79	48.15	50.37	2.47	30.55	36.41	1.85
Essential	42.90b	47.21a	0.74	27.35	28.8	1.4	15.55	18.41	1.04
Non-essential	35.80b	39.56a	0.43	20.8	21.56	1.1	15	18	1.06

a,b: means in rows with different letters were different ($P < 0.05$) within TAA, FAA and SAA, respectively.

[1]Observation number.

[2]C=control.

[3]T=treatment.

[4]Standard error of means.

[5]SAA=TAA-FAA.

MANUSCRIPT II

THE EFFECTS OF LIGNOSULFONATE, WATER, ALCOHOL AND HEAT TREATMENT ON THE RUMEN DEGRADATION AND POST-RUMEN ENZYME DIGESTION OF CANOLA MEAL PROTEIN

ABSTRACT

Canola meal (CM) was heat treated with lignosulfonate (LS), water or alcohol to protect the protein from ruminal degradation. The four factors: LS, 0%, 4%, 6.5% and 9% CM DM; temperature and time, 100°C/1 h, 125°C/0.5 h and 150°C/0.5 h; water, no added water and added-water to moisture of 20% of CM DM with or without LS added; and alcohol, 0%, 5%, 10% and 15% of CM DM with or without LS added were tested in a 4x3x2x4 factorial design. The increasing LS levels up to 6.5% increased digestible undegradable intake protein (DUIP) of CM protein. Temperature and time levels did not differ in their effects on DUIP and alcohol addition did not increase DUIP in relative to untreated CM. Addition of water decreased DUIP. Four CM treatments, i.e. control, 129°C/32 min/6.5% LS, 154°C/32 min, and 154°C/32 min/ 6.5% LS, were tested further using a 4 x 4 latin square with four rumen cannulated heifers over four periods. Alcohol or water addition were not used in the treatment. Heating CM to 154°C with LS increased the in situ effective degradability of CM protein, and thus increased the amount of CM protein available for digestion in the lower gastrointestinal (GI) tract over the control.

Key words: Canola meal, heat, lignosulfonate, alcohol, in situ, protein degradation.

INTRODUCTION

Reducing sugars such as xylose, glucose, fructose or lactose have been used with heat treatments to protect dietary protein from rumen degradation (Cleale et al. 1987 a, b, c; Wallace and Falconer 1992; Mosimanyana and Mowat 1992; McAllister et al. 1993). The mechanism of protection with the reducing sugars and heat treatments involve principally the initial stage of the Maillard reaction. The reaction is between the free amino group of lysine in protein and the aldehyde group of reducing sugars (Hurrell and Finot 1985). The products of this initial stage of the Maillard reaction, namely the Schiff's base and intermediate products are reversible and thus were bio-available to animals (Finot et al. 1977). Heating may enhance the formation of these reversible products which are more stable at a neutral pH while liable in an acidic environment. Thus the reducing sugars and heat treatments stabilize protein and render it less degradable in the rumen (pH 5.5 to 7.5) while maintaining digestibility in the abomasum (pH < 3.0).

Lignosulfonate (LS), a byproduct of the wood industry, has been used successfully as a rich source of reducing sugar in heat treatment to protect soybean meal (SBM) protein (Stern 1984; Windschitl and Stern 1988; Nakamura et al. 1992) and canola meal (CM) protein (McAllister et al. 1993). Alcohol solution was shown to decrease the rumen degradation of SBM in lambs when alcohol soaked SBM (unheated) (van der Aar et al. 1982), and alcohol and heat treated SBM (Lynch et al. 1987) was fed. The objective of this research was to investigate the potential of the combination of LS, water, alcohol and

heat treatments in reducing CM protein rumen degradation and improving the availability of CM protein in the lower gastro-intestinal (GI) tract.

MATERIAL AND METHODS

Trial One

Canola meal treatments and experimental design

One source of CM (38.8% crude protein (CP), dry matter (DM) basis, from a local feed mill was exposed to the following treatment combination involving four factors: LS (Ligno Tech USA, Inc., WI) consisting of 65 % DM, 70% of reducing sugar and 40% xylose (DM basis); temperature and time of heating (TT); alcohol (ALC, 95% v/v ethanol); and water (W) via a 4x3x4x2 factorial design. The levels of these four factors were: LS, 0, 4, 6.5 and 9% of original CM DM; TT, 100°C/1 h, 125°C/0.5 h and 150°C/0.5 h; ALC, 0, 5, 10, 15% of CM DM with or without LS added; and W, no added-water treatment with moisture at 12% (standard deviation = 1%) of CM DM with or without LS added on average, and added-water treatment with moisture at 20% of CM DM with or without LS added. The LS was first added to the original CM (150 g DM) to the assigned LS level and then alcohol was added. Finally, water was added to reach the moisture level assigned to the added-water. The moisture level of CM treatment was calculated as the following:

Moisture (% of DM of CM with or without LS added) =

(moisture in original CM, g + moisture in added LS, g + moisture in added alcohol, g + added water, g) / (DM of CM with or without LS added, g) x 100.

The CM mixtures were put in an 18 x 18 cm aluminum tray covered with aluminum foil to prevent the evaporation of moisture from the CM mixture. The covered tray was heated in a forced-air oven to the assigned temperature and time level. After heating the tray was retrieved from the oven, and the mixture was mixed and cooled without a cover and left to air dry at room temperature for two days. There was no replication for each CM treatment of the combination of each level of the four factors.

In situ nylon bag incubation

The ninty-six treated CM samples were randomly divided into 5 sets (18 - 20 samples per set) and were incubated in the rumen of a dry dairy cow to determine the nylon bag protein disappearance of the samples on five separate testing days, i.e. 1st, 3rd, 9th, 17th and 20th day in a 21-day experimental period. No reference standard of feedstuffs was used. Three grams of each treated CM sample were placed in a 10 x 10 cm nylon bag (50 um porosity, Telco Industries Ltd., Concord, ON). This resulted in 15 mg sample per cm^2 of nylon bag (Michalet-Doreau and Ould-Bah 1992). On any given test day duplicate sample bags were held separately in two large 25 x 45 cm nylon bags with 2 x 3 mm mesh containing 18 - 20 small sample bags. The large nylon bags were tied to the rumen cannula (Bar Diamond

4", Bar Diamond, Inc., Parama, ID) by a 45 cm nylon cord and were weighed down in the rumen with a bottle of sand.

The rumen cannulated mature dry Holstein cow was fed a lactation diet free choice with 50 : 50 (DM basis) forage and concentrate twice a day (9:00 and 16:00). The cow was adapted to the diet 10 days prior to the start of the experimental period. Bags were incubated in the rumen for 16 h from 17:00 of the first day to 9:00 of the next day and removed before the morning feeding. Incubated sample bags were retrieved from rumen and washed with cold water in a wringer type washing machine for 10 min followed by another 5 min washing in clean water. The washed bags were dried in a forced-air oven at 60°C for 48 h. The dried bags were weighed. The original and the treated CM, and the residues in the bags were sampled and analysed for CP content. The nylon bag disappearance (NBD) of CM protein in the treatments was calculated as follows:

NBD (% of CM protein) = (the difference of CP contents in the nylon bag before and after rumen incubation, g) / (CP contents in the nylon bag before rumen incubation, g) x 100.

The mean of the two NBDs calculated from two duplicated nylon bags was used to represent the NBD value of each CM treatment sample.

In vitro pepsin and pancreatin digestion

A pepsin and pancreatin digestion procedure (modified from Akeson and Stahmann 1964)

was used to investigate the effects of treatment on the in vitro post-rumen digestibility of CM protein in vitro. A half gram of sample (about 167 mg crude protein) was incubated with 25 ml, 10% (w/v) pepsin (No. P-7000, Sigma) HCl (0.1 N) solution in a 250 ml flask (covered with aluminum foil) at 39°C for 3 h in a incubator. After the pepsin digestion, the mixture was neutralized with 12.5 ml 0.2 N NaOH. A pancreatin solution which was made of 6.67 mg pancreatin (8049-47-6, Sigma) in 12.5 ml pH 8.0 phosphate buffer was added into the mixture. A 0.25 ml of 1% thimerosal solution (T 5125, Sigma) was added into the incubation mixture right before the pancreatin digestion to prevent microbial growth in the culture. The mixture was covered with aluminum foil and was incubated further for 24 hours at 39°C in an incubator. Five ml of 80% t-butyl alcohol solution was added to the digestion mixture upon the finish of pancreatin digestion to stop enzyme activity. The final digestion mixture was filtered through a filter paper (Whatman No 541) and the residue was analysed for CP content. The pepsin and pancreatin digestibility (PPD) of CM protein, i.e. the CM protein that had not been incubated in the rumen, was measured as the following:

PPD (% of CM protein) = (the difference of crude protein content between the input of CM and the residue after the pepsin and pancreatin incubation, g) / (CM protein input, g) x 100.

Each CM treatment sample was measured for PPD value twice and the mean of these two values was used to represent the PPD value of the treatment sample.

The post-rumen digestibility of CM protein was assumed to be unaltered by rumen

fermentation. Thus the measured PPD of CM protein was used to represent the post-rumen digestibility of CM protein that would escape rumen degradation. The percentage of digestible undegradable intake protein (DUIP, % of CM protein) of treated CM protein can be calculated from the following formula:

DUIP (% of CM protein) = (PPD, % of CM protein) x (100 - (NBD, % of CM protein)) / 100.

The NBD and PPD in the formula were the means of the values of two duplicated measurements as mentioned above.

Trial Two

Lignosulfonate and heat treated CM

Canola meal was heat-treated (Sureleen-Albion Agra Inc. West, Alrdrie, AB) together with or without LS in a Jet-Pro heating apparatus adapted for meal (Jet-Pro Company, Springfield, OH) in April 1995. For the LS treated CM, the CM was mixed with 6.5% LS before heating. The CM or its LS mixture (about 450 kg) was heated to reach the assigned exit temperature of 129 or 154°C in 2 min 34 sec. The heated CM remained in a holding chamber for 32 min (i.e. steeping) without further heating. The temperature dropped to about 27°C during steeping before exiting the holding chamber. Water (79°C) was added at 2.73 kg/min to the treatment in the beginning of steeping to make up the moisture lost during the whole treatment process. Four treatments were made up of: I

(control), original untreated CM as a control; II (129C+LS), CM heated to 129°C and held for 32 min with the addition of LS at 6.5% of original CM DM; III (154C), CM heated to 154°C and held for 32 min without LS added; and IV (154C+LS), CM heated to 154°C and held for 32 min with the addition of LS at 6.5% of original CM DM. Samples of these four CM treatments were taken with a sample probe and were analysed for DM, CP, NDF, ADF and ADIN.

Animals

Four Holstein heifers, averaging 507 kg body weight (standard deviation = 51 kg) and about 2.5 month before calving, were fitted with rumen cannula (Bar Diamond 4", Bar Diamond, Inc., Parama, ID) and duodenum T-shaped cannula (IC2, Ankom, Fairport, NY; at the proximal duodenum).

Diet

The four heifers were fed the same diet free choice prior to and during the whole trial. The diet was formulated to meet the NRC (1989) requirements using a least cost ration program (TriLogic Systems, Des Moines, IA) for a 500 kg lactating cow producing 40 l milk (3.6% butter fat) daily. The diet consisted of barley grain 22.3%, CM 13.7%, tallow 2.05%, vitamin and mineral premix 0.53%, limestone 0.18%, dynamate 0.05%, salt 0.12%, alfalfa hay (18% CP) 17.5% and corn silage (eared) 43.2% (as fed), which

resulted in a diet of 53% concentrate and 47% roughage (DM basis). Heifers were housed in pens in the Animal Science Research Unit (University of Manitoba) and were fed twice a day at 9:00 and 16:00. The concentrate was fed to heifers mixed with the roughage and weigh backs were taken before the morning feeding. The quantity of diet fed to a heifer was based on the amount of diet that heifer consumed the day before plus about 2 kg.

Experimental design

Heifers were used in a 4 x 4 latin square design to measure the in situ protein degradation and mobile bag digestion over four periods. There was a five days adjustment with an experimental period of nine days for period one, three and four, and with an experimental period of seven days for period two. One heifer aborted in the beginning of period four and was milking during the testing period.

Nylon bag incubation in rumen

The sample bags (3 g of sample in a 10 x 10 cm nylon bag, 50 μm pore size, Ankom, Fairport, NY) were prepared as indicated in trial one. Ten rumen incubation time points, i.e. 0.32, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h were assigned to each cow x treatment observation during each period. Duplicate sample bags were incubated in the rumen to represent each cow x diet x time point observation. Duplicate bags for two time points were held in a large 25 x 45 cm nylon mesh bag with 2 x 3 mm mesh which was tied to

the rumen cannula by a 45 cm nylon cord and was weighed down with a bottle of sand in the rumen. The sample bags were inserted into the rumen in a reverse time point sequence, such that all the nylon bags (five large mesh bags in total, with four sample bags residing in each large mesh bag) in each cow was retrieved from the rumen at the same time upon the completion of rumen incubation. For the time point 0.32 h, nylon bags were inserted into the rumen 19 min before they were retrieved together with other nylon bags which were inserted into the rumen earlier. The incubated sample bags were washed, dried and contents analysed for CP, and the NBD of CM protein was calculated as in trial one. The nylon bag data was not available in one cow during period three due to the failure of the rumen cannula.

Outflow rate of CM supplement from the rumen

The outflow rate (k) of CM from the rumen of each cow was measured by the administration of Cr mordanted CM (Moshtaghi Nia 1994) as an ingestible marker via diet. During the third experimental period, cows were held in stalls and 65 g of Cr mordanted CM (6.57% of Cr, as fed) was mixed with a portion of grain (1 kg) in the daily ration and fed to each cow before the remaining ration was fed at 9:00 am of day one. Feces was collected and sampled every three hours in the first one and a half days and every twelve hours in the remaining three and half days. The collected fecal samples (around 500 g) were stored frozen (-20°C). The fecal samples were thawed at room temperature and dried at 60°C for two days in a forced-air oven. The dried fecal samples

were grounded through a 2 mm screen and were analysed for DM and Cr (Williams et al. 1962) contents. There were 19 fecal samples for three cows and only the first 16 fecal samples were available for one cow due to the loss of rumen content caused by the failure of the rumen cannula during the fecal collection.

The *k* values were measured by using the following linear regression model:

$$\ln y = a + b*t$$

where

ln = natural logarithm,

y = the peak and post-peak value of Cr concentration (ppm),

t = the average duration of time after the administration of Cr-mordanted CM through the diet to the collection of feces (h),

a, b = constant.

The absolute value of constant b in the above model represented the outflow rate *k* (%/h) in which the CM supplement was passing out of the rumen (Hartnell and Satter 1979).

Effective degradability of canola meal protein

The following model was used to describe the protein disappearance from the nylon bag (Ørskov and McDonald 1979):

$$p = a + b*(1 - e^{-c*t})$$

where

t = incubation time of nylon bag (with CM in the bag) in the rumen (h),

p = the percentage of protein disappeared from the nylon bag (i.e. NBD) by the time t of ruminal incubation (%),

a, b, c = constant.

The constant a, b and c in the model were estimated for each treatment in each cow by analysing for the protein disappearances (the mean estimated from the two bags in each time point) from nylon bags at ten time points with the non-linear model (NLIN) procedures of SAS system (SAS 1989).

The constant a, b and c were interpreted to represent the rapidly-soluble protein fraction of CM protein, the degradable protein fraction of CM protein and the fractional-rate constant at which the degradable protein fraction of CM protein degrades in the rumen in situ, respectively (Ørskov 1992).

The in situ effective degradability of CM protein in treatments was thus calculated from the following equation, considering both the degradation and outflow of CM protein in an actual rumen environment (Ørskov 1992):

$$ED = a + b*c/(c + k)$$

where

ED = in situ effective degradability (% of CM protein),

a = rapidly-soluble protein faction (% of CM protein),

b = degradable protein fraction (% of CM protein),

c = fractional-rate constant at which degradable protein fraction of CM protein degrades in the rumen (%/h),

k = outflow rate of CM protein (%/h).

Sample Analysis

The DM, CP, ADF and ADIN content in the sample was analysed according to method number 934.01, 976.06, 973.18, and 973.18 and 973.06, respectively, published by AOAC (1990). The NDF content of sample was measured by using the method of Goering and Van Soest (1970).

Statistical Analysis

All the data were analysed statistically by using GLM procedures in SAS system (SAS 1989).

Trial one was a factorial design. The effects of the four factors, i.e. LS, TT, ALC, and

W, and the interaction effect among these four factors were evaluated using the following model:

$$Y_{ijkl} = \mu + LS_i + TT_j + ALC_k + W_l + LS_i*TT_j + LS_i*ALC_k + LS_i*W_l + TT_j*ALC_k + TT_j*W_l + ALC_k*W_l + LS_i*TT_j*ALC_k + LS_i*TT_j*W_l + TT_j*ALC_k*W_l + e_{ijkl}$$

where

Y_{ijkl} = observation,

μ = overall mean,

LS_i = lignosulfonate effect (i = 1, 2, 3, 4),

TT_j = temperature and time effect (j = 1, 2, 3),

ALC_k = alcohol effect (k = 1, 2, 3),

W_l = water effect (l = 1, 2),

LS_i*TT_j, LS_i*ALC_k, LS_i*W_l, TT_j*ALC_k, TT_j*W_l, ALC_k*W_l, $LS_i*TT_j*ALC_k$, $LS_i*TT_j*W_l$, $TT_j*ALC_k*W_l$ = interaction among factors,

e_{ijkl} = residue.

The statistical significance of the differences among means of the main effects, i.e. LS, TT, ALC, and W, was tested by using Tukey's studentized range test (SAS 1989). The statistical significance of the difference among a set of means in the interaction effect was tested by using Poly-difference test (SAS 1989).

Trial two was a Latin square design. The treatment effects on the in situ estimated rapidly-

soluble protein fraction (a), degradable protein fraction (b), the fractional-rate constant at which degradable protein fraction degrades in the rumen (c), effective degradability (ED) and the undegradable intake protein of CM protein were evaluated by using the following model:

$$Y_{ijk} = \mu + T_i + C_j + P_k + e_{ijk}$$

where

Y_{ijk} = observation,

μ = overall mean,

T_i = treatment effect (i = 1, 2, 3, 4),

C_j = cow effect (j = 1, 2, 3, 4),

P_k = period effect (k = 1, 2, 3, 4),

e_{ijk} = residue.

One observation, i.e. Y_{333}, was missing in trial two due to the failure of the rumen cannula. The statistical significance of the differences among means of the treatment effect was tested by using Tukey's studentized range test (SAS 1989).

RESULTS AND DISCUSSION

Trial One

Disappearance of CM protein from the nylon bag at 16 h incubation was decreased ($P <$

0.05) by 14.9% (62.9 vs. 53.5%, SEM = 1.0%) when the LS levels were increased from 0 to 4% with a further decrease ($P < 0.05$, SEM = 1.0%) of 16.2% to 44.9% for the 6.5% LS treatment (Table 2.1). These results were consistent with the finding of McAllister et al. (1993) who also demonstrated that increasing the LS (20% reducing sugar) concentration from 0 to 5% and from 5 to 10% decreased the effective dagradability of CP in treated CM. The pepsin and pancreatin digestibility of CM protein was not affected by the addition of 4% of LS in CM treatment (Table 2.1). However, 6.5% LS decreased ($P < 0.05$) the PPD of CM protein by 6% (79.1 vs. 84.2%, SEM = 0.5%) compared with the control. The DUIP in CM protein was increased ($P < 0.05$) by 25.1% (38.5 vs. 30.8%, SEM = 0.8%) and 12.8% (43.5 vs. 38.5%, SEM = 0.8%) when LS levels were increased from 0 to 4% and from 4 to 6.5% in the treatments, respectively. However, the increase of LS levels from 6.5 to 9% did not altere the NBD, PPD and DUIP of CM protein. Thus, the addition of 6.5% of LS in the CM DM was sufficient to reduce the CM protein degradability in the rumen and improve the availability of protein in the lower GI tract.

Increasing treatment temperature from 100 to 125°C while decreasing exposive time from 1 to 0.5 h did not affect the NBD and DUIP of CM protein, although the pepsin and pancreatin digestibility was increased ($P < 0.05$) slightly (82.1 vs.84.2%, SEM = 0.5%, Table 2.1). When the treatment temperature was increased to 150°C from 125°C (heating time 0.5 h), the NBD and PPD of CM protein were decreased ($P < 0.05$, Table 2.1) with no significant change in DUIP. The three temperature and time combinations thus were

not different in improving the the availability of CM protein in the lower GI tract. However the higher temperature (i.e. 125 or 150°C) and short heating time (30 min) treatment will be more desirable than the lower temperature (100°C) and longer heating time (1 h) treatment as the former saved more time and space in producing product than the latter.

The LS and TT resulted in significant interaction effects on NBD, PPD and DUIP of CM protein. With 4 and 6.5% LS application, the NBD (Figure 2.1) and DUIP (Figure 2.3) of CM protein was not different among the three levels of TT in each LS level, although the PPD was lower for the 150°C treatment relative to 125°C treatment at 4% LS or the 100°C treatment at 6.5% LS (Figure 2.2). However, the interaction effect among LS, TT and W (Figure 2.4) showed that the DUIP was highest ($P < 0.05$) in CM treated at 100°C for 1 h with 4% LS and water added or in CM treated with 6.5% LS and no water added as compared to CM treated at 125°C for 0.5 h with water and the same level of LS added. Increasing the LS level from 6.5 to 9%, CM treated at 125°C for 0.5 h had a higher ($P < 0.05$) PPD than that of CM treated at 100°C for 1 h or CM treated at 150°C for 0.5 h (Figure 2.2), and had a higher ($P < 0.05$) DUIP than that of CM treated at 150°C for 0.5 h (Figure 2.3). The interaction of water and LS at the level of 9% (Figure 2.4), however, diminished the difference of the effects of three temperature and time combination on the DUIP in CM protein at 9% LS (Figure 2.4).

Without the presence of LS in the treatments, the heating temperature needed to be

increased to 150°C to decrease ($P < 0.05$) the NBD of CM protein to a higher level (Figure 2.1). Although the PPD of CM protein was decreased ($P < 0.05$, Figure 2.2), the DUIP was further increased ($P < 0.05$) when CM was treated at 150°C for 0.5 h compared to the TT levels when the LS was absent from the treatment (Figure 2.3). Data further showed that only the treatment with no added water had a significantly higher DUIP in CM treated at 150°C compared to the that in CM treated at lower temperature when LS was absent (Figure 2.4). Thus the higher temperature of 150°C (for half an hour with no added-water) was needed for CM to increased the level of rumen escape protein if LS was not included in the treatment. This result is in agreement with the recommended heating condition (exit temperature 146°C and steeping time 30 min) of soybean to achieve optimum protein protection (Satter et. al 1994).

Alcohol levels showed no effect on the NBD, PPD and DUIP of CM protein (Table 2.1). However, there is a significant interaction between water and alcohol on NBD, PPD and DUIP. Without the presence of water, 5 and 15% of alcohol decreased ($P < 0.05$) the NBD (Figure 2.5) and PPD (Figure 2.6) over the control. Alcohol did not alter ($P > 0.05$) DUIP in both water levels (Figure 2.7). Lynch et al (1987) treated SBM (800 g) with a 70% (v/v) ethanol solution (2000 ml) at 78°C for 1 h. They reported, in agreement with the present study, that the treatment decreased ($P < 0.05$) the in situ nitrogen disappearance (0 to 18 h ruminal incubation) of SBM compared to the untreated SBM. The rumen NH_3 concentration was decreased ($P < 0.05$), the N retained in the body was increased ($P < 0.05$), and the apparent N digestibility was unaltered when treated SBM

was fed to lambs in replacing the untreated SBM. It is noteworthy that in their trial a large quantity of ethanol (140 ml/100 g SBM) and a low temperature were used in the treatment comparing to those in the present trial. From the results of the present study, alcohol will not be recommended for use in CM treatment.

The addition of water to CM increased ($P < 0.05$) NBD and PPD, but decreased ($P < 0.05$) DUIP of CM protein (Table 2.1). Cleale et. al (1987a) however reported that the amount of ammonia released from treated SBM (heated at 150°C for 30 min, at pH 8.5 and three mole of xylose for each of mole SBM-lysine) in an in vitro system was increased drastically when the moisture level in SBM was decreased from 20% to 10%. The reason for the difference between their trial and the present trial, regarding the effect of moisture on the protein rumen degradability is not apparent. The water levels in CM treatments however had different effects on the PPD of CM protein among the three TT combinations. At high temperature (150°C for 0.5 h), adding water reduced ($P < 0.05$) the damaging effect of the treatment on the PPD of CM protein (Figure 2.8). However this decline was significant only when 5 or 15% of alcohol was presented in the treatment (Figure 2.9). This result thus suggested that the decrease ($P < 0.05$) of PPD in the no added-water level in relation to the added-water level at high temperature treatment (Figure 2.8) was mainly caused by the presence of 5 and 15% of alcohol and water can reduce ($P < 0.05$) this decline (Figure 2.9). Water together with alcohol will not be recommended to be used in the CM treatment.

In general, the treatment of 6.5% of LS in CM DM, 125 or 150°C heating temperature and 30 min heating time with no added water or alcohol is recommended for use in reducing the rumen degradability of CM protein and improving the availability of CM protein in the lower GI tract.

Trial Two

The nutrient compositions of untreated (control) and three LS-heat treatment combinations for CM are shown in Table 2.2. The regression curves and regression models of the NBDs (% of CM protein) of four CM treatments against rumen incubation times (h) are presented in Figure 2.10. The values of constant a, b, and c representing the rapidly-soluble protein fraction, the degradable protein fraction, and the fractional rate at which degradable protein fraction degrades in the rumen, respectively, are shown in Table 2.3. The rapidly-soluble protein fraction of CM protein decreased ($P < 0.05$) when the CM was treated at 154°C with 6.5% LS compared to the control (19.5 vs. 40.2%, SEM = 3.4%, Table 2.3). Although the other two treatments were intermediate in the rapidly-soluble protein fraction, the differences were not significant ($P > 0.05$) among the three treated CMs (Table 2.3). The heat treatment at 154°C with LS tended to increase ($P < 0.10$) the degradable protein fraction in CM protein compared to the control (75.3 vs. 54.5%, SEM = 3.8%) with the other treatments being intermediate in value. The fractional-rate constant at which degradable protein fraction degraded in the rumen was decreased ($P < 0.05$) when the CM was treated at 154°C with or without 6.5% LS

compared to control. The regression curves of NBD of protein for each of four treatments against rumen incubation times (Figure 2.10) showed that the CMs heated to 129°C with 6.5% LS and CM heated to 154°C without LS resulted in the NBD curves being similar and midway between the control and 154°C with 6.5% LS treatment. In general, high temperature (154°C) with LS added decreased ($P < 0.05$) the rapidly-soluble fraction and reduced the ruminal degradation rate of CM protein in situ over the control CM.

The outflow rates (k) of CM supplement were estimated at 3.1, 4.5, 3.9 and 3.6 %/h for cow 1, 2, 3 and 4, respectively (Figure 2.11). The average of the estimated k value (3.7%/h) is thus very close to the k value (4%/h) estimated (Moshtaghi Nia 1994) in steers fed a 50% concentrate dairy diet. The estimated in situ effective degradability of CM protein was decreased ($P < 0.05$) when the CM was treated at 154°C with 6.5% LS compared to the control (Table 2.3), and the estimated undegradable intake protein of CM protein was increased ($P < 0.05$) by 64% over the control.

The estimated in situ effective degradability of untreated CM protein (74.7%, Table 2.3) was close to the in situ estimates by McAllister et al. (1993, 69.9% on average) and Moshtaghi Nia (1994, 69.9%) and in agreement with the in vivo estimates in Rooke et al. (1983, 75%) and NRC (1989, 72%). Moshtaghi Nia (1994) reported that moist heat treatment at 127°C for 15 to 45 min decreased ($P < 0.05$) the rapidly-soluble protein fraction, increased ($P < 0.05$) degradable protein fraction with no significant effect on fractional-rate constant at which degradable protein fraction degraded in rumen, and thus

decreased the in situ effective degradability of CM protein by 47% (37% on average vs. 69.9%) compared to untreated. This reduction in the in situ effective degradability in CM protein through the moist heat treatment is more significant than those in the present trial (Table 2.3) through the LS and heat treatment. McAllister et al. (1993) treated CM with water, 5 or 10% lignosulfonate (20% reducing sugar), or 2% xylose at 100°C for 1 to 2 h in an oven, which reduced the in situ effective degradability to 64.9, 55.9, 48.9 and 50.2%, respectively, compared to 69.9% for the untreated CM. These results are consistent with the results from the present trial (Table 2.3) regarding the effects of LS. In comparison with the low heat (100°C) and long treatment duration (1 to 2 h) in McAllister et al. (1993), the high heating temperature (154°C) and short treatment time (32 min) resulted in a similar level of ruminal protection to CM protein (Table 2.3). In the present trial the curve for decrease in temperature during steeping is not known, but the exit temperature after 32 min was 27°C. Heating to 154°C with 6.5% LS and steeping for 32 min significantly decreased the in situ ruminal degradability of CM protein.

In conclusion, LS addition up to 6.5% of CM DM is sufficient to reduce the ruminal degradability of protein and improve the protein availability in the lower GI tract. Temperature and time levels at 100°C/1 h, 125°C/0.5 h and 150°C/0.5 h in the presence of LS do not differ in their effects on the availability of CM protein in the lower GI tract. The addition of alcohol or water to the heated CM will not be beneficial in improving the protein availability in the lower GI tract. In the absence of LS, the 150°C/0.5 h treatment will improve the CM protein availability by 21% and 24% in the lower GI tract over the

100°C/1 h and 125°C/0.5 h treatments, respectively. The optimum treatment combination in this trial was to 154°C with LS added at 6.5% of CM DM, held for 32 min (with no alcohol and added-water) will be an effective method in protecting CM protein from rumen degradation, increasing the amount of CM protein available for digestion in the lower GI tract, and improving the nutrient value of CM protein for dairy cow.

Table 2.1. The effects of lignosulfonate (LS), time and temperature (TT), alcohol (ALC) and water (W) application during processing on the nylon bag disappearance (NBD) at 16 h ruminal incubation, the in vitro pepsin and pancreatin digestibility (PPD) and the estimated digestible undegradable intake protein (DUIP) of canola meal (CM) protein.

Item	Level	n[1]	NBD (%[2])	PPD (%[2])	DUIP (%[2])
LS (%)[3]	0	24	62.9a	84.2a	30.8c
	4	24	53.5b	83.1a	38.5b
	6.5	24	44.9c	79.1b	43.5a
	9	24	41.9c	78.0b	45.2a
	SEM[4]		1.0	0.5	0.8
TT	100C/1h	32	50.8ab	82.1b	40.1
	125C/.5h	32	54.1a	84.2a	38.5
	150C/.5h	32	47.5b	77.0c	40.0
	SEM		0.9	0.5	0.7
ALC (%)[5]	0	24	52.3	82.2	39.0
	5	24	50.8	81.0	39.4
	10	24	50.1	80.9	40.0
	15	24	50.0	80.3	39.6
	SEM		1.0	0.5	0.8
W	No added-water[6]	48	49.0b	80.0b	40.3a
	added-water[7]	48	52.6a	82.2a	38.7b
	SEM		0.6	0.1	0.5
Interaction	LS*TT	8	*	*	*
	LS*ALC	6	NS	NS	NS
	LS*W	12	NS	NS	NS
	TT*ALC	8	NS	NS	NS
	TT*W	16	NS	*	NS
	ALC*W	12	*	*	*
	LS*TT*ALC	2	NS	NS	NS
	LS*TT*W	4	NS	NS	*
	LS*ALC*W	4	NS	NS	NS
	TT*ALC*W	4	NS	*	NS

a, b, c: means in columns for each item with different letters are different ($P < 0.05$).
NS: non-significant ($P > 0.05$).
*: significant ($P < 0.05$).
[1]observation number.
[2]Percentage of the CM CP.
[3]Percentage of original CM DM.
[4]Standard error of means.
[5]Percentage of the CM DM with or without LS added.
[6]Moisture level was 12% (standard deviation = 1%) of CM DM with or without LS added in average.
[7]Moisture level was 20% of CM DM with or without LS added.

Table 2.2. Nutrient composition of four canola meal (CM) treatments.[1]

Item	Treatment			
	Control[2]	129C/LS[3]	154C[4]	154C/LS[5]
CP[6] (% DM)	36.6	36.8	39.1	37.0
ADIN[7] (% N)	5.5	5.8	5.3	9.3
NDF[8] (% DM)	29.8	27.1	28.3	29.2
ADF[9] (% DM)	22.4	23.1	22.9	25.7
Amino acid (% DM)				
Aspartic acid	2.9	2.8	2.7	2.6
Threonine	1.7	1.6	1.6	1.5
Serine	1.8	1.7	1.7	1.6
Glutamic acid	6.3	6.0	6.0	5.6
Proline	2.4	2.2	2.5	2.1
Glycine	1.9	1.9	1.8	1.7
Alanine	1.8	1.6	1.7	1.6
Valine	1.6	1.4	1.5	1.3
Isoleucine	1.2	1.1	1.1	1.0
Leucine	2.6	2.5	2.5	2.3
Tyrosine	0.9	0.9	0.9	0.8
Phenylalanine	1.4	1.4	1.4	1.3
Histidine	1.0	0.9	0.9	0.8
Lysine	2.0	1.8	1.9	1.7
Arginine	2.1	2.0	2.0	1.8
Cystine	0.8	0.8	0.8	0.8
Methionine	0.7	0.8	0.8	0.7
Essential AA	14.3	13.4	13.7	12.4
Non-Essential AA	18.7	17.9	18.1	16.8
Total AA	33.0	31.3	31.7	29.2

[1]Average of the values of two measurements.
[2]Untreated CM.
[3]CM heated to 129°C with LS added at 6.5% of original CM dry matter (DM).
[4]CM heated to 154°C without LS added.
[5]CM heated to 154°C with LS added at 6.5% of original CM DM.
[6]Crude protein.
[7]Acid detergent insoluble nitrogen.
[8]Neutral detergent fiber.
[9]Acid detergent fiber.

Table 2.3. The effects of the heat treatment with or without lignosulfonate (LS) on the estimated in situ effective degradability (ED) of protein, undegradable intake protein (UIP), rapidly-soluble protein faction (i.e. constant a), degradable protein faction (i.e. constant b), fractional-rate constant at which degradable protein fraction degrades (i.e. constant c) for control and treated CM ($n^1 = 4$).

Item	Treatment				SEM[6]
	Control[2]	129C/LS[3]	154C[4]	154C/LS[5]	
ED (% of CM protein)	74.7a	68.0ab	65.7ab	58.4b	2.1
UIP (% of CM protein)	25.4b	32.0ab	34.3ab	41.6a	2.1
Rapidly-soluble protein faction (% of CM protein)	40.2a	27.9ab	30.4ab	19.5b	3.4
Degradable protein faction (% of CM protein)	54.5	66.9	66.3	75.3	3.8
Fractional-rate constant at which degradable protein fraction degrades (%/h)	7.3a	5.6ab	4.3b	4.1b	0.5

a, b: least squares means in rows with different letters are different ($P < 0.05$).
[1]Observation numbers = 4 for all treatments but control, for which n = 3.
[2]Untreated CM.
[3]CM heated to 129°C with LS added at 6.5% of original CM DM.
[4]CM heated to 154°C without LS added.
[5]CM heated to 154°C with LS added at 6.5% of original CM DM.
[6]Standard error of means.

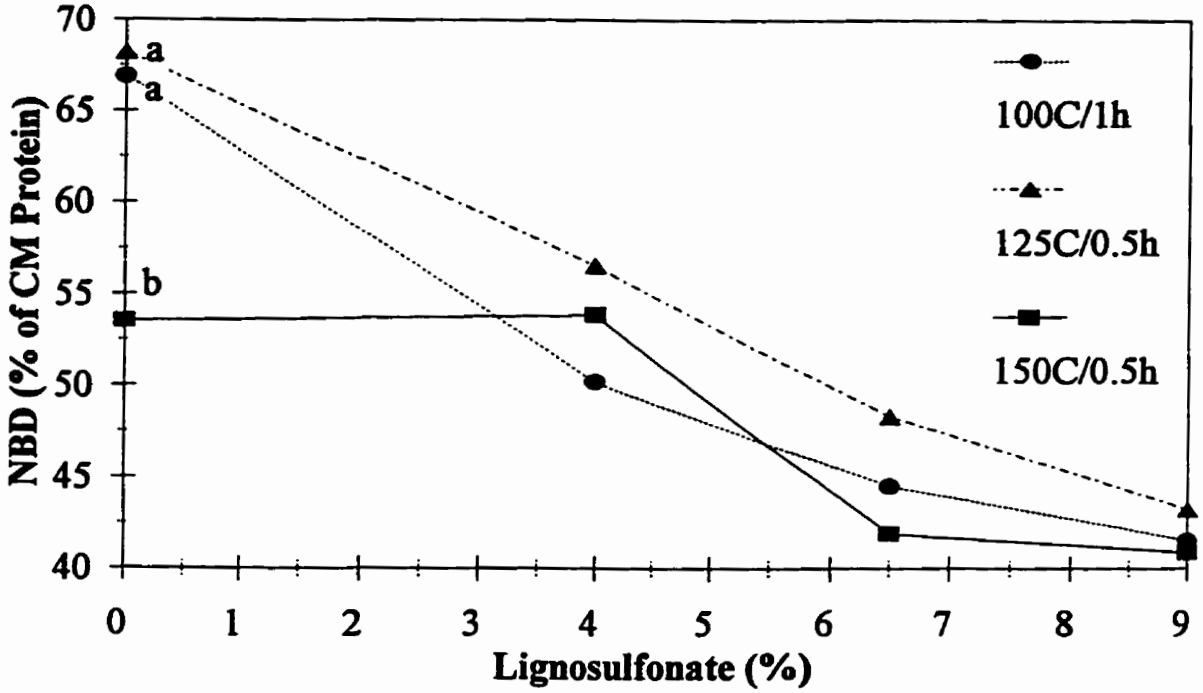


Figure 2.1. The interaction effects of lignosulfonate (0, 4, 6.5 and 9%) and temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) levels on the nylon bag disappearance (NBD) of canola meal (CM) protein (the means bearing different labels in each lignosulfonate level are different, $P < 0.05$). ($n = 8$, SEM = 1.81%)

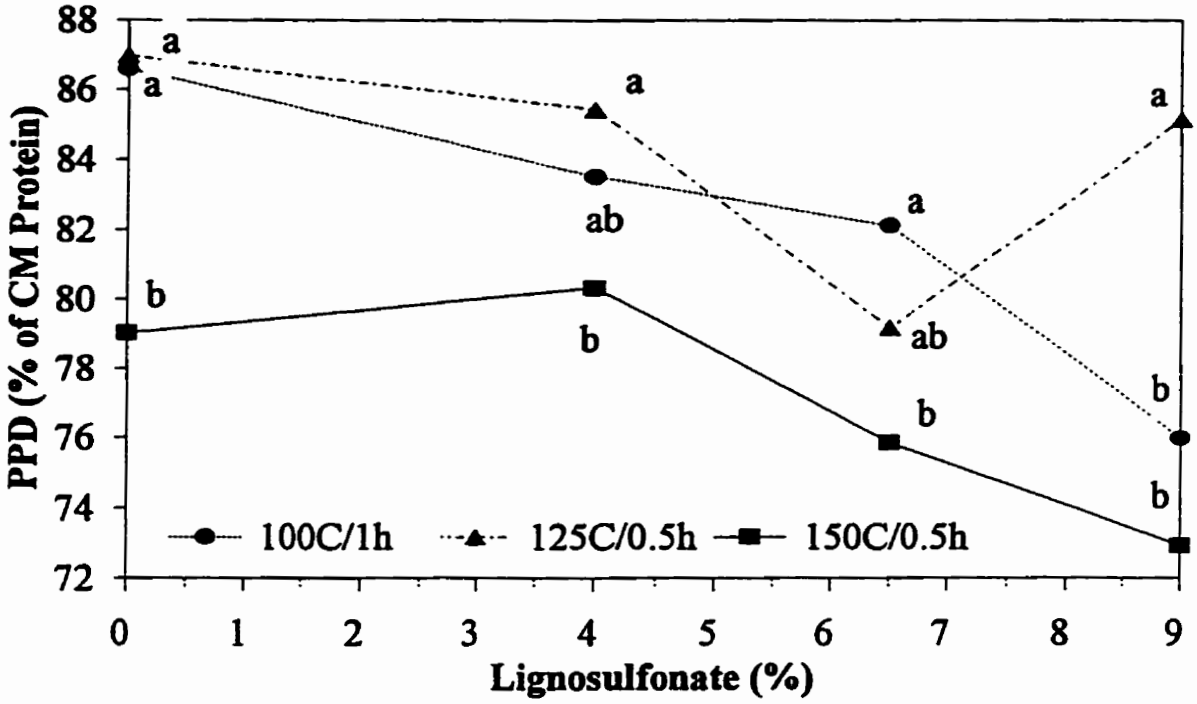


Figure 2.2. The interaction effects of lignosulfonate (0, 4, 6.5 and 9%) and temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) levels on the pepsin and pancreatin digestibility (PPD) of canola meal (CM) potein (the means bearing different labels in each lignosulfonate level are different, $P < 0.05$). (n = 8, SEM = 0.92%)

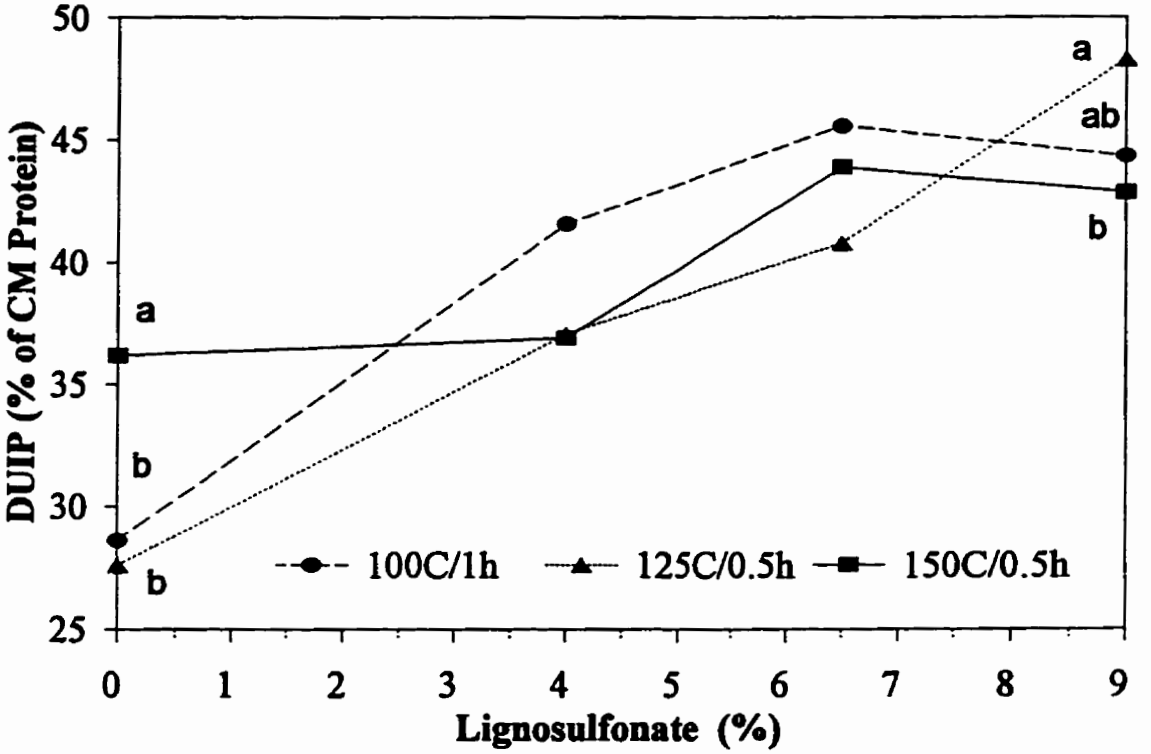


Figure 2.3. The interaction effects of lignosulfonate (0, 4, 6.5 and 9%) and temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) levels on the digestible undegradable intake potein (DUIP) of canola meal (CM) protein (the means bearing different labels in each lignosulfonate level are different, $P < 0.05$). ($n = 8$, SEM = 1.31%)

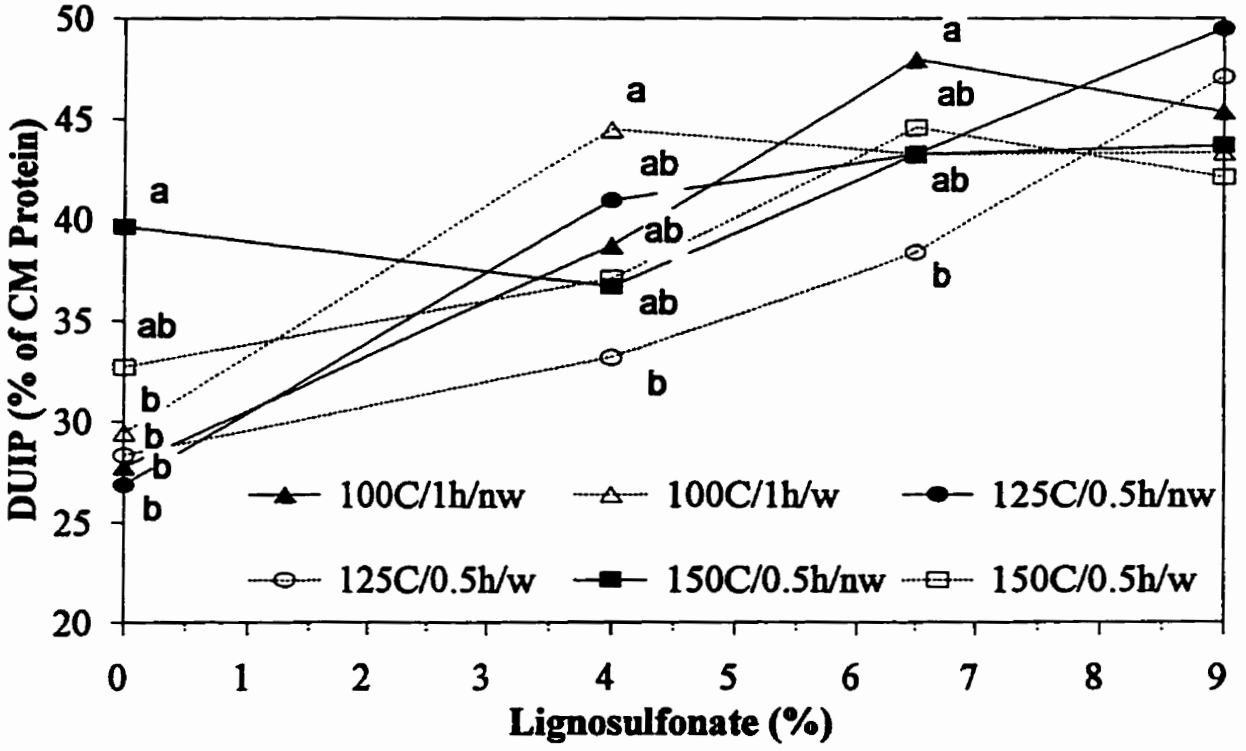


Figure 2.4. The interation effects of lignosulfonate (0, 4, 6.5 and 9%) and temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) and water (no added-water (nw) or added-water (w)) levels on the digestible and degradable intake protein (DUIP) of canola meal (CM) treatments (the means bearing different labels in each lignosulfonate level differed significantly, $P < 0.05$). ($n = 4$, SEM = 1.85%)

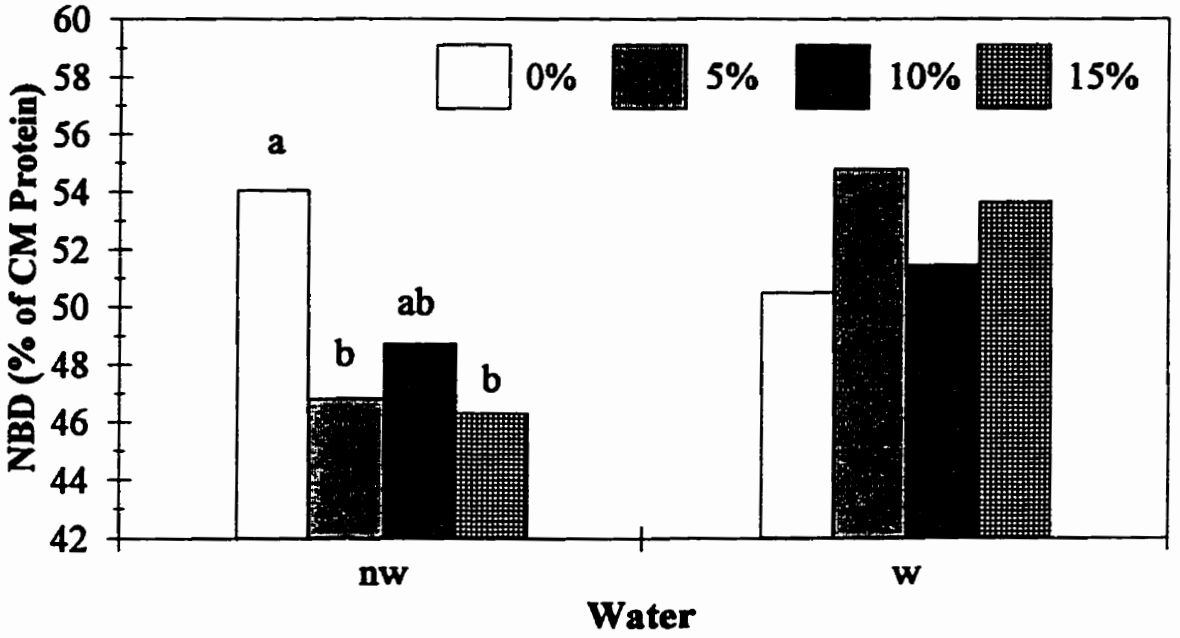


Figure 2.5. The interaction effects of water (no added-water (nw) and added-water (w)), and alcohol (0, 5, 10 and 15%) levels on the nylon bag disappearance (NBD) of canola meal (CM) protein (the means bearing different labels in each water level are different, $P < 0.05$). ($n = 12$, SEM = 1.48%)

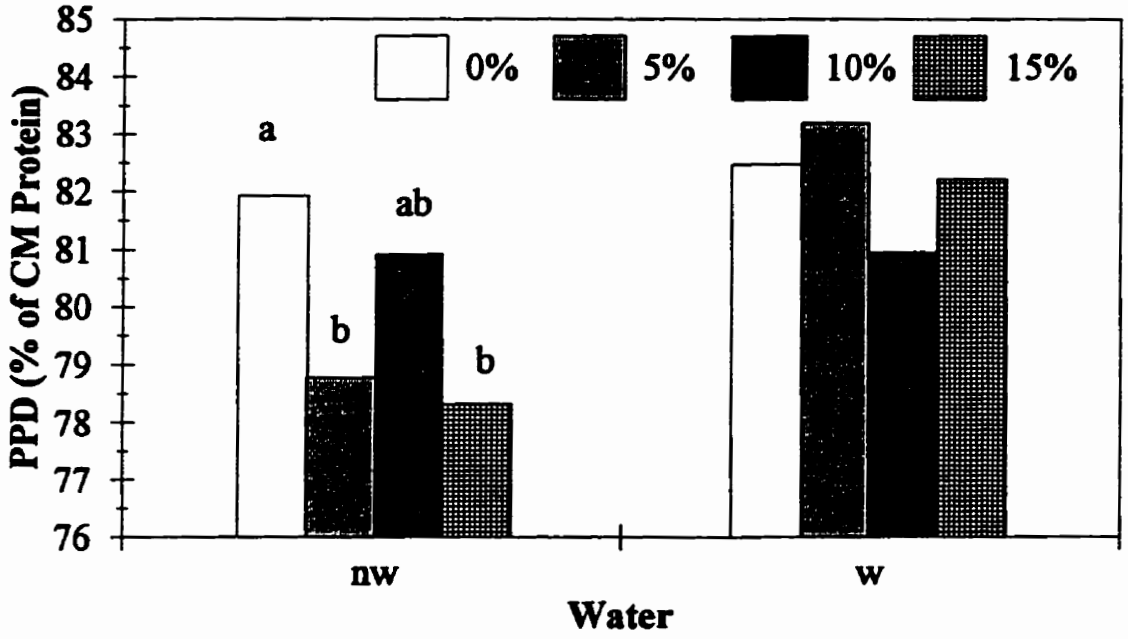


Figure 2.6. The interaction effects of water (no added-water (nw) and added-water (w)), and aclcohol (0, 5, 10 and 15%) on the pepsin and pancreatin digestibility (PPD) of canola meal (CM) protein (the means bearing different labels in each wter level are different, $P < 0.05$). (n = 12, SEM = 0.75%)

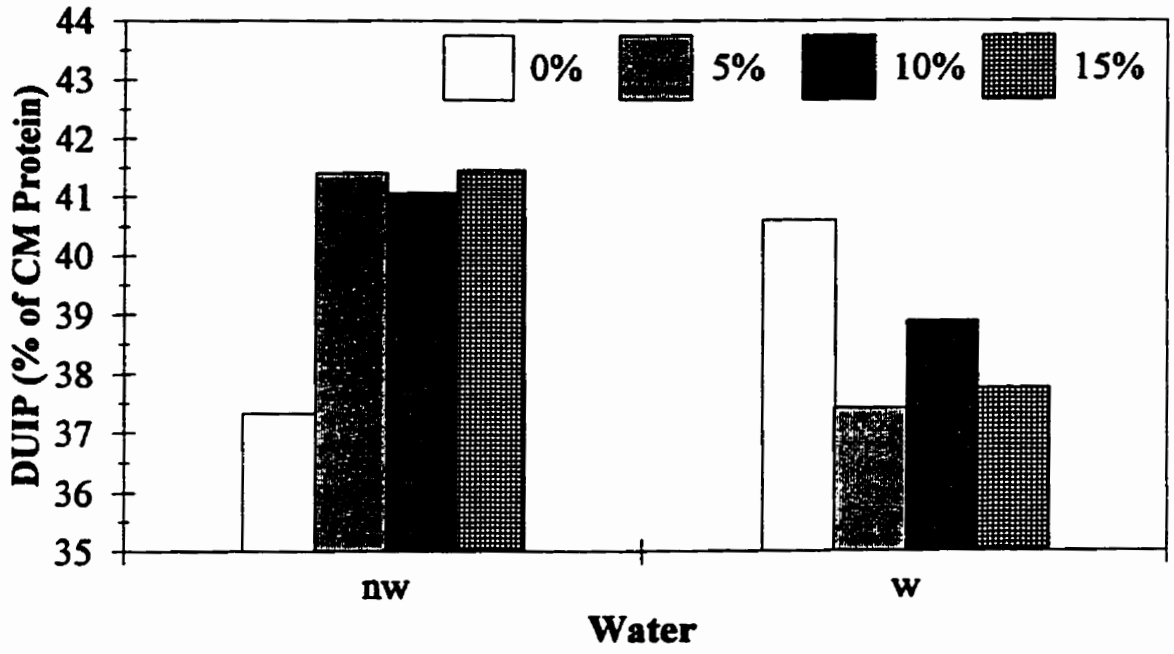


Figure 2.7. The interaction effects of water (no added-water (nw) and added-water (w)) and aclcohol (0, 5, 10 and 15%) levels on the digestible undegradable intake protein (DUIP) of canola meal (CM) protein. (n = 12, SEM = 1.07%)

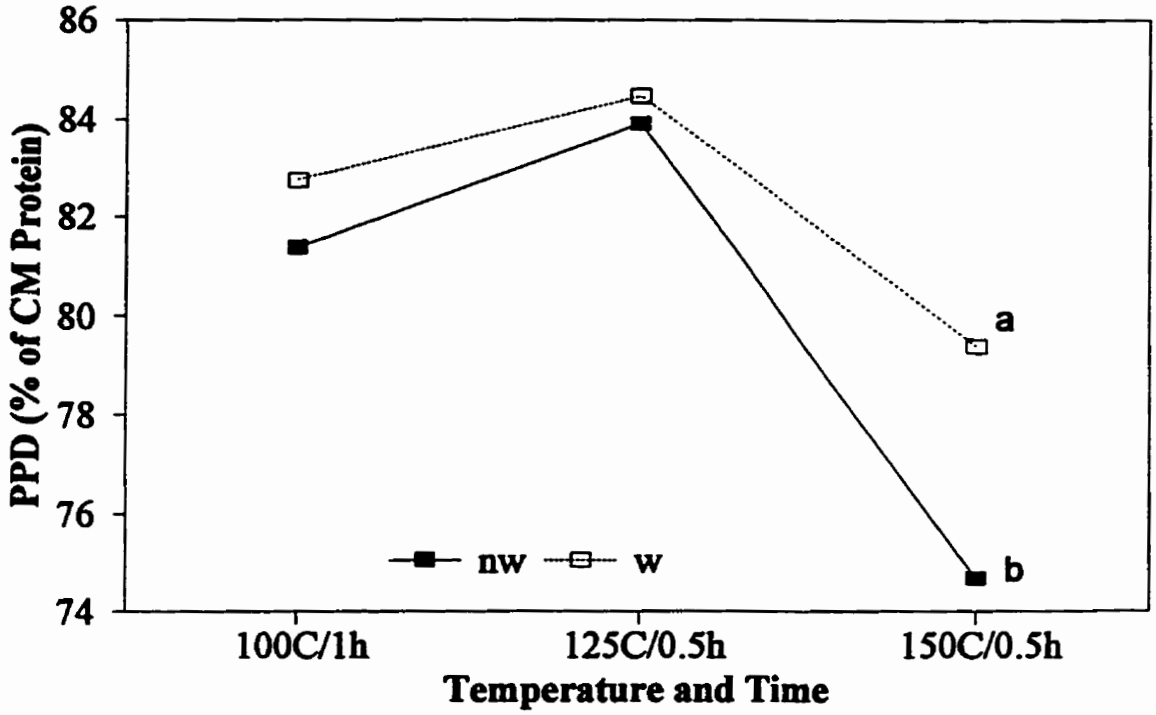


Figure 2.8. The interaction effects of water (no added-water (nm) and added-water (w)) and temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) levels on the pepsin and pancreatin digestibility (PPD) of canola meal (CM) protein (the means bearing different labels in each temperature and time level are different, $P < 0.05$). ($n = 48$, SEM = 0.65%)

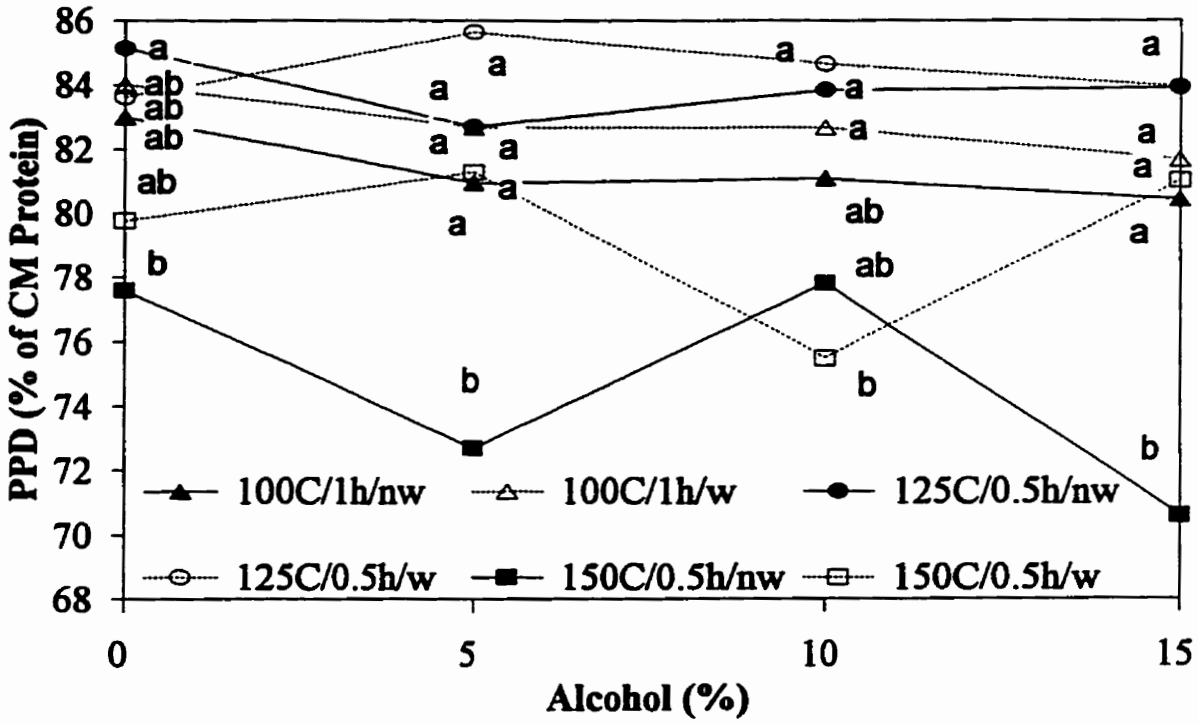


Figure 2.9. The interation effects of temperature and time (100C/1h, 125C/0.5h and 150C/0.5h) , alcohol (0, 5, 10 and 15%) and water (no added-water (nw) and added-water (w)) levels on the pepsin and pancreatin digestibility (PPD) of canola meal (CM) protein (the means bearing different labels in each alcohol level are different, $P < 0.05$). ($n = 4$, SEM = 1.3%)

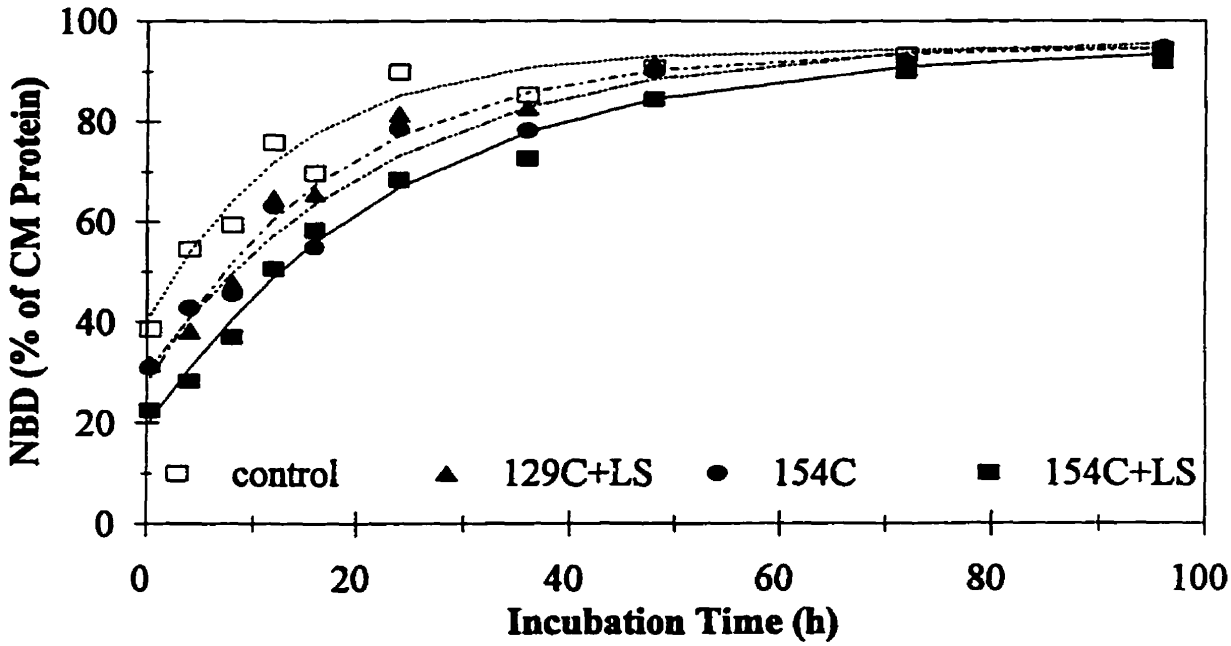


Figure 2.10. Plot of the value and the regression curves of the value of nylon bag disappearance (NBD) of canola meal (CM) protein of four treatments (control, 129C+LS, 154C and 154C+LS) against incubation time (t) in the rumen (n = 4 for all treatments but control, for which n = 3). The models of the regressions are:

control:	NBD = 40.2 + 54.5e^(-0.073t)	(r = 0.96),
129C+LS:	NBD = 27.9 + 66.9e^(-0.056t)	(r = 0.98),
154C:	NBD = 30.0 + 66.3e^(-0.043t)	(r = 0.96),
154C+LS:	NBD = 19.5 + 75.3e^(-0.041t)	(r = 0.98).

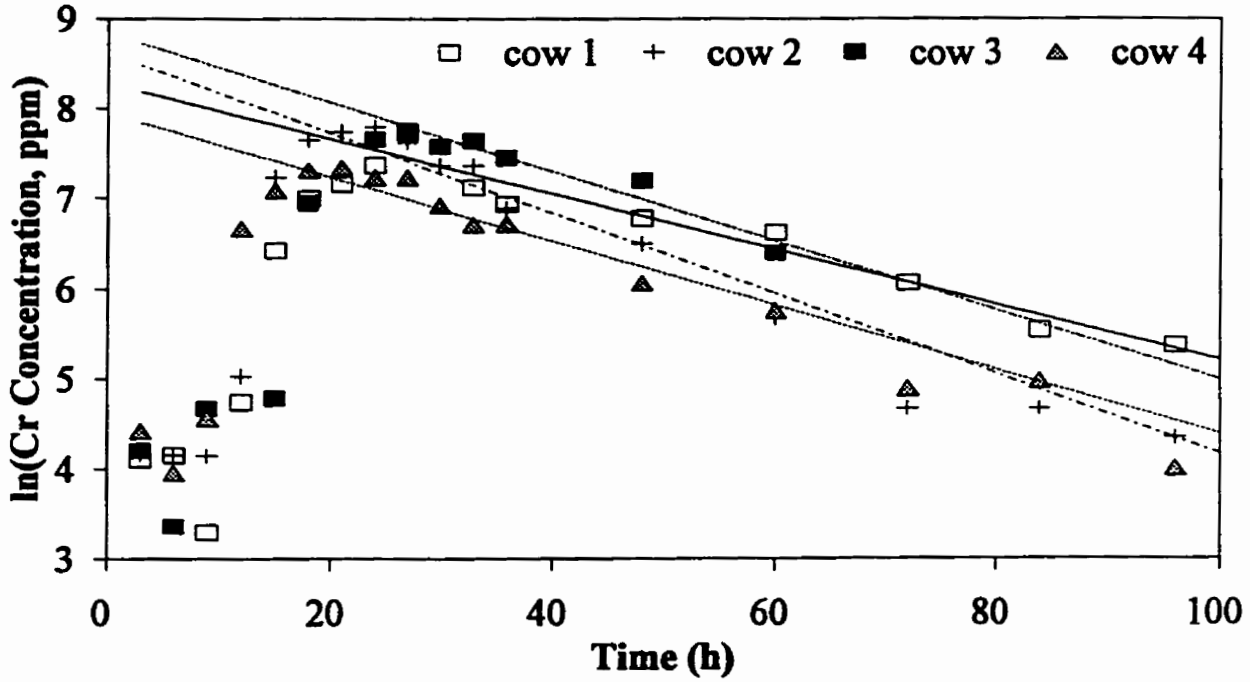


Figure 2.11. Plot of ln(Cr concentration in feces) against time (t) after Cr-mordanted canola meal was dosed in the rumen and the linear regression curves of ln (y, peak and post-peak value of Cr concentration in feces) against time for four cows. The models for the linear regression are:

cow 1:	$y = 8.28 - 0.031t$	$(n = 10,\ r = 0.98)$,
cow 2:	$y = 8.62 - 0.045t$	$(n = 12,\ r = 0.95)$,
cow 3:	$y = 8.84 - 0.039t$	$(n = 6, r = 0.94)$,
cow 4:	$y = 7.95 - 0.036t$	$(n = 13, r = 0.93)$.

MANUSCRIPT III

EFFECT OF LIGNOSULFONATE AND HEAT TREATMENT ON CANOLA MEAL PROTEIN DEGRADATION IN THE RUMEN, POST-RUMEN DIGESTION AND PRODUCTION

ABSTRACT

Canola meal (CM) was heat treated with or without lignosulfonate (LS) to protect protein from ruminal degradation. Four CM treatments: untreated; heated to 129°C with 6.5% LS; 154°C without LS; and 154°C with 6.5% LS were used to formulate four diets which were fed to four Holstein dairy cows cannulated at the rumen and the proximal duodenum over four 21-d periods in a 4 x 4 Latin square design. The in situ undegradable intake nitrogen (N), amino acid and dry matter; and the estimated true digestibility of N in the lower gastrointestinal (GI) tract were increased; while the estimated true digestibility of N and DM in the total GI tract were not decreased in treated CMs compared to those in untreated CM. The concentrations of acetic acid, butyric acid and the total volatile fatty acid tended to be lower in cows fed CM heated to 154°C with LS than those in cows fed the untreated CM. The in vivo undegradable intake N was increased in the diet containing CM heated to 154°C with LS compared to that in a diet containing untreated CM or CM treated at 129°C with LS. The in vivo apparent digestibility of dietary N and DM in the lower or total GI tract, and milk production were not altered by the inclusion of treated CM in the diet. Heating to 154°C with 6.5% LS thus reduced the calculated ruminal degradability of CM protein by 49% without decreasing the post-ruminal digestibility.

Key words: Canola meal, heat, lignosulfonate, degradation, digestion, VFA, milk production, dairy cow.

INTRODUCTION

Canola meal (CM) is a by-product resulting from the crushing and the oil extraction of canola seed. Canola is defined as rapeseed containing less than 2% of total fatty acids in oil as erucic acid and less than 30 μmol of alkenyl glucosinolates per gram of oil-free dry matter (DM). Canola meal has a high protein content (Zinn 1993; Manuscript II) and it has a more favorable amino acid (AA) profile than soybean meal (SBM),, as CM has a lysine/methionine ratio (Bell and Keith 1991; Manuscript II) which is closer to those of milk and beef muscle (Ørskov 1992) than SBM (Zinn 1993) . In western Canada where canola is planted most widely, CM is used extensively as a protein source for ruminants because of its competitive price. The CM, however, has a relatively high rumen protein degradation rate (72%, NRC 1989) which is close to that of SBM (70%, NRC 1989). The degraded protein in the rumen can be used to generate rumen microbial protein, passing to lower gastrointestinal (GI) tract or otherwise lost as urea in milk (Clark et al. 1978) or in urine (Nolan and Leng 1972). To use CM protein more efficiently in dairy rations it would be desirable to reduce the ruminal protein degradability of CM protein and thus increase the percentage of CM protein or AA escaping rumen degradation and passing to the lower GI tract for digestion by the host animal.

Heating has been used extensively as a method to reduce the dietary protein degradability in the rumen (Sherrod and Tillman 1964; Stern 1984; Faldet et al.1991; Moshtaghi Nia and Ingalls 1992, 1995 a,b; Satter et al. 1994; Onyango and Ingalls 1994; Onyango 1995).

The mechanism of protection in heat treatment involves principally the initial stage of Maillard reaction. The reaction is between the free amino group of lysine in protein and the aldehyde group of reducing sugars such as glucose and fructose which were present in the diet (Hurrell and Finot 1985). The Schiff's base and intermediate products of this initial stage of the Maillard reaction are reversible and thus are bio-available to animals (Finot et al. 1977). Heating may enhance the formation of these reversible products which are more stable at a neutral pH while liable in an acidic environment. Heat treatment thus is assumed to stabilize the dietary protein and render it less degradable in the rumen (pH 5.5 to 7.5) and, thus increase the flow of dietary protein to the abomasum (pH < 3.0) for further digestion by the animal. Previous work (Manuscript II) demonstrated CM moist heat heated to 110°C for 1 h increased the AA concentration in deproteinized plasma and improved the feed efficiency when the treated CM was fed to growing lambs in replacing the untreated CM.

Recently, reducing sugars such as xylose, glucose, fructose or lactose were tested to enhance the heat treatment effects on protein protection from rumen degradation (Cleale et al. 1987 a,b,c; Wallace and Falconer 1992; Moismanyana and Mowat 1992; McAllister et al. 1993). Lignosulfonate (LS), a byproduct of the wood industry and a rich source of xylose (20-40% of DM), has been used successfully in heat treatment to protect SBM (Stern 1984; Windschitl and Stern 1988; Nakamura et al. 1992) and CM (McAllister et al. 1993) proteins from rumen degradation. Previous research (Manuscript II) showed that LS (40% xylose, DM basis) applied at 6.5 % of original CM DM was sufficient, and the

heating temperature of 125 and 150°C and heating time of 0.5 h were effective in reducing the in situ ruminal degradability of CM protein and improving the estimated digestible undegradable intake protein of CM in lower GI tract. The objectives of this research were: to investigate the effect of LS and heat treatments (Manuscript II) on the in situ ruminal degradation and the estimated post-ruminal digestion of the CM and to investigate the in vivo effect of these CM treatments on the rumen condition, ruminal degradability of dietary nitrogen (N), AA and DM, the post-ruminal apparent digetibility of the dietary N and DM, the plasma AA and urea N level, and the milk production in dairy cows fed diets containing these CM treatments.

MATERIAL AND METHODS

Lignosulfonate and Heat treatment of CM

Four CM treatments were made up of: I (control), original untreated CM as a control; II (129C+LS), CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM DM; III (154C), CM heated to 154°C and held for 32 min without LS added; and IV (154C+LS), CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM. The treatment procedure and the nutrient content, i.e. DM, crude protein (CP), neutral detergent fibre (NDF), the acid detergent fibre (ADF) and the acid detergent insoluble N (ADIN) of these CM treatments were reported in a previous study (Manuscript II).

Animals

Four first lactation Holstein cows, averaging 91 days (standard deviation = 37 day) in milk and 558 kg (standard deviation = 52 kg) body weight, were used. Each cow was fitted with a rumen cannula (Bar Diamond 4", Bar Diamond, Inc., Parama, ID) and a duodenal T-shaped cannula (IC2 1", Ankom, Fairport, NY) at the proximal duodenum.

Diets and feeding

Four diets were formulated to include the four CM treatments. About 36% of dietary CP came from CM protein. The diets were formulated to meet NRC (1989) nutrient requirement for cows weigh 550 kg and producing 35 kg of milk with 3.4% milk fat using Trilogic Systems Ration Package (1990, Trilogic Systems, Des Moines, IA). The ratio of concentrate : forage was 61 : 39 (DM basis).

Cows were housed in a tie stall barn at the Glenlea Agricultural Research Station (University of Manitoba, Winnipeg, MB) together with the main herd. Cows were given the diet as a total mixture ration free choice once a day at 10:00 am. Concentrate was mixed with silage in the manger at the time of feeding. The quantity of silage used was adjusted weekly according to the DM percentage in silage. The amount of ration fed to each cow was based on the amount of diet that cow consumed the day before plus 2 kg of extra. Cows were on rubber mats and bedded with chopped straw except for sample collection days when bedding was removed.

Experiment Design

Cows were used in a 4 x 4 Latin square design to test the four diets over four periods. Each period (21 day) consisted of a 13-d adaptation period and an 8-d sample collection period.

In Situ Measurements

Samples (1 g) of CM were heat-sealed in 3.5 x 5.5 cm nylon bags (50 μm porosity, Ankom, Fairport, NY). Two sets of sample bags were prepared so that one set was used for the determination of in situ ruminal DM and N degradabilities and the other set for the determination of the estimated true digestibility of CM DM and N in the lower and total GI tract. These two sets of sample bags (4 replication bags for each set) for each CM treatment were incubated in the rumen of the cow that was receiving that diet. The incubation was conducted on day 6 of the 8-d sample collection period. All the sample bags (eight) were held in a large nylon bag (25 x 45 cm size, 2 x 3 mm mesh) which was tied to the rumen cannula by a nylon cord (45 cm long) and weighed down in the rumen with a bottle of sand. Bags were retrieved from the rumen after 16 h incubation and were placed on ice immediately to stop microbial activity. The bags of the first set were stored frozen (-20°C) until the determination of DM, N and AA disappearance from the bags in the rumen. The second set of bags was incubated in a pepsin-HCl solution (1 g pepsin per liter of 0.01 N HCl, 20 ml/bag) for 3 h at 39°C (Moshtaghi Nia and Ingalls 1992). The bags were stored frozen (-20°C) after the pepsin digestion. On day 8 of the 8-d sample collection period, these pepsin digested bags (four replications for each cow x diet) were thawed at room temperature and inserted into the duodenum via a duodenal cannula at 20 min intervals. Bags were collected from feces until 32 h after the insertion of the first bag. The bags were separated from feces by washing with a high pressure tap hose on a metal screen (about 5 x 5 mm mesh) and the collected bags were stored frozen (-20°C).

All frozen bags were thawed at room temperature and washed with cold water in a wringer-type washing machine for 10 min followed by another 5 min in clean water. All the washed bags were dried in a forced-air oven at 60°C for 48 h. The dried bags were weighed. The dried bags of the second set and half of the first set were further analyzed for N content, and the dried bags of the other half of first set were analyzed for AA content in the residue.

The following equations were used in the calculation of in situ measurements:

In situ undegradable intake DM, N or AA of CM (% of CM DM, N or AA)

= (Residue DM, N or AA content in the bag after 16 h ruminal incubation, g) / (Initial DM, N or AA content in the bag, g) x 100;

Estimated true digestibility of DM or N in the total GI tract of CM (% of CM DM or N)

= (Residue DM or N content in the bag recovered from feces, g) / (Initial DM, N or AA content in the bag, g) x 100;

Estimated true digestibility of DM or N in the lower GI tract of CM (% of intake CM DM or N)

= (Estimated true digestibility of DM or N in the total GI tract of CM, % of CM DM or N) - (100 - (In situ undegradable intake DM or N of CM, % of CM DM or N));

Estimated true digestibility of DM or N in the lower GI tract of CM (% of DM or N of rumen escape CM after 16 h ruminal incubation)

= (Estimated true digestibility of DM or N in the lower GI tract of CM, % of intake CM DM or N) / (In situ undegradable intake DM or N of CM, % of CM DM or N) x 100.

In Vivo Measurements

Diet

The amount of concentrate and silage intake, and weigh-back was recorded daily during the 8-d sample collection period. The weigh-back, concentrate and silage samples were taken every two days, composited and subsampled for each cow x diet combination. The silage and weigh-back samples were dried at 60°C for 48 h in a forced-air oven to determine the moisture content in the original wet sample and to obtain the related dry samples of silage and weigh-back. All the concentrate, and the dry silage and weigh-back samples were ground (2 mm screen) and analyzed for DM, N and Cr content, and the concentrate and the dry silage samples were further analyzed for AA, NDF, ADF and ADIN content.

Rumen fluid

A rumen fluid sample was collected every 8 h in a day for four consecutive days in each cow. Cows were arranged to be sampled two hours apart during this 8 h. Four sample collection patterns (i.e. sample collected at: I, 1:30, 9:30 and 17:30; II, 3:30, 11:30 and 19:30; III, 5:30, 13:30 and 21:30; and IV, 7:30, 15:30 and 23:30 in each sample collection day) were assigned to four cows over the four consecutive days following a 4 x 4 Latin square design. Thus the samples (12 in total) obtained from each cow over 4 days represented every two h of a day (24 h).

Rumen contents (500 ml) were collected and strained through cheesecloth to result in a 150 ml rumen fluid sample. The residue was returned to the rumen after the fluid was collected. Rumen fluid was measured for pH and was then acidified with 0.5 ml H_2SO_4 (7.2 N). Acidified fluid was split into two parts and stored frozen (-20°C), one (50 ml) as a spare sample and the remainder (100 ml) for further analysis. Rumen fluid was thawed at room temperature and centrifuged at 25,000 x g for 20 min to obtain a clear supernatant which was stored frozen (-20°C). The ammonia (NH_3) concentration in rumen fluid supernatant thawed to room temperature was measured with an NH_3 electrode (model 95-10, Orion Research Inc., Cambridge, MA). Ten ml of rumen fluid supernatant and 0.6 ml of NH_3 pH-adjusting ISA (5 M NaOH, 10% methanol, color indicator, 0.05 M disodium EDTA) were used. Rumen fluid samples for every two samples of adjacent time points were composited to represent samples at six time points of a day (i.e. 0.5, 4.5, 8.5,

12.5, 16.5 and 20.5 h of post-feeding time) for each cow x diet. The composited samples also were analyzed for volatile fatty acids (VFA) by gas chromatography (Erwin et al. 1961).

Rumen microbes

Rumen content (750 ml) was sampled from each cow at 6:00, 14:00 and 22:00 on day 8 of the 8-d sample collection period. The rumen content samples were stored frozen (-20°C) for further isolation of rumen microorganisms. The samples were thawed at room temperature and blended in a blender (Model K5-AC, The Harbart MFG Co., Troy, OH) for 3 min at speed 4 to release the microorganisms adhered to feed particles. The blended samples were strained through 4-layers of cheesecloth to obtain rumen fluid. Bacteria were isolated from rumen fluid via differential centrifugation (Lardy et al. 1993). The isolated bacterial samples were lyophilized (30°C for 72 h, Model Genesis LE 25, The Virtis Company, Gardiner, NY) and the samples of the three time points were further composited for each cow x diet combination and ground (1 mm screen). The composited sample was analyzed for AA, purine, DM and N contents.

Duodenal digesta

Duodenal digesta was sampled half an hour later than rumen fluid samples in each cow through a duodenal cannula following the same collection pattern and sequence conducted

in the rumen fluid collection. The collected duodenal digesta (150 ml) was split into two parts and stored frozen (-20°C), one (50 ml) as a spare sample and the remainder (100 ml) for further analysis. The samples were lyophilized (30°C for 72 h, Model Genesis LE 25, The Virtis Company, Gardiner, NY) and then ground (1 mm screen). Every two samples of adjacent time points were composited to obtain six samples for each cow x diet. These composited samples were analyzed for DM, Cr, N, NH_3 N and AA. The six sample times of duodenal digesta were further composited and the composited sample was measured for purine content for each cow x diet combination.

Feces

The gutter was arranged for fecal collection. Feces was collected for 24 h from each cow during the 3rd, 5th and 7th days of the 8-d sample collection period. Feces were weighed and a subsample of about 5% of total feces was taken for each day and stored frozen (-20°C) until analysis. Feces samples were thawed at room temperature and dried in a forced-air oven for three days at 60°C. Feces were ground (2 mm screen) and further composited for each cow x diet combination. The composited sample was analyzed for DM, Cr, and N content.

Calculations and assumptions

The following equations were used in calculation of duodenal digesta flows:

Duodenal DM flow (g/d)

= (Cr intake, g/d) / (Cr concentration, % of Duodenal DM);

Duodenal N, NH_3 N, or AA flow (g/d)

= (Duodenal DM flow, g/d) x (N, NH_3 N, or AA concentration, % of duodenal DM)

Duodenal non-NH_3 N (NAN) flow (g/d)

= (Duodenal N flow, g/d) - (Duodenal NH_3 N flow, g/d);

Duodenal microbial N flow (g/d)

= (Duodenal DM flow, g/d) x (Purine content, % of duodenal DM) / (Purine : N of rumen microbe);

Duodenal endogenous N flow (g/d)

= 0.004 x (Dietary DM intake, g/d) (Tamminga et al. 1979);

Duodenal endogenous AA flow (g/d)

= (Duodenal endogenous N flow, g/d) / (Duodenal NAN flow, g/d) x (Duodenal AA flow, g/d);

Duodenal dietary N flow (g/d)

= (Duodenal N flow, g/d) - (Duodenal NH_3 N flow, g/d + Duodenal microbial N flow, g/d + Duodenal endogenous N flow, g/d);

Duodenal dietary AA flow (g/d)

= (Duodenal AA flow, g/d) - (Duodenal microbial AA flow, g/d + Duodenal endogenous AA flow, g/d).

The in vivo undegradable intake N (UIN) and undegradable intake AA (UIAA) were calculated from the following equation:

UIN or UIAA (% of dietary N or AA)

= (Duodenal dietary N or AA flow, g/d) / (Dietary N or AA intake, g/d).

The in vivo UIN of treated CMs included in the diets was calculated by the following four-step procedure.

Step one, an equation was established for each of four treatment diets as following:

UIN of diet (% of dietary N)

= ((UIN of CM in diet, % of CM N) x (CM N concentration, % of dietary N) + (UIN of dietary source other than CM, % of N of dietary source other than CM) x (Concentration of N of dietary source other than CM, % dietary N)) x 100.

Step two, the in vivo UIN of untreated CM in the control diet was assumed to be 28% of CM N (NRC 1989). The values of CM N concentration (% of dietary N) and the concentration of N of dietary source other than CM (% dietary N) in the above equation were known for each diet from the dietary ingredient (Table 3.1), and the diet (Table 3.1) and CM (Manuscript II) N composition. Thus the value of UIN of dietary source other than CM (% of N of dietary source other than CM) can be derived in the above equation for the control diet.

Step three, the value of UIN of dietary source other than CM in diet, % of N of dietary source other than CM in diet was assumed to be constant in all of the four treatment diets.

Step four, the UIN of treated CM in the diet (% of treated CM N) thus can be calculated by substituting the value of UIN of dietary source other than CM (% of N of dietary source other than CM) derived for the control diet to the equation established in step one for each of other diets containing treated CMs.

The quantity of fecal DM and N excretion, and apparent digestibilities of dietary DM and N were calculated as follows:

Fecal DM excretion (g/d) = (Cr intake, g/d) / (Cr concentration, % of fecal DM);

Fecal N excretion (g/d)

= (Fecal DM excretion, g/d) x (N concentration, % of fecal DM);

Apparent digestibility of dietary DM or N in the lower GI tract (% of dietary DM or N intake)

= ((Duodenal DM or N flow, g/d) - (fecal DM or N excretion, g/d)) / (Dietary DM or N intake, g/d);

Apparent digestibility of dietary DM or N in the lower GI tract (% of duodenal DM or N flow, g/d)

= ((Duodenal DM or N flow, g/d) - (fecal DM or N excretion, g/d)) / (Duodenal DM or N intake, g/d);

Apparent digestibility of dietary DM or N in the lower GI tract (% of dietary DM or N intake)

= ((Dietary DM or N intake, g/d) - (fecal DM or N excretion, g/d)) / (Dietary DM or N intake, g/d).

Plasma

Blood samples (15 ml) were taken 4:30, 10:30, 16:30 and 22:30 h from the coccygeal vein/artery during each rumen fluid and duodenum digesta sampling day. The four cows

were assigned to these four time points over four sample collection days in each period following the same latin square design for rumen fluid and duodenum digesta sampling. The blood sample was collected in two 10 ml heparinized vacuum tubes. Blood was centrifuged at 2000 x g for 10 min to obtain plasma (10 ml). The plasma was stored frozen (-20°C). Three ml of plasma (thawed at room temperature) was deproteinized by the addition of a 75 mg 5-sulphosalicylic acid and the resulting mixture was centrifuged at 27,750 x g for 10 min to obtain a deproteinized plasma. The deproteinized plasma was lyophilized and then hydrolyzed to determine the AA content following the same procedure reported in a previous study (Manuscript I). The AAs in deproteinized plasma are comprised of free AAs and short peptide AAs (McCormick and Webb 1982).

Milk production

Cows were milked twice daily at 4:30 and 16:00. Milk samples (about 50 ml) were collected during each milking from 16:00 of day 1 to 4:30 of day 7 in the 8-d sample collection period. Samples were preserved with Brotab-"10" (30% 2-bromo-2-nitropropane-1,3-diol and 1.4% pimaricin) and stored at 4°C. The three 4:30 samples from day 2 to 4 and the other three 4:30 sample from day 5 to 7, and three 16:00 samples from day 1 to 3 and the other three 16:00 samples from day 4 to day 6 were composited and subsampled separately to obtain four milk samples (two 4:30 and two 16:00 samples) for each cow x diet combination. These composited milk samples (50 ml) were sent to Manitoba Milk Recording Corporation (MMRC) for analysis of CP (33.2.11, AOAC

1995), fat and solid-non-fat (Foss MS300 Infra-red Spectroscopy Analyzer, Milk-O-Scan Model 203B Type 17920, Fosselectric Cornwall, ON) contents. The remaining milk samples were stored frozen (-20°C) until analysis. They were thawed at room temperature and composited (at same volume basis) in the same way conducted before and were analyzed for the milk non-protein N (33.2.12, AOAC 1995), milk true protein (33.2.14, AOAC 1995) and milk urea N afterwards.

Sample Analysis

The DM, N, ADF ADIN content in the sample was analyzed according to the method number 93401, 976.06, 973.18, and 973.18 plus 976.06 in AOAC (1990), respectively. The Cr, NH_3 N, NDF, purine, and AA (excluding AA of the deproteinized plasma) content in the sample was determined by using the method of Williams et al. (1962), Bremner and Keeney (1965), Goering and Van Soest (1970), Zinn and Owens (1986) and Moshtaghi Nia and Ingalls (1995a), respectively. The essential AAs (EAA) measured are comprised of threonine, valine, isoleucine, leucine, phenylalanine, histidine lysine, arginine and methionine. The non-essential AAs (NEAA) measured are comprised of aspartic acid, serine, glutamic acid, proline, glycine, alanine, tyrosine and cystine. The total AA includes both EAA and NEAA. The urea N in the plasma and milk was analyzed by using Sigma Diagnostics Kit (number 535, 1990).

Statistical Analysis

All the data were analyzed statistically by using GLM (general linear models) procedures of SAS System (SAS 1989). The statistical significance of the differences among treatment means were tested by using Tukey studentized range test excepting those treatment means of dietary intake of degradable N (Table 3.3) and the diet UIN (Table 3.9). ANOVA F-test indicated that treatment had a significant effect on the dietary intake of degradable N and the diet UIN. Tukey's studentized range test, however, did not show any significant difference among treatment means. Least significant difference test (with a higher experimental-wise Type I error rate, SAS 1989) was used to compare treatment means of dietary intake of degradable N and the diet UIN. The statistical significance of the difference among a set of means in the interaction effect was tested by using Poly-difference test (SAS 1989).

The following model was used in evaluating the factors affecting the rumen fluid pH and NH3 concentration (two observations were missing in the pH observation):

$$Y_{ijklmn} = \mu + T_i + C_j + P_k + T_i*C_j*P_k + CT_i + S_m + D_n + CT_i*S_m + T_i*CT_i*S_m + e_{ijklmn}$$

where

$Y_{ijklmnp}$ = observation,

μ = overall mean,

T_i = treatment effect (i = 1, 2, 3, 4),

C_j = cow effect (j = 1, 2, 3, 4),

P_k = period effect (k = 1, 2, 3, 4),

CT_l = sample collection pattern effect (l = 1, 2, 3, 4),

S_m = sample collection sequence effect (m = 1, 2, 3),

D_n = sample collection day effect (n = 1, 2, 3, 4),

$T_i*C_j*P_k$, CT_l*S_m, $T_i*CT_l*S_m$ = interaction effect,

e_{ijklmn} = residue.

The factors affecting the rumen fluid VFA and the percentage of milk components were evaluated by the following model (eight observations were missing for the percentage of each milk components):

$$Y_{ijkl} = \mu + T_i + C_j + P_k + T_i*C_j*P_k + TM_l + T_i*TM_l + e_{ijkl}$$

where

Y_{ijkl} = observation,

μ = overall mean,

T_i = treatment effect (i = 1, 2, 3, 4),

C_j = cow effect (j = 1, 2, 3, 4),

P_k = period effect (k = 1, 2, 3, 4),

TM_l = sampling time effect (l = 1, 2, ..., 6),

$T_i*C_j*P_k$, T_i*TM_l = interaction effect,

e_{ijkl} = residue.

Factors affecting the urea N and AA concentration in plasma were evaluated in the following model (one observation was missing for both of urea N and AA concentration:

$$Y_{ijkl} = \mu + T_i + C_j + P_k + T_i*C_j*P_k + TM_l + D_m + T_i*TM_l + e_{ijkl}$$

where

Y_{ijkl} = observation,

μ = overall mean,

T_i = treatment effect (i = 1, 2, 3, 4),

C_j = cow effect (j = 1, 2, 3, 4),

P_k = period effect (k = 1, 2, 3, 4),

TM_l = sampling time effect (l = 1, 2, ..., 6),

D_m = sampling day effect (m = 1, 2, 3, 4)

$T_i*C_j*P_k$, T_i*TM_l = interaction effect,

e_{ijkl} = residue.

In all the three models discussed above, the $T_i*C_j*P_k$ was used as an error term in testing T_i, C_j and P_k. Only the treatment effect (i.e. T_i) and the interaction effect between treatment and sampling time (i.e. $T_i*CT_i*S_m$ or T_i*TM_l) were of interest and discussed.

Factors affecting the remaining estimates were tested in the following model (one observation was missing for both in situ undegradable intake DM and N):

$$Y_{ijk} = \mu + T_i + C_j + P_k + e_{ijk}$$

where

Y_{ijk} = observation,

μ = overall mean,

T_i = treatment effect (i = 1, 2, 3, 4),

C_j = cow effect (j = 1, 2, 3, 4),

P_k = period effect (k = 1, 2, 3, 4),

e_{ijk} = residue.

RESULTS AND DISCUSSION

Ingredients and nutrient compositions of diets are presented in Table 3.1. The variations of the determined CP content among diets might have been caused by sampling variations. The CP content in CM treatments with added LS would be expected to be diluted to 96% of the CP content of the original CM due to the addition of LS (65% DM) at the level of 6.5% of original CM DM. However this dilution effect did not show up in the determined CP content of the diet containing CM treated with LS compared to the determined CP content of control diet (Table 3.1).

In Situ Measurements

In situ ruminal degradability

Rumen 16 h incubation of treated and untreated canola meals showed that heating to 129°C with LS or to 154°C with or without LS increased ($P < 0.05$) the in situ undegradable intake N, AA (excepting methionine) and DM in CM compared to the untreated CM (Table 3.2). These results are consistent with the findings of other researchers on heat treatment (McKinnon et al. 1990; Moshtaghi Nia and Ingalls 1992, 1995 a,b; Onyango 1995) and heat plus LS treatment (McAllister 1993) on CM protein.

Heating CM to 129°C with LS did not increase ($P > 0.05$) the in situ undegradable intake

methionine in treated CM compared to that in the untreated CM (Table 3.2). In the presence of LS, increasing exit temperature from 129°C to 154°C further increased the in situ undegradable intake N in CM and increased the in situ undegradable intake aspartic acid and glycine in CM (Table 3.2). Heating to 154°C without LS had the same effect as heating to 154°C or 129°C with LS on reducing N, total and individual AA (excepting proline) and DM in situ ruminal degradability of CM (Table 3.2). The in situ undegradable intake proline was lower in CM heated to 154°C without LS compared to that with LS (Table 3.2).

In general, heat treatment (to 129 or 154°C) with or without LS decreased the in situ ruminal degradability of N, total AA and DM in CM. In the presence of LS, increasing exit temperature from 129°C to 154°C further reduced the in situ ruminal degradability of N in CM. Addition of LS in the high temperature (i.e. 154°C) heat treatment did not alter the in situ ruminal degradability of N, total AA and DM in CM. The CM heated to 129°C with LS was not different from the CM heated to 154°C without LS in reducing in situ ruminal degradability of N, AA and DM in CM.

Estimated true digestibility

The estimated true digestibility of CM N and DM in the lower GI tract was increased ($P < 0.05$) in treated CM compared to untreated (Table 3.2) but was not different among treated CMs. The estimated true digestibility of CM N and DM in the total GI tract was

not affected ($P > 0.05$) by the three different heat treatments with or without LS, excepting that heating to 129°C with LS increased the estimated true digestibility of CM DM in the total GI tract compared to that of the untreated CM (Table 3.2). Heating to 154°C with or without LS was not different ($P < 0.05$) from the heating to 129°C with LS or the untreated on the estimated true digestibility of CM DM in the total GI tract (Table 3.2). Thus it can be concluded that heat treatment with or without LS caused the shift of digestion of CM N and DM from the rumen to the lower GI tract by reducing the ruminal degradability of N and DM and this shift did not reduce the digestibility of CM N and DM in the lower and the total GI tract.

Moshtaghi Nia and Ingalls (1992) demonstrated that moist heating at 127°C increased the lower GI tract digestibility of the CM protein and DM compared to the untreated. They also showed that the CM protein and DM digestibility in the total tract were not significantly decreased until the heating time was over 30 min at 127°C. Dakowski et al. (1996) roasted rapeseed meal to 130, 140 and 150°C (allowing a short heating time of approximately 5 min). They reported that rapeseed meal treated to 130°C had a similar estimated true digestibility of protein in the total tract (92 vs. 93%) with a higher estimated true digestibility of the rumen escape protein in lower GI tract (81 vs. 73%) compared to those of untreated rapeseed meal. However they observed that heating temperatures over 130°C decreased the estimated true digestibility of rapeseed meal protein in the lower and total GI tract compared to that of the untreated rapeseed meal protein. This reduction in the protein digestibility may have resulted from the longer exposure time (i.e. 5 min) to

high heat in reaching the heating temperature of 140 and 150°C compared to that (2 min and 34 seconds in reaching 154°C) in the present study. Extensive exposure of protein meal to high heat thus might cause damage to the protein and reduce protein digestibility significantly.

In conclusion, the in situ incubation data showed that heat treatment with or without LS increased undegradable intake N, total AA and DM in CM without sacrificing the estimated true digestibility of CM N and DM in the lower and the total GI tract. The proportion of N and DM of CM digested in the lower GI tract was thus increased due to the protective effect of heat treatment to the CM. The treatment of heating to 154°C with or without LS was sufficient in reducing the in situ ruminal degradability of CM protein.

In Vivo Measurements

Daily intake

The DM, N, AA and Cr daily intakes were not different among cows fed different diets (Table 3.3). These results support the findings of many other studies which also reported that heat treatment (to SBM) had little effect on dietary DM or N (or CP) intake in steers (Plegge et al. 1985; Coomer et al. 1993; Aldrich et al. 1995; Demjanec et al. 1995; Ludden and Cecava 1995) and in lambs (Cleale et al. 1987b,c). Due to the treatment effect of LS and heat, cows fed a diet containing CM heated to 154°C with LS was 14%

lower ($P < 0.05$) in the intake of degradable N than cows fed diets containing CM heated to 129°C with LS and the untreated CM (Table 3.3), and tended to be 47% higher ($P = 0.06$) in the undegradable AA intake than the cow fed a control diet (Table 3.3). Some variations occurred in the individual AA consumption calculated from intake and feed analysis, but total AA, EAA or NEAA intakes were not different among diets (Table 3.3). Thus CM heat treatment with or without LS did not alter the total dietary DM, N and AA intakes compared to the untreated CM in dairy cows.

Rumen fluid and rumen microbes

The rumen pH and NH_3 concentration were not different ($P < 0.05$) among cows fed different diets (Table 3.4). In agreement with the present study, many other studies also found no effect of heat treatment on the ruminal NH_3 concentration when heat treated SBM (Coomer et al. 1993) or soybean (Aldrich et al. 1995) was fed to steers. Plegge et al. (1985) however reported that roasted SBM (at 115 to 145°C) gave a significantly lower ruminal NH_3 concentration in steers (6.3 on average vs. 8.9 mg/dl) compared to that of the untreated SBM. Demjanec et al. (1995) further showed that SBM roasted at 165°C for 75 to 210 min decreased ($P < 0.05$) the ruminal NH_3 concentration in sheep in a linear fashion. Consistent with the present trial, the rumen pH was unaltered by the heat treatment in most of the studies (Demjanec et al. 1995; Plegge et al. 1985; Coomer et al. 1993).

The total VFA, acetic acid and butyric acid levels in the rumen fluid tended to be lower ($P < 0.10$) in cows fed a diet containing CM heated to 154°C with LS compared to those in the cows fed control diet (Table 3.4). This tendency to have a decreased VFA level might be caused by the decrease ($P < 0.05$) in the dietary intake of degradable N (Table 3.3) available for rumen metabolism in cows fed diet containing CM heated to 154°C with LS compared to the cows fed control diet. Stern (1984) also observed a decrease in VFA production when a diet containing SBM pelleted with calcium lignosulfonate (8 g/100 g SBM) was fed to a continuous culture compared to that of the control diet. Demjanec et al. (1995) reported that roasted SBM (at 165°C for 75 to 210 min) decreased ($P < 0.05$) the total VFA concentration in rumen fluid of sheep by 14% compared to that of sheep fed the untreated SBM. The authors speculated that this reduction in VFA level might reflect the decrease of the quantity of fermentable substrate available in SBM roasted at higher temperature.

No interaction between treatment and rumen fluid sampling time was observed for rumen pH, NH_3 concentration and VFA level.

The purine (ribonucleic acid equivalent) concentration and the purine : N ratio of mixed microbes (harvested from the rumen content) were not different among cows fed different diets (Table 3.4). The purine : CP ratio of microbes was 0.80 : 1 on average and thus is in agreement with the ratios reported by Cecava et al. (1990, 0.82 on average) and Firkins et al. (1987, 0.80 : 1 on average), while lower than the ratio (0.90 : 1) reported by

Titgemeyer et al. (1988).

It is concluded that the inclusion of CM heat treated with or without LS in replacing the untreated CM in the dairy diet did not alter the rumen fluid pH and NH_3 concentration, and the rumen microbial purine concentration and purine : N ratio in cows. The total VFA, acetic acid and butyric acid levels in the rumen fluid were decreased in cows fed CM heated to 154°C plus LS compared to those in cows fed untreated CM.

Duodenal DM and N flow

The Cr_2O_3 was employed as a single marker in estimating the duodenal digesta DM flow in the present study. The Cr_2O_3 has been used in several studies (Overton et al. 1995; Streeter and Mathis 1995; Zinn 1993; Demjanec et al. 1995). Rohr et al. (1984) reported that the mean recovery of Cr_2O_3 from the duodenal digesta (total collection) was 96 to 98% when the Cr_2O_3 mixed with wheat flour was given to dairy cows in four portions through the rumen. However Cr_2O_3 has been criticized for not closely associating itself with either the solid (MacRae 1974) or liquid phase (Merchen 1988) in digesta. Attempts had been made to include two or more markers in representing both solid and liquid phases or other phases (MacRae 1974; Faichney 1980, Doyle et al. 1988; Armentano and Russell 1985). However, there was not direct evidence indicating that two or more markers were actually superior to a single marker (Cr_2O_3) in estimating duodenal digesta flow. The duodenal DM and N flows estimated by using Cr_2O_3 as a single marker were demonstrated to be in

close agreement with those obtained through the total duodenal digesta collection via re-entrant cannula (Rohr et al. 1984; Corse and Sutton 1971).

The duodenal digesta DM, N, NAN, calculated endogenous N, microbial N and dietary N daily flows, and Cr and purine concentrations were not different ($P > 0.05$) among cows fed different diets (Table 3.5). The duodenal NH_3 N flow tended to be higher ($P = 0.09$) in cows fed a diet containing CM heated to 154°C with LS than that in cows fed diets containing CM heated to 129°C with LS, although the increase seems too small to be biologically significant.

Many other studies showed, in agreement with the present study, that protein meal heat treatment did not change ($P > 0.05$) duodenal organic matter flow (Plegge et al. 1985), and duodenal microbial or dietary N flow (Plegge et al. 1985; Coomer et al. 1993; Aldrich et al. 1995; Ludden and Cecava 1995). Demjanec et al. (1995) reported that SBM roasted at an increasing time from 75 to 210 min (at 165°C) increased ($P < 0.05$) the duodenal N flow linearly when the treated SBM was fed to sheep. The heating decreased ($P < 0.05$) the bacterial N flow while increasing ($P < 0.05$) the non-bacterial N flow in the duodenum of sheep when the heated SBM was fed to sheep in replacing the untreated SBM (Demjanec et al. 1995). Lack of change in duodenum microbial N flow (Table 3.5) indicated that the rumen microbial growth was not limited in cows fed heat treated CMs compared to the control in the present study. The NH_3 concentration of rumen fluid was between 32 to 35 mg/dl (Table 3.4) and was well above the observed minimal NH_3

concentration (24 mg/dl) for maximal rate of ruminal fermentation (Mehrez et al. 1977). Thus the calculated degradable protein content in the diets (Table 3.1) supported a maximal rate of microbial protein production in the present trial.

The percentages of microbial N in the duodenal NAN flow were not different ($P > 0.05$) among cows fed different diets (Table 3.5). The percentage of calculated endogenous N tended to be lower ($P < 0.10$), while the percentage of dietary N tended to be higher ($P < 0.10$) in the duodenal NAN flow of cows fed diet containing CM heated to 154°C with LS compared with the cows fed control diet or diet containing CM heated to 129°C with LS.

The daily flow of duodenal N as a percentage of intake was not different among cows fed different diets (Table 3.5). Duodenal N flow represented 87% of intake for control to 103% of intake for 154°C plus LS (Table 3.5). Overton et al. (1995) also reported high N flow through the duodenum as a percentage of intake (90 to 103%) in lactating cows fed diets (50% concentrate, on DM basis) containing different proportions of corn and barley starches. Data from Demjanec et al. (1995) showed that the quantity of duodenal N flow was increased linearly from 81 to 106% of N intake when wethers were fed diets containing SBM roasted at 165°C for 0 to 210 min. These data are consistent with the findings in the present study. The results of the present trial and those of Demjanec et al. (1995) indicate that the increase of duodenal N flow as a percentage of intake is the direct result of an increase of rumen escape dietary N due to the protective effect of the heat

treatment with a relatively unaltered microbial N production.

Nolan and Leng (1972) studied the dynamic aspects of NH_3 and urea metabolism in sheep fed lucerne hay (18.5% CP as feed). They reported that 60% of dietary N was degraded in the rumen, 9% of intake N was absorbed as NH_3 through the rumen wall and 5% of the intake N or 7% of urea N produced in the body was recycled to the rumen. The ruminal NH_3 N contributed only 11% of the urea N produced in the body. The duodenal N flow was 92% of the N intake in their study. These data thus suggested that the amount of NH_3 N recycled to rumen or wasted in the body was relatively insignificant compared to the amount of NH_3 N and the degraded dietary N reused by microbes in microbial N production. This same situation may also exist in the present study.

Tamminga et al (1979) reported that, in dairy cows fed a diet of 12.9 kg DM/d, the increase of CP percentage in diet from 13.6 to 19.4 or 24.5% decreased the duodenal N flow from 138.6 to 108.5 or 87.5% of N intake. These data suggest the recycled N contribution to the total duodenal N flow became more significant as dietary N contribution decreased. In agreement with these findings, Doyle et al. (1988) reported that the increase of the N level from 3.6 to 8.2, 12.5 and 15.5% of organic matter decreased the total ruminal N content as a percentage of N intake from 300 to 178, 105 and 81%, and decreased the abomasal N flow as a percentage of N intake from 200 to 137, 109 and 94%, respectively in lambs. The higher duodenal or abomasal N flow as a percentage of N intake may mean a higher efficiency in the utilization of both dietary and recycling N.

In conclusion, the inclusion of CM heat treated with or without LS in replacing the untreated CM in the dairy diet did not alter significantly the total DM and N flows in the duodenum. The percentage of dietary N in the duodenal NAN flow, however, tended to be increased by the substitution of CM heated to 154°C plus LS to the untreated CM in the dairy diets.

Duodenal AA flow

The total AA and the total endogenous, microbial and dietary AA daily flows (Table 3.5); and the individual AA (Table 3.6), microbial (Table 3.7) and dietary AA (Table 3.8) flows in the duodenum were not different ($P > 0.05$) among cows fed different diets.

Demjanec at al. (1995) showed that SBM roasted at 165°C for 75 to 210 min improved ($P < 0.05$) non-bacterial AA flow by 114% (130 on average vs. 60 g/d), while decreased bacterial AA flow by 15% (130 on average vs. 152 g/d) in duodenum in wethers. The higher temperature and longer heating duration might account for the more significant effects of heating on the duodenal digesta AA flows in their trial compared to the present study, although the difference between the responses to heat treated protein meal from the different animals (i.e. wethers vs. dairy cows) was unknown.

The percentage of lysine and methionine in the EAA of duodenal digesta (Table 3.6) were 14.5 and 4.3% on average, respectively, which is slightly lower than the recommended

level of 15 and 5.3%, respectively, required for the maximum milk yield and the maximum production of milk components (Schwab 1995).

The total duodenal AA (Table 3.5), EAA, NEAA, and individual AA, excepting cystine and methionine (Table 3.8) flow as a percentage of intake were not different among cows fed different diets. In cows fed CM heated to 129°C with LS, the duodenal cystine flow as a percentage of intake tended to be lower ($P < 0.10$) than that in cows fed CM heated to 154°C with LS and the duodenal methionine flow as a percentage of intake tended to be lower ($P < 0.10$) than that in cows fed CM heated to 154°C without LS (Table 3.8).

The total duodenal AA (Table 3.5), EAA, NEAA and individual AA (Table 3.8) flow as a percentage of intake were below 100% because rumen AA degradation surpassed microbial AA synthesis (Table 3.3 and 3.5). The glycine in duodenal digesta, however, was 144 to 166% of intake (Table 3.8). The duodenal flows of tyrosine, in all the diets, and some other AA such as methionine and lysine in the control diet and diets containing CM treated to 154°C with or without LS were close to 100% of intake (Table 3.9). Santos et. al. (1984) reported that the individual AA flows to duodenal digesta were generally higher than dietary intakes in dairy cows fed various protein supplements. The duodenal AA level approaching or surpassing the intake level indicated an active microbial AA synthesis or a significant endogenous influx of AA or both which in quantity approximated or surpassed that degraded in the rumen.

The CM heat treatment with or without LS thus did not change the duodenal digesta AA flow compared to the untreated CM when fed in dairy cows.

Undegradable intake N in Diet and CM

The in vivo dietary UIN was higher ($P < 0.05$) in diets containing CM heated to 154°C with LS than the diets containing untreated CM or CM heated to 129°C with LS (Table 3.9). While the UIN of diet containing CMs heated to 129°C plus LS or at 154°C without LS were not different ($P > 0.05$) from that of control diet (Table 3.9). The in vivo undegradable intake N in the CM heated to 154°C with LS was estimated to be 225% of that of the untreated CM (Table 3.9). Thus the higher heating temperature (154°C) together with LS (6.5%) were necessary to treat CM to increase the UIN level in the diet in which the CM was used as a main protein source.

Cleale et al. (1987b) treated SBM at 150°C for 30 min with xylose (3 mol/ mol SBM-lysine). They reported that treated SBM had a 157% higher in vivo rumen escape N (33.7 vs. 13.1%) than the untreated SBM had in lambs. Xylose is also the main active reducing reagent in LS. The results of the present study thus supported their findings. Plegge et al. (1985) found that the percentage of SBM-N escaping rumen degradation was 15, 30, 36 and 47% for SBM non-roasted and roasted to 115, 130 and 145°C in proximally 2 min, respectively. In contrast to their trial, the low temperature heat treatment (at 129 C) with LS did not reduce the ruminal degradability of CM protein in the present study (Table

3.9).

The in situ method in the present study predicted that the UIN of original CM was 22.9% (Table 3.2). This is close to the assumed in vivo UIN of untreated CM (28%, NRC 1989) and that (25%) of Rooke et al (1983). However, the in situ prediction of UIN of CM heated to 129°C with LS (Table 3.2) was much higher (40 vs. 27%), while the prediction of CM heated to 154°C with LS (Table 3.2) was much lower (45 vs. 63%) than the in vivo estimate (Table 3.9). A previous study (manuscript II) estimated that the undegradable intake protein (calculated from the effective degradability of protein) of CM untreated, heated to 129°C plus LS, 154°C or 154°C plus LS were 25, 32, 34 and 42%, respectively. These estimates employed the nylon bag disappearance and the ruminal outflow rate in estimating the dietary UIN. These in situ estimates still over-estimated the protein undegradability of CM heated to 129°C with LS, while under-estimated that of CM heated to 154°C with or without LS compared to the in vivo estimates (Table 3.9). Thus the in situ estimates in predicting the rumen protein degradability shall be interpreted cautiously in relating to the actual feeding practice.

In conclusion, CM heated to 154°C with LS increased the UIN of dietary N compared to the untreated CM in dairy cows when fed.

Undegradable intake AA in diet

The UIAAs of total, essential, non-essential and individual (excepting cystine and glycine) AA were not different ($P > 0.05$) among the diets (Table 3.9). In the presence of LS, increasing the heating temperature from 129°C to 154°C tended ($P < 0.10$) to increase the undegradable intake cystine. The diet containing CM heated to 154°C with LS also tended to have higher ($P < 0.10$) undegradable intake glycine than the control diet.

The undegradable intake glycine was the one of the highest among the UIAAs in the diets (Table 3.9). The in situ data showed that the undegradable intake glycine was medium high among the individual undegradable intake AAs in CM (Table 3.2). The undegradable intake glycine of the dietary sources other than CM was unlikely to be very high. Thus it was speculated that free glycine or glycine containing compounds might be secreted into the digesta and passed to the duodenum in a substantial amount. However, the exceptional accumulation of glycine compared to other AA in duodenal digesta was not previously reported in the literature. Scheifinger et al. (1976) observed two species of rumen bacteria isolated from the pure culture of rumen bacteria that used AA as an energy source but did not use glycine. The significance of these rumen bacteria in the dietary glycine degradation is unclear.

Dietary isoleucine and proline were close to complete degradation in the rumen with the exception of the isoleucine in the diet containing CM heated to 154°C with LS and proline

in the diet containing CM heated to 129°C with LS (Table 3.9). The cystine and methionine were relatively stable among AAs as was glycine in diets excepting that methionine was close to complete degradation in the diet containing CM heated to 129°C with LS (Table 3.9). After evaluating the in vivo ruminal degradability of AA in SBM, corn gluten meal, blood meal and fish meal in steers, Titgemeyer et al. (1988) reported threonine, valine and isoleucine were more resistant to ruminal degradation; methionine, cysteine, histidine and arginine were more extensively degraded of EAA. The different protein sources and methods (incremental vs. difference method) used in the studies may have resulted in the differences between the results obtained in their and the present trial. In the incremental method, the effects of different levels of N or AA intake on the level of endogenous N or AA flow in duodenal digesta were not considered and thus might cause a bias in the measuring of undegradable intake AA of feedstuffs.

The undegradable intake total AA was about 10 to 13 percentage points below that of N in diets for dairy cows (Table 3.9). This data suggested that the degradability of N is higher than AA in dairy diets. This difference in degradability might account for a lower percentage of dietary AA in total duodenal AA flow than the percentage of dietary N in the total duodenal N (Table 3.5).

In general, CM heat treatment with or without LS did not improve the undegradable intake total AA and essential AA in the diet compared to the untreated CM when fed to cows.

Fecal excretion and diet apparent digestibility

The total fecal DM and N excretions were not different among cows fed different diets, although the fecal N concentration was higher ($P < 0.05$) in cows fed CM heated to 154°C with LS compared to cows fed control diet (Table 3.10).

The apparent digestibility of DM and N in the lower and total GI tract were not different among diets suggesting lack of heat damage on apparent protein digestion of CM (Table 3.4). High temperature heat treatment shifting the digestion of CM DM and N from rumen to the small intestine without reducing the apparent digestibility of CM DM and N thus should improve the nutritive value of CM for ruminants. In agreement with the present study, Demjanec et al. (1995) showed that the percentage of intake N disappearing in the total tract was not affected by the roasting treatment to SBM, while the percentage of intake SBM-N disappearing in the small intestine was improved by 89% on average (55 vs. 28%) compared to the untreated.

The in situ measurements showed that treated CM had significantly higher estimated true digestibility of CM DM and N in the lower GI tract over untreated CM (Table 3.2). This increase however was not indicated in the in vivo measurements of lower GI tract apparent digestibility of diets containing related CMs. It may be noteworthy that the estimate measured by the mobile bag technique was considered as a true digestibility (de Boer et al. 1987) while the in vivo estimate is an apparent digestibility. Bias might exist in the

estimated true digestibility as the method used in the determination (i.e. mobile bag technique) assumed that the nutrients which disappeared from the nylon bags were either degraded in the rumen or digested in the lower GI tract. For the practical feeding situation, the in vivo estimates will be valuable in evaluating the degradability and digestibility of diet.

The inclusion of CM heat treated with or without LS in replacing the untreated CM thus did not alter the apparent digestibility of dietary DM and N when fed to cows.

Plasma Urea N and AA

The urea N level, which might serve as an index of the quality and quantity of protein and AA absorbed from the intestine (DePeters and Ferguson 1991), and the AA composition of deproteinized plasma were similar ($P > 0.05$) for cows fed different diets (Table 3.11). The interaction effect between treatment and plasma sampling time on the urea N and AA level in the deproteinized plasma was not significant.

A previous study (manuscript I) showed that the concentration of AA (total AA and most of the individual AA in the deproteinized plasma were increased ($P < 0.05$) in lambs fed moist heat (110°C for 60 min) treated CM compared to the control. It is unclear if the responses of plasma AA level to the heat treated CM were different between dairy cows and lambs or because of treatment differences. Red blood cells were suggested to be

involved in the transport of AA to tissues (Hanigan et al. 1991) and might carry more peptides than plasma (Matthews 1991). The role of red blood cells in the transportation of AA in animals was not examined in the present study.

The CM heat treatment with or without LS did not affect the urea N and AA concentrations in the plasma of dairy cows compared to the untreated CM when fed.

Milk Production

The percentage of non-protein-N (NPN), true protein (TP), fat and solid-non-fat (SNF) in milk, and the efficiency of the milk TP produced from the CP intake and the milk yield produced from the DM intake were not different among cows fed different diets (Table 3.12). However, in the presence of LS, increasing the heating temperature of CM 129°C to 154°C tended to decrease ($P = 0.054$) milk yield by about 8%. This decrease in milk yield caused a significant reduction ($P < 0.05$) in both milk TP and SNF production and a trend towards reduced ($P < 0.10$) efficiency of milk yield from the DM intake in cows fed the diet containing CM heated to 154°C with LS compared to cows fed the diet containing CM heated to 129°C with LS. The interaction between treatment and milking time on milk component concentration excepting milk SNF concentration was not observed. The SNF concentration in the milk taken in the first three mornings (i.e. the morning sample of day 2 , 3 and 4 of the 8-d sample collection period) was higher in cows fed control diet than that in cows fed diet containing CM heated to 154°C with LS

(8.8 vs. 8.4%, SEM = 0.07%, data was not presented in a tabular form). The treatment at lower temperature (129°C) with LS thus might retain more available nutrients in CM compared to the treatment at higher temperature (154°C) with LS.

However, all the three diets containing treated CM were not different ($P > 0.05$) from control diet on milk production. Data from many other studies also showed that heat treated SBM had no significant effect on milk production (Ahrar and Schingoethe 1979; Netemeyer et al. 1982; Chen et al. 1987). To detect the heat treatment or protein protection effect on milk production, the levels of dietary protein or AA supplied to the animal need to be examined carefully. The effects of the increase of rumen escape protein or AA on milk production may not be significant if the quantity of protein or AA passing to the small intestine is already in excess to the requirement for milk production in both experimental and control diets.

The intensive sample collections in the present digestion trial would increase animal stress. The production data generated from the trial thus may not be applicable to a normal production situation. The experimental design of this trial may be better in detecting the change in the nutrient composition of digesta throughout the animal digestive tract rather than in reflecting changes in the animal production.

In conclusion, heat treatment with or without LS increased the in situ rumen escape of N, AA and DM of CM, and improved the percentage of CM N, AA and DM digested in

lower GI tract, without sacrificing the estimated digestibility of CM N or DM in the lower or total GI tract. The CM heated to 154°C plus LS tended to increase the percentage of dietary N in the duodenal NAN flow compared to the untreated CM in dairy cows. The in vivo undegradable intake N in the diet containing CM heated to 154°C with LS was significantly higher than that in the diet containing untreated CM. The in vivo apparent digestibility of N and DM in the total or lower GI tract were not affected by the inclusion of the treated CM in the dairy diet. Heating to 154°C plus 6.5% LS and steeping for 32 min showed the maximum effect in improving the rumen escape of protein without damaging the N or DM digestibility of CM, and thus improved the nutritive value of CM to the dairy cows.

Table 3.1. The ingredients and composition of the four treatment diets fed to lactating dairy cows.

Item	Diet			
	Control[1]	129C+LS[2]	154C[3]	154C+LS[4]
Ingredient (% DM)				
Barley-rolled	24.8	24.9	24.9	24.9
CM (control)	18.6	—	—	—
CM (129C+LS)	—	18.7	—	—
CM (154C)	—	—	18.7	—
CM (154C+LS)	—	—	—	18.7
Sunflower seeds	5.8	5.8	5.8	5.8
Tallow	0.3	0.3	0.3	0.3
Beet pulp	9.1	8.9	8.9	8.9
Beet molasses	0.5	0.5	0.5	0.5
Chromic oxide	0.3	0.3	0.3	0.3
Limestone	0.9	0.9	0.9	0.9
Vitamin Mix [5]	0.25	0.24	0.24	0.24
Mineral premix [6]	0.5	0.5	0.5	0.5
Corn silage	17.9	17.9	17.9	17.9
Alfalfa silage	21.1	21.1	21.1	21.1
Composition[7]				
NE(L) (Mcal/kg)	1.73	1.73	1.73	1.73
CP (% DM)	18.7	19.4	19.7	20.0
UIP (% DM)	5.8	5.9	7.5	8.6
DIP (% DM)	12.9	13.4	12.2	11.3
NDF (% DM)	36.5	36.5	36.6	38.1
NDIN (% nitrogen)	19.8	22.6	24.4	29.1
ADF (% DM)	20.9	20.5	21.4	21.6
ADIN (% nitrogen)	6.0	6.5	6.0	7.8

[1]Contains untreated canola meal (CM).

[2]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).

[3]Contains CM heated to 154°C and held for 32 min without LS added.

[4]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[5]Vitamin mix includes vitamin premix (4409.2 kIUs vitamin A, 1102.3 kIUs vitamin D, and 11023.0 IUs vitamin E) and 300 g niacin per 500 kg of the concentrate.

[6]Mineral premix includes: potassium iodide 75 mg, copper sulphate 1,875 mg, zinc oxide 7,560 mg, manganese oxide 81 g and salt 819 g per kg of mineral premix.

[7]NE(L) (net energy for lactation) value was estimated from NRC (1989), UIP (undegradable intake protein) and DIP (degradable intake protein) were determined by the in vivo dietary protein ruminal degradability data of the present study, and NDF (neutral detergent fiber), NDIN (neutral detergent insoluble nitrogen), ADF (acid detergent fiber) and ADIN (acid detergent insoluble nitrogen) were determined by laboratory analysis.

Table 3.2. The effects of lignosulfonate (LS) and heat treatment on in situ undegradable intake nitrogen (N), amino acid (AA) and dry matter (DM) of canola meal (CM) in the rumen, and the estimated true digestibility of CM N and DM in the lower and total gastrointestinal (GI) tract in dairy cows.

Item			n[1]	Canola meal				SEM[6]
				Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
In situ undegradable intake (% of intake)								
N[7]			8[8]	22.9c	40.2b	41.9ab	44.9a	1.2
DM[7]			8[8]	25.5b	33.7a	35.6a	36.4a	1.0
AA	Aspartic acid		8	20.5c	33.6b	36.7ab	41.5a	1.8
	Threonine		8	20.4b	35.4a	36.4a	41.1a	2.1
	Serine		8	25.0b	42.2a	44.1a	50.0a	2.2
	Glutamic acid		8	13.7b	25.4a	27.0a	28.4a	1.9
	Proline		8	17.4c	31.4ab	29.2b	36.3a	1.6
	Glycine		8	19.8c	33.5b	36.6ab	41.3a	1.7
	Alanine		8	21.3b	40.2a	41.2a	44.7a	2.1
	Valine		8	20.9b	39.1a	39.6a	43.0a	2.0
	Isoleucine		8	19.3b	38.7a	39.4a	42.8a	2.2
	Leucine		8	19.4b	35.3a	36.7a	41.5a	1.8
	Tyrosine		8	27.9b	50.2a	51.3a	59.9a	2.8
	Phenylalanine		8	22.1b	39.6a	40.9a	46.9a	2.1
	Histidine		8	16.3b	32.8a	32.9a	39.8a	1.8
	Lysine		8	19.0b	34.5a	35.0a	36.1a	1.6
	Arginine		8	17.7b	35.3a	35.7a	40.6a	1.9
	Cystine		8	17.4b	24.8a	28.5a	30.6a	1.5
	Methionine		8	21.9b	29.3ab	38.9a	37.5a	2.5
	Essential AA		8	19.6b	35.8a	37.1a	41.0a	1.8
	Non-Essential AA		8	18.5b	32.4a	33.9a	37.9a	1.7
	Total		8	19.0b	33.9a	35.3a	39.3a	1.7
Estimated true digestibility (in lower GI tract)								
% of intake		N	4	14.9b	32.7a	31.9a	36.6a	1.8
		DM	4	7.2b	16.9a	18.1a	19.4a	1.8
% of rumen escape		N	4	65.6b	81.0a	81.6a	81.5a	2.0
		DM	4	26.7b	50.0a	52.4a	53.3a	4.5
Estimated true digestibility (in total GI tract)[7]								
% of intake		N	10[9]	92.2	92.5	92.5	91.5	0.6
		DM	10[9]	81.6b	83.4a	82.7ab	83.1ab	0.4

a, b, c: means or least squares means in rows with different letters are different ($P < 0.05$).

[1]Observation number.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM DM.

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Standard error of means.

[7]Least squares means.

[8]Obsevation number = 8 for all treatments but control, for which n = 7.

[9]Average of ns of all treatments with n = 11 for control and 129C+LS, and n = 9 for 154C+LS.

Table 3.3. The daily Cr intake and the effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the dry matter (DM) undegradable and degradable dietary nitrogen (N) and amino acid (AA) intake by cows ($n^1 = 4$).

	Intake (g/d)	Diet				SEM[6]
		Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
DM		18,678	18,207	17,965	18,884	759
N	Total	556.2	556.8	566.2	597.8	26.5
	Undegradable[7]	169.3	167.9	214.5	263.0	25.5
	Degradable[8]	386.9a	388.8a	351.7ab	334.8b	12.0
AA	Total	2,991	2,732	2,789	3,090	127
	Undegradable[7]	513.7	542.7	603.3	968.7	157.3
	Degradable[8]	2,477	2,189	2,186	2,122	75
	Aspartic acid	275.8	256.1	257.6	284.2	11.6
	Threonine	131.6	119.2	117.7	132.8	5.5
	Serine	189.4	172.9	176.3	191.2	7.7
	Glutamic acid	538.2	483.8	511.6	555.5	22.7
	Proline	273.9	266.4	247.9	278.2	11.2
	Glycine	161.8	152.5	157.5	173.4	7.1
	Alanine	193.7	181.5	182.9	200.7	8.2
	Valine	151.3	129.0	127.9	154.7	6.7
	Isoleucine	105.2ab	90.2b	92.9ab	116.1a	5.2
	Leucine	226.4	207.0	214.9	241.2	10.1
	Tyrosine	88.3	74.2	82.2	93.8	4.0
	Phenylalanine	150.0	128.1	133.5	144.8	5.9
	Histidine	79.2	72.1	74.1	85.5	3.6
	Lysine	166.1	149.5	155.3	166.5	6.7
	Arginine	155.2	135.0	149.4	156.8	6.4
	Cystine	62.7	62.9	60.8	61.4	2.3
	Methionine	42.0b	51.4ab	46.7ab	53.4a	2.2
	Essential	1,272	1,144	1,167	1,308	54
	Non-Essential	1,718	1,588	1,622	1,782	73
Cr		36.4	35.4	42.6	48.8	3.5

a and b: means in rows with different letters are different ($P < 0.05$).

[1]Observation number.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM DM.

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Average of the standard error of means of the four CM treatments.

[7]Equal to duodenum dietary flow.

[8]Equal to the dietary intake less duodenum dietary flow.

Table 3.4. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on pH, ammonia (NH_3) and volatile fatty acid (VFA) concentrations in rumen fluid; and the purine concentration and purine : nitrogen ratio of rumen bacteria in dairy cows.

Item	n[1]	Diet				SEM[6]
		Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Rumen fluid						
pH[7]	48[8]	5.63	5.74	5.73	5.69	0.04
NH_3 (mg/dl)	48	34.9	34.9	35.2	32.0	2.5
VFA (mM)						
Total[9]	24	107.4	98.9	97.4	88.3	3.8
Acetic Acid	24	65.4	61.4	60.3	53.6	2.5
Propionic Acid	24	25.6	22.7	22.6	21.6	1.2
Butyric Acid	24	11.9	10.8	10.4	9.6	0.5
Rumen bacteria						
Purine[10] (% dry matter)	4	5.6	5.6	5.5	5.8	0.2
Purine[10] : nitrogen	4	0.78 : 1	0.78 : 1	0.80 : 1	0.83 : 1	0.03 : 1

[1]Observation numbers.
[2]Contains untreated CM.
[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).
[4]Contains CM heated to 154°C and held for 32 min without LS added.
[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.
[6]Standard error of means.
[7]Least squares means.
[8]Observation number = 48 for all treatments but 154C and 154C+LS, for which n = 47.
[9]Total VFA includes major VFAs, i. e. acetic acid, propionic acid and butyric acid , and minor VFAs, i. e. isobutyric acid isovaleric acid and valeric acid.
[10]Ribonucleic acid equivalent.

Table 3.5. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the composition and quantity of daily duodenal digesta flows in dairy cows ($n^1 = 4$).

Duodenal Digesta Flow			Diet				SEM[6]
			Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
DM		(g/d)	10,528	10,114	11,208	12,945	903
		(% intake)	56.0	56.4	62.0	68.3	3.9
Cr		(%)	0.34	0.35	0.38	0.38	0.01
Purine[7]		(%)	1.68	1.71	1.72	1.68	0.07
Nitrogen	Total	(g/d)	487.5	476.9	548.1	622.5	40.6
		(% intake)	87.6	86.3	96.2	103.0	4.0
	Ammonia	(g/d)	15.5	14.9	16.7	19.3	1.1
	NAN[8]	(g/d)	472.0	462.0	531.5	603.2	40.3
	Endogenous[9]	(g/d)	74.7	72.8	72.0	75.5	3.0
		(% NAN)	15.9	15.7	13.8	12.9	0.8
	Microbial	(g/d)	228.0	221.3	245.1	264.6	20.0
		(% NAN)	47.8	47.7	45.5	43.9	2.0
	Dietary[10]	(g/d)	169.3	168.0	214.5	263.0	25.5
		(% NAN)	36.3	36.7	40.8	43.3	2.0
Amino acid	Total	(g/d)	2002	1965	2138	2521	182
		(% intake)	66.3	72.8	76.5	80.9	4.0
	Endogenous[11]	(g/d)	307.0	301.0	280.6	307.0	16.7
	Microbial	(g/d)	1171	1112	1245	1235	113
	Dietary[12]	(g/d)	513.7	542.7	603.3	968.7	157.3

[1]Observation numbers.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Standard error of means.

[7]Ribonucleic acid equivalent.

[8]Non-ammonia-nitrogen: equals to total duodenal nitrogen (N) flow (g/d) less duodenal ammonia flow (g/d).

[9]Assumed to be 4 g per 1000 g of dietary DM intake (Tamminga et al. 1979).

[10]Equals to duodenal NAN flow (g/d) less duodenal microbial (g/d) and endogenous (g/d) flow.

[11]Assumed that the AA composition of the endogenous flow was the same as that of total duodenal flow, and thus the endogenous AA flow (g/d) was calculated by the total duodenal AA flow (g/d) times the percentage of endogenous N in NAN (%).

[12]Equals to duodenal total AA flow (g/d) less duodenal endogenous AA flow (g/d) and microbial AA flow (g/d).

Table 3.6. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the duodenal daily amino acid (AA) flow and the lysine and methionine content as a percentage of essential AA in duodenal digesta flow of dairy cows ($n^1 = 4$).

Item	Diet				SEM[6]
	Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Duodenal AA flow (g/d)					
Aspartic acid	195.6	188.1	207.6	247.4	17.0
Threonine	92.6	88.3	101.1	116.2	9.0
Serine	124.5	119.7	133.1	157.6	12.8
Glutamic acid	267.9	251.6	285.9	342.8	29.7
Proline	101.1	143.0	105.3	128.6	23.1
Glycine	230.8	227.4	236.2	282.7	13.8
Alanine	142.4	141.9	148.6	181.9	13.3
Valine	97.5	94.9	101.8	121.6	9.5
Isoleucine	69.7	68.0	77.9	89.9	7.2
Leucine	148.3	143.4	160.5	188.0	15.0
Tyrosine	82.9	80.1	88.3	105.7	9.5
Phenylalanine	91.0	85.6	100.0	112.9	8.8
Histidine	50.3	44.6	49.6	58.4	5.0
Lysine	131.1	124.9	141.8	160.7	11.9
Arginine	98.7	92.6	109.7	130.5	11.6
Cystine	39.5	37.4	42.8	48.9	3.7
Methionine	38.0	33.7	47.9	47.3	4.7
Essential AA	900	865	958	1128	84
Non-Essential AA	1102	1100	1180	1394	100
Lysine (% Essential AA)	14.6	14.4	14.8	14.2	0.1
Methionine (% Essential AA)	4.3	3.9	4.9	4.1	0.3

[1]Observation numbers.
[2]Contains untreated CM.
[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).
[4]Contains CM heated to 154°C and held for 32 min without LS added.
[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.
[6]Standard error of means.

Table 3.7. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the duodenenal microbial amino acid (AA) daily flow of dairy cows ($n^1 = 4$).

Microbial AA Flow (g/d)	Diet				SEM[6]
	Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Individual					
Aspartic acid	123.2	124.8	135.3	137.3	10.9
Threonine	62.7	62.4	66.7	66.4	5.6
Serine	71.8	65.7	77.0	78.5	7.3
Glutamic acid	153.0	145.5	162.3	164.2	114.6
Proline	56.9	48.6	53.2	61.5	7.2
Glycine	66.7	64.2	72.2	71.8	6.4
Alanine	96.0	89.9	103.2	103.3	9.3
Valine	64.8	60.2	68.4	63.2	5.9
Isoleucine	60.3	55.0	61.7	54.2	5.6
Leucine	89.6	86.5	93.3	89.8	7.3
Tyrosine	56.1	52.7	61.5	59.8	6.3
Phenylalanine	61.2	56.4	64.3	62.5	6.6
Histidine	23.3	21.8	25.6	24.0	2.4
Lysine	87.3	84.3	94.6	92.4	8.3
Arginine	62.8	58.5	65.7	67.6	7.2
Cystine	14.2	14.3	15.6	17.0	1.3
Methionine	21.3	21.0	25.4	23.1	2.5
Essential	586.4	559.3	629.7	612.5	55.6
Non-Essential	584.8	552.4	615.7	622.9	57.1

[1]Observation numbers.
[2]Contains untreated CM.
[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).
[4]Contains CM heated to 154°C and held for 32 min without LS added.
[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.
[6]Standard error of means.

Table 3.8. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the duodenal daily amino acid (AA) flow as the percentage of the dietary AA intake in dairy cows ($n^1 = 4$).

Duodenal AA Flow (% of dietary intake)	Diet				SEM[6]
	Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Individual					
Aspartic acid	70.3	74.2	80.3	86.5	4.4
Threonine	69.6	74.9	85.6	86.3	4.9
Serine	65.4	70.1	75.5	81.4	4.7
Glutamic acid	49.1	52.5	55.9	60.8	3.8
Proline	36.3	53.6	42.5	45.5	7.5
Glycine	143.9	151.1	150.1	165.7	6.7
Alanine	72.8	79.2	80.9	90.0	5.3
aline	62.9	74.5	79.2	78.1	5.6
Isoleucine	64.8	76.3	83.6	76.5	5.4
Leucine	64.6	70.3	74.6	77.1	4.7
Tyrosine	93.0	110.9	107.4	111.6	9.0
Phenylalanine	59.8	68.0	74.5	77.1	4.8
Histidine	63.1	63.2	66.7	67.6	4.6
Lysine	77.9	84.5	90.8	95.7	5.7
Arginine	62.6	69.6	73.2	81.3	5.1
Cystine	62.7	60.1	70.2	79.0	4.4
Methionine	89.8	66.1	101.1	86.9	7.5
Essential	69.9	76.8	81.8	85.5	5.1
Non-Essential	63.6	70.0	72.6	77.5	3.3

[1]Observation numbers.
[2]Contains untreated CM.
[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).
[4]Contains CM heated to 154°C and held for 32 min without LS added.
[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.
[6]Standard error of means.

Table 3.9. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the undegradable intake nitrogen (UIN) and amino acid (UIAA) of the diet fed to dairy cows and the effects of this treatment on the UIN of the CM included in the diet ($n^1 = 4$).

Item		Diet				SEM[6]
		Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Diet	UIN[7] (% of dietary intake)	30.9b	30.6b	38.2ab	43.2a	2.7
	UIAA[7] (% of dietary intake)					
	Total amino acid	17.1	20.2	22.3	30.3	3.8
	Individual amino acid					
	Aspartic acid	15.0	13.7	17.9	27.5	3.5
	Threonine	11.5	10.6*	18.4	25.0	4.7
	Serine	17.6	20.4	22.5	29.9	4.4
	Glutamic acid	13.2	13.9	17.1	23.2	3.4
	Proline	10.1*	26.4	15.6*	17.3*	7.1
	Glycine	80.2	85.0	84.6	102.8	5.0
	Alanine	12.3	16.4	14.6	26.6	4.9
	Valine	10.6*	15.7	16.2	27.0	5.4
	Isoleucine	-2.0*	3.0*	7.9*	19.3	5.7
	Leucine	15.1	16.8	21.9	29.7	3.7
	Tyrosine	15.6*	21.1	19.5	33.0	7.7
	Phenylalanine	10.1*	12.5	17.3	23.6	4.2
	Histidine	23.9	22.6	23.9	30.4	5.1
	Lysine	13.8	14.2	18.7	27.5	4.9
	Arginine	12.9	14.7	20.0	27.7	4.1
	Cystine	30.3	27.8	35.4	41.8	3.0
	Methionine	25.7	14.5	34.9	32.6	7.2
	Essential amino acid	13.3	15.3	17.8	27.4	4.6
	Non-essential amino acid	19.9	23.7	25.5	32.4	3.3
CM	UIN[8] (% of CM nitrogen)	28.0	26.9	47.6	63.1	N/A

a and b: means in rows with different letters are different ($P < 0.05$).

N/A: value is not available as the UIN data was derived from a simple calculation as indicated in the text.

*Mean is not different from zero ($P > 0.05$).

[1]Observation numbers.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Standard error of means.

[7]Equals to duodenal dietary flow (g/d) divided by dietary intake (g/d) times 100.

[8]UIN of untreated CM was assumed to be 28% of total N of CM (NRC 1989). The UIN of treated CM was adjusted based on the changes in total diet UIN for treatment vs. control diets.

Table 3.10. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the fecal excretion of dry matter (DM) and nitrogen (N), the concentration of N and Cr in feces, and the apparent digestibility of dietary DM and N in the lower and total gastrointestinal (GI) tract in dairy cows ($n^1 = 4$).

Item		Diet				SEM[6]
		Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Feces						
DM	(g/d)	6,665	6,186	6,796	7,746	435
N	(% of DM)	2.68b	2.76ab	2.77ab	2.86a	0.02
	(g/d)	168.9	158.4	178.5	209.8	13.2
Cr	(% of DM)	0.57	0.63	0.66	0.67	0.02
Apparent digestibility (lower GI tract)						
% of intake[7]	N	57.7	57.9	65.0	68.4	2.9
	DM	20.8	22.3	24.6	27.5	2.1
% of duodenal flow[8]	N	65.8	67.0	67.4	66.5	1.1
	DM	37.1	39.3	39.5	40.1	1.6
Apparent Digestibility (total GI tract)						
% of intake[9]	N	70.1	71.6	68.8	65.4	1.7
	DM	64.8	65.9	62.5	59.1	2.2

a and b: means in rows with different letters are different ($P < 0.05$).

[1]Observation numbers.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM DM.

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Standard error of means.

[7]Equals to the duodenal flow (g/d) less fecal excretion (g/d) divided by dietary intake (g/d) times 100.

[8]Equals to the duodenal flow (g/d) less fecal excretion (g/d) divided by duodenal flow (g/d) times 100.

[9]Equals to the dietary intake (g/d) less fecal excretion (g/d) divided by dietary intake (g/d) times 100.

Table 3.11. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal (CM) on the urea nitrogen and amino acid (AA) concentration in the deproteinized plasma of dairy cows ($n^1 = 16$).

Plasma composition (mg/dl)	Diet				SEM[6]
	Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Urea nitrogen	14.1	14.0	13.5	14.2	0.4
Amino acid					
Total	149.4	147.8	139.3	145.8	7.9
Individual					
Aspartic acid	12.7	12.3	12.2	12.3	0.9
Threonine	6.0	5.8	5.6	5.9	0.5
Serine	11.8	11.1	10.7	11.2	1.2
Glutamic acid	25.3	26.2	24.4	24.6	1.7
Proline	7.2	6.9	5.9	6.5	0.5
Glycine	7.3	7.2	7.0	7.1	0.5
Alanine	10.4	10.8	10.1	10.5	0.6
Valine	8.4	8.0	7.7	8.8	0.3
Isoleucine	2.8	3.1	2.8	3.0	0.2
Leucine	12.9	13.3	12.6	13.3	0.9
Tyrosine	5.6	5.4	5.2	5.3	0.3
Phenylalanine	5.8	6.4	5.7	5.9	0.4
Histidine	4.5	4.7	4.6	4.6	0.3
Lysine	12.0	12.3	11.5	12.3	0.8
Arginine	8.7	8.8	7.8	9.0	0.6
Cystine	3.5	3.3	3.1	3.4	0.2
Methionine	2.2	2.3	2.3	2.1	0.2
Essential	64.4	64.7	60.7	64.9	3.5
Non-Essential	83.8	83.1	78.6	81.0	4.9

[1]Observation numbers = 16 for all treatments but 154C+LS, for which n = 15.
[2]Contains untreated CM.
[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM dry matter (DM).
[4]Contains CM heated to 154°C and held for 32 min without LS added.
[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.
[6]Standard error of means.

Table 3.12. The in vivo effects of lignosulfonate (LS) and heat treatment of canola meal on the milk yield; the concentration of milk urea nitrogen (MUN); the production and the concentration of non-protein nitrogen (NPN), true protein (TP), fat, and solid non-fat (SNF) of daily milk; the efficiency of daily milk yield (kg/d) from daily dry matter (DM) intake (kg/d) (Milk yield/DM intake); and the efficiency of the daily milk TP (g/d) produced from daily dietary crude protein (CP) intake (g/d) (TP/CP intake) in dairy cows.

Item		n[1]	Diet				SEM[6]
			Control[2]	129C+LS[3]	154C[4]	154C+LS[5]	
Milk yield	(kg/d)	4	26.8	28.7	26.4	26.3	0.5
MUN	(mg/dl)	16	13.3	13.2	13.6	14.1	0.3
NPN	(% of daily milk)	16	0.03	0.03	0.03	0.03	0.0007
	(g/d)	4	8.7	9.5	8.6	9.0	0.2
TP	(% of daily milk)	14	2.96	2.89	2.94	2.89	0.04
	(g/d)	4	787.2ab	821.9a	757.6ab	742.2b	13.4
Fat	(% of daily milk)	14	2.9	2.8	2.8	2.9	0.1
	(g/d)	4	784.2	818.2	723.6	746.6	22.3
SNF	(% of daily milk)	14	8.57	8.56	8.61	8.51	0.04
	(g/d)	4	2302ab	2449a	2261ab	2226b	44
Yield/DM intake		4	1.43	1.57	1.46	1.41	0.04
TP/CP intake		4	0.23	0.23	0.22	0.21	0.09

a and b: means in rows with different letters are different ($P < 0.05$).

[1]Observation numbers.

[2]Contains untreated CM.

[3]Contains CM heated to 129°C and held for 32 min with LS added at 6.5% of original CM DM.

[4]Contains CM heated to 154°C and held for 32 min without LS added.

[5]Contains CM heated to 154°C and held for 32 min with LS added at 6.5% of original CM DM.

[6]Standard error of means.

GENERAL DISCUSSION

The general purpose of heat treatment is to decrease the degradability of dietary protein and amino acids (AA) in the rumen and to increase the amount of dietary protein and AA passing through the rumen to the abomasum and small intestine for animal digestion.

The present study showed that canola meal (CM) moist heat treated at 110°C for 1 h increased the AA level in the deproteinized plasma and improved the weight gain efficiency in growing lambs compared to the untreated CM. These improvements were caused by the decrease of the ruminal degradability of CM protein and the increase of the quantity of CM AA available for animal digestion and absorption in the lower gastrointestinal (GI) tract due to the moist heat treatment effects as indicated in Onyango (1995). These results were consistent with the finding of Onyango and Ingalls (1994) whose data showed an increase in milk yield in primparous cows fed moist heat treated CM compared to that of the control. These results were also consistent with the observations of Broderick and Craig (1881) who autoclaved cottonseed meal at 121°C for 60 min and found that the treatment increased the percentage of undegraded digestible protein to a maximum in the cottonseed meal. The moist heat treatment in the preliminary work reported here however was a time consuming process (i.e. 60 min). A relative shorter duration of heat treatment will be more desirable for an industrial scale production of high rumen escape protein CM.

A laboratory scale CM heat treatment was set up in the present study. The heating

temperature and duration, and the addition of lignosulfonate (LS), water and alcohol during the heating process were screened to find an effective method to reduce the heating duration and enhance the effects of heat treatment on protecting CM protein from ruminal degradation.

Results showed that the heat treatment of 150°C for 0.5 h increased significantly the in situ protein ruminal undegradability and the estimated digestible undegradable intake protein content in CM compared to those of the treatment of 100°C for 1 h or 125°C for 0.5 h when LS was not added during the heating process. This finding was in a good agreement with the observation of Satter et al. (1994) who reported that the optimum heat treatment condition in protection of soybean protein from ruminal degradation was roasting soybeans to 146°C and steeping for 30 min without cooling. It thus can be expected that increasing the heating temperature to 150°C with a shorter steeping duration of 30 min in the moist heat treatment will not only save the heat processing time, but also will further reduce the degradability of CM protein and thus further improve the quantity of dietary AAs available for digestion in the lower GI tract.

The addition of LS (4 to 9% of CM DM) during the heat treatment improved the in situ rumen escape protein and the estimated digestible undegradable intake protein in the CM. This result thus confirmed the finding of other researches on the protective effect of reducing sugars on the protein in the rumen (Stern 1984; Cleale et al. 1987a,b,c; Nakamura et al. 1992; Wallace and Falconer 1992; McAllister et al. 1993). Thus it also can be expected that the addition of LS at the level of 6.5% of CM DM in the moist heat treatment will further

increase the undegradable intake protein content in the CM and improve the availability of CM protein and AA to the lower GI tract digestion in ruminants. Results further showed that the CMs treated at three different levels of heating temperature and time (i.e. 100°C/1 h, 125/0.5 h and 150/0.5 h) were not different from each other in the in situ ruminal protein degradability (16 h incubation) and the estimated digestible undegradable intake protein content when LS was added at 4 to 6.5% of CM DM during the heat treatment.

The present study showed, in agreement with the finding of Lynch et al. (1987), that the addition of alcohol in the CM heat treatment (in the absence of water) decreased the in situ ruminal protein degradability, but the alcohol treatment was not beneficial for the improvement of the estimated digestible undegradable intake protein content in the CM. Increasing moisture level in the CM heat treatment was shown to cause an increase in the in situ ruminal protein degradability and a decrease in the estimated digestible undegradable intake protein content in CM. This result contradicted the finding of Cleale et al. (1987a) who reported that the amount of ammonia released from SBM heat treated with xylose (which also was a major reducing sugar agent in LS) in an in vitro system was decreased when the moisture level was increased in the treatment. However, the reason for this difference, regarding the effect of moisture on the protein ruminal degradation, is unclear.

Based on the above findings, further research was conducted to investigate the in situ and in vivo effects of CM heat treatments in which CM was treated on an industrial scale for a short heating duration (held for 32 min) at a medium high temperature (heating to 129°C) with

6.5% LS, or at a higher temperature (heating to 154°C) with or without 6.5% LS which approximated the treatment conditions of CM with heat and LS in the laboratory scale.

The present study showed that only the treatment of heating to 154°C with 6.5% LS and holding for 32 min reduced significantly the in situ effective degradability of CM protein compared to the control. The in situ 16 h incubation results however showed that all three heat treated CMs were improved in the in situ protein and AA undegradability (16 h incubation) compared to the control. CMs treated under the three different treatment conditions were not different among themselves in both the in situ effective degradability of protein and the in situ 16 h incubation undegradabilities of protein and total AA. The in situ 16 h incubation undegradability of protein, total AA, lysine and methionine of CM heated to 154°C with 6.5% LS were measured to be 196, 207, 190 and 171% of those of untreated CM, respectively.

The in vivo results further demonstrated that only the CM heated to 154°C with 6.5% LS and held for 32 min was sufficient in improving the in vivo ruminal protein undegradability of a lactation diet compared to the untreated CM when fed to dairy cows using CM as the main protein source. This finding is consistent with the in situ results of the effective degradability of protein in CM treatments indicated above. The in vivo results showed that, in the presence of 6.5% LS, increasing heating temperature from 129 to 154°C in the CM treatment improved the undegradable intake protein content in the dairy diet containing these treated CMs as the main protein source. This improvement however was not predicted by the in situ

measurements of the ruminal protein degradability of these treated CMs in the present study. The reason for this difference was not apparent. The duodenal total AA, lysine and methionine flows, and the dietary AA flow in cows fed CM heated to 154°C with LS were 126, 123, 124 and 289% of those in cows fed the untreated CM, respectively.

The in vivo ruminal protein undegradability of CM heated to 154°C with LS was estimated to be 225% of that of the untreated CM when fed to the dairy cows as a main protein source. In agreement with the present study result, Cleale et al. (1987b) reported that the in vivo ruminal protein escape was increased by 157% in soybean meal roasted at 150°C for 30 min with xylose and NaOH compared to that of untreated SBM in steers. The improvement in the in vivo ruminal protein undegradability of CM heated to 154°C with LS relative to that of the untreated CM however is higher than that predicted by the in situ effective degradability which showed a 64% increase in the percentage of undegradable intake protein in CM protein or that predicted by the in situ 16 h incubation undegradability which showed a 96% increase in the pecentage of undegradable intake protein in CM protein.

The estimated in vivo ruminal protein undegradability of CM heated to 154°C without LS and CM heated to 129°C with 6.5% LS was 170 and 96% of those of untreated CM, respectively. The low estimated in vivo ruminal protein undegradability of CM heated to 129°C with 6.5% LS on an industrial scale was not predicted by the in situ degradability of CM treated at the proximal heating condition on a laboratory scale which showed that the ruminal protein degrtadability (16 h incubation) of CM heat treated with LS was improved over that of CM

heat treated without LS. This inconsistency may be caused by the differences between the methods employed in treating CMs in the laboratory and industrial scale heat treatments.

The present study demonstrated that the heat treatment (with or without LS added) reduced the ruminal degradability of CM protein and increased the amount of CM protein and AA available for the post-ruminal digestion and thus improved the efficiency of CM protein utilization in ruminants. This finding is consistent with the results of other researches regarding the protective effect of heat treatment (with or without reducing sugar added) on the dietary protein in the rumen (Sahlu et al. 1984; Stern 1984; Cleale et al. 1987a,b,c; McKinnon et al. 1990; Moshtaghi Nia and Ingalls 1992, 1995a,b; McAllister et al. 1993; Satter et al. 1994; Hsu and Satter 1995; Onyango 1995). Results from the growth of lambs fed the treated CM, the estimated true digestibility of protein and dry matter (DM) of the treated CMs, and the measured apparent digestibility of protein and DM of the diet containing the treated CMs as the main protein sources demonstrated that heat treatment (with or without LS) at the present study levels did not cause damage to the digestibility of CM protein and DM in the lower GI tract of ruminants. This finding is also in agreement with the observations of other studies on the effects of heat treatment (with or without reducing sugar added) on the protein digestibility in the lower GI tract of ruminants (Plegge et al. 1982, 1985; Cleale et al. 1987b; Moishtaghi Nia and Ingalls 1992, 1995a,b; Wallace and Falconer 1992; Coomer et al. 1993; Aldrich et al. 1995; Onyango 1995).

As the digestibility of CM was not affected by the heat treatment in the present study, it

suggests that the Maillard reaction (Finot et al. 1977) induced through the heat treatment under the heating condition of the present study was controlled to the initial reversible stages in which a Schiff's base and it's intermediates were produced from the addition reaction between the free amino group of AA in protein and the aldehyde group of reducing sugar. The present study thus support the finding that the Schiff's base and its intermediates were reversible and bio-available (Finot et al. 1977). These data supported the hypothesis that the Schiff's base and its intermediates produced through the Maillard reaction during the heat treatment were stable in the rumen neutral condition and thus protected dietary protein from microbial proteolysis in the rumen. The Schiff's base and its intermediates could be hydrolyzed back to the original protein and reducing sugar in the acidic condition when they passed the rumen to the abomasum, and thus the heat treated dietary protein remained digestible for the animals.

It is concluded that heating to 154°C with 6.5% LS and holding for 32 min on an industrial treatment scale was effective in reducing the ruminal degradability of CM protein and improving the amount of CM protein and AA (essential AA especially) passing the rumen to the lower GI tract for ruminant animal digestion and absorption.

CONCLUSIONS

Canola meal (CM) moist heat treated at 110°C for 60 min increased ($P < 0.05$) the weight gain efficiency in lambs compared to the untreated CM. This treated CM also improved the amino acid (AA) absorption in the lower gastrointestinal (GI) tract in lambs over the control as measured by change in blood levels. Moist heat treatment thus improved the nutritional value of CM for the growth of lambs by increasing the passage of nutrients such as essential AA to the lower GI tract.

The addition of up to 6.5% lignosulfonate (LS) in CM during heat treatment decreased ($P < 0.05$) the in situ nylon bag protein disappearance, while slightly increasing ($P < 0.05$) the in vitro pepsin and pancreatin digestibility of CM protein, and increased ($P < 0.05$) the percentage of estimated digestible undegradable intake protein (DUIP) in CM. Three temperature and time levels (i.e. 100°C/1 h, 125°C/0.5 h and 150°C/0.5 h) were not different ($P > 0.05$) in improving the percentage of DUIP in CM in the presence of 4 and 6.5% of LS during the heat treatment. However the heating temperature of 150°C (heated for o.5 h) was needed in treating CM to increase its rumen escape protein and the DUIP in CM, if LS was not added to the heat treatment. Generally adding water or alcohol did not improved the DUIP percentage in CM during the heat treatment in the present study.

Compared to the other two treatments (i.e. 129°C/32 min with 6.5% LS and 154°C/32 min without LS), 154°C/32 min with 6.5% LS decreased ($P < 0.05$) the in situ effective

degradability of CM protein compared to the control. The heat treatment with or without LS increased ($P < 0.05$) the estimated true digestibility of CM nitrogen (N) and dry matter (DM) in the lower GI tract, while they did not decrease the estimated true digestibility of CM N and DM in the total GI tract compared to the control.

The rumen pH and ammonia concentration was not altered ($P < 0.05$) by substituting untreated CM with treated CM in the dairy diets. The inclusion of CM treated to 154°C for 32 min with 6.5% LS however tended to decrease ($P < 0.10$) the acetic, butyric and total volatile fatty acids concentration, and tended to decrease ($P < 0.10$) the percentage of endogenous N in the duodenal non-ammonia-N (NAN) flow, while tended to increase ($P < 0.10$) the percentage of dietary nitrogen in the duodenal NAN flow. However, the total N, NAN, endogenous N, microbial N, dietary N, and the total, endogenous, microbial and dietary AA flows in the duodenum were not different ($P > 0.05$) among cows fed different diets. Diet containing CM treated to 154°C for 32 min with 6.5% LS had a 40% higher ($p<.05$) undegradable intake N content than the control diet. From these data the undegradable intake N of CM heated to 154°C and held for 32 min with 6.5% LS was estimated to be about 225% of that of untreated CM.

The apparent digestibility of N and DM passed to the lower GI tract, the apparent digestibility of dietary N and DM in the total GI tract, and milk production were not affected by the inclusion of treated CM in a dairy diet in place of untreated CM.

Heating to 154°C and held for 32 min with 6.5% LS reduced the degradability of N in CM and increased the percentage of dietary N in the duodenal NAN flow in dairy cows fed this treated CM compared to those in the dairy cows fed untreated CM, and thus improved the nutritional value of CM protein.

For a further study, it would be of interest to try and improve the protective effect of heat and LS treatment. Maintaining the steeping temperature more constant through out the whole steeping process (32 min) at the exiting temperature level (i.e. 154°C) would be of interest, as the steeping temperature decreased from 154°C in the beginning of steeping to 27°C at the end of 32 min. Other methods such as the use of rumen fermentation manipulating agent ionophores and microbial feed additives also would be of interest to investigate further to improve the dietary protein and AA supply to the lower GI tract in ruminants.

REFERENCE

Ahrar, M. and Schingoethe, D. J. 1979. Heat-treated soybean meal as a protein supplement for lactating cows. J. Dairy Sci. 62:932-940.

Akeson, W. R. and Stahmann, M. A. 1964. A pepsin pancreatin digest index of protein quality evaluation. J. Nutr. 83:257-261.

Aldrich, C. G., Merchen, N. R., Nelson, D. R. and Barmore, J. A. 1995. The effects of roasting temperature applied to whole soybeans on site of digestion by steers: II. Protein and amino acid digestion. J. Anim. Sci. 73:2131-2140.

Allen, S. A. and Miller, E. L. 1976. Determination of nitrogen requirement for microbial growth from the effect of urea supplementation of a low N diet on abomasal N flow and N recycling in wethers and lambs. Br. J. Nutr. 36:353-368.

AOAC (Association of Official Analytical Chemists) 1990. Official Methods of Analysis. ed. Kenneth Helrich. 15th edition. Association of Official Analytical Chemists, Inc., Arlington, Virginia.

AOAC (Association of Official Analytical Chemists) 1995. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, Virginia.

Armentano, L. E. 1994. Impact of metabolism by extra-gastrointestinal tissues on secretory rate of milk proteins. J. Dairy Sci. 77:2809-2820.

Armentano L. E. and Russell, R. W. 1985. Method for calculating digesta flow and apparent absorption of nutrients from nonrepresentative samples of digesta. J. Dairy Sci. 68:3067-3070.

Asplund, J. M. 1994. Structure and function of the ruminant digestive tract. Pages 5-27 in Principles of Protein Nutrition of Ruminants. Ed. J. M. Asplund. CRC Press, Inc., Boca Raton, FL.

Atwal, A. S. 1995. Protected Proteins in Dairy Cattle Nutrition. Pages 59-70 in Livestock Production for the 21st Century: Priorities and Research Needs. Ed. P. A. Thacker. Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan.

Atwal, A. S., Mahadevan, S. and Wolynetz, M. S. 1995. Increased milk production of cows in early lactation fed chemically treated soybean meal. J. Dairy Sci. 78:595-603.

Atwal, A. S., Milligan, L. P. and Young, B. A. 1974. Effects of volatile fatty acid treatment on the protection of protein in the rumen. Can. J. Anim. Sci. 54:393-401.

Bailey, C. B. and Hironaka, R. 1984. Estimation of the rumen degradability of nitrogen and of nonprotein organic matter in formaldehyde-treated and untreated canola meal. Can. J. Anim. Sci. 64:183-185.

Barry, T. N., Manley, T. R. and Duncan, S. J. 1986. The role of condensed tannins in the nutritional value of Lotus peunculatus for sheep. Br. J. Nutr. 55:123-137.

Bartley, T. B., Avery, T. B., Watt, N. B. R., Davidovich, A. Galitzer, S. and Lassman, B. 1981. Ammonia Toxicity in cattle. V. Ammonia concentration of lymph and portal, carotid and jugular blood after the ingestion of urea. J. Anim. Sci. 53:494-515.

Bell, J. M. 1993. Factors affecting the nutritional value of canola meal: A review. Can. J. Anim. Sci. 73:679-697.

Bell, J. M. and Keith M. O. 1991. A survey of variation in the chemical composition of commercial canola meal produced in Western Canadian crushing plants. Can. J. Anim. Sci. 71:469-480.

Bergen, W. B. and Bates, D. B. 1984. Ionophores: their effect on production efficiency and mode of action. J. Anim. Sci. 58:1465-1483.

Bradbury, J. H. 1973. The structure and chemistry of keratin fibers. Adv. Protein Chem. 27: 111-211.

Bremner, J. M. and Keeney, D. R. 1965. Steam distillation methods for determination of ammonia, nitrate and nitrite. Anal. Chem. Acta. 32:485-495.

Broad, T. E. and Dawson R. M. C. 1975. Phospholipid biosynthesis in the anaerobic protozoan *Entodinium caudatum*. Biochem. J. 146:317-328.

Brock, F. M., Forsberg, C. W., Buchanan-Smith, J. G. 1982. Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. Appl. Environ. Microbiol. 44:561-569.

Broderick, G. A. 1978. In vivo procedures for estimating rates of ruminal protein degradation and protein escapeing the rumen undegraded. J. Nutr. 108:181-190.

Broderick, G. A. and Balthrop, Jr., J. E. 1979. Chemical inhibition of amino acid deamination by ruminal microbes in vitro. J. Anim. Sci. 49:1101-1111.

Broderick, G. A. and Craig, W. M. 1980. Effect of heat treatment on ruminal degradation and escape, and intestinal digestibility of cottonseed meal protein. J. Nutr. 110:2381-2389.

Broderick G. E. and Merchen N. R. 1992. Markers for quantifying microbial protein synthesis in the rumen. J. Dairy Sci. 75:2618-2632.

Broderick G. A., Wallace R. J. and Ørskov E. R. 1991. Control of rate and extent of protein degradation. Pages 541-592, in Physiological Aspects of Digestion and Metabolism in Ruminants. Ed. T. Tsuda, Y. Sasaki and R. Kawashima. Academic Press, Inc., San Diego, CA.

Bruckental, I., Oldham, J. D. and Sutton, J. D. 1980. Glucose and urea kinetics in cows in early lactation. Br. J. Nutr. 44:33-45.

Bryant, M. P. 1974. Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract. Am. J. Clin. Nutr. 27:1313-1319.

Bryant, M. P. and Robinson, I. M. 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. J. Dairy Sci. 46:150-154.

Calsamiglia, S. and Stern, M. D. 1995. A three-step in vitro procedure for estimating intestinal digestion of protein in ruminants. J. Anim. Sci. 73:1459-1465.

Cecava, M. J., Hancock, D. L. and Parker, J. E. 1993. Effects of zinc-treated soybean meal on ruminal fermentation and intestinal amino acid flows in steers fed corn silage-based diets. J. Anim. Sci. 71:3423-3431.

Cecava, M. J., Merchen, N. R., Gay, L. C. and Berger, L. L. 1990. Composition of ruminal bacteria harvested from steers as influenced by dietary energy level, feeding frequency, and isolation techniques. J. Dairy Sci. 73:2480-2488.

Chen, G. and Russell, J. B. 1989. More monensin-sensitive, ammonia-producing bacteria from the rumen. Appl. Environ. Microbiol. 55:1052-1057.

Chen, G. and Russell, J. B. 1990. Effect of monesin and a protonophore on protein degradation, peptide accumulation, and deamination by mixed ruminal microorganisms in vitro. J. Anim. Sci. 69:2196-2203.

Chen, G., Sniffen, C. J. and Russell, J. B. 1987. Concentration and estimated flow of peptides from the rumen of dairy cattle: Effects of protein quantity, protein solubility, and feeding frequency. J. Dairy Sci. 70:983-992.

Chen, M. and Wolin, M. J. 1979. Effect of monensin and lasalocid-sodium on the growth of methanolgenic and rumen saccharolytic bacteria. Appl. Environ. Microbial. 38:72-77.

Church, D. C. 1976. Digestive physiology. Pages 99-114 in Digestive Physiology and Nutrition of Ruminants. Volume I. 2nd ed. O & Books, Corvallis, Or.

Clark, J. H., Davis, C. L. and Hatfield, E. E. 1974. Effects of formaldehyde treated soybean meal on nutrient use, milk yield and composition, and free amino acids in the lactating bovine. J. Dairy Sci. 57:1031-1036.

Clark, J. H., Klusmeyer, T. H. and Cameron, M. R. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304-2323.

Clark J. H., Spires, H. R. and Davis, C. L. 1978. Uptake and metabolism of nitrogenous components by the lactating mammary gland. Fed. Proc. 37:1233-1238.

Cleale IV, R. M., Klopfenstein, T. J., Britton, R. A., Satterlee, L. D. and Lowry, S. R. 1987a. Induced non-enzymatic browning of soybeen meal. I. Effects of factors controlling non-enzymatic browning on in vitro ammonia release. J. Anim. Sci. 65:1312-1318.

Cleale IV, R. M., Britton, R. A., Klopfenstein, T. J., Bauer, M. L., Harmon, D. L. and Satterlee, L. D. 1987b. Induced non-enzymatic browning of soybean meal. II. Ruminal escape and net portal absorption of soybean protein treated with xylose. J. Anim. Sci. 65:1319-1326.

Cleale IV, R. M., Klopfenstein, T. J., Britton, R. A., Satterlee, L. D. and Lowry, S. R. 1987c. Induced non-enzymatic browning of soybean meal. III. Digestibility and efficiency of protein utilization by ruminants of soybean meal treated with xylose or glucose. J. Anim. Sci. 65:1327-1335.

Coleman, G. S. 1986. The distribution of carboxymethylcellulase between fractions taken from the rumens of sheep containing no protozoa or one of five different protozoal populations. J. Agric. Sci., Camb. 106:121-127.

Coomer, J. C., Amos, H. E., Froetschel, M. A., Ragland, K. K. and Williams, C. C. 1993. Effects of supplemental protein source on the ruminal fermentation, protein degradation, and amino acid absorption in steers and on growth and feed efficiency in steers and heifers. J. Anim. Sci. 71:3078-3086.

Corse, D. A. and Sutton, J. D. 1971. Methods of measuring flow of duodenal digesta in sheep. Proc. Nutr. Soc. 30:18A-19A.

Cotta, M. A. and Hespell, R. B. 1986. Protein and amino acid metabolism of rumen bacteria. Pages 122-136 in Control of Digestion and Metabolism in Ruminants. Ed. L. P. Milligan, W. L. Grovumand and A. Dobson. Prentice-Hall, Englewood Cliffs, NJ.

Crawford, Jr., R. J. and Hoover, W. H. 1984. Effects of particle size and formaldehyde treatment of soybean meal on milk production and composition for dairy cows. J. Dairy Sci. 67:1945-1952.

Crooker, B. A., Sniffen, C. J., Hoover, W. H. and Johnson, L. L. 1978. Solvents for soluble nitrogen measurements in feedstuffs. J. Dairy. Sci. 61:437-447.

Czerkawski, J. W. and Breckenridge, G. 1977. Design and development of a long-term rumen simulation technique (Rusitec). Br. J. Nutr. 38:371-384.

Dakowski, P. Weisbjerg, M. R., Hvelplung, T. 1996. The effect of temperature during processing of rapeseed meal on the amino acid degradation in the rumen and the digestion in the intestine. Anim. Feed Sci. Technol. 58:213-226.

Dannelly, C. C. 1980. Rumen-stable pellets. U.S. 4,181,709 (Cl. 424-21; A61K9/24).

Dawson, K. A. 1990. Designing the yeast culture of tomorrow - mode of action of yeast culture for ruminants and non-ruminants. Pages 59-75 in Biotechnology in the Feed Industry. Ed. T. P. Lyons. Alltech Technical Publications, Nicholasville, Kentucky.

Dawson, K. A., Newman, K. E. and Boling, J. A. 1990. Effects of microbial supplements containing yeast and lactobacilli on roughage - fed ruminal microbial activities. J. Anim. Sci. 68:3392-3398.

de Boer, G., Murphy, J. J., Kennelly, J. J. 1987. Mobile nylon bag for estimating intestinal availability of rumen undegradable protein. J. Dairy Sci. 70:977-982.

Demeyer, D. I. and Van Nevel, C. J. 1979. Effect of defaunation on the metabolism of rumen micro-organisms. Br. J. Nutr. 42:515-524.

Demjanec, B., Merchen, N. R., Cremin, Jr., J. D. Aldrich, C. G. and Berger, L. L. 1995. Effect of roasting on site and extent of digestion of soybean meal by sheep: I. Digestion of nitrogen and amino acids. J. Anim. Sci. 73:824-834.

Dennis, S. M. and Nagaraja, T. G. 1986. Effect of lasalocid, monensin and thiopeptin on rumen protozoa. Res. Vet. Sci. 41:251-256.

DePeters, E. J. and Cant, J. P. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. J. Dairy Sci. 75:2043-2070.

DePeters, E. J. and Ferguson, J. D. 1992. Nonprotein nitrogen and protein distribution in the milk of cows. J. Dairy Sci. 75:3192-3209.

Doyle, P. T., Dove, H., Freer, M. Hart, F. J., Dixon, R. M. and Egan, A. R. 1988. Effects of a concentrate supplement on the intake and digestion of a low-quality forage by lambs. J. Agric. Sci., Camb. 111:503-511.

Driedger, A. and Hatfield, E. E. 1972. Influence of tannins on the nutritive value of soybean meal for ruminants. J. Anim. Sci. 34:465-468.

El Hassan, S. M., Newbold, C. J. and Wallace, R. J. 1993. The effect of yeast culture on rumen fermentation: Growth of the yeast in the rumen and the requirement for viable yeast cells. Anim. Prod. 56:463. (Abstr.)

Ensminger, M. E. and Parker, R. O. 1986. Page 377 in Sheep & Goat Science. The Interstate Printers & Publishers, Inc. Danville, Illinois.

Erasmus, L. J., Botha, P. M. and Kistnser, A. 1992. Effect of yeast culture supplement on production, rumen fermentation, and duodenal nitrogen flow in dairy cows. J. Dairy Sci. 75:3056-3065.

Erfle, J. D., Sauer, F. D., Mahadevan, S. and Teather, R. M. 1986. Response of lactating dairy cows to formaldehyde-treated soybean meal when fed with control or urea-treated corn silage. Can. J. Anim. Sci. 66:85-95.

Erwin, E. S., Manco, G. J. and Emery, E. M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas and liquid chromatography. J. Dairy Sci. 44:1768-1771.

Evans, R. J. and Butts, H. A. 1949. Inactivation of amino acid by autoclaving. Science 109:569-572.

Faichney, G. J. 1980. The use of markers to measure digesta flow from the stomach of sheep fed once daily. J. Agric. Sci., Camb. 94:313-318.

Faldet, M. A., Voss, V. L., Broderick, G. A. and Satter, L. D. 1991. Chemical, in vitro, and in situ evaluation of heat-treated soybean protein. J. Dairy Sci. 74:2548-2554.

Finlayson, H. J. and Armstrong, D. G. 1986. The effect of methanal (formaldehyde) treatment of casein on its digestion in vivo and in vitro. J. Sci. Food Agric. 37:742-752.

Finot, P.-A., Bujard, B., Mottu, F. and Mauron J. 1977. Availability of the true Schiff's bases of lysine. Chemical evaluation of the Schiff's base between lysine and lactose in milk. Pages 343-365 in Protein Crosslinking: Nutritional and Medical Consequences

(Advances in Experimental Medicine and Biology Volume 86b). Ed. Mendel Friedman. Plenum Press, New York.

Firkins, J. L., Berger, L. L. , Merhen, N. R., Fahey, Jr., G. C. and Mulvaney, R. L. 1987. Ruminal nitrogen metabolism in steers as affected by feed intake and dietary urea concentration. J. Dairy Sci. 70:2302-2311.

Forsberg, C. W., Lovelock, L. K. A., Krumholz, L. and Buchanan-Smith, J. G. 1984. Protease activities of rumen protozoa. Appl. Environ. Microbiol. 47:101-110.

Friedrich, M. 1989. Physiology of intestinal digestion and absorption. Pages 218-272 in Protein Metabolism in Farm Animals: Evaluation, Digestion, Absorption and Metabolism. Ed. H. D. Bock, B. O. Eggum, A. G. Low, O. Simon and T. Zebrowska. Oxford University Press (Oxford) and VEB Deutscher Landwritschaftsverlag (Berlin).

Fulghum, R. S. and Moore, W. E. C. 1963. Isolation, enumeration and the characteristics of proteolytic ruminal bacteria. J. Bacteriol. 85:808-815.

Gibb, D. J., Klopfenstein, T. J., Britton, R. A. and Lewis, A. J. 1992. Plasma amino acid response to graded levels of escape protein. J. Anim. Sci. 70:2885-2892.

Glenn, B. P., Ely, D. G. and Boling, J. A. 1977. Nitrogen metabolism in lambs fed lipid-coated protein. J. Anim. Sci. 46:871-877.

Goering, H. K., and Van Soest, P. J. 1970. Forage fibre analysis (apparatus, reagents, procedures and some applications). USDA-ARS Agricultural Handbook 379. U.S. Government Printing Office, Washington, D. C.

Goodrich, R. D., Garrett, J. E., Gast, D. R., Kirick, M. A., Larson, D. A. and Meiske, J. C. 1984. Influence of monensin on the performance of cattle. J. Anim. Sci. 58:1484-1498.

Hanigan, M. D., Calvert, C. C., DePeters, E. J., Reis, B. L. and Baldwin, R. L. 1991. Whole blood and plasma amino acid uptakes by lactating bovine mammary gland. J. Dairy Sci. 74:2484-2490.

Hannson, T. L. and Klopfenstein, T. 1979. Monensin, protein source and protein levels for growing steers. J. Anim. Sci. 48:474-479.

Harris, L. E., Learl, L. C. and Fonnesbeck, P. V. 1972. Use of regression equations in predicting availability of energy and protein. J. Anim. Sci. 35:653-680.

Harrison, G. A., Hemken, R. W., Dawson, K. A., Harmon, R. J. and Barker, K. B.

1988. Influence of addition of yeast culture supplement to diets of lactating cows on ruminal fermentation. and microbial populations. J. Dairy Sci. 71:2967-2975.

Hartnell, G. F. and Satter, L. D. 1979. Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. J. Anim. Sci. 48:381-392.

Hawke, J. C. and Silcock, W. R. 1988. The in vivo rates of lipolysis and biohydrogenation in rumen contents. Biochem. Biophys. Acta 218:201-212.

Hespell, R. B. and O'Bryan-Shah, P. J. 1988. Esterase activities in *Butyrivibio fibrisolvens* strains. Appl. Environ. Microbiol. 54:62-68.

Hino, T. and Russell, J. B. 1985. Effect of reducing-equivalent disposal and NADH/NAD on deamination of amino acids by intact rumen microorganisms and their cell extracts. Appl. Environ. Microbiol. 50:1368-1382.

Hino, T. and Russell, J. B. 1987. Relative contributions of ruminal bacteria and protozoa to the degradation of protein in vitro. J. Anim. Sci. 64:261-270.

Hobson, P. N. and Wallace, R. J. 1982. Microbial ecology and activities in the rumen: Part II. CRC Crit. Rev. Microbiol. 9:253-320.

Hoover, W. H. and Stokes, S. R. 1991. Balancing carbohydrates and proteins for optimum rumen microbial yield. J. Dairy Sci. 74:3630-3644.

Hoover, W. H., Crooker, B. A. and Sniffen 1976. The effects of differential solid liquid removal rates on protozoa numbers in continuous culture of rumen contents. J Anim. Sci. 39:528-534.

Hsu, H. W., Valak, D. L., Satterlee, L. D. and Miller, G. A. 1977. A multienzyme technique for estimating protein digestibility. J. Food Sci. 42:1269-1273.

Hussein, H. S., Demjanec, B., Merchen, N. R. and Aldrich, C. G. 1995. Effect of roasting on site and extent of digestion of soybean meal by sheep: II. Digestion of artifacts of heating. J. Anim. Sci. 73:835-842.

Huntington, G. B., Zetina, E. J., Whitt, J. M. and Potts, W. 1996. Effects of dietary concentrate level on nutrient absorption, liver metabolism, and urea kinetics of beef steers fed isonitrogenous and isoenergetic diets. J. Anim. Sci. 74:908-916.

Hurrell, R. F. and Carpenter, K. J. 1977. Nutritional significance of cross-link formation during food processing. Pages 225-238 in Protein Crosslinking: Nutritional and Medical

Consequences (Advances in Experimental Medicine and Biology Volume 86b). Ed. M. Friedman. Plenum Press, New York.

Hurrell, R. F. and Finot, P. A. 1985. Effects of food processing on protein digestibility and amino acid availability. Pages 233-247 in Digestibility and Amino Acid Availability in Cereals and Oilseeds. Ed J. W. Finley and D. T. Hopkins. American Association of Cereal Chemists, St. Paul, Minn.

Hvelplund, T. Weisbjerg, M. B. and Anderson, L. S. 1992. Estimation of the true digestibility of rumen undegraded dietary protein in the small intestine of ruminants by the mobile bag technique. Acta Agric. Scand., Sect. A., Anim. Sci. 42:34-39.

Ingalls, J. R., McKirdy and Sharma, H. R. 1980. Nutritive value of fababeans in the diets of young Holstein calves and lactating dairy cows. Can. J. Anim. Sci. 60:689-698.

Ivan, M., Mahadevan, S. and Dayrell, M. de S. 1996. Duodenal flow of microbial and feed nitrogen in sheep fed normal soybean meal or soybean meal treated with modified zein. J. Dairy Sci. 79:121-126.

James, G. L. and Olson, J. D. 1995. Using milk urea nitrogen to evaluate diets and reproductive performance of dairy cattle. Pages 155-167 in 4-State Applied Nutrition and Management Conference. The Conference, The La Crosse Centre, La Crosse, WI.

Jones, W. T., Anderson, L. B. and Ross, M. D. 1973. Bloat in cattle. N.Z. J. Agric. Res. 16:441-446.

Jones, W. T. and Mangan, J. L. 1977. Complexes of the condensed tannin of Sainfoin (*Onobrychis viciifolia Scop.*) with fraction 1 leaf protein and with submaxillary mucoprotein, and their reversal by polyethylene glycol and pH. J. Sci. Food Agric. 28:126-136.

Kendall, E. M. 1988. Insacco Rumen Degradation, and Digestibility in the Lower Digestive Tract of Ruminants, of Canola Meal and Soybean Meal. Msc. Thesis, University of Manitoba, Winnipeg, Manitoba, Canada.

Kendall, E. M., Ingalls, J. R. and Boila, R. J. 1991. Variability in rumen degradation and postruminal digestion of the dry matter, nitrogen and amino acids of canola meal. Can. J. Anim. Sci. 71:739-754.

Kennedy, P. M. 1980. The effects of dietary sucrose and the concentrations of plasma urea and ruminal ammonia on the degradation of urea in the gastrointestinal tract of cattle. Br. J. Nutr. 43:125-140.

Krebs, H. A. and Henseleit, K. 1932. Untersuchungen über die harnstoffbildung im tierkörper. Hoppe-Seyler's Z. Physiol. Chem. 210:33-66.

Krishnamoorthy, U., Sniffen, C. J., Stern, M. D. and van Soest, P. V. 1983. Evaluation of a mathematical model of rumen digestion and an in vivo simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. Br. J. Nutr. 50:555-568.

Lardy, G. P., Catlett, G. E., Kerley, M. S. and Paterson, J. A. 1993. Determination of ruminal escape value and duodenal amino acid flow of rapeseed meal. J. Anim. Sci. 71:3096-3104.

Legay-Carmier, F. and Bauchart, D. 1989. Distribution of bacteria in rumen contents of dairy cows given a diet supplemented with soya-bean oil. Br. J. Nutr. 61:725-740.

Little, C. O., Burroughs, W. and Woods, W. 1963. Nutritional significance of soluble nitrogen in dietary proteins for ruminants. J. Anim. Sci. 22:358-363.

Luchini, N. D., Broderick, G. A. and Combs, D. K. 1996. Characterization of the proteolytic activity of commercial proteases and strained ruminal fluid. J. Anim. Sci. 74:685-692.

Ludden P. A. and Cecava, M. J. 1995. Supplemental protein sources for steers fed corn-based diets: I. Ruminal characteristics and intestinal amino acid flows. J. Anim. Sci. 73:1466-1475.

Lynch, G. L., Berger, L. L. and Fahey, Jr., G. C. 1987. Effects of ethanol, heat, and lipid treatment of soybean meal on nitrogen utilization by ruminants. J. Dairy Sci. 70:91-97.

Mackie R. I. and White B. A. 1990. Recent advances in rumen microbial ecology and metabolism: Potential impact on nutrient output. J. Dairy Sci. 73:2971-2995.

MacRae, J. C. 1974. The use of intestinal markers to measure digestive function in ruminants. Proc. Nutr. Soc. 33:147-154.

Madsen, J. and Hvelplund, T. 1985. Protein degradation in the rumen. A comparison between in vivo, nylon bag, in vitro and buffer measurements. Acta Agric. Scand. Suppl. 25:103-124.

Madsen, J. and Hvelplund, T. 1994. Prediction of in situ protein degradability in the rumen. Results of a European ring test. Livestock Prod. Sci. 39:201-212.

Mahadevan, S. 1990. A new process for protecting soybean meal from rumen degradation. Pages 54-57 in 1990 Dairy Research Report. Agriculture Canada, Ministry of agriculture and food, Ontario. O.A.C. Publication No. 1290.

Mahadevan, S., Erfle J. D. and Sauer, F. D. 1980. Degradation of soluble and insoluble proteins by Bacteroides Amylophilus protease and by rumen microorganism. J. Anim. Sci. 50:723-728.

Mahadevan, S., Sauer, F. D. and Erfle, J. D. 1987. Preparation of protease from mixed rumen microorganisms and its use for the in vitro determination of the degradability of true protein in feedstuffs. Can. J. Anim. Sci. 67:55-64.

Makkar, H. P. S., Singh, B. and Dawra, R. K. 1988. Effect of tannin-rich leaves of oak (*Quercus incana*) on various microbial enzyme activities of the bovine rumen. Br. J. Nutr. 60:287-296.

Marquardt, R. R. 1988. Dietary effects of tannins, vicine and convicine. Pages 141-155 in Recent Advances of Research in Antinutritional Factors in Legume Seeds: Animal Nutrition, Feed. Ed. J. Huisman, T. F. B. van der Poel, and I. E. Liener. Pudoc, Wageningen, Netherlands.

Martin, A. A. and Nisbet, D. J. 1992. Effect of direct-fed microbials on rumen microbial fermentation. J. Dairy Sci. 75:1736-1744.

Matthews, D. M. 1991. Protein Absorption: Chapter 8 in Development and Present State of the Subject. Wiley-Liss, Inc. New York.

McAllan and Smith R. H. 1973. Degradation of nucleic acids in the rumen. Br. J. Nutr. 29:331-345.

McAllister, T. A., Cheng, K.-J., Beauchemin, K. A., Bailey, D. R. C., Pickard, M. D. and Gilbert, R. P. 1993. Use of lignosulfonate to decrease the rumen degradability of canola meal protein. Can. J. Anim. Sci. 73:211-215.

McCormick, M. E. and Webb, Jr., K. E. 1982. Plasma free erythrocyte free and plasma peptide amino acid exchange of calves in steady state and fasting metabolism. J. Nutr. 112:276-282.

McKinnon, J. J., Olubobokun, J. A., Christensen, D. A. and Choen, R. D. H. 1990. The influence of heat and chemical treatment on ruminal disappearance of canola meal. Can. J. Anim. Sci. 70:1161. (Abstr.)

Mehrez A. Z. and Ørskov E. R. 1977. A study of artificial fibre bag technique for

determinating the digestibility of feeds in the rumen. J. Agric. Sci., Camb. 88:645-650.

Mehrez, A. Z., Ørskov, E. R. and McDonald, I. 1977. Rates of rumen fermentation in relation to ammonia concentration. Br. J. Nutr. 38:437-443.

Merchen, N. R. 1988. Digestion, absorption and excretion in ruminants. 1988. Pages 172-201 in The Ruminant Animal Digestive Physiology and Nutrition. Ed. D. C. Church. Prentice Hall, Englewood Cliffs, New Jersey.

Michalet-Doreau, B. and Cerneau P. 1991. Influence of foodstuff particle size on in situ degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35:69-81.

Michalet-Doreau, B. and Ould-Bah, M. Y. 1992. In vitro and in sacco methods for the estimation of dietary nitrogen degradability in rumen: A review. Anim. Feed Sci. Technol. 40:57-86.

Miller, E. L., Hartley, A. W. and Thomas, D. C. 1965. Availability of sulphur animo acids in protein foods. 4. Effect of heat treatment upon total amino acid content of cod muscle. Br. J. Nutr. 19:565-573.

Mir, Z., MacLeod, G. K., Buchanan-Smith, J. G., Grieve, D. G. and Grovum, W. L. 1984. Methods for protecting soybean and canola proteins from degradation in the rumen. Can. J. Anim. Sci. 64:853-865.

Moismanyana, B. M. and Mowat, D. N. 1992. Rumen protection of heat-treated soybean proteins. Can. J. Anim. Sci. 72:71-81.

Morita, O., Ota, M., Shimaguchi, N. and Ito, K. 1986. Feed additives for ruminants. Jpn. Kokai Tokkyo Koho JP 61 88,843 [86 88,843].

Moshtaghi Nia, S. A. 1994. Improving Rumen Undegraded Intake Protein by Means of Moist Heat Treatment and Time of Feeding. Ph. D. Thesis, University of Manitoba, Winnipeg, MB.

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

Moshtaghi Nia, S. A. and Ingalls, J. R. 1995a. Influence of moist heat treatment on ruminal and intestinal disappearance of amino acids from canola meal. J. Dairy Sci. 78:1552-1560

Moshtaghi Nia, S. A. and Ingalls, J. R. 1995b. Evaluation of moist heat treatment on

digestion in rumen, small intestine, large intestine and total digestive tract of steers. Can. J. Anim. Sci. 75:279-283.

Murphy, T. A., Loerch, S. C. and Dehority, B. A. 1994. The influence of restricted feeding on site and extent of digestion and flow of nitrogenous compounds to the duodenum in steers. J. Anim. Sci. 72:2487-2496.

Nagaraja, T. G., Towne, G. and Beharka, A. A. 1992. Moderation of ruminal fermentation by ciliated protozoa in cattle fed a high-grain diet. Appl. Environ. Microbiol. 58:2410-2414.

Nakamura, T., Klopfenstein, T. J. Owen, F. G., Britton, R. A., Grant, R. J. and Winowiski, T. S. 1992. Nonenzymatically browned soybean meal for lactating dairy cows. J. Dairy Sci. 75:3519-3523.

Netemeyer, D. T., Bush, L. J., Ward, J. W. and Jafri, S. A. 1982. Effect of heating soybean meal for dairy cows. J. Dairy Sci. 65:235-241.

Newbold, C. J., Wallace, R. J. and McKain, N. 1990. Effects of the ionophore tetronasin on nitrogen metabolism by ruminal microorganisms in vitro. J. Anim. Sci. 68:1103-1109.

Nocek, J. E. 1985. Evaluation of specific variables affecting in situ estimates of ruminal dry matter and protein digestion. J. Anim. Sci. 60:1347-1358.

Nocek J. E. 1988. In situ and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71:2051-2069.

Nolan, J. V. 1989. Implications of protozoa and fungi for the protein nutrition of ruminants. Pages 211-221 in The Roles of Protozoa and Fungi in Ruminant Digestion. Ed. J. V. Nolan , R. A. Leng and D. I. Demeyer. Penambul Books, Armidale, Australia.

Nolan, L. V. and Leng, R. A. 1972. Dynamic aspects of ammonia and urea metabolism in sheep. Br. J. Nutr. 27:177-194.

Nolan, J. V., Norton, B. W. and Leng, R. A. 1976. Further studies of the dynamics of nitrogen metabolism in sheep. Br. J. Nutr. 35:127-147.

Norton, B. W., Moran, J. B. and Nolan, J. V. 1979. Nitrogen metabolism in *Brahman cross*, buffalo, banteng and shorthorn steers fed on low-quality roughage. Aust. J. Agric. Res. 30:341-351.

NRC. 1985a. Nutrient Requirements of Sheep. National Academy Press, Washington, D. C.

NRC 1985b. Ruminant Nitrogen Usage. National Academy Press, Washington, D. C.

NRC 1989. Nutrient requirements for dairy cattle. 6th ed. Natl. Acad. Sci., Washington, D. C.

Odle, J. and Schaefer, D. M. 1987. Influence of rumen ammonia concentration on the rumen degradation rates of barley and maize. Br. J. Nutr. 57:127-138.

Oldham, J. D. and Tamminga, S. 1980. Amino acid utilization by dairy cows. I. Methods of varying amino acid supply. Livestock Prod. Sci. 7:437-452.

Onyango, T. A. 1995. Heat Treatment of Canola Meal and Subsequent Availability of Rumen Escape Protein and Amino Acids in Ruminants. Ph. D. thesis, Univ. of Manitoba, Winnipeg, MB.

Onyango, T. A. and Ingalls, J. R. 1994. Feed intake and lactation response of cows fed heat treated canola meal and high undegradable protein supplements in early lactation. J. Anim. Sci. 72 (suppl. 1)/ J. Dairy Sci. 77 (suppl. 1):237. (Abstr.)

Ørskov, E. R. 1992. Protein Nutrition in Ruminants. Academic Press Limited. London, UK.

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

Ørskov, E. R., Mills, C. L. and Robinson, J. J. 1980. The use of whole blood for the protection of organic materials from degradation in the rumen. Proc. Nutr. Soc. 39:60A.

Overton, T. R., Cameron, M. R. Elliott, J. P. and Clark J. H. 1995. Ruminal fermentation and passage of nutrients to the duodenum of lactating cows fed mixtures of corn and barley. J. Dairy Sci. 78:1981-1998.

Owens, F. N. and Hanson C. F. 1992. External and internal markers for appraising site and extent of digestion in ruminants. J. Dairy Sci. 75:2605-2617.

Plegge, S. D., Berger, L. L. and Fahey, Jr., G. C. 1982. Effect of roasting on utilization of soybean meal by ruminants. J. Anim. Sci. 55:395-401.

Plegge, S. D., Berger, L. L. and Fahey, Jr., G. C. 1985. Effect of roasting temperature on the protection of soybean meal nitrogen escaping degradation in the rumen. J. Anim. Sci. 61:1211-1218.

Pocius, P. A., Clark, J. H. and Baumrucker, C. R. 1981. Glutathione in bovine blood: Possible source of amino acids for milk protein synthesis. J. Dairy Sci. 64:1551-1554.

Poore, M. H., Moore, J. A., Eck, T. P. and Swingle, R. S. 1991. Influence of passage model, sampling site, and marker dosing time on passage of rare earth-labeled grain through Holstein cows. J. Anim. Sci. 69:2646-2654.

Poos, M., Klopfenstein, T. and Britton, R. A. 1985. Evaluation of laboratory techniques for predicting ruminal protein degradation. J. Dairy Sci. 68: 829-839.

Poos, M., Klopfenstein, T. Britton, R. A. and Olson, D. G. 1980. An enzymatic technique for determining ruminal degradation. J. Dairy Sci. 63 (suppl. 1):142. (Abstr.)

Punia, B. S., Leibholz, J. and Faicheny, G. J. 1987. The role of rumen protozoa in the utilization of paspalum (Paspalum dilatatum) hay by cattle. Br. J. Nutr. 87:395-406.

Raab, L., Cafantaris, B., Jilg, T. and Menke, K. M. 1983. Rumen protein degradation and biosynthesis. 1. A new method for determination of protein degradation in rumen fluid in vitro. Br. J. Nutr. 50:569-582.

Radcliffe, B. C., Hynd, P. I., Benevenga, N. J. and Egan, A. R. 1985. Effects of cysteine ethyl ester supplements an wool growth rate. Aust. J. Agric. Res. 36:709-715.

Rae, R. C., Ingalls, J. R. and McKirdy, J. A. 1983. Response of dairy cows to formaldehyde-treated canola meal during early lactation. Can. J. Anim. Sci. 63:905-915.

Roe, M. B., Chase, L. E. and Sniffen, C. J. 1991. Comparison of in vitro techniques to the in situ technique for estimation of ruminal degradation of protein. J. Dairy Sci. 74:1632-1640.

Roffler, R. E., Schwab, C. G. and Satter, L. D. 1975. Relationship between ruminal ammonia and nonprotein nitrogen utilization by ruminants. III. Influence of intraruminal urea infusion on ruminal ammonia concentration. J. Dairy Sci. 59:80-84.

Rohr, K., Brandt, M., Lebzien, P. and Schafft, H. 1984. Measurement of duodenal flow in dairy cows by either total collection or spot sampling, using a special cannula. Can. J. Anim. Sci. 64 (suppl.):116-117.

Rooke, J. A. 1985. The nutritive values of feed proteins and feed protein residues resistant to degradation by rumen microorganisms. J. Sci. Food Agric. 36:629-637.

Rooke, J. A., Brookes, I. M. and Armstrong. 1983. The digestion of untreated and formaldehyde-treated soya-bean and rapeseed meals by cattle fed a basal silage diet. J.

Agri. Sci., Camb. 100:329-342.

Roseler, D. K. Ferguson, J. D., Sniffen, C. J. and Herrema, J. 1993. Dietary protein degradability effects on plasma and milk urea nitrogen and milk nonprotein nitrogen in Holstein cows. J. Dairy Sci. 76:525-534.

Rulquin, H. 1994. Protected lysine and methionine in dairy cow rations. Feed Mix 2:24-27.

Rulquin, R., Guinard, V. G. and Pisulewski, P. M. 1995. Dairy cows' requirements for amino acid. Pages 143-160 in Animal Research and Development: Moving Toward a New Century. Ed. M. Ivan. Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, ON.

Russell, J. B. 1987. A proposed mechanism of monensin action in inhibiting ruminal bacterial growth: Effects on ion flux and protonmotive force. J. Anim. Sci. 64:1519-1525.

Russell, J. B. and Strobel, H. J. 1987. Concentration of ammonia across cell membranes of mixed rumen bacteria. J. Dairy Sci. 70:970-976.

Russell, J. B. and Strobel, H. J. 1989. Effect of Ionophores on ruminal fermentation. Appl. Environ. Microbial. 55:1-6.

Russell, J. B.; Strobel, H. J. and Chen, G. 1988. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Appl. Environ. Microbiol. 55:872-877.

Sahlu, T., Schingoethe, D. J. and Clark, A. K. 1984. Lactational and chemical evaluation of soybean meals heat-treated by two method. J. Dairy Sci. 67:1725-1738.

Salunkhe, D. K., Chavan, J. K. and Kadam, S. S. 1990. Nutritional consequence of dietary tannins. Pages 113-146 in Dietary Tannins: Consequences and Remedies. CRC Press, Boca Raton, Fla.

Santos, K. A., Stern, M. D. and Satter, L. D. 1984. Protein degradation in the rumen and amino acid absorption in the intestine of lactating dairy cattle fed various protein sources. J. Anim. Sci. 58:244-255.

SAS 1989. SAS® User's Guide: Statistics, Version 6 Edition. SAS Institute Inc., Cary, NC.

Satter, L. D. 1986. Protein supply from undegraded dietary protein. J. Dairy Sci.

69:2734-2749.

Satter, L. D., Dhiman, T. R. and Hsu, J. T. 1994. Use of heat processed soybean in dairy rations. Pages 19-28 in Proc-Cornell-Nutr-Conf-Feed-Manuf. Ithaca, NY.

Sauer, W. C., Jorgensen, H. and Berzins, R. 1983. A modified nylon bag technique for determining apparent digestibilities of protein in feedstuffs for pigs. Can. J. Anim. Sci. 63:233-237.

Scheifinger, C., Russell, N. and Chalupa, W. 1976. Amino acid utilization by pure cultures of rumen bacteria. Fed. Proc. 34:902 (Abstr.)

Schwab, C. G. 1995. Current status on amino acid requirements of lactating dairy cows. Pages 17-33 in 4-State Applied Nutrition and Management Conference. The Conference, The La Crosse Centre, La Crosse, WI.

Sherrod, L. B. and Tillman, A. D., 1964. Further studies on the effects of different processing temperatures on the utilization of solvent-extracted cottonseed protein by sheep. J. Anim. Sci. 23:510-516.

Shirley, R. L. 1986. Nitrogen and Energy Nutrition of Ruminants. Academic Press, Inc. Orlando, Florida.

Sibbald, I. R. 1987. Estimation of bioavailable amino acids in feedstuffs for poultry and pigs: A review with emphasis on balance experiments. Can. J. Anim. Sci. 67:221-301.

Siddons, R. C., Paradine, J., Gale, D. L. and Evans, R. T. 1985. Br. J. Nutr. 54:545-561.

Sklan, D. 1989. In vitro and in vivo rumen protection of proteins coated with calcium soaps of long-chain fatty acids. J. Agric. Sci. Camb. 112:79-83.

Sklan, D. and Tinsky, M. 1993. Production and reproduction response by dairy cows fed varying undegradable protein coated with rumen bypass fat. J. Dairy Sci. 76:216-233.

Slyter, L. L. and Putnam, P. A. 1967. In vivo vs. in vitro continuous culture of ruminal microbial populations. J. Anim. Sci. 26:1421-1427.

Slyter, L. L., Nelson, W. O. and Wolin, M. J. 1964. Modifications of a device for maintenance of the rumen microbial population in continuous culture. Appl. Microbiology. 12:374-377.

Smith, R. H. 1989. Nitrogen metabolism in the ruminant stomach. Pages 165-203 in

Protein Metabolism in Farm Animals: Evaluation, Digestion, Absorption and Metabolism. Ed. H. D. Bock , B. O. Eggum, A. G. Low, O. Simon and T. Zebrowska. Oxford University Press (Oxford) and VEB Deutscher Landwritschaftsverlag (Berlin).

Smith, S. I. and Boiling, J. A. 1984. Lipid coating as a mode of protecting free methionine from ruminal degradation. J. Anim. Sci. 58:187-233.

Smith, R. H. and McAllan, A. B. 1970. Nucleic acid metabolism in the ruminant. 2. Formation of microbial nucleic acids in the rumen in relation to the digestion of food nitrogen and the fate of dietary nucleic acids. Br. J. Nutr. 24:545-556.

Streeter, M. N. and Mathis M. J. 1995. Effect of supplemental fish meal protein on site and extent of digestion in beef steers. J. Anim. Sci. 73:1196-1201.

Stephenson, R. G., Suter, G. R. and Howitt, C. J. 1991. Wool growth responses to DL-methionine administration and factors affecting the value of supplementation. Aust. J. of Experimental Agric. 31:471-477.

Stern, M. D. 1984. Effect of lignosulfonate on rumen microbial degradation of soybean meal protein in continuous culture. Can. J. Anim. Sci. 64 (suppl.):27-28.

Stern M. D. and Satter L. D. 1980. In vivo estimation of protein degradability in the rumen. Pages 57-71 in Protein Requirements for Cattle: Symposium. Ed. F. N. Owen. Oklahoma State University, Stillwater, OK.

Stern, M. D., Calsamiglia, S. and Endres, M. I. 1995. Estimates of ruminal degradability and post-ruminal digestibility of proteins. Pages 1-15 in 4-State Applied Nutrition and Management Conference. The Conference, The La Crosse Centre, La Crosse, WI.

Sticker, L. S. Bunting, L. D., Wyatt, W. E. and Wolfrom, G. W. 1991. Effect of supplemental lysocellin and tetronasin on growth, ruminal and blood metabolites, and ruminal proteolytic activity in steers grazing ryegrass. J. Anim. Sci. 69:4273-4278.

Swanson, E. W. 1982. Estimation of metabolic protein requirements to cover unavailable losses of endogenous nitrogen in maintenance of cattle. Pages 183-197 in Protein Requirements of Cattle: Symposium. Ed. F. N. Owens. Oklahoma State University, Stillwater, OK.

Tamminga, S., Van der Koelen, C.J. and Van Vuuren, A. M. 1979. Effect of level of feed intake on nitrogen entering the small intestine of dairy cows. Livestock Prod. Sci. 6:255-262.

Tilley, J. M. A. and Terry, R. A. 1963. A two stage technique for in vitro digestion of

forage crops. J. Br. Grassl. Soc. 18:104-711.

Titgemeyer, E. C., Merchen, N. R. and Berger, L. L. 1988. Evaluation of soybean meal, corn gluten meal, blood meal and fishmeal as sources of nitrogen and amino acids disappearing from the small intestine of steers. J. Anim. Sci. 67:262-275.

Udén, P., Parra, R. and Van Soest, P. J. 1974, Factors influencing reliability of the nylon bag technique. J. Dairy Sci. 57:622. (Abstr.)

Ueda, S., Nagai, T., Kobayashi, T., Okada, H., Miyake, M. and Matsuzawa, S. 1990. Ruminant feed additives coated with synthetic polymers. Jpn. Kokai Tokkyo Koho JP 0436,154 [92 36,154].

Ushida, K., Jouany, J. P. and Thivend, P. 1986. Role of rumen protozoa in nitrogen digestion in sheep given two isonitrogenous diets. Br. J. Nutr. 56:407-419.

van der Aar, P. J., Berger, L. L., and Fahey, Jr., G. C. 1982. The effects of alcohol treatments on solubility and in vitro and in situ digestibilites of soybean meal protein. J. Anim. Sci. 55:1179-1189.

Van Soest, P. J. 1994. Nitrogen Metabolism. Paged 290-311 in Nutritional Ecology of the Ruminant. 2nd ed. Cornell University Press, Ithaca, New York.

Veira, D. M. 1986. The role of ciliate protozoa in nutrition of the ruminant. J. Anim. Sci. 63:1547-1560.

Waghorn, G. C., Ulyatt, M. J., John, A. and Fisher, M. T. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus L.* Br. J. Nutr. 57:115-126.

Waldo, D. R. and Goering, H. K. 1979. Insolubility of products in ruminant feeds by four methods. J. Anim. Sci. 49:1560-1568.

Walker, J. F. 1964. Pages 299-404 in Formaldehyde. 3rd ed. Reinhold Publishing Corporation, New York, NY.

Wallace, R. J. 1988. Ecology of rumen microorganism: protein use. Pages 99-122 in Aspects of Digestive Physiology in Ruminants. Ed. A. Dobson and M. J. Dobson, Cornell University Press, Ithaca, N.Y.

Wallace, R. J. 1989. Protection of proteins from degradation in rumen fluid by heating in the presence of sugars. Anim. Prod. 48:629. (Abstr.)

Wallace R. J. 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. J. Anim. Sci. 72:2992-3003.

Wallace, R. J. 1996. The mode of action of yeast culture in modifying rumen fermentation. Pages 217-232 in Biotechnology in the Feed Industry. Ed. T. P. Lyons and K. A. Jacques. Nottingham Univ. Press. Nottingham, UK.

Wallace, R. J. and Cotta M. A. 1988. Metabolism of nitrogen-containing compounds. Pages 217-249 in The rumen Microbial Ecosystem. Ed. P. N. Honson. Elsevier Applied Sci., London, England.

Wallace, R. J. and Falconer, M. L. 1992. In vitro studies of conditions required to protect protein from ruminal degradation by heating in the presence of sugars. Anim. Feed Sci. Technol. 37:129-141.

Wallace, R. J. and Newbold, C. J. 1993. Rumen fermentation and its manipulation: The development of yeast cultures as feed additives. Pages 173-192 in Biotechnology in the Feed Industry. Ed. T. P. Lyons. Alltech Technical Publications, Nicholasville, Kentucky.

Wallace, R. J., Broderick, G. A. and Brammall, M. 1987. Microbial protein and peptide metabolism in rumen fluid from faunated and ciliate-free sheep. Br. J. Nutr. 58:87-93.

Weakley, D. C., Stern, M. D. and Satter, L. D. 1983. Factors affecting disappearance of feestuffs from bags suspended in the rumen. J. Anim. Sci. 56:493-507.

Webb, Jr., K. E. and Bergman E. N. 1991. Amino acid and peptide absorption and transport across the intestine. Pages 111-128 in Physiological Aspects of Digestion and Metabolism in Ruminants. Ed. T. Tsuda, Y. Sasaki and R. Kawashima. Academic Press, Inc. San Diego, CA.

Webb, Jr., K. E., Dirienzo, D. B. and Matthews, J. C. 1993. Recent developments in gastrointestinal absorption and tissue utilization of peptides: A review. J. Dairy Sci. 76:351-361.

Webb, Jr. K. E. and Matthews, J. C. 1994. Absorption of amino acids and peptides. Pages 127-146 in Principles of protein nutrition of ruminants. Ed. J. M. Asplund. CRC Press, Inc., Boca Raton, FL.

Webb, Jr., K. E., Matthews, J. C. and Dirienzo, D. B. 1992. Peptide absorption: A review of current concepts and future perspectives. J. anim. Sci. 70:3248-3257.

Whitelaw, F. G., Milne, J. S. and Wright S. A. 1991. Urease (EC 3.5.1.5) inhibition in

the sheep rumen and its effect on urea and nitrogen metabolism. Br. J. Nutr. 66:209-225.

Williams, A. G. 1989. Metabolic activities of rumen protozoa. Pages 97-126 in The Role of Protozoa and Fungi in Ruminant Digestion. Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer. Penambul Books, Armidale, Australia.

Williams A. G. and Coleman G.S. 1988. The rumen protozoa. Pages 77-128 in The Rumen Microbial Ecosystem. Ed. P. M. Hobson. Elsevier Applied Science, London, England.

Williams, C. H., David, D. J. and Lisma, O. 1962. The determination of chromic oxide in fecal sample by atomic absorption analysis. J. Agric. Sci. 59:381-385.

Williams, P. E. V., Tait, C. A. G., Innes, G. M. and Newbold, C. J. 1991. Effects of the inclusion of yeast culture (*Saccharomyces cerevisiae* plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. J. Anim. Sci. 69:3016-3029.

Windschitl, P. M. and Stern, M. D. 1988. Effects of urea supplementation of diet containing lignosulfonate-treated soybean meal on bacterial fermentation in continuous culture of ruminal contents. J. Anim. Sci. 66:2948-2958.

Wood, R. D., Bell, M. C., Grainger, R. B. and Teekell, R. A. 1963. Metabolism of labeled linoleic-1-C^{14} acidin the sheep rumen. J. Nutr. 79:62-68.

Wright, D. E. 1967. Metabolism of peptides by rumen microorganisms. Appl. Microbiol. 15:547-550.

Wu, S. H. W., Kirk, S. K., Perry, K. P., Smith, E. P. Chang, Y. H. and Jenkins, W. L. 1989. Rumen-stable pellets coated with polymer films. US 4,837,004 (Cl. 424-438; A61K9/22).

Wu, S. H. W. and Sandhu, M. A. 1984. Rumen-stable pellets. PCT Inc. Appl. WO 84 00, 282 (Cl. A23K1/00).

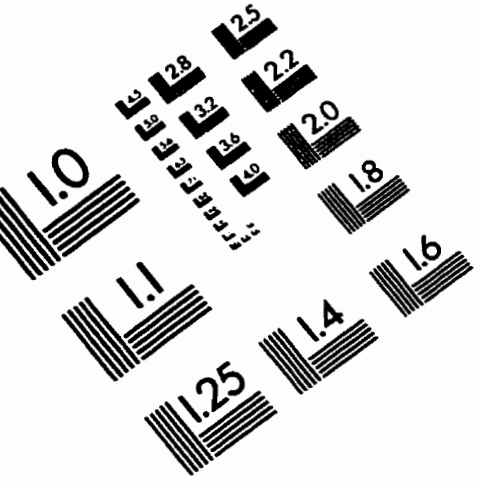
Zinn R. A. 1993. Characteristics of ruminal and total tract digestion of canola meal and soybean meal in a high-energy diet for feedlot cattle. J. Anim. Sci. 71:796-801.

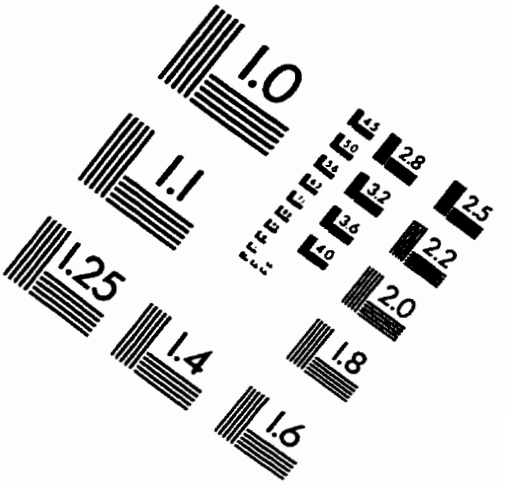
Zinn, R. A. and Owens, F. N. 1982. Predicting net uptake of nonammonia N from the small intestine. Pages 133-140 in Protein Requirements for Cattle: Symposium. Olkahoma State University, Stillwater, Oklahoma.

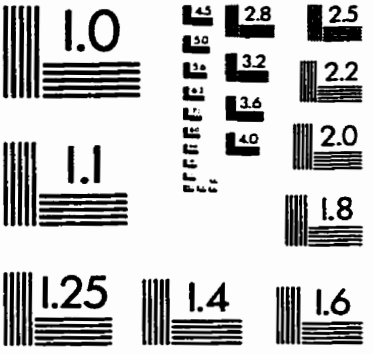
Zinn, R. A. and Owens, F. N. 1986. A rapid procedure for purine measurement and its

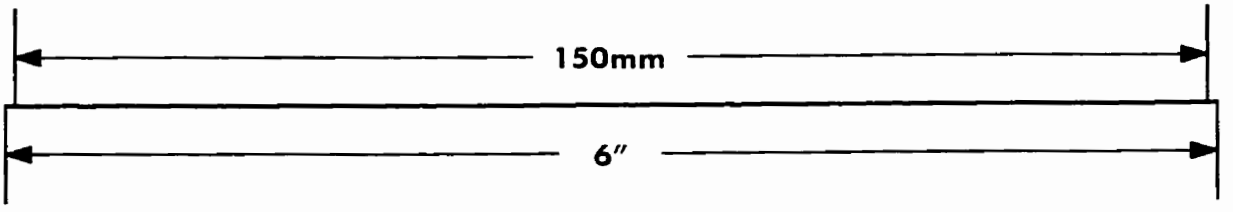
use for estimating net ruminal protein synthesis. Can. J. Anim. Sci. 66:157-166.

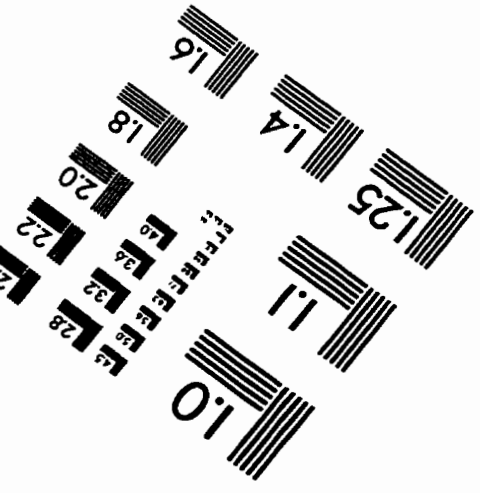
IMAGE EVALUATION
TEST TARGET (QA-3)

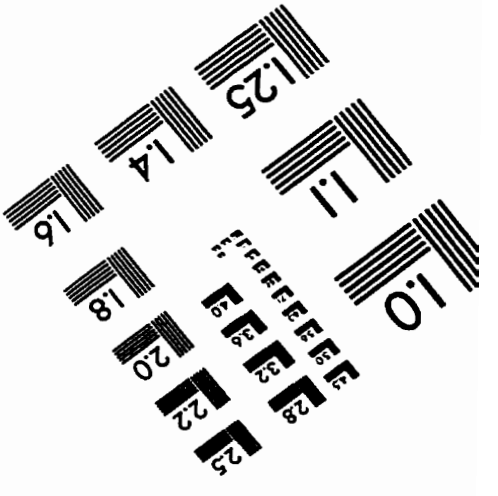












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989