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IMPROVING RUMEN UNDEGRADED INTAKE PROTEIN BY MEANS OF MOIST HEAT TREATMENT AND TIME OF FEEDING

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Seyed Ahmad Moshtaghi Nia

In Partial Fulfilment of the

Requirements for the Degree

of

Doctor of Philosophy

Department of Animal Science

May, 1994

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ISBN 0-612-13384-2

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IMPROVING RUMEN UNDEGRADED INTAKE PROTEIN BY MEANS OF MOIST HEAT TREATMENT AND TIME OF FEEDING

BY

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

DOCTOR OF PHILOSOPHY

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ABSTRACT

The effect of moist heat treatment on canola meal (CM) nutrient degradability in the rumen and digestion in the small and large intestines and total digestive tract was determined using three mature Angus steers fitted with large rumen cannulae and three Holstein steers fitted with T-shaped duodenal cannulae in the proximal duodenum and reentrant ileal cannulae in the terminal ileum. Commercially available canola meal was moist heat-treated at 127°C with steam-pressure of 117 kPa for 15 and 45 min. Rumen degradability was estimated by incubating sample-filled nylon bags in the rumen of steers and lower GI tract digestibility was estimated using a sequence of ruminal in situ incubation, in vitro incubation in an acid-pepsin solution and a mobile nylon bag technique. There was a significant reduction in soluble N, sucrose and glucosinolate content while the concentration of pepsin insoluble N, acid detergent insoluble N (ADIN) and neutral detergent insoluble N was increased. The effective ruminal degradabilities of dry matter (DM) and nitrogen (N) were reduced from 60.5 to 42.5 and 37.2% and from 69.9 to 40.2 and 33.7% for untreated, 15 and 45 min treated CM, respectively. Treatment significantly reduced the degradation of DM, N and amino acids (AA) in the rumen, while the digestibility of DM, N and AA in the small intestine increased with both treatments and was proportional to the decreased rumen degradation. Although heat treatment reduced the concentration of lysine and arginine in CM, the availability of lysine and arginine in the small intestine with 15 min treatment were increased by 75 and 131% respectively compared to 0 min treatment with 16 h rumen incubation. Heat treatment had no effect on DM and N digestibility at the large intestine site. Heat

treatment for 45 min tended to increase AA digestion in the large intestine by 5 percentage points compared to 0 min treatment. Total tract disappearance of DM, N, AA, acid detergent fibre (ADF) and ADIN were not affected by 15 min heating, however at 45 min heating, disappearance was reduced for DM, N and AA but increased for ADF and ADIN. Moist heat treatment was effective in increasing rumen escape of CM protein without adverse effect on protein digestibility in the lower digestive tract.

Twenty-two Holstein cows in late lactation were used in a 16-wk lactation period to study the influence of feeding sequence of protein supplement (PS) on ruminal fermentation and milk production. One mixed ration was offered twice daily at 08:00 h and 18:00 h and the PS was offered in one meal per day at either 08:30 h or 12:30 h. Half of the cows were assigned to each of the two dietary treatments designated DAY (08:30 h) or NIGHT (12:30 h). Eight cows fitted with rumen cannulae were used in the production study to examine treatment effects on rumen fermentation and nutrient disappearance from the rumen. Time of feeding PS had no effect on DM intake, body weight and condition score. Milk yield and milk composition were not influenced by treatment. Treatment had no significant effect on DM and CP disappearance and rate of passage of PS from the rumen. Feeding PS at 12:30 h increased rumen concentration of acetate, propionate, isobutyrate, isovalerate and total volatile fatty acid (VFA) and decreased the butyrate and caproate. All measured VFA concentrations were higher during the period of 24:00-10:00 h for cows fed the PS at night. Time of feeding a PS can alter ruminal fermentation characteristic without influencing animal performance.

DEDICATION

To my wife, Sandy, and my children, Kyra and Shareen. Without their support, understanding and patience I would not have completed this undertaking.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. J.R. Ingalls, for his guidance, support, and patience throughout this program. Sincere thanks are extended to my committee members Drs. T.J. Devlin, T.D. Galloway, A.G. Castell for the discussions and suggestions during the committee meeting and to the external reviewer, Dr. A.S. Atwal, for reading the thesis.

Appreciation is also extended to Dr. G.H. Crow for statistical advice, Dr. N.E. Stanger for surgical implantation of cannulas, Dr. B. Slominski and Mr. P. Mills for sample analysis, Mrs. T. Garner for sample collection, staff of Metabolism Unit, the University of Manitoba and staff of Dairy Research Unit, the University of Alberta for care of the animals.

Financial assistance was provided by the Canola Council of Canada, the Manitoba Department of Agriculture, the Natural Science and Engineering Research Council of Canada and Cyril L. Anderson Feed-Rite Graduate Fellowship.

FOREWORD

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

1. 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.

2. Moshtaghi Nia, S.A. and Ingalls, J.R. 1994. Evaluation of moist heat treatment of canola meal on digestion in the rumen, small intestine, large intestine and total digestive tract of steers. Can. J. Anim. Sci. (accepted).

3. Moshtaghi Nia, S.A. and Ingalls, J.R. 1994. Influence of moist heat treatment on ruminal and intestinal disappearance of amino acids from canola meal. J. Dairy Sci. (accepted).

4. Moshtaghi Nia, S.A., Robinson, P.H., Gill, M., Ingalls, J.R. and Kennelly, J.J. 1994. Influence of time of feeding the dietary protein supplement on milk production and composition in lactating dairy cows. (to be submitted).

ABBREVIATIONS

AA	- Amino acid
ADF	- Acid detergent fibre
ADIN	- Acid detergent insoluble nitrogen
BBM	- Brush border membrane
CM	- Canola meal
CP	- Crude protein
CSM	- Cottonseed meal
DM	- Dry matter
DIP	- Degradable intake protein
EAA	- Essential amino acid
EDDM	- Effective degradability of dry matter
EDN	- Effective degradability of nitrogen
GI	- Gastrointestinal
N	- Nitrogen
NAAN	- Non amino acid nitrogen
NAN	- Non ammonia nitrogen
NDF	- Neutral detergent fibre
NDIN	- Neutral detergent insoluble nitrogen
NH_3N	- Ammonia nitrogen
NPN	- Nonprotein nitrogen

PIN	- Pepsin insoluble nitrogen
PS	- Protein supplement
SBM	- Soybean meal
TAA	- Total amino acid
TMR	- Total mixed rations
UIP	- Undegradable intake protein
VFA	- Volatile fatty acid

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INTRODUCTION

The use of ruminant animals for meat, milk and wool production is based on their ability to digest fibrous feeds as energy sources and their ability to use nonprotein nitrogen (NPN) to supply the host with microbial protein of high biological value. Although all the compartments of the digestive tract play a part in ruminant nitrogen (N) metabolism, the supply of amino acids for protein synthesis and other metabolic purposes is determined mainly by what happens in the rumen. Processes in the rumen are dominated by the activities of its very complex and variable microbial population and the N nutrition of these organisms is an important ingredient of the N nutrition of the host animal.

Requirements of ruminants for amino acids are met from microbes grown in the rumen and digested in the small intestine and from dietary protein that is not degraded in the rumen (bypass protein) but is intestinally digestible. However, ruminal digestion of protein may introduce inefficiencies for amino acid (AA) utilization at high levels of milk production or growth. The supply of protein from microbial synthesis plus bypass protein is frequently inadequate to meet the requirements of ruminants for high levels of production. Moreover, when ruminants are fed diets containing high quality protein, microbial digestion is wasteful because a large proportion of the NH_3 released by deamination is not assimilated into microbial protein since energy production for microbial growth is not synchronized with NH_3 release. The NH_3 can not then be assimilated and it diffuses across the rumen wall to be lost ultimately as urea. An objective of research in ruminal protein metabolism is, therefore, to minimize ruminal protein breakdown, so that the released N by protein breakdown exactly balances

microbial N requirements, and to enable extra dietary protein to escape to the lower gastrointestinal tract for hydrolysis and absorption. Reduction in ruminal degradation of dietary protein supplement such as canola meal by heat treatment as well as feeding the dietary protein supplement at different times of the day when ruminal rate of passage is high, was investigated.

LITERATURE REVIEW

PHYSICAL CONDITION IN THE RUMEN

The content of the rumen is a heterogeneous pool. The mass in a fully fed animal can be described as a mat or boat floating on a liquid (Sniffen 1987). The upper part of the mat contains the longest particles and the particles become finer as you move down through the mat. The material at the top of the mat is generally low density and material toward the bottom of the mat is higher density. Trapped within the mat, including the top of the mat, are heavy density fine particles (Sniffen and Robinson 1984). After forage is consumed, it tends to move into the top of the mat. As the rumen contracts, the ingested material is slowly moved toward the rear of the rumen and down into the middle of the mat. This material will slowly be moved, through rumen contractions, to a point in the reticulum area where it can be regurgitated. A bolus is formed through squeezing the liquids and some of the fine material out, which is swallowed. The bolus is then chewed and particle size is reduced. After rumination, the material is swallowed. The smaller particles will have the cells fractured and the hydrated density will be increased (Welch 1986). The particles move to a lower level in the mat. The fractured

cells will release any soluble nutrients and the microbial mass attaches to the hydrated particulate matter and digests it (Van Soest 1982).

PROTEIN DIGESTION IN THE RUMEN

Degradation of protein in the rumen involves a sequence of microbial enzymes, starting with proteases and continuing with peptidases and deaminases until eventually ammonia is liberated. Microbial proteases are generally cell-associated rather than free-floating enzymes in the rumen fluid (Broderick 1989). Protein degradation is accomplished by both bacteria and protozoa, however; proteolytic fungi also have been found in the rumen (Wallace 1988).

Although protozoa are known to be involved in engulfment and degradation of bacterial protein in the rumen, their importance in ruminal degradation of soluble dietary protein is generally considered minor. A number of researchers have tried to clarify the role played by protozoa in rumen function by comparing faunated and defaunated animals. Removal of protozoa reduced fiber digestion in the rumen, but increased efficiency of microbial protein synthesis and increased total amino acid flow to the duodenum (Veira 1986). Ushida et al. (1986) in experiments using defaunated and refaunated sheep concluded that decreased dietary protein degradation might play a part in causing this amino acid flow.

Whether protozoa contribute appreciably to protein degradation remains uncertain. Bacteria are generally regarded as being mainly responsible for degrading dietary protein,

but it appears that protozoa degrade the protein in bacteria and in those feed particles that they are able to engulf (Nugent and Mangan 1981). In most bacteria, the proteases responsible are firmly associated with the microbial cell and proteolysis is facilitated by adsorption of protein on to the cell surface (Nugent and Mangan 1981). No single species of bacteria has been indentified with certainty as being of overwhelming importance, and it is believed that a mixed population is necessary to account for the proteolytic activity found in rumen contents (Russell and Hespell 1981). Generally, rumen bacterial proteases fall into four classes: (1) serine proteases, (2) cysteine proteases, (3) metal-ion dependent proteases, and (4) aspartate proteases (Broderick 1989).

The sequence of events in the process of ruminal protein degradation is as follows (Cotta and Hespell 1986). Intact soluble and insoluble dietary proteins are first attacked by proteases associated with the rumen microbes. This step generates large polypeptides which are soluble in the fluid phase in the rumen. Degradation of soluble intact proteins and polypeptides, and possibly the insoluble proteins as well, involves rapid absorption of protein onto the microbial cell (Nugent and Mangan 1981; Wallace 1988). Polypeptides are progressively hydrolyzed to smaller molecular weight peptides by further action of proteolytic enzymes. Although little research has been directed toward this point, it is generally believed that the small peptides are largely transported into bacterial cells for final hydrolysis to free amino acids. The free amino acids are then used directly for protein synthesis, deaminated to ammonia, or are effluxed from the cells. There is some evidence that the peptide uptake may be important in determining

the overall rate of degradation of dietary protein to ammonia.

Whether microbial degradation activity adapts to specific dietary proteins remains controversial. Orskov et al. (1983) reported that protein supplements of animal origin are generally broken down rapidly but incompletely, whereas plant proteins are degraded more slowly, but potentially completely. Thus good quality fish meal has a characteristically low degradability and will always be a useful protein supplement for ruminants because the undegraded protein will bypass the rumen over a wide range of retention times. The escape of the potentially fully degradable soybean protein, on the other hand, will depend much more on the ruminal proteolytic activity and particle outflow rate that result from other components of the diet. If the rumen environment provides near-optimum conditions (pH and temperature) for the expression of proteolytic activity, it appears that variation in the rate and extent of degradation of dietary protein will be related primarily to the properties of that protein.

PROTEIN DEGRADATION

The rate at which feed protein is digested in the rumen is complicated by numerous factors which involve the nature of the feed, the individual cow, and the total ration being fed. Consumed protein can be partitioned into pools that are soluble or insoluble in rumen fluid and into pools that are degradable or undegradable by ruminal microorganism. In general, proteins soluble in the rumen liquid phase are more rapidly and completely degradable than those that are insoluble and it often is assumed for practical purposes that N solubility in buffer solution is the same as degradability of the

protein meal in the rumen. However, soluble proteins such as serum albumin, ovalbumin, chloroplast protein extract, and soluble proteins from soybean meal and rapeseed meal have variable resistance to degradation (Leng and Nolan 1984).

MacGregor et al. (1978) reported that in 14 of 18 feeds tested, insoluble proteins contained a greater proportion of essential amino acids than the soluble proteins. Rumen microorganisms which degrade soluble proteins do not have the same essential amino acid requirements as host animals, therefore the ratio of essential to nonessential amino acids in soluble protein may have less relevance to N metabolism of dairy cows.

Insoluble protein may or may not be degraded by ruminal microbes. Protein associated with acid detergent fiber (ADIN) is thought to be unavailable to ruminal microorganisms. Insoluble digestible protein is that portion that is available for microbial digestion, and the digestion rate is dependent on amino acid composition and structure of proteins. Proteins which have no terminal amino or carboxyl groups(i.e., ovalbumin) and those with excessive crosslinking (i.e., ribonuclease A) have major influence on degradability of proteins by rumen microorganism (Chalupa 1984).

Proteins from various feedstuffs and within feedstuffs have widely different digestion rates. Proteins can be divided into various fractions: NPN (nonprotein nitrogen) (A), soluble true protein (B1), insoluble rapidly degraded (B2), insoluble slowly degraded (B3) and unavailable protein (C). The extent of protein degradation of a feed will vary depending on the proportions of these fractions, the rate of degradation of the proteins, and the rate at which the feed passes through the rumen (Sniffen 1987).

Proteins are also classified as either degradable (DIP) or undegradable (UIP).

The degradable protein fraction contains NPN, peptides, and proteins which are digested in the rumen. This includes all of fraction A and variable amounts of fractions B1, B2, and B3. Undegradable protein, which is also referred to as "escape" or "bypass" protein, is comprised of protein which is not digested by the microbes but can be either available once it arrives at the small intestine or unavailable to the animal. This includes small amounts of the B1 fraction, variable amounts of the B2 and B3 fractions, and all of fraction C (NRC 1989).

The protein fractions vary considerably among feedstuffs. High moisture silage can have 60-70% of the protein in fraction A and hays only 25-35% in fraction A. In contrast, corn gluten meal and brewers grains have only 2-4% of the total protein in the A fraction. The rate and extent of digestion of the B fractions are also variable. Forages vary from 80-100% and concentrates vary from 30-90% of the B fraction being degraded in the rumen. Generally large particle material will be extensively digested and proteins high in NPN and albumin and globulin will be extensively degraded in the rumen (NRC 1989; Sniffen and Robinson 1984). Ensiled forages and a few feeds such as high moisture corn are high in NPN, which, because of the rapid microbial digestion rate, can lead to excess quantities of rumen ammonia and blood urea, contributing to a reduction in animal efficiency, especially if the diets are balanced to meet crude protein requirements.

The proportion of dietary protein degraded in the rumen can be described by a first order disappearance model. With this model, feed protein is divided into fractions A + B + C. Fraction A is the proportion of total N already degraded at zero-time, B

is the fraction of total protein which is potentially digestible or degradable, and C is the protein fraction which is indigestible. The proportion of total protein disappearing in the rumen is determined by the fractional rates for degradation and turnover (Broderick 1989). When both degradation and turn over account for removal, the rate of disappearance of potentially degradable protein B is expressed by kd/(kd + kr) where kd and kr are rate constants for ruminal degradation and turn over of ruminal dry matter. Total protein degradation in this model is given by the equation:

$$\text{Degraded protein} = A + B(kd/(kd + kr))$$

The fraction of total protein escaping undegraded is given by the complementary equation:

$$\text{Escaped protein} = B(kr/(kd + kr)) + C$$

Although fraction C will escape the rumen, it is indigestible and will not contribute to the animal's amino acid supply.

Fraction A can be quantified as the proportion of the buffer soluble N which is not precipitated by protein denaturants such as trichloroacetic acid. The remaining N can be separated between the other two protein fractions by determining C, the indigestible N, then B by difference. Indigestible N frequently has been estimated as ADIN. However, Nocek et al. (1986) showed that ADIN of some feedstuffs could be degraded in the rumen. Krishnamoorthy et al. (1983) used enzyme preparations to estimate indigestible protein. They suggested that use of proteolytic enzymes, rather than chemical extractions, is a more logical approach for estimating the amount of protein which is biologically unavailable.

PROTEIN PROTECTION

The amount of protein degraded in the rumen determines the quantity of rumen undegraded intake protein (UIP), commonly referred to as escape or by-pass protein, entering the small intestine. In rapidly growing ruminants or dairy cows in early lactation, production may be limited by a low dietary UIP. In these situations, microbial protein synthesis is not sufficient to meet the animal's protein requirements. Different approaches are taken when protein requirements are in excess of that required by rumen microbes. One approach is the addition of slowly degraded protein sources such as corn gluten meal, dried brewers grains, fish meal, meat and bone meal and dehydrated alfalfa, which contain relatively slowly degraded proteins (de Boer et al. 1987b; Ha and Kennelly 1984; Loerch et al. 1983).

The next approach is by feeding the protein supplement at different times of the day. While there has been a fair amount of work on timing of feeding concentrates relative to forage, this tends to have been restricted to feeding high-grain concentrates frequently, and to feeding concentrate and forage in totally mixed rations (TMR). Although rapid consumption of large quantities of concentrates alters both the microbial population and acid production in the rumen, this is usually accompanied by a marked reduction in ruminal pH which tends to depress fiber digestion and feed intake. The practice of feeding total mixed rations (TMR) overcomes the inherent problems associated with feeding concentrates and forage separately. Feeding TMR forces the cow to consume forages and concentrates together which results in consuming feed in a more uniform pattern throughout the day. However, the TMR feeding ignores the fact that

animals have 24-hour (diurnal) cycles and that there may be advantages to feeding supplements at specific times of the day. The timing of feeding protein supplement in relation to TMR does not appear to have been the subject of detailed investigation.

Ruminant animals normally prefer to eat during the hours of daylight (rather than during darkness (Deswysen et al. 1984)) and have the hours of darkness to digest the feed and ensure its onward passage in time for the intake of fresh feed the next morning. The diurnal cycles in the weight of contents in the rumen and rumination were also observed to be greater at night (Ulyatt et al. 1984). Further, an increase in the muscular activity of the rumen wall has also been observed in young cattle offered forage diet (Sissons et al. 1986). The muscular activity of the rumen is at a maximum at night and if feed could pass from the rumen faster during the night, then offering the protein supplement (PS) to coincide with maximum passage rate could be a means of decreasing the negative effects of fermentation on the PS by the rumen microorganism. If PS is retained in the rumen for long periods of time, the microorganisms will ferment it to ammonia-N which is absorbed and excreted in the urine. However, if the dietary PS were to leave the rumen rapidly, the PS would be digested in the intestines to amino acids which would be absorbed and be available to the animal. Robinson et al. (1993) investigated changing the sequence of feeding a PS in relation to mixed ration, and suggested that rates of rumen passage of DM, NDF and CP were lower in the day feeding cycle (08:00-18:00 h) for cows fed the PS at 08:30 h and were higher in the evening feeding cycle for cows fed the PS at 12:30 h. If PS rate of passage from the rumen can be increased by feeding at times of the day when overall rate of passage is high then the undegradable protein

(UIP) value of the dietary protein should be increased.

The other approach has been to alter the rate of ruminal degradation of rapidly degraded protein sources such as canola meal (CM), soybean meal (SBM) and cottonseed meal (CSM). These protein sources have been altered both chemically and physically in attempts to increase the amount of undegraded digestible dietary protein flowing to the small intestine. Chemical treatments have centered on addition of tannins (Hartnell and Satter 1978) and formaldehyde (Rae et al. 1983; Erfle et al. 1986), while physical treatment has primarily been the addition of heat (Arieli et al. 1989; Kibelolaud et al. 1993; McKinnon et al. 1990).

Although it is easy to reduce degradability by such means, the difficulty is to combine protection in the rumen with increased digestibility of the escape protein in the small intestine and to avoid destruction or irreversible bonding of the more labile essential amino acids, that is, to avoid over-protection. Heat treatment decreases the solubility of protein by creating crosslinkages both within and among peptide chains and to carbohydrates (Van Soest 1982), thus lowering their susceptibility to ruminal degradation. However, high temperature and extended heating times render protein less digestible through the Maillard reaction between sugar aldehyde groups and free amino groups, thus lowering digestibility of constituent amino acids.

Traditionally, heat damage of the protein has been measured as acid detergent insoluble nitrogen (ADIN). However, ADIN is partially digestible (Arieli et al. 1989; Pena et al. 1986; Rogers et al. 1986; Weiss et al. 1989) and heat damage can be greatly overestimated (Britton et al. 1987; Van Soest and Mason 1991; Weiss et al. 1989) if

ADIN is used as a measure of protein damage. Each of these researchers have examined distillers' by-products of grains and similar material subjected to sufficient heat and moisture to induce the Maillard reaction. Such by-products contain more ADIN in absolute terms than the grain from which they were derived. Chaudhry and Webster (1993) have suggested that this added ADIN from Maillard reaction products can be degraded in part in the rumen and so contribute to microbial protein synthesis.

However, heat-damaged protein has been evaluated mostly through N digestibilities, and few studies have been conducted to relate heat-damage to animal production. Kung and Huber (1983) showed that diets containing NPN coupled with heated soybean meal (140° C for 2.5 h) supported greater milk production than diets containing regular soybean meal and corn silage. Ahrar and Schingoethe (1979) and Sahlu et al. (1983) found that milk production of cows past peak lactation is not greatly increased by feeding heated soybean meal as compared to regularly processed soybean meal. A recent study by Nakamura et al. (1994) showed that some of the nitrogen in heat-damaged protein was absorbed postruminally but was not used for growth by growing ruminants.

NITROGEN RECYCLING IN THE RUMEN

Urea formed as an end-product of N metabolism in the liver of the ruminant can leave the body-fluid pool by means of urine or enter back into the rumen. Urea enters the rumen via the saliva and through the rumen wall. The conversion of this urea to ammonia is important in supporting the growth of bacteria when animals are fed diets

low in nitrogen. Cereal diets also may contain an excess of fermentable energy relative to N and therefore similarly promote the use of endogenous N by the microbes. However, when fermentation and microbial protein synthesis are limited by lack of fermentable carbohydrate, the urea recycled to the rumen will simply be reabsorbed as ammonia and ultimately excreted as urinary urea. Animals on sub-maintenance roughage rations with limited nitrogen, utilize tissue protein for energy (Orskov and Ryle 1990). The oxidation of protein by the liver produces urea, and adds to the amount of blood urea available for recycling to the rumen. The urea is then reincorporated into microbial protein, which is digested and reabsorbed as essential and non-essential amino acids.

In studies with dairy cows in the first month of lactation, as little as 25% of the urea synthesized by the cow was excreted in urine (Oldham 1984). Apparently there can be extensive recycling of urea to the gastrointestinal tract even at this stage of lactation. From estimates of rates of urea transfer by the different routes (rumen wall vs saliva) indicated that entry of urea into the rumen from saliva is small (less than 20-30%) in relation to total urea production (Egan et al. 1986). However, Kennedy and Milligan (1980) showed that urea from saliva could account for most of the urea entering the rumen for animals on high-fiber intake with high-N contents and little readily available carbohydrates. In a number of experiments, much greater amounts of urea have been shown to enter the rumen than can be accounted for in salivary flow, and there may be additional entry across the rumen wall. This has been shown with normal N diets which contained substantial amounts of readily degradable carbohydrate (Kennedy and Milligan 1978) and marked increases in movement of urea into the rumen have been shown with

the addition of sugars to forage diets (Kennedy 1980; Kennedy et al. 1981).

Urea is a variable part of the total endogenous N entering the rumen; however, the amounts of non-urea endogenous N may also be appreciable at this site (Egan et al. 1986). Such non-urea N includes mucoprotein in saliva and the dead layers of keratinized cells known to be continually sloughed off from the wall of the rumen. These materials together probably contribute the main part of the non-urea endogenous N fraction entering the rumen which was shown by Siddons et al. (1985) to form as much as 60-70% of the total rumen endogenous N in sheep receiving dried grass or silage.

In summary, a number of factors influence recycling of urea to the rumen. High concentrate, low N diet would be likely to lead to high recycling to the rumen, while roughage diets lacking available carbohydrate and not low in N probably would not. This may be important when the value of non protein N supplements for different diets is being considered.

TECHNIQUES FOR STUDYING INTESTINAL UPTAKE OF NITROGENOUS COMPOUNDS

In vitro techniques

The use of rings of everted intestinal sacs to study active transport provided an easy in vitro technique. By using this technique, researchers were able to measure simultaneously amino acid disappearance from mucosal fluids, appearance in serosal fluids and acumulation in tissue. This method has suffered methodologically from the

overlapping of many cell types with both brush border membrane (BBM) and basal lateral membrane. Although the development of the technique to isolate BBM or basal lateral membranes is a significant accomplishment in intestinal physiology, the difficulty of isolating pure membrane preparations and changes that occur with isolation, are an indication that this system is not without problems (Hopfer 1984).

In vivo techniques

The two main methods used to measure amino acid absorption in vivo are based on the concept of concentration differences mutiplied by flow rate. In the first method, the concentration of amino acids or other nitrogenous compounds in duodenal and ileal digesta and digesta flow are measured with markers such as Cr-EDTA (Uden et al. 1980). The second method involves measurement of concentration in arterial (supply) and hepatic portal venous (drainage) blood or plasma, and measurement of blood flow by dilution with an inert marker such as p-aminohippuric acid (Bergman 1986). Therefore, the first method measures disappearance from the lumen of the small intestine, and the second method measures appearance in blood draining the portal-drained viscera.

The major limitation of the disappearance method is the inability to account directly for endogenous nitrogen from sloughed cells, intestinal bacteria, pancreatic secretions, and urea transferred from blood to the lumen of the small intestine. The appearance method measures the absorptive rates for portal-drained viscera and not just the small intestine. This method measures the net effects of true absorption, use of a

given amino acid by visceral tissue from arterial supply, and endogenous secretion or losses by the pancreas and the rest of the portal-drained viscera. Thus, the latter method accounts for endogenous losses on a net basis.

A different technique, used recently by a number of workers, involves sealing a protein source in a porous nylon bag, introducing this into the duodenum, and recovering it from the terminal ileum or from the feces (de Boer et al. 1987a; Kendall et al. 1991; Frydrych 1992). Disappearance from the washed bag is regarded as a measure of digestibility in the small intestine. Preliminary treatment with pepsin is unnecessary (Vanhatalo and Aronen 1991) and the recovery from ileum or feces makes little difference to the results (Hvelplund 1985). In a comparative study, different samples were ranked in the same order as in direct digestibility measurements, but correlation was poor, and, on average, the bag method gave higher values by more than 10 per cent (Hvelplund 1985). However, the proportions of N disappearing in the small intestine were reduced when the bags were incubated initially in the rumen and the difference with the two methods became less with increasing rumen fermentation time.

Amino Acids Transport System

The transport of amino acids by the intestinal epithelium (enterocytes) occurs by simple diffusion, facilitated diffusion (Na-independent) and active transport (Na-dependent) processes (Stevens et al. 1984). The relative significance of each route is highly dependent on the concentration of substrate present (Webb 1990). Quantitatively, active transport is the most important process. The uptake of amino acids across the enterocyte

into portal blood involves the following steps: (1) transport from the intestinal lumen across the BBM, (2) diffusion through the enterocyte cytoplasm where metabolism and protein synthesis occur, and (3) transport across the basal lateral membrane (Baumrucker et al. 1989).

Moe et al. (1987) and Guerino and Baumrucker (1987) demonstrated that Na-independent lysine, methionine and phenylalanine uptake was greater than Na-dependent uptake, while Na-dependent uptake was greater for alanine. Furthermore, methionine uptake was not affected by physiological concentrations of competing amino acids. Moe et al. (1987) reported that methionine inhibits the uptake of other amino acids (alanine, lysine, phenylalanine and proline). This may be important because lysine has been thought of as one of the possible limiting amino acids for both growth and lactation in ruminants (Chalupa and Sniffen 1989). The competition for absorption is an indication of the need for ruminant nutritionists and physiologists to be aware of the interactions among amino acids before attempting to manipulate their supply.

ABSORPTION OF NITROGENOUS COMPOUNDS BY THE RUMINANT INTESTINE

Dietary proteins form the largest components of nitrogenous compounds in the rumen. Microbial protein produced by the ruminal flora is a major source of protein for ruminants. Endogenous protein enters the intestine in the form of enzymes, bile, mucus, serum albumin, lymph, epithelial cells and other degradable products from the gastrointestinal lining. Endogenous protein secreted into the intestines may equal dietary

escape protein entering the duodenum, but 80 to 90% of the endogenous protein is reabsorbed (Chalupa and Sniffen 1989).

Digestive enzymes

The principal proteases of bovine pancreatic juice are trypsin, chymotrypsin, elastase and the carboxypeptidases A and B. Trypsin, chymotrypsin and elastase are endopeptidases which attack centrally located peptide bonds, and the carboxypeptidases A and B are classed as exopeptidases which cleave only the terminal bonds of protein and peptides (Smith and Zebrowska 1989). These enzymes are secreted as inactive zymogens. Trypsinogen is activated to trypsin by the action of enterokinase located in the intestinal mucosa and trypsin activates all other pancreatic proteases (Alpers 1986). All the proteases show maximal activity at a pH of 7.5 or above.

The usual result of pancreatic protease action is the production of single amino acids and a series of small peptides (two to four amino acids). Some peptide bonds are resistant to pancreatic and brush-border digestion, and rapid intestinal transit may not permit the time necessary for hydrolysis to amino acids and small peptides to accur. The rate of digestion of protein may also vary depending on whether dietary proteins has been processed. Feed processing (heating, storage) may create new amide or ester linkages between peptide bonds, causing resistance to hydrolysis (Van Soest 1989).

Site of absorption

Amino acid absorption in the ruminant small intestine varies with intestinal location, so

that the affinities for the transport systems differ among sites. Phillips et al. (1979) reported that the highest amount of threonine and valine absorption occurred in the ileum, and the amount of methionine absorbed was about equal from both the jejunum and ileum. These amino acids were not absorbed from the duodenum. Wilson and Webb (1990) reported that total methionine and lysine uptake was higher by ileal brush border membrane vesicles than by jejunal brush border membrane vesicles.

The differences in the amino acid absorption sites are not clear. Species of animal, diet and physiological state seem to be important in determining the predominant site of amino acid absorption. Under normal conditions, digesta entering the duodenum are consistently very acid (pH 2-3) due to the copious and continuous flow of digesta through the pylorus and the weakly alkaline nature of the pancreatic secretions. Thus the ruminant generally maintains a rather low pH for a considerable distance (the first 3-4 m in sheep) into the small intestine. Ben-Ghedalia et al. (1976) demonstrated that the pH of the intestinal content increased gradually along the intestine, but remained acidic even at a distance of more than 3 m from the pylorus. Bondi (1987) suggested that the slow rise in pH in the proximal section of the small intestine may extend the activity of abomasal pepsin, but delay that of the pancreatic proteases, since activation of the zymogens requires a pH above 5. The optimal pH for most intestinal proteases is in the range of pH 7.2 to 9.0 (Bondi 1987). The contents of the ruminant small intestine usually do not reach this pH range until near the latter one half to one-third of the small intestine. Because the luminal environment of the ileal region of the small intestine of the ruminant is more compatible with optimal proteolytic activity, the transport

capabilities would be expected to be concentrated there.

Nitrogen absorption

The extent of protein digestion has been the subject of a considerable number of investigations in recent years, using a variety of techniques. The most frequently used method involves animals equipped with pairs of re-entrant cannulae inserted into the proximal duodenum and terminal ileum. Digestibilities of total N, non-ammonia N (NAN), total AA or values for individual amino acids are calculated as differences between amounts entering and leaving the small intestine expressed as proportions of the amounts entering the small intestine. Since these values include the endogenous N entering the small intestine, they are essentially apparent digestibility values. In a number of experiments, using a variety of diets, digestibility coefficients of about 0.55-0.70 have commonly been reported.

Estimates of true absorption of N in the small intestine have been made by examining the disappearance of N-labelled material (Salter and Smith 1977) and by examining ileal flows following incremental addition of different protein sources to the abomasum (Storm et al. 1983; Hvelplund 1985). The digestibility coefficients for rumen bacterial total N and total AA were 0.80 and 0.85 respectively. Using similar methods to investigate the feed protein digestibility, total N and total AA coefficients were obtained between 0.75 and 0.87 for wheat leaf protein, rapeseed meal, and soyabean meal. Treatment of soyabean meal with formaldehyde at a high level and heat-damaged fish meal reduced digestibility significantly (Hvelplund 1985).

In summary, microbial protein, which is the main protein component entering the ruminant duodenum, is digestible in the small intestine to a reasonably constant and known degree. Digestibilities of supplementary UIP can vary markedly. Proteins protected by treatments against rumen degradation are often of lower digestibility than the untreated material.

Amino acids absorption

In numerous studies with sheep and cattle, the relative absorption rates of essential amino acids from equimolar mixtures of 18 common dietary amino acids, were greater than for nonessential amino acids (Armstrong et al. 1977; Christiansen and Webb 1990a; Christiansen and Webb 1990b). In the above studies, absorption from the small intestine was greater for methionine than other amino acids.

The complex pattern of amino acid absorption probably is attributable to differences in the affinities of carrier systems for individual amino acids and because competition for transport is greater among amino acids for which a carrier has a greater affinity. Moe et al. (1987) and Guerino and Baumrucker (1987) reported that methionine inhibits the uptake of lysine. More methionine was absorbed than either threonine or valine and when different mixtures of methionine, valine and threonine were perfused through isolated segments (Phillips et al. 1979), methionine was absorbed in the greatest amounts and was a strong inhibitor of the absorption of both valine and threonine.

Not all the interactions that are observed among amino acids in the absorption process are negative. Some amino acids stimulate the transport of others. For examples,

alanine, phenylalanine and leucine increase the intestinal transport of the basic amino acids arginine, lysine and ornithine.

It is now well established that amino acids that are absorbed from the small intestine in the forms of peptides (di- and tri-) as well as in the free form. The peptides are split at the surface of the mucosal cells or within the cells so that most amino acids enter the circulation in the free form. When labelled amino acids were infused into the duodenum and entered into the portal vein, glutamine virtually disappeared during this process while leucine and lysine were partially deaminated (Smith et al. 1987). Tagari and Bergman (1978) showed that most glutamic acid was metabolized while alanine was partially metabolized during the absorption process.

Although the net disappearance of amino acids from the small intestine has been measured in sheep receiving a wide variety of diets, there have been much fewer studies measured the net appearance of amino acids in portal blood. There are indications that portal appearance of amino acids is substantially less than that expected from the rate of disappearance from the small intestine. Tagari and Bergman (1978) simultaneously measured amino acid absorption using both techniques in the same animal. The portal appearance was, for most amino acids, less than 50% of that expected on the basis of intestinal disappearance. There were differences with different diets (medium and high protein) but in general little or no glutamine, glutamic acid, or aspartic acid entered the portal plasma, while only 30-80% of the amount of essential amino acids that disappeared from the intestine could be detected in portal plasma. The loss of essential amino acids between gut lumen and blood may be explained by their use for protein synthesis by the

gut itself. Another possibility is that appreciable amounts enter the blood as peptides rather than free amino acids (Webb 1986).

Other factors may be involved in amino acid absorption. Fasting reduces absorption of amino acids (Lomax and Baird 1983) and absorption increases with increased intake (Huntington and Prior 1985). Although dietary N intake has an influence on intestinal loss and portal appearance of amino acids, the ruminal degradability of dietary nitrogen has little effect on net appearance of amino acids in portal blood (Huntington 1987).

The disappearance of a particular pattern of amino acids from the gut does not mean that the appearance of a similar pattern in the blood will be assumed. Some principles of absorption have been defined using both disappearance and net appearance techniques: (1) endogenous secretion and use by intestinal tissues play a significant role in amounts and profiles of absorbed compound that are available for post-absorptive metabolism; (2) the amount of dietary protein, but not degradability in the rumen, is reflected in absorption rates; (3) the small intestine is the predominant site of amino acid absorption.

Peptide absorption

Many studies have led to the realization that intact peptides are absorbed by enterocytes. Phillips et al. (1979) were able to account for only 19-63% of amino acids disappearing from the mucosal side of an in vitro system with sheep intestine. Tagari and Bergman (1978) reported that only 30-80% of the amino acids leaving the intestinal lumen could

be accounted for in portal plasma of sheep. In each of these studies, metabolism of amino acids by intestinal mucosa and (or) analytical inaccuracies were suggested as potential reasons for these discrepancies. None of the authors suggested the potential contribution of peptide absorption.

It is currently believed that peptides are transported from the lumen, hydrolyzed within the epithelial cells, and released into the bloodstream as free amino acid (Alpers 1986). Webb (1986) has recently stated that about 70% of the amino acids appearing in portal blood were associated with peptides, and also suggested that peptide absorption has significant quantitative implication for ruminant nutrition.

Because peptides were absorbed more rapidly than free amino acids, Hara et al. (1984) suggested that independent transport systems for peptides may exist. Both peptides and amino acids can be absorbed against a concentration gradient. The energy-dependent transport of amino acids is linked with the co-transport of Na; for peptides, energy-dependent transport is linked with the co-transport of proton (Webb 1990).

The amino acid composition of the peptide influences absorption. Glutamic acid was absorbed at nearly twice the rate when it was present as glutaminlytyrosine rather than glutaminylmethionine. Not only do the particular amino acids present influence absorption, but whether an amino acid is in the N- or C- terminal position also is important. Lysine was absorbed much more rapidly when it was present in the N-terminal position in a dipeptide with glycine than when it was in the C- terminal position. Conversely, lysine was absorbed most rapidly when it was in the C- terminal position in a dipeptide with glutamic acid (Webb 1990).

Although peptides compete with one another for transport, there may be some stimulatory effects of one peptide on the absorption of another. Carrier specificity, therefore exits in peptide transport which will influence the nature of absorption from a mixture of peptides.

Nonprotein nitrogen absorption

Nucleic acid absorption

The composition of digesta presented to the duodenum of ruminant animals is high in nucleic acids because of the contribution from rumen microbial N compounds. Nucleic acids form 8-15% of digesta total N at this site (Smith and Zebrowska 1989). The gross composition of the microbial biomass which enters the small intestine is quite variable. The N content of bacteria can vary from 50 to 120 g/kg dry matter and values for protozoa fall in the range of 45-83 g N/kg dry matter (Lindsay and Armstrong 1982). Some of the variability in microbial biomass composition will be due to variations in the proportion of bacteria to protozoa present in the duodenal digesta.

The ruminant animal has a wide spectrum of nucleases and related enzymes involved in nucleic acid degradation. Proportionate digestion of nucleic acid N entering the small intestine lies in the range 0.75-0.95. Pancreatic ribonuclease, which is particulary abundant in the ruminant, acts as an endonuclease, attacking internucleotide bonds of RNA. Pancreatic deoxyribonuclease is also an endonuclease which hydrolyses DNA and has a pH optimum in the range of 6.8-8.2. Two phosphodiesterases (I and II) from intestinal mucosa are exonucleases, both attack polyribonucleotides and

oligodeoxyribonucleotides. They have pH optima of 9.3 and 7.0 respectively.

RNA and DNA disappear to extents of about 85-90% and 75-85% respectively between the abomasum and the ileum of calves and sheep. Nucleic acid degradation in the upper small intestine was accompanied by the transient appearance of adenosine, guanosine, and pyrimidine nucleosides. Subsequent metabolism of the pyrimidines, uracil and cystosine yield ß-alanine, and thymine yields ß-amino isobutyric acid. Both of the products formed are capable of further degradation within the body to yield carbon substrates which enter the metabolic pool, with the N being converted (via NH_3) to urea and either excreted in the urine or recycled to the digestive tract.

Ammonia nitrogen (NH_3N) and urea N absorption

Ruminants can survive on diets high in NPN or very low in total N by microbial conversion of NPN to amino acids that are absorbed, and recycling urea N through the gut that might otherwise be excreted in the urine.

Several sources contribute to the NH_3N available for absorption, including the diet, proteolysis and deamination by microbes in the gut, hydrolysis of urea that enters the gut from blood or saliva, and endogenous secretions of ammonia or protein (Chalupa 1984). Ruminal ammonia concentration rises after consumption of a meal, and net absorption is positively correlated with ruminal concentration (Huntington 1986). Ammonia transfer to and from the lumen of the gut appears to be primarily a passive process.

Urea N transfer from blood to the gut is a significant factor because of the

capacity to hydrolyze and recycle urea N as NH_3N or amino N. Transfer of urea to saliva or through gut epithelium appears to be by diffusion. Factors that indirectly affect transfer by altering salivary flow include intraruminal infusion of urea or ammonium chloride, intake of urea, dietary dry matter content, and time since last feeding. Factors that increase urea N transfer to the gut include intraruminal infusion of volatile fatty acids or CO_2, increased dietary content of readily fermentable carbohydrate or urea, and increased N intake (Huntington 1986).

Transfer of endogenous urea N to the rumen ranges from 0.4 to 12.7 g/day for sheep and from 17 to 20 g/day for cattle (Kennedy et al. 1981). Transfer of endogenous urea N to the entire gut ranges from 6.2 to 13.4 g/day for sheep (Norton et al. 1982) and 10.5 to 44.7 g/day for cattle (Kennedy 1980). Urea N transfer to the rumen as a percentage of N intake ranges from 7.5 to 26% for sheep and from 15 to 25% for cattle (Kennedy and Milligan 1980).

The net uptake and transfer of nitrogenous compounds from portal-drained viscera can be affected by animal and dietary factors. Huntington (1986) summarized data to provide estimates of net daily uptake and transfer for sheep and cattle. First, NH_3N makes a subtantial contribution to net uptake of N from portal-drained viscera. Net NH_3N uptake ranges from 16 to 80% of N intake (mean 40%) and the ratio of net uptake of NH_3N to α-amino N (AAN) ranges from 0.4:1 to 6.5:1 (mean 1.9:1). Second, like NH_3N, endogenous urea N loss to portal-drained viscera is a substantial factor in total N metabolism. As a percentage of N intake, net urea N loss ranges from 10 to 42% (mean 23%). The ratio of net urea N loss to net AAN uptake ranges from 1:1.2 to 1:1.4

(mean 1:1.3).

In summary, urea N and NH_3N are substantial factors over a wide range of diets, intakes, and productive states. Postruminal transfer of urea N plays a major if not predominant role. Sites of NH_3N uptake appear to vary the most and are dependent on several factors including species, diet, intake, and productive state.

Nitrogen digestion in the large intestine

Nitrogen compounds entering the large intestine with digesta flow from the ileum consist of undigested dietary and rumen microbial materials together with residues of sloughed cells and endogenous secretions. The latter originate in part from enzymic or mucous secretions, but also include urea and/or ammonia released from urea. Concentration of ileal (urea and ammonia) N and plasma urea N are linearly related in a manner that implies passive diffusion of urea into the lumen of the sheep intestine (Dixon and Nolan 1986).

The extent to which microbial protein synthesized within the cecum of the ruminants provides a further source of amino acids for the host animal is uncertain at the present time. It is unlikely that amino acids themselves are absorbed. However, appreciable amounts of ammonia-N are absorbed from this site of the tract and this N can presumably contribute to body synthesis of non-essential amino acids.

The fermentation of residual carbohydrate in this organ, leading to the synthesis of microbial biomass could, however, be of potential importance in relation to N metabolism and it is necessary to have information on exchanges of N compounds across

the large intestinal wall. Although considerable amounts of blood urea may enter the postruminal alimentary tract, very little generally enters directly into digesta across the wall of the large intestine. Dixon and Milligan (1984) showed that the majority of the urea metabolized in the large intestine of sheep was accounted for in ileal flow and net flow of urea into the cecum/colon of sheep on a low-N diet was about 2 g/day. However, it is possible that a greater amount of urea passed across the intestinal wall but was partially hydrolyzed by ureolytic activity and reabsorbed before it mixed with the digesta pool (Dixon and Nolan 1986).

Dixon and Nolan (1986) showed that ammonia was rapidly absorbed across the wall of the large intestine and made a substantial contribution to plasma urea production. Rate of ammonia incorporation into microbial protein as a N source appears to be small and may vary with energy and other nutrients available, but there appears to be a substantial entry of endogenous non-urea non-ammonia N into the cecum (Dixon and Milligan 1984). Growth of bacteria in the large intestine may depend to a major extent on peptides and amino acids derived from this source or from rumen microbial debris or other proteins (Dixon and Nolan 1986). Rumen microbial debris appears to be extensively degraded in the cecum while at the same time an approximately similar amount of new microbial growth takes place. As a result of these changes, mainly new microbial N makes up approximately one half of cecal digesta (Dixon and Nolan 1986).

THE FATE OF AMINO ACIDS BEYOND THE GASTROINTESTINAL TRACT

Amino acids do not simply move out from the digestive tract to nourish tissues. Instead,

the gut epithelium actually metabolizes a portion of amino acids during absorption. Further, the liver is positioned so that it can remove or even interconvert some of the absorbed amino acids.

After transport across the intestinal wall, amino acids are degraded, metabolized to other amino acids, incorporated into proteins, or released unaltered in portal blood. The intracellular pool of amino acids can be replenished by degradation of protein or by intracellular synthesis. Normally this is an equilibrium between synthesis and degradation of proteins, and metabolism and resynthesis of amino acids, so that the intracellular amino acid pool remains constant.

In many tissues (such as liver and muscle), protein and amino acid metabolism are under hormonal regulation and depend on the nutritional state, while the intestine is much less sensitive to such regulation (Alpers 1986). The relative independence from hormonal regulation might be related to the need to maintain an intact absorbing surface, or the intestine utilizes high concentrations of luminal amino acids at all times.

Of all the amino acids disappearing from the lumen of the small intestine of sheep, only alanine and serine appear in portal blood in amounts approaching (72-103%) of that disappearing from the intestine (Tagari and Bergman 1978). Glutamine, glutamate, and aspartate either were absorbed in only negligible net amounts or were actually removed from the blood by the gut tissues. Glutamine is consistently consumed by the small intestinal mucosa for energy production or transamination. A significant percentage of this ammonia produced by deamination of glutamine is released into the intestinal lumen (Nixon and Mawer 1970). Much of the ammonia is reabsorbed from

the lumen for use by other tissues. Other amino acids had net rates of appearance in portal plasma of only 32-64% of their rates of intestinal disappearance (Tagari and Bergman 1978).

The absorbed amino acids are directed in large part into the portal blood for use by other tissues. However, an important portion of the luminal (intracellular) degradation products of protein is used for maintenance of the rapidly renewed enterocyte population and for the considerable amount of secreted protein that is released by the small intestine. Lindsay and Armstrong (1982) calculated that the total protein synthesized by the gastrointestinal tract is about 50-100 g/day in sheep and 500-1500 g/day in cattle which reflects the sloughing and renewing of cells of the gastrointestinal tract.

Transport of amino acids by plasma and whole blood differs. Plasma transport of amino acids (removal or release) across four different tissues (portal-viscera, liver, kidneys, and hindquarters) of sheep was less than that of whole blood and thus these amino acids must be partly transported by blood cells (Bergman 1986). In fact, for most amino acids the plasma transport rate averaged close to 77% of that of blood. Measurements of plasma amino acid fluxes qualitatively reflect true amino acid transport but, in most cases, underestimate the total transport to about the same extent as the packed cell volume (Bergman 1986). Therefore, the experimental approach to sample whole blood or plasma has to be considered carefully.

The flow of amino acids from the gastrointestinal tract to peripheral tissues is substantially modified by the action of the liver. The liver removes approximately the same quantity as that added by the portal-drained viscera (Bergman 1986). At the same

time, both the kidneys and hindquarters remove some amino acids and release others. For adult animals fed a maintenance diet, this should be expected in that peripheral tissue theoretically will be in nitrogen balance and the ultimate fate of most nitrogenous compounds is urea formation in the liver.

There is substantial variation in the fraction of amino acids extracted by the liver and, on this basis, the amino acids fall into three classes. The first group are mostly nonessential amino acids with extraction of 10-30%; the second group are mostly essential, with extraction normally from 6-10%; the third group includes the branched-chain amino acids, for which extraction is low (Lindsay and Armstrong 1982).

All the amino acids taken up by the liver are catabolized. The gluconeogenic amino acids alanine and glycine are absorbed in the greatest amounts, but even larger quantities are removed by the liver. The balance is then made up mostly by a large release primarily from the muscles, fat, skin, and bones. The kidneys also release glycine, but unlike the hindquarters, they remove alanine in significant amounts for gluconeogenesis (Bergman 1986). Other amino acids contribute nitrogen or carbon skeletons for alanine and glycine formation for transport mainly to the liver (Lindsay 1982).

The liver also removed more arginine than was absorbed but it released most of this back into the blood as citrulline and ornithine. In contrast, both kidney and hindquarters released arginine and removed citrulline and ornithine (Bergman 1986). Therefore, urea is produced in the liver, and arginine, like alanine and glycine, must carry nitrogen away from peripheral tissues in a relatively nontoxic form. This probably

is advantageous to the body in that amino acids can be catabolized in peripheral tissues without the risk of ammonia toxicity.

The branched-chain amino acids (leucine, isoleucine, and valine) also are absorbed in large quantities. However, 40% was removed by the liver and the remaining 60% removed mainly by the muscles and other tissues such as adipose tissue, and lungs (Bergman 1986). Their terminal metabolic fate in the liver thus probably was for plasma protein synthesis (e.g. albumin) with subsequent export to the blood (Bergman et al. 1984). In muscle, however, they eventually are deaminated to their respective ketoacids. A portion of the ketoacids in muscle then can be oxidized to CO_2 (Lindsay 1982), but the remainder is returned to the blood for removal by the liver. Amino groups derived from deaminated amino acids in muscle must then be transferred to form alanine, glycine, glutamine, or arginine. In the liver, most of the ketoacids seem to be oxidized to CO_2 and ketone bodies but small amounts could be converted to the original amino acid by transamination (Bergman 1986). The kidneys released some branched-chain amino acids, which possibly could arise from hydrolysis of plasma proteins.

The metabolism of glutamine and glutamate differs from that of other amino acids. Glutamine is removed by both gut and liver in large quantities and this removal is balanced by a net movement out of the kidneys and other peripheral tissues (Bergman 1986). Glutamate is removed from the blood in only small amounts by the gut but is released by liver and removed by peripheral tissues. Glutamine and glutamate thus are interconvertible and the amount of glutamate transported depends on the quantity of glutamine that is available.

The fact that peptides may be absorbed intact raises the question of the fate of these in the animal. High concentrations of circulating peptides were observed in the plasma of calves fed a conventional diet (McCormick and Webb 1982). When soybean protein supplied the N in the diet, peptides appeared to be removed from plasma by hind limb tissues. When urea supplied the N in the diet, peptide removal by hind limb tissues was small. Dietary status may influence peptide utilization by tissues.

In summary, the amounts of individual amino acids appearing in portal blood are different from amounts disappearing from the gut lumen. Some are absorbed in amounts equal to that disappearing but most are absorbed in lesser quantities because of intestinal metabolism. Further, the liver removes absorbed amino acids and synthesizes plasma proteins, urea, and glucose. Peripheral tissues exchange amino acids with protein for normal turnover but also use amino acids for oxidation and transamination. Alanine, glutamine, glycine, and arginine are important in transporting nitrogen out of peripheral tissues in a nontoxic form. Branched-chain amino acids are removed by both liver and peripheral tissues mainly for plasma protein and ketoacid formation, respectively.

MANUSCRIPT I:

EFFECT OF HEATING ON CANOLA MEAL PROTEIN DEGRADATION IN THE RUMEN AND DIGESTION IN THE LOWER GASTROINTESTINAL TRACT OF STEERS

ABSTRACT

Commercially available canola meal (CM) was moist heat treated at 127°C with steam-pressure of 117 kPa for 15, 30, 45, 60 and 90 min. The effect of moist heat treatment of CM on rumen degradability and lower gastrointestinal (GI) tract digestibility of rumen escape dry matter (DM) and nitrogen (N) was determined using three rumen and two duodenal cannulated steers. Rumen degradability was estimated by incubating sample-filled small nylon bags in the rumen of steers for 10 and 16 h. Lower GI tract digestibility was estimated using a sequence of ruminal in situ incubation, in vitro incubation in an acid-pepsin solution and a mobile nylon bag technique. DM and N degradation of treated CM were significantly ($P<0.01$) reduced in the rumen while the digestibility of DM and N in the lower GI tract was increased at all treatment times. At 16 h incubation, N disappearance declined in the rumen from 74.4 to 18.9% and increased in the lower GI tract from 16.2 to 64.2% for control and for CM heat treated for 45 min respectively. There was a significant reduction ($P<0.01$) in the soluble N in heat-treated CM, while the concentration of pepsin insoluble N, acid detergent insoluble N and neutral detergent insoluble N were increased as heating time increased. These increases in general were not associated with a decrease in N disappearance in the lower GI tract. Moist heat treatment was effective in increasing rumen escape of CM protein without adverse effect on protein digestibility in the lower GI tract.

KEY WORDS: Canola meal, rumen degradability, post ruminal digestion, moist heat treatment, insoluble nitrogen

INTRODUCTION

Canola meal (CM) is a protein supplement that contains up to 40% crude protein on a dry matter (DM) basis. When CM is fed to ruminants, a large part of the protein is degraded in the rumen (de Boer et al. 1987a). Heat treatment (baking and roasting) has been used to decrease the rumen degradability of rapidly degraded protein sources such as CM and soybean meal (Plegge et al. 1985; Mir et al. 1984; McKinnon et al. 1990; Ahmadi 1988). The increase in protein escape through heating may be a result of protein denaturation and reduction in solubility and degradation rate which favours more rumen escape of intact undegraded proteins to the lower gastrointestinal (GI) tract (Van Soest 1982). There is very limited research available concerning the effect of moist heat treatment on rumen escape of protein and the resulting availability of the rumen escape protein for absorption in the small intestine. Moist heat is applied to CM during the oil extraction process at the crushing plants. Additional moist heat treatment may be a simple method to improve the value of CM for high producing cows or young growing ruminant animals.

The objective of this study was to measure the effect of moist heat treatment of CM on rumen degradability of DM and nitrogen (N) and on the availability of that rumen escape DM and N in the lower GI tract of steers.

MATERIALS AND METHODS

Experimental Protocol

Three mature Angus steers with an average weight of 675 kg, each fitted with a large diameter rumen cannulae (Bar Diamond, Parma, ID) and 2 Holstein steers averaging 350 kg, each fitted in the proximal duodenum with a T-shaped cannulae made of plastisol (F.H. and Sons Manufacturing Ltd., Rexdale, ON) were used for rumen degradation and lower GI tract studies, respectively. The diets consisted of 50% dairy concentrate and 50% long alfalfa hay for the ruminal cannulated steers or 50% of dehydrated alfalfa pellets for the duodenal cannulated steers. The dairy concentrate was formulated for high producing dairy cows (National Research Council, 1989). Diet amounts were established on the basis of what the steers would consume. Animals were maintained on these diets for at least 3 wk prior to data collection. Composition of the concentrate, alfalfa hay and dehydrated alfalfa pellets are presented in Table 1. The mean daily DM intakes were 8.6 and 5.9 kg for rumen and duodenal cannulated steers respectively. Feed was provided to both groups of steers twice a day at 08:00 h and 16:00 h.

Commercially available CM (CSP Foods Ltd., Altona MB) was spread in a tray to a depth of 1 cm and autoclaved at 127°C with steam-pressure of 117 kPa for 15, 30, 45, 60 and 90 min. Meal temperature was monitored by means of an autoclave thermometer which was inserted into the meal during heating. After heating, CM was allowed to cool to ambient temperature and then stored in plastic bags at room temperature.

Table 1. Composition of the forages and concentrate fed to cannulated steers (dry matter basis)

	Alfalfa hay	Alfalfa pellets	Dairy concentrate[z]
Dry matter (%)	87.9	94.9	93.2
Crude protein (%)	18.8	15.3	17.6
ADF (%)[y]	32.0	38.5	12.5
NDF (%)[x]	42.8	51.4	24.1
Soluble N (%)	0.94	0.48	0.75
ADIN (%)[w]	0.42	0.69	1.51
NDIN (%)[v]	1.05	1.97	2.13
Energy (kJ g^{-1})	-	-	17.0

[z]Contained (%): Barley 41.2, oats 12.8, canola meal 14.7, distillers dried grain 8.2, beet pulp 12.0, dehydrated alfalfa 5.3, tallow 1.2, urea 0.3, biophos 0.73, $CaCO_3$ 1.34, cobalt-iodized salt 1.0 and vitamin-mineral premix 1.23 which provides (per kg concentrate): 10000 IU vitamin A, 2000 IU vitamin D3, 69 IU vitamin E, 15 mg copper, 60 mg zinc, 61 mg manganese, 0.2 mg selenium, and 649 mg magnesium.
[y]Acid detergent fibre.
[x]Neutral detergent fibre.
[w]Acid detergent insoluble nitrogen.
[v]Neutral detergent insoluble nitrogen.

In Situ and Mobile Nylon Bag Technique

Samples (1 g) of each treatment were placed in small (3.5 X 5.5 cm) heat-sealed nylon bags of porosity 50 μm (Felco Industries Ltd., Concord, ON) and incubated in the rumen of 3 Angus steers for 10 and 16 h. The incubation times of 10 and 16 h were chosen to approximate 10 and 6% rumen turnover rate respectively. Three bags per steer per treatment per incubation time as suggested by Orskov (1982) plus two empty bags per steer per incubation time were used. The empty bags served as blanks to correct for feed particles and microorganisms that adhered to the bags. The small nylon bags were held in large 2 x 3 mm mesh nylon bags weighted down with stones in a bottle during the rumen fermentation period. All bags were removed from the rumen at the same time (Nocek 1988) and immediately washed with cold water in a wringer-type washing machine for 10 min followed by 5 min in clean water and then dried in a forced air oven at 60°C for 48 h in preparation of analyses.

The effect of moist heat treatment of CM on the digestibility of DM and N in the lower GI tract and total tract (rumen plus lower GI tract) was examined by incubation of a separate set of bags in the rumen of the steers for 10 and 16 h (incubated at the same time as rumen degradation study). Two bags per steer per treatment per incubation time plus two empty bags per steer per incubation time were used. These bags were not washed after ruminal incubation and were kept frozen (-20°C) until the day of insertion into duodenum. On the day of insertion into duodenum, the bags were thawed at room temperature and incubated in a pepsin-HCl solution (1 g pepsin per litre of 0.01 N HCl) for 3 h at 39°C (Sauer et al. 1983; Kirkpatrick and Kennelly 1984; Kendall et al. 1991).

Nylon bags were then kept on ice at 4°C waiting random insertion into the duodenum of the two steers through the duodenal cannulae at the rate of two bags per h and collected in the feces. Bags recovered from the feces were treated similarly to the bags removed from the rumen. The transit time for each bag was approximately 12±2 h from duodenum to feces.

Chemical Analysis

All nylon bags, empty and containing samples from the ruminal incubation and mobile bag technique, were analyzed for N and DM. The entire bag plus sample was analyzed for N content by Micro-Kjeldahl method (Method 47.023, Association of Official Analytical Chemists, AOAC 1984) using Tecator 1030 analyzer and DM by method 7.013, AOAC 1984. Pepsin insoluble nitrogen (PIN) and neutral detergent fibre (NDF) concentration of the CM samples after autoclaving were determined according to the procedure of Goering and van Soest (1970). Acid detergent fibre (ADF) determined by method 7.076, AOAC 1984 and soluble N determination was based on Crooker et al. (1978). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) were performed on ADF and NDF residues by Micro-Kjeldahl method.

Calculation and Statistical Analysis

The DM and N contents of the blanks were subtracted from the DM and N residue in each bag respectively. Percentage disappearance of DM and N from samples in the nylon bags at each incubation time was calculated from their respective amounts

remaining after incubation in the rumen. The amount of digestible DM and N in the lower GI tract was calculated on each individual sample by the difference between the sample from total tract digestion and a random sample from the rumen degradation study. Data were analyzed separately for rumen incubation and lower GI tract digestion trials. Least square means of DM and N disappearance in the rumen and digestion in the lower GI tract were analyzed in the model that included treatment and ruminal cannulated steers, and treatment, ruminal and duodenal cannulated steers respectively (Snedecor and Cochran 1980). Statistical differences between the treatments were tested using the Newman-Keuls Test (Snedecor and Cochran 1980). Analyses of variance were completed using the General Linear Models Procedure of the Statistical Analysis System, Inc. (1988).

RESULTS AND DISCUSSION

Heat treatment had no significant ($P>0.05$) effect on CM total N content while it reduced the N solubility significantly ($P<0.01$) at all treatment times (Table 2). The 15 min treatment resulted in the largest reduction (78%) of the soluble N, with no additional reduction for 30 to 90 min heating. The gradual increase of soluble N as the heating time increased is unexplained. Heating of CM at 45, 60 and 90 min increased ($P<0.01$) ADIN content by 265%, 396% and 669%, respectively. Heating for 15 and 30 min tended to increase ADIN but not significantly ($P>0.05$). Heat treatment resulted in a gradual increase in PIN with an increase of 79% for the 90 min heat treatment. These findings are in agreement with those of others on the

Table 2. Effect of moist heat treatment on nutrient composition of canola meal

	Heat treatment (min)						
Item	0	15	30	45	60	90	SEM
Total N (%)	6.44	6.49	6.44	6.48	6.55	6.58	0.04
ADF (%)[z]	15.8d	14.2d	15.0d	23.3c	28.3b	36.4a	0.55
NDF (%)[y]	24.9f	35.3e	40.1d	48.3c	52.2b	55.7a	0.76
N composition (% of total N)							
ADIN[x]	5.35d	6.57d	6.98d	19.51c	26.54b	41.12a	0.66
PIN[w]	13.3d	15.3d	16.1cd	18.3bc	20.3b	23.8a	0.68
NDIN[v]	8.4f	26.3e	35.2d	45.5c	58.4b	70.2a	1.87
Soluble N	32.4a	7.0c	7.6bc	8.1bc	9.3bc	9.7b	0.50

a-f Values within a row with different letters differ ($P < 0.01$).
[z]Acid detergent fibre.
[y]Neutral detergent fibre.
[x]Acid detergent insoluble nitrogen.
[w]Pepsin insoluble nitrogen.
[v]Neutral detergent insoluble nitrogen.

effects of heat treatment on N solubility, ADIN and PIN contents (Ahmadi 1988; Plegge et al. 1985; Pena et al. 1986; Arieli et al. 1989). The increase in NDIN content with treatment ($P<0.01$) was even greater than ADIN and progressively increased by 736% of the original meal with 90 min heating. Heating of CM resulted in increased ADF and NDF content in a pattern similar to the increased ADIN and NDIN content. The percentage increase over the control for ADF and NDF were 130% and 124% at 90 min heating, respectively. Lindberg et al. (1982) reported a similar effect of heat treatment on the increased content of ADF and NDF in rapeseed meal. The difference in the ADF and NDF content with increasing heat can be attributed to the proteins which are less soluble and some of the heat denatured protein are recovered in NDF fraction. Therefore the N content of NDF is greatly increased by heating, while ADF requires the Maillard reaction or condensation of protein with carbohydrate to render protein recoverable in the ADF fraction (Van Soest 1982).

Treatment significantly ($P<0.01$) reduced DM and N disappearance in the rumen at both incubation times (Table 3). At 10 h rumen incubation time, moist heat treatment on average (mean of all treatment times) reduced DM and N disappearance in the rumen by 51% and 74% respectively, while it increased the proportion of DM and N digested in the lower GI tract by 169% and 222%, respectively. Therefore the treatment shifted the digestion of DM and N from rumen to the lower GI tract. Increased heating time did not decrease the amount of DM digestion in the lower GI tract ($P>0.05$). Ninety min heating decreased the amount of N digested in the lower GI tract, compared with 45 min of heating. Heating for 45 min appeared to maximize the amount of DM and N digested

Table 3. Effect of moist heat treatment of canola meal on dry matter (DM) and nitrogen (N) disappearance (%) from the rumen, lower gastrointestinal (GI) tract and total tract of steers

Item	Incubation time (h)	Heat treatment (min) 0	15	30	45	60	90	SEM
				Rumen (n=9)				
DM	10	63.2a	34.8b	32.3c	29.8d	29.4d	29.0d	0.48
	16	67.8a	42.7b	38.0c	35.4d	34.2d	33.1d	0.70
N	10	70.2a	23.4b	19.3c	16.5c	15.7d	16.5d	0.72
	16	74.4a	30.1b	21.3c	18.9cd	17.3d	17.4d	0.98
				Lower GI tract (n=6)				
DM	10	11.5c	27.6b	25.5b	40.7a	34.1ab	32.9ab	2.74
	16	10.1b	35.0a	34.3a	33.0a	30.2a	34.8a	2.16
N	10	18.8c	57.4b	58.4b	68.0a	63.6ab	58.8b	2.09
	16	16.2b	56.5a	64.7a	64.2a	61.6a	64.5a	2.51
				Total tract (n=6)				
DM	10	74.8a	62.4bc	59.7c	71.4ab	63.7bc	62.8bc	2.49
	16	77.9a	77.1ab	72.3abc	68.4bc	65.2c	68.5bc	2.36
N	10	89.0a	80.8bc	78.1bc	85.1ab	79.2bc	76.0c	1.84
	16	90.5a	88.2ab	85.9abc	83.2bc	79.8c	82.4bc	1.62

a-d Values within a row with different letters differ ($P<0.01$).

in the lower GI tract. Total tract digestibility of DM and N was similar between control and heat treated samples at 45 min. However, the total tract digestibility of DM and N declined ($P<0.01$) with 15, 30, 60 and 90 min heat treatment compared to control.

At 16 h of rumen incubation, moist heat treatment for 15, 30 and 45 min progressively reduced ($P<0.01$) DM disappearance in the rumen (Table 3). Similarly, the N disappearance was reduced ($P<0.01$) from 74.4% to 30.1%, 21.3% and 18.9% after heating for 15, 30 and 45 min respectively. Heating for 45 min or more had no further effect ($P>0.05$) on DM and N disappearance in the rumen. These results are consistent with finding of Ahmadi (1988) and Mir et al. (1984), who showed that dry heat treatment of CM reduced DM and N disappearance in the rumen. Mir et al. (1984) reported that heating at 120°C for 20 min reduced protein degradability of CM from 47.6 to 41.0%. Ahmadi (1988) reported that rumen disappearance of N decreased from 79.6 to 61.4% for a 16 h rumen incubation period when dry heat at 128°C was applied to CM.

At 16 h rumen incubation, moist heat treatment increased ($P<0.01$) DM digested in the lower GI tract from 10.1% for the untreated meal to an average of 33.0% for all treated meals (Table 3) with no significant ($P>0.05$) difference among the heat treated samples. The N digested in the lower GI tract increased from 16.2% for the control to an average of 62.0% for all heat treatment times. There were no differences ($P>0.05$) in total tract digestibility of DM and N between control and heat treated CM for 15 and 30 min. However, the total tract digestibility of DM and N declined ($P<0.01$) from 77.9 to an average of 67.4 and from 90.5 to an average of 81.8%, respectively, with

heating times of 45 to 90 min compared to control. The use of mobile nylon bag technique for estimating the total tract disappearance of DM and N of unheated CM was also reported by de Boer et al. (1987a), Deacon et al. (1988) and Kendall et al. (1991). The DM and N disappearance reported by de Boer et al. (1987a) and Deacon et al. (1988) was slightly higher (2-4 percentage points) at 12 h of incubation. The lower DM and N values (4-5 percentage points) reported by Kendall et al. (1991) may be because the bags recovered from the feces were not washed with cold water as compared to the present study.

Tests for ADIN and PIN have been suggested as estimates of unavailable N (Thomas et al.1982; Van Soest 1982). In the present study, the total tract disappearance of N was negatively correlated with ADIN ($r=-0.81$, $P=0.002$) and PIN ($r=-0.86$, $P=0.004$). However, the N digestibility of heated CM in the lower GI tract was positively correlated with ADIN ($r=+0.43$, $P=0.16$) and PIN ($r=+0.59$, $P=0.04$). If moist heat treatment is effective in increasing the proportion of N digestion occurring in the lower GI tract by more than 300%, as indicated by this study, an increase in ADIN or PIN could be tolerated for ruminant diets that require additional rumen escape protein.

Moist heat treatment of CM at 127°C was effective in increasing the ruminally undegradable protein fraction of CM at all treatment times. Heat treatment increased by more than 300% the amount of rumen escape protein that disappeared in the lower GI tract. The increase in ADIN and PIN was not effective in predicting change in lower GI tract digestion of N. However, the total tract digestibility of DM and N in CM was reduced with the longer heating periods. The increased rumen undegradable protein and

the availability of this rumen escape protein for absorption in the lower GI tract could benefit rapidly growing calves and high producing dairy cows.

MANUSCRIPT II:

EVALUATION OF MOIST HEAT TREATMENT OF CANOLA MEAL ON DIGESTION IN THE RUMEN, SMALL INTESTINE, LARGE INTESTINE AND TOTAL DIGESTIVE TRACT OF STEERS

ABSTRACT

The effect of heat treatment on canola meal (CM) dry matter (DM), nitrogen (N), acid detergent fibre (ADF) and acid detergent insoluble nitrogen (ADIN) degradability in the rumen and digestion in the small and large intestine and total digestive tract of steers was determined. CM was moist heat treated at 127°C for 15 and 45 min. The degradation of DM and N was estimated using small nylon bags in the rumen for 0.1, 4, 8, 12, 16, 24, 48 and 72 h. Rumen outflow rate was measured using chromium-mordanted CM. Small and large intestines and total digestive tract digestion of nutrient were estimated using separate sets of bags in a sequence of ruminal in situ incubation (0.1, 8, 16 and 24 h), in vitro incubation in an acid-pepsin solution and a mobile bag technique. There was a significant reduction in soluble N, sucrose and glucosinolate content, while the concentration of ADIN increased. The effective ruminal degradabilities of DM and N were reduced from 60.5 to 42.5 and 37.2% and from 69.9 to 40.2 and 33.7% for untreated, 15 and 45 min treated CM, respectively. Treatment significantly reduced the degradation of DM and N in the rumen while the digestibility of DM and N in the small intestine increased with both treatments which was proportional to the decreased rumen degradation. Heat treatment had no effect on DM and N digestibility at the large intestine site. Total tract disappearance of DM, N, ADF and ADIN was not affected by 15 min heating, however at 45 min heating, disappearance was reduced for DM and N but increased for ADF and ADIN. Moist heat treatment was effective in reducing rumen degradation of CM nitrogen while it increased the N digestion in the small intestine.

Key words: Heat treatment, canola meal, ruminant intestine, protein digestion

INTRODUCTION

Dairy cows in early lactation or young growing calves are in need of large quantities of protein (CP) at the small intestine. Microbial protein can be supplemented by feeding undegradable CP or by protecting CP from excessive ruminal degradation. Canola meal (CM) is a protein supplement which is readily available and used extensively in western Canada but has relatively high rumen degradability. Heat treatment of protein reduces ruminal degradation of CP with a corresponding increase in the lower gastrointestinal (GI) tract digestibility (Kibelolaud et al. 1993; Moshtaghi Nia and Ingalls 1992; Arieli et al. 1989). Heat treatment of protein sources decreases the solubility by creating crosslinkage between peptide chains and carbohydrates, therefore lowering their degradation in the rumen. Although moderate heat may increase the CP flow to the lower GI tract, excessive heat may decrease and lower the digestibility of amino acids in the small intestine (Van Soest 1982). The beneficial effects of decreased rate of protein degradation in the rumen appeared to be greater than the decreased protein digestibility in total GI tract caused by limited heat damage (Moshtaghi Nia and Ingalls 1992).

The objectives of this study were to examine the effect of moist heat treatment of CM on rumen degradation of DM and N and on the digestibility of DM, N, ADF and ADIN in the small and large intestines and total digestive tract of steers.

MATERIAL AND METHODS

Animals and Diets

The experiment consisted of two parts. The first part involved the incubation of nylon bags in situ in the rumen of three steers, each fitted with a large-diameter rumen cannulae (Bar Diamond, Parma, ID). The steers were held in individual crates. The second part involved a sequence of ruminal in situ incubation, in vitro incubation in an acid-pepsin solution and exposure to intestinal digestion using the mobile nylon bag technique in three Holstein steers, each fitted with a T-shaped duodenal cannulae in the proximal duodenum and a re-entrant cannulae in the terminal ileum. The cannulae were made of plastisol (F.H. and Sons Manufacturing Ltd., Rexdale, ON). The intestinal cannulated steers were housed in individual pens.

Diets formulated for high-producing dairy cows (National Research Council 1989) were fed to each of the rumen cannulated steers (weighing 675 kg) with a daily allotment of 5.0 kg of dairy concentrate (93.2% DM) plus 5.0 kg of alfalfa hay (87.9% DM). Intestinal cannulated steers (weighing 350 kg) received daily dry matter intake of 5.9 kg which consisted of 50% dairy concentrate and 50% of dehydrated alfalfa pellets (94.9% DM). Feed was provided to both ruminal and intestinal cannulated steers twice daily at 08:00 h and 16:00 h. Diet compositions for steers have been previously reported (Moshtaghi Nia and Ingalls 1992).

Canola Meal Treatment

Canola meal (CM), as received from the processing plant, was spread in a stainless steel

tray (30 x 42 cm) to a depth of one cm and autoclaved at 127° ± 1° C with a steam-pressure of 117 kPa for 15 and 45 min. CM temperature was monitored by a thermometer which was inserted into the meal during heating. CM was then allowed to cool to room temperature and stored in plastic bags at room temperature.

In Situ Technique

Small (3.5 x 5.5 cm) heat-sealed nylon bags of porosity 50 μm (Felco Industries Ltd., Concord, ON) were each filled with a one g sample of untreated or treated CM. The bags were incubated in the rumen for 0.1, 4, 8, 16, 24, 48 and 72 h. Nine sample-filled bags (three steers x three replicates) per treatment per incubation time plus six empty bags (three steers x two replicates) per incubation time were used. The empty bags were used to correct for DM and N contributed by feed particles and microorganisms that adhered to the bags. The small nylon bags were held in large 2 x 3 mm mesh nylon bags weighted down with stones in a bottle during the rumen fermentation period. The bags within each set of incubations were placed in the rumen of three steers in order of decreasing length of incubation such that all bags were removed from the rumen at the same time. After incubation, all bags were rinsed under cold tap water to remove extra material outside the bags and then washed with cold water in a wringer-type washing machine for 10 min followed by 5 min in clean water. The washed bags were dried in a forced-air oven at 60°C for 48 h or to constant weight in preparation of analysis.

The fractional outflow rate from the rumen was measured by treatment of unheated CM with sodium dichromate according to the method of Uden et al. (1980).

Seventy-five g of mordanted materials as a single dose were mixed with the dairy concentrate of the three ruminal cannulated steers and were fed on AM feeding on the day of rumen incubation time. Feces were sampled from each dropping and time of sampling was recorded from 0-110 h at approximately 4 h intervals (Grovum and Williams 1973) after the addition of the mordanted material to the feed. Fecal samples were kept frozen (-20°C) until analyzed.

Mobile Nylon Bag Technique

The effect of heat treatment of CM on the digestibility of rumen escape DM, N, ADF and ADIN in the small and large intestine and total tract was measured by incubation of three separate sets of bags initially in the rumen of ruminal cannulated steers and then inserted into duodenum of intestinal cannulated steers. The first set of bags incubated in the rumen for 0.1, 8, 16 and 24 h (incubated at the same time as the rumen degradation study) was treated similarly to the bags from the in situ study. The second and third sets of bags were incubated in the rumen the same as the first set but were not washed and were then frozen (-20°C) until the day of insertion into the duodenum. Nine bags (3 steers x 3 replicates) per treatment per incubation time for N and ADF analysis and three empty bags per incubation time for each segment of the digestive tract were used. On the day of insertion into the duodenum, the bags were thawed at room temperature and incubated in a pepsin-HCl solution (one g pepsin per litre of 0.01 N HCl) for three h at 39°C. The nylon bags were then kept on ice at 4°C until they were randomly inserted into the duodenum of three steers through the duodenum cannulae at

the rate of two bags per h. The second set of bags was inserted into the duodenum and collected at the terminal ileum on day one. The ileal re-entrant cannulas were kept open and digesta and bags were collected in a bucket, bags were removed and the digesta were injected back as soon as possible into the terminal ileum using a 50 ml syringe. The following day, the third set of bags was inserted into the duodenum while the reentrance ileal cannulae were closed allowing bags to be collected in the feces. Bags recovered from the terminal ileum and the feces were treated similarly to the bags removed from the rumen. The transit time for bags from duodenum to terminal ileum and feces was approximately 7 $\pm$ 1 and 16 $\pm$ 1 h, respectively.

Chemical Analysis

Dry matter and nitrogen determination were carried out on both empty bags and bags containing residues from the ruminal incubation and mobile bag technique. The entire bag plus residue was analyzed for N content by the micro-kjeldahl method (Method 47.023, Association of Official Analytical Chemists (AOAC) 1984 using a Tecator 1030 analyzer and for DM by Method 7.013 (AOAC 1984). Acid detergent fibre (ADF) was determined by Method 7.076 (AOAC 1984) and acid detergent insoluble nitrogen (ADIN) analysis was performed on ADF residue using the Micro-kjeldahl method. Soluble N determination of CM samples was based on Crooker et al. (1978). Individual glucosinolates content of CM samples was determined using the GLC procedure outlined by Slominski and Campbell (1987). Chromium in feces was determined by atomic absorption spectrophotometry following the procedure described by Williams et al.

(1962). Sucrose determination of CM samples was based on Slominski et al. (1994).

Calculation and Statistical Analysis

All bags containing DM and N residues were corrected for DM and N content of the respective blanks. Percentage disappearance of DM, N, ADF and ADIN from the nylon bags at each incubation time was calculated from their respective amounts remaining after each incubation in the rumen. The amount of digestible DM, N, ADF and ADIN in the small intestine was calculated on each individual bag by the difference between the bag from ileum and a random bag from the rumen degradation study. The per cent disappearance of DM, N, ADF and ADIN in the large intestine was calculated by the difference between the bag from total tract and a random bag from the ileum. The total tract DM, N, ADF and ADIN disappearance from each of the nylon bags was calculated from their respective amount remaining after collection in the feces. Thus ruminal and total tract disappearance were direct measurements while small and large intestine disappearance were measured by difference.

Percentage disappearance of DM and N in the rumen at each incubation time was fitted to the nonlinear regression equation of McDonald (1981), which incorporates a lag time:

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

where p is the disappearance of nutrients from the rumen at time (t); a is the soluble fraction; b is the potentially degradable fraction; c is the rate of degradation; and d is the lag time before the start of degradation of the potentially degradable fraction. The

nonlinear parameters *b*, *c* and *d* were estimated by an iterative least square procedure, based on Marquardt's methods (1963). The expression of $a + b$ represents the maximum extent of degradation which was constrained so that $a + b$ did not exceed 100%. The constant *a* was the mean disappearance of DM and N measured after 0.1 h of rumen incubation.

The effective degradabilities were estimated using the equation of Orskov and McDonald (1979) modified to include lag time:

$$ED = a + bc/\{c + (k^{-1}-d)^{-1}\}$$

where *ED* is the effective degradability of DM or N; *a*, *b*, *c* and *d* are the parameters described in the preceding paragraph and *k* is the outflow rate for solid from the rumen. The fractional outflow rate h^{-1} (*k*) was calculated from the descending concentration of chromium in the feces which was transformed to natural logarithms and a series of linear regressions were then performed on the post-peak values (Hartnell and Satter 1979). The slope with the best fit was then taken as the outflow rate from the rumen. *EDs* were also estimated at rumen particulate outflow rates of 0.08 and 0.06 h^{-1} (Agricultural Research Council 1984).

Data were analyzed separately for rumen incubation and lower GI tract digestion trials. Least square means of DM and N degradation in the rumen and digestion of DM, N, ADF and ADIN in the lower GI tract were analyzed in the model that included treatment and ruminal cannulated steers; and treatment, ruminal and duodenal cannulated steers, respectively (Snedecor and Cochran 1980). Statistical differences among the treatment least square means were tested using the Bonferroni test (Statistical Analysis

System Institute, SAS 1988). Analyses of variance were completed using the General Linear Models Procedures (SAS 1988).

RESULTS AND DISCUSSION

Chemical Composition

Moist heat treatment for 15 and 45 min had no effect on the concentration of CP, while it reduced the N solubility by 78 and 75%, respectively (Table 4). The ADF and ADIN contents of CM heat treated for 15 and 45 min appeared to be increased by 11.1, 37.4% and 31.0, 169%, respectively. These results are consistent with the finding of others on the effects of heat treatment on N solubility, ADF and ADIN contents (Kibelolaud et al. 1993; Moshtaghi Nia and Ingalls 1992; Arieli et al. 1989; Lindberg et al. 1982).

Heat treatment applied in commercial canola seed processing plants during oil extraction and preparation of the meal results in the breakdown of glucosinolates (Campbell and Slominski 1990). Heat treatment in this study decreased glucosinolate content of CM in comparison to untreated (commercial-CSP, Altona, MB) meal (Table 4), and the effect was most marked for 45 min heating. The decrease was probably due to thermal decomposition of the glucosinolates. Stedman and Hill (1987), in an attempt to reduce glucosinolate contents in a lamb feeding trial, also found significant reduction in glucosinolate contents with steam heat treatment at 121°C for 30 min. In laboratory trials, Slominski and Campbell (1987) demonstrated that little or no decomposition of

Table 4. Effect of moist heat treatment on nutrient composition and glucosinolate contents of canola meal (dry matter basis)

	Heat treatment (min)		
Item	0	15	45
Dry matter (%)	90.7	89.7	87.3
Protein (% DM)	40.3	40.6	40.5
ADF (%)[Z]	17.1	19.0	23.5
N composition (% of total N)			
ADIN[Y]	4.2	5.5	11.3
Soluble N	32.4	7.0	8.1
Sucrose (% DM)	6.83	5.32	4.25
Glucosinolate (μmol g)$^{-1}$			
Allyl	0.11	ND	ND
3-Butenyl	3.91	0.22	ND
4-Pentenyl	0.55	ND	ND
2-Hydroxy-3-butenyl	8.16	0.39	ND
2-Hydroxy-4-Pentenyl	0.17	ND	ND
4-Hydroxy-benzyl	3.14	0.17	ND
3-Indolylmethyl	0.17	ND	ND
4-Hydroxy-3-indolylmethyl	2.87	ND	ND
Total	19.07	0.78	ND

[Z]Acid detergent fibre.

[Y]Acid detergent insoluble nitrogen.

ND=not detected.

glucosinolates occurred due to dry heat treatment (100°C), while substantial breakdown was caused by moist heat treatment (100°C).

Sucrose content of CM also decreased after 15 and 45 min heating (Table 4). High temperature and extended heating times increases the ADIN contents through the Maillard reaction between sugar and free amino acids. Sucrose contains glucose and fructose, the latter of which is unstable in the presence of heat. The glucose from sucrose decomposition could have contributed to this reaction, as the increase in ADIN and the decrease in sucrose contents were negatively correlated ($r=-0.89$, $P=0.02$). Yang et al. (1993) also found significant negative relationships of ADIN and sugar in heat treated alfalfa hay.

Rumen degradation of DM and N

Disappearance rates for both DM and N were most rapid for untreated; intermediate for 15 min and lowest for 45 min treated CM. Moist heat treatment effectively reduced the rate of rumen degradation of CM (Fig. 1), therefore increasing the amount of dietary N available for digestion in the lower gastrointestinal (GI) tract. Similar effects of moist heat on degradation of DM and N of CM in the rumen have been reported (Vanhatalo and Aronen 1991). The effective degradabilities of DM and N (EDDM and EDN, respectively) values obtained from ruminal disappearance data, for the measured rumen outflow rate of 0.04 h^{-1} and outflow rates of 0.06 and 0.08 h^{-1} are shown in Table 5. Treatment significantly ($P<0.01$) reduced EDDM and EDN compared with that of untreated CM at all outflow rates. Although increasing the heating from 15 to 45 min

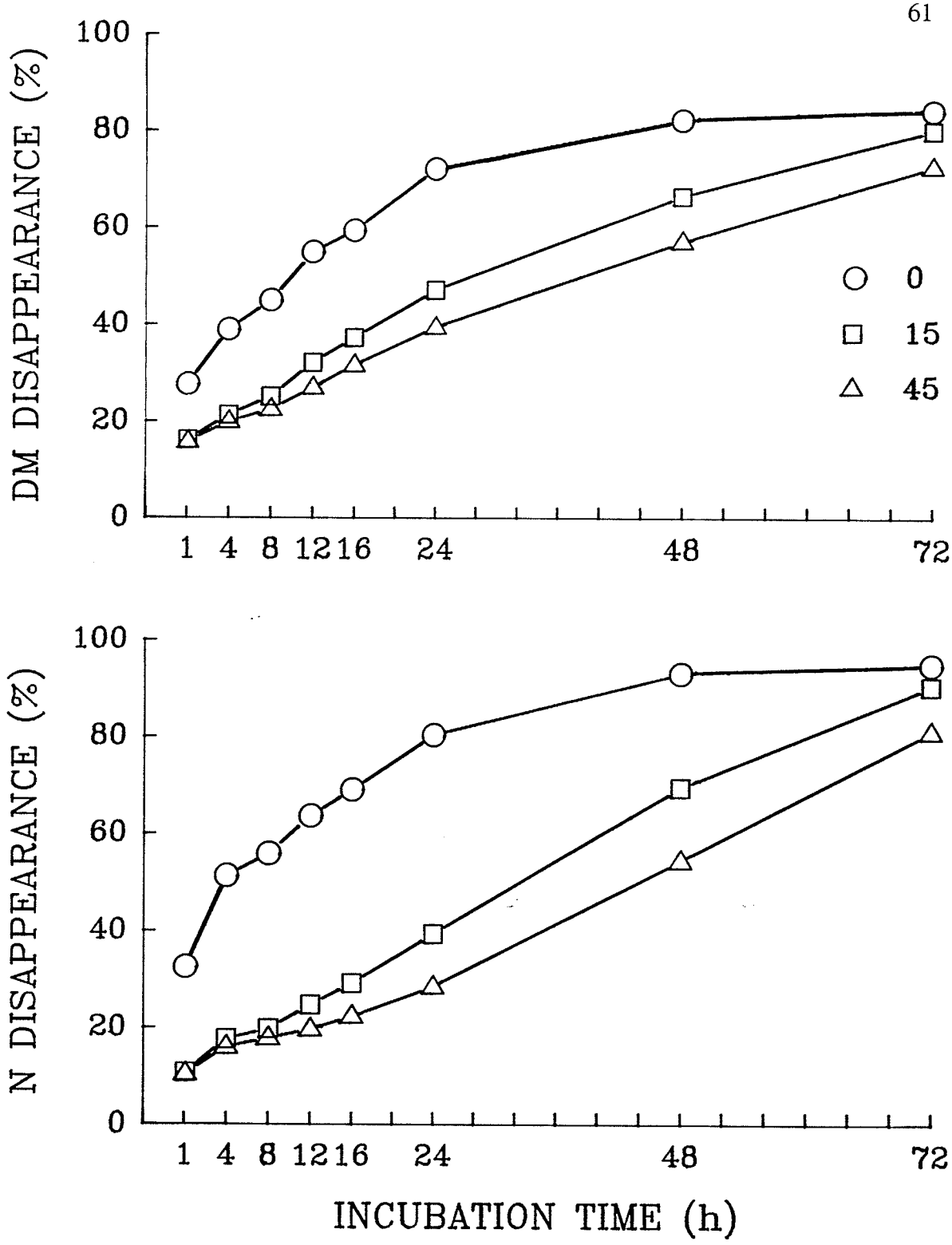

Figure 1. Effect of 0, 15 and 45 min of moist heat treatment of canola meal on dry matter and nitrogen disappearance from nylon bags incubated in the rumen of steers.

Table 5. The effect of moist heat treatment of canola meal on dry matter and nitrogen disappearance parameters and estimated degradabilities in the rumen of steers

	Disappearance parameters[z]				Effective degradabilities		
	a	*b*	*c*	*d*	k=0.04[y]	k=0.06	k=0.08
Treatment	(%)	(%)	(h)	(h)	(%)	(%)	(%)
Dry matter							
0	27.7*a*	59.6*b*	0.05	0.26	60.5*a*	54.9*a*	50.7*a*
15	16.2*b*	80.9*a*	0.02	1.06	42.5*b*	35.9*b*	31.6*b*
45	15.7*b*	82.3*a*	0.02	1.29	37.2*b*	31.4*b*	27.7*b*
SEM	0.20	1.16	0.005	0.29	0.90	0.86	0.79
Nitrogen							
0	32.5*a*	64.1*b*	0.06	0.0*b*	69.9*a*	63.9*a*	59.4*a*
15	10.7*b*	89.3*a*	0.02	2.22*a*	40.2*b*	32.4*b*	27.3*b*
45	10.4*b*	89.6*a*	0.02	2.73*a*	33.7*b*	26.9*b*	22.6*b*
SEM	0.37	0.92	0.005	0.24	1.29	1.18	1.06

[z]Disappearance parameters: *a*, soluble fraction; *b*, potentially degradable fraction; *c*, rate of degradation; *d*, lag time.

[y]Measured rumen outflow rate.

a-c Values within a column of each variable with different letters differ ($P<0.01$).

SEM=standard error of the mean (n=9).

decreased EDDM and EDN, the differences were not significant ($P>0.01$). The EDN was positively correlated with the N solubility in buffer ($r=+0.98$, $P=0.001$) at all outflow rates and between buffer soluble N and the rapidly soluble fraction a ($r=+0.99$, $P=0.001$), however the buffer soluble N was negatively correlated with slowly degradable fraction b ($r=-0.99$, $P=0.001$). A correlation between buffer soluble N and EDN was observed in heat-treated white lupin seeds (Kibelolaud et al. 1993).

Lower GI Tract Digestion

The disappearance of DM and N in each segment of the digestive tract as a per cent of initial level is presented in Tables 6 and 7. Regardless of treatment time, increasing ruminal incubation time resulted in a linear decrease in disappearance of DM and N in the small intestine. As the per cent disappearance of DM and N increased in the rumen with increasing incubation time, the disappearance in the small intestine decreased, thus reducing the amount of DM and N available in the small intestine. Treatment significantly ($P<0.01$) increased DM and N disappearance in the small intestine at 8, 16 and 24 h incubation times (Table 6 and 7). As the degradation of DM and N was decreased in the rumen by heat treatment, the digestion of DM and N increased in the small intestine. The treatment apparently shifted the disappearance of DM and N from rumen to the small intestine. Heating increased N disappearance in the small intestine by 2.4-2.5 times with 16 h rumen incubation and 3.9-4.7 times with 24 h rumen incubation. Others (Kibelolaud et al. 1993; Moshtaghi Nia and Ingalls 1992) have

Table 6. The effect of moist heat treatment of canola meal on dry matter disappearance (% of initial) in the digestive tract of steers

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	27.7*a*	45.0*a*	59.4*a*	72.1a
15	16.2*b*	25.1*b*	37.3*b*	47.0b
45	15.7*b*	22.5*b*	31.6*c*	39.5c
SEM	0.20	0.63	0.75	0.44
	Small intestine			
0	42.9*b*	30.6*b*	21.6*b*	10.6*c*
15	48.5*a*	45.8*a*	39.2*a*	33.4*b*
45	44.7*ab*	44.0*a*	39.6*a*	38.1*a*
SEM	1.15	1.26	1.11	0.78
	Large intestine			
0	3.2	0.0	-0.3*b*	0.3
15	3.7	4.2	2.7*a*	0.5
45	4.2	3.6	1.8*ab*	1.5
SEM	1.37	1.31	0.63	0.72
	Total tract			
0	73.6*a*	75.6	79.6*a*	82.8*a*
15	72.2*a*	75.1	78.2*a*	80.4*ab*
45	65.7*b*	73.0	74.6*b*	78.6*b*
SEM	1.01	0.85	0.79	0.65

a-c Values within a column of each variable with different letters differ ($P < 0.01$).

SEM=standard error of the mean (n=18).

Table 7. The effect of moist heat treatment of canola meal on nitrogen disappearance (% of initial) in the digestive tract of steers

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	32.5*a*	55.9*a*	69.1*a*	80.3*a*
15	10.7*b*	19.8*b*	29.2*b*	39.4*b*
45	10.4*b*	17.8*b*	22.4*c*	28.5*c*
SEM	0.37	0.78	1.42	0.74
	Small intestine			
0	58.2*b*	36.1*b*	25.6*b*	13.5*c*
15	75.2*a*	68.0*a*	61.7*a*	52.9*b*
45	70.5*a*	66.8*a*	64.4*a*	63.2*a*
SEM	1.01	1.50	1.53	0.74
	Large intestine			
0	-0.5	-0.3	-0.4	0.1
15	3.3	2.5	0.5	1.0
45	-1.3	1.6	1.8	-0.7
SEM	1.74	1.37	0.89	0.64
	Total tract			
0	90.3*a*	92.2	92.8	93.9*a*
15	90.3*a*	90.8	91.4	93.4*a*
45	81.1*b*	88.5	89.8	91.1*b*
SEM	0.75	0.96	0.62	0.37

a-c Values within a column of each variable with different letters differ ($P < 0.01$).

SEM = standard error of the mean (n = 9).

observed shifting of N disappearance from rumen to lower GI tract with heat treatment.

With one exception, there were no significant effects of incubation time or treatment on disappearance of DM and N in the large intestine (Tables 6 and 7). Although the extent of DM and N digestion of CM in the large intestine was small in this study, Ulyatt et al. (1975) indicated a considerable potential of microbes to digest cellulose, hemicellulose, energy and protein in the large intestine.

Increasing rumen incubation time appears to increase the total tract DM and N disappearance for untreated and treated CM. Total tract N disappearance for untreated and 15 min exceeded 90% even for the 0.1 h of ruminal incubation time. However, with 45 min heating there appeared to be an increase in total tract N disappearance from 88 to 91% at incubation times of 8 to 24 h vs 81% at 0.1 h. Nitrogen disappearance of untreated and treated CM from total tract exceeded DM disappearance at each rumen incubation time. The total tract disappearance data of CM are in agreement with that of Deacon et al. (1988). Although the 15 min treatment had little effect on total tract DM and N disappearance, the 45 min treatment resulted in the classical decrease ($P < 0.01$) in disappearance found with prolonged heating. The difference of 3 to 4 percentage points for 8 to 24 rumen incubated samples was small relative to the increased small intestine disappearance of N with heating.

The amount (g N/100 g CM) of N available for absorption in the small intestine for each treatment was calculated using the disappearance value of N in the small intestine (Table 4) multiplied by the concentrations of N in canola meal after heating (Table 1). Regardless of treatment, the amount of N available for absorption in the small

intestine appeared to decrease as incubation time increased. The amounts for 0 min were 3.75, 2.33, 1.65 and 0.87; for 15 min were 4.88, 4.41, 4.00 and 3.43; and for 45 min were 4.57, 4.33, 4.17 and 4.10 g N/100 g CM for incubation time of 0.1, 8, 16 and 24 h, respectively.

The disappearance of ADF and ADIN in the digestive tract of steers is presented in Tables 8 and 9, respectively. The disappearance of ADF and ADIN in the rumen was negative and thus unrealistic (Appendix I). Analyses were redone several times with the same results. The reason(s) for the negative rumen values are not known. The set of bags recovered from the ileum were used as a combined rumen and small intestine disappearance of ADF and ADIN (Tables 8 and 9). Heat treatment tended to increase ADF disappearance at all incubation time in the combined rumen and small intestine compared to 0 min, the ADIN disappearance was not affected by heat treatment. Although there was no significant difference among treatments in the disappearance of ADF in the large intestine, treatment generally increased the disappearance of ADIN in the large intestine. Total tract disappearance of ADF and ADIN increased with heat treatment; the effect was most marked at 45 min compared to the control. It is customary to relate ADIN to heat-damaged protein and to assume that ADIN represents unavailable N in the small intestine of ruminants (Van Soest 1982). In the present study, the added ADIN of moist heat treated CM was digested in the total digestive tract of steers, which supports other findings that ADIN can be digested (Arieli et al. 1989; Pena et al. 1986). Two thirds to three quarters of ADIN can be digested by ruminants. These results are supported by steer gains when diets were supplemented with distillers

Table 8. The effect of moist heat treatment of canola meal on acid detergent fibre disappearance (% of initial) in the digestive tract of steers

	Rumen incubation time (h)			
Treatment	0.1	8	16	24
	Rumen and small intestine			
0	2.3*b*	12.1	22.1	31.5
15	10.8*ab*	18.0	29.7	32.3
45	16.1*a*	26.5	28.8	32.8
SEM	2.66	3.84	2.28	1.59
	Large intestine			
0	7.1	-2.3	-2.5	-1.7
15	0.8	-4.5	-1.9	-3.9
45	6.4	-6.2	2.1	5.47
SEM	3.76	3.36	3.85	3.17
	Total tract			
0	8.0*b*	7.3*c*	19.6*b*	29.8
15	10.5*b*	18.0*b*	27.9*ab*	28.1
45	22.5*a*	25.9*a*	30.6*a*	36.3
SEM	2.06	2.00	2.13	1.88

a-c Values within a column of each variable with different letters differ ($P<0.01$).

SEM=standard error of the mean (n=9).

Table 9. The effect of moist heat treatment of canola meal on acid detergent insoluble nitrogen disappearance (% of initial) in the digestive tract of steers

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen and small intestine			
0	67.2*a*	69.6*a*	71.7*a*	76.2*a*
15	61.6ab	59.0*b*	60.7*b*	72.3*b*
45	58.3*b*	67.4*ab*	72.6*a*	79.2*a*
SEM	1.63	1.84	1.66	0.97
	Large intestine			
0	-3.6*b*	-3.6*b*	1.8*b*	0.7
15	5.4*ab*	13.9*a*	14.2*a*	-0.5
45	13.6*a*	7.0*a*	4.1*ab*	4.3
SEM	2.13	1.92	2.32	1.53
	Total tract			
0	63.3*b*	66.0*b*	73.6	76.9*b*
15	66.2*ab*	72.4*a*	75.3	71.7*c*
45	71.9*a*	75.9*a*	77.3	83.0*a*
SEM	1.73	1.20	1.09	0.94

a-c Values within a column of each variable with different letters differ ($P<0.01$).

SEM = standard error of the mean (n = 9).

dried grain with high levels of ADIN (Klopfenstein and Britton 1987).

Moist heat treatment was effective in increasing the ruminally undegradable protein fraction of CM with corresponding increased digestion in the small intestine. The increase in ADIN content after heating was not effective in predicting the change in small intestine digestion of N. Nitrogen digestion in the small intestine at 16 h rumen incubation time was increased from 1.65 for 0 min to 4.00 g N/100 g CM for 15 min heating. Heating of CM increased the ADIN content of CM and increased the total tract disappearance of ADIN. The increased N available for absorption in the small intestine by moist heat treatment of CM could benefit high producing dairy cows and rapidly growing calves.

MANUSCRIPT III:

INFLUENCE OF MOIST HEAT TREATMENT ON RUMINAL AND INTESTINAL DISAPPEARANCE OF AMINO ACIDS FROM CANOLA MEAL

ABSTRACT

Three mature Angus steers fitted with large ruminal cannulae and three Holstein steers each fitted with a T-shaped cannulae in the proximal duodenum and reentrant cannulae in the terminal ileum were used to study the effect of moist heat treatment on canola meal (CM) amino acid (AA) digestion in each segment of the digestive tract. Canola meal was treated with moist heat at 127°C for 15 or 45 min. Degradation of AA in the rumen was estimated using small nylon bags incubated at 0.1, 8, 16, and 24 h in the rumen. Digestion of AA in the small and large intestine and total tract were estimated using separate sets of bags in a sequence of ruminal in situ incubation, in vitro incubation in an acid-pepsin solution, and a mobile bag technique. Heat treatment for 15 or 45 min reduced concentrations of lysine by 15.9 and 29.2% and arginine by 8.0 and 15.2% respectively. Heat-treatment for 15 or 45 min significantly reduced the degradation of AA in the rumen; while the availability of AA in the small intestine increased proportionally to the decreased ruminal degradation. Although heat treatment reduced the concentration of lysine and arginine in CM, the availability of lysine and arginine in the small intestine with 15 min of treatment was increased by 75 and 131%, respectively, compared to no heat treatment with 16 h of ruminal incubation. Heat treatment for 45 min tended to increase AA digestion in the large intestine by 5 percentage points compared to no heat treatment. Apparent digestion of AA in the total tract was unaffected by 15 min of heating; however, 45 min of heating reduced digestion. Moist heat treatment for 15 min effectively increased ruminal escape of AA in CM, resulting in large increases in AA available for digestion in the small intestine ranging, from 75%

for lysine to 154% for histidine with 16 h of ruminal incubation.

Key words: canola meal, moist heat treatment, amino acid, small intestine, digestion

INTRODUCTION

Canola meal, which contains up to 40% CP on a DM basis, is used as a supplemental protein in lactation diets primarily because of its low cost versus competitive protein supplements. Much of the dietary CM protein that enters the rumen is degraded (de Boer et al. 1987b). The extent to which a dietary protein is digested in the rumen depends on incubation time and the physical and chemical nature of the protein (Cotta and Hespell 1986). Heat-treatment reduced ruminal degradation of protein supplement with a corresponding increase in postruminal digestion in CM (Moshtaghi Nia and Ingalls 1992; Moshtaghi Nia and Ingalls 1994), soybean meal (Broderick et al. 1990), horsebean (Cros et al. 1992b), and white lupin (Cros et al 1992a; Kibelolaud et al. 1993; Singh et al. 1991). Heat treatment of protein decreases the solubility and degradation rate in the rumen. However, high temperature and extended heating time increase the ADIN contents through Maillard reaction between sugars and amino acids (AA). Although moderate heat may increase the protein flow to the small intestine, excessive heat may decrease quantity of some AA and lower the digestibility of protein in the small intestine.

The objectives of this study were to examine the effect of moist heat treatment of CM on ruminal degradation of AA and on the digestibility of escape AA in the small and large intestines and total digestive tract of steers.

MATERIAL AND METHODS

The experiment involved the incubation of nylon bags in situ in the rumen of three Angus steers, each fitted with a ruminal cannula (Bar Diamond, Parma, ID), in vitro incubation in an acid-pepsin solution and exposure to intestinal digestion with a mobile nylon bag in three Holstein steers, each fitted with a T-shaped duodenal cannulae in the proximal duodenum and reentrant cannulae in the terminal ileum. The cannulae were made of plastisol (F.H. and Sons Manufacturing Ltd., Rexdale, ON). Diet composition and amounts fed have been previously reported (Moshtaghi Nia and Ingalls 1992).

Commercially available CM (CSP Foods Ltd., Altona, MB) was autoclaved at 127±1°C with a steam pressure of 117 kPa for 15 or 45 min. The temperature of the CM was monitored by a thermometer, that was inserted into the CM during heating.

Small (3.5 X 5.5 cm) heat-sealed nylon bags of 50 μm porosity (Felco Industries Ltd., Concord, ON) were each filled with one gram of untreated or treated CM. Three separate sets of bags were used. The first set was incubated in the rumen for 0.1, 8, 16, and 24 h to measure ruminal degradation of AA. The digestibility of ruminally undegraded AA in the small and large intestines and total tract, was examined by incubation of the second and third set of bags initially in the rumen of ruminally cannulated steers for 0.1, 8, 16, and 24 h (incubated at the same time as the ruminal degradation study). These bags were recovered from the rumen but were not washed and were then kept frozen (-20°C) until insertion into the duodenum. On the day of insertion into the duodenum, bags were thawed at room temperature and incubated in a pepsin-HCl solution (one g pepsin per litre of 0.01 N HCl) for three h at 39°C. The nylon bags

were then kept on ice at 4°C until they were randomly inserted into the duodenum of three steers through the duodenum cannulae at the rate of two bags per h. The second set of bags was inserted into the duodenum and collected at the terminal ileum on day one. The ileal reentrant cannulae were kept open, digesta and bags were collected in a bucket. Bags were removed, and the digesta were reinjected into the terminal ileum using a 50 ml syringe. The following day, the third set of bags was inserted into the duodenum and collected in the feces. The in situ technique and washing procedures used after removal of the bags from each segment of digestive tract have been described previously (Moshtaghi Nia and Ingalls, 1994). The washed bags were dried in a forced-air oven at 60°C for 48 h. The transit time for bags from duodenum to terminal ileum and feces was approximately 7 ± 1 and 16 ± 1 h, respectively.

Samples of CM before and after autoclaving and residues from bags in each segment of the digestive tract were analyzed for AA. One hundred mg of sample were analyzed for AA content using a LKB 4151 Alpha AA Analyzer (LKB Biochrom Ltd., Cambridge, England). Samples were prepared by acid hydrolysis using the method of Association of Official Analytical Chemists (1984) as modified by Mills et al. (1989). Acid hydrolysis involved digestion in 4 ml of 6 N HCl, in vacuo, for 16 h at 121°C. For analysis of methionine and cystine, samples were prepared using performic acid oxidation followed by acid hydrolysis. Non-amino acid nitrogen (NAAN) content of untreated and heat treated CM was calculated by difference between total N and N measured as AA.

Nine sample bags (three steers X three replications) per treatment per incubation

time for each segment of digestive tract were used. The percentage of disappearance of AA from the nylon bags at each incubation time was calculated from their respective amounts remaining after each incubation in the rumen. The amount of digestible AA in the small intestine was calculated for individual bags by the difference between the bag from ileum and a random bag from the ruminal degradation study. The per cent disappearance of AA in the large intestine was calculated by the difference between the bag from total tract and a random bag from the ileum. The disappearance of apparent total tract AA from each of the nylon bags was calculated from their respective amount remaining after collection in the feces. Thus ruminal and apparent total tract disappearance involved direct measurements, but disappearance from the small and large intestines was measured by difference.

Data were analyzed separately for each segment of digestive tract. Least square means of AA disappearance in the rumen and digestion in the lower GI tract were analyzed in the model that included treatment and ruminally cannulated steers; and treatment, and ruminally and duodenally cannulated steers, respectively (Snedecor and Cochran, 1980). Statistical differences among the treatment least squares means were tested using the Bonferroni test and analyses of variance were performed using the General Linear Models Procedure (SAS 1988).

RESULTS AND DISCUSSION

Heat treatment did not change the individual AA composition of CM greatly except for the essential amino acids (EAA) lysine and arginine (Table 10). Increasing heat treatment progressively reduced lysine and arginine contents of CM heat-treated for 15 and 45 min respectively. The loss may be a result of the Maillard reaction to advanced degradation stages in which some of the lysine and arginine nitrogen is no longer accounted for in routine AA analysis. Heat treatment caused no major changes in AA composition of soybean and sunflower meal (Schingoethe and Ahrar 1979). However, Craig and Broderick (1981) found that available lysine decreased with increasing time of autoclaving of cottonseed meal. The non-amino acid nitrogen (NAAN) content increased linearly with increased heat treatment, by 1.6 and 3.6% of total N over control with 15 and 45 min of heat treatment, respectively.

The disappearance of individual EAA in each segment of the digestive tract at 0.1, 8, 16, and 24 h of incubation are presented in Figures 2, 3, 4, and 5, respectively. Because disappearance of individual essential EAA in each section of digestive tract (rumen, small intestine, large intestine and feces) were measured separately from different sets of bags, the cumulative disappearance of these four sections does not equal exactly 100% (Figures 2 to 5). For example, total tract disappearance of methionine at 8 h of ruminal incubation with 15 min heat treatment (figure 3) appears to be 101.7% (rumen, 17.2%; small intestine, 69.8%; large intestine, 7.5%; and feces, 7.2%). Moist heat treatment at 15 and 45 min significantly ($P < .01$) reduced disappearance of all EAA in the rumen at all incubation times, except for valine and isoleucine at 0.1 h. The

Table 10. Effect of moist heat treatment on canola meal amino acid concentrations (g/16 g N).

	Treatment (min)		
	0	15	45
Essential			
Threonine	4.24	4.29	4.27
Valine	4.12	4.17	4.20
Methionine	2.23	2.19	2.17
Isoleucine	3.20	3.11	3.04
Leucine	7.07	7.10	7.11
Tyrosine	2.70	2.69	2.69
Phenylalanine	4.34	4.39	4.30
Histidine	2.78	2.59	2.64
Lysine	5.78	4.83	4.07
Arginine	5.88	5.37	4.96
Non-essential			
Aspartic acid	7.81	7.84	7.56
Serine	4.64	4.73	4.67
Glutamic acid	36.71	37.84	37.65
Proline	6.20	6.34	6.49
Glycine	5.41	5.42	5.43
Alanine	4.42	4.41	4.49
Cystine	2.41	2.29	2.12
Non-amino acid nitrogen (% of total N)	10.70	12.33	14.35
Total N (% DM)	6.45	6.49	6.48

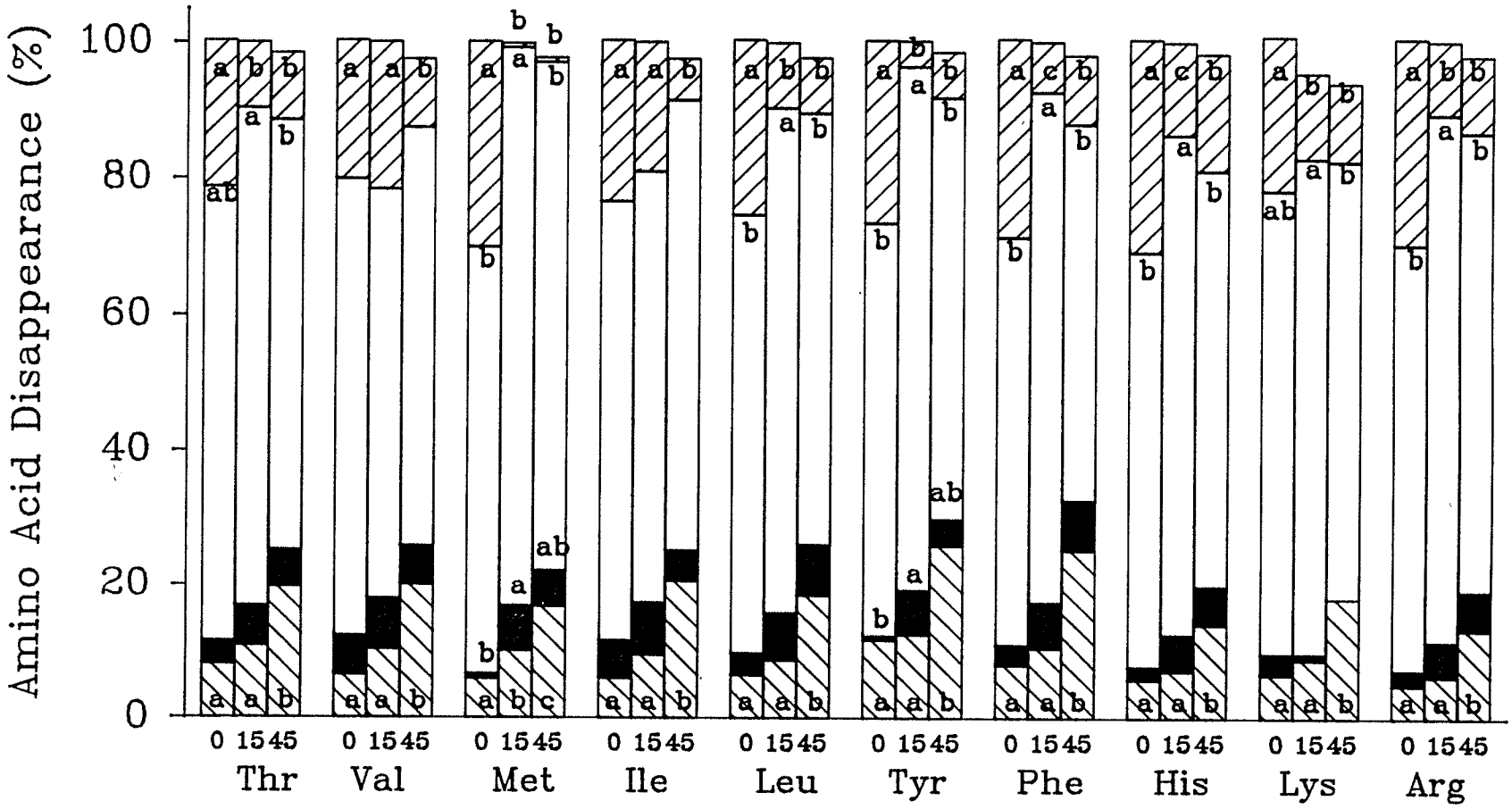

Figure 2. Effect of 0, 15 and 45 min moist heat treatment of canola meal on essential amino acids disappearance in the rumen ▨, small intestine □, large intestine ■ and fecal ▧ content of samples incubated 0.1 h in the rumen of steers.
a-c means within an amino acid within each segment of digestive tract, not having the same letter are different ($P < .01$). The letters above solid bar indicate the significant difference for the large intestine.

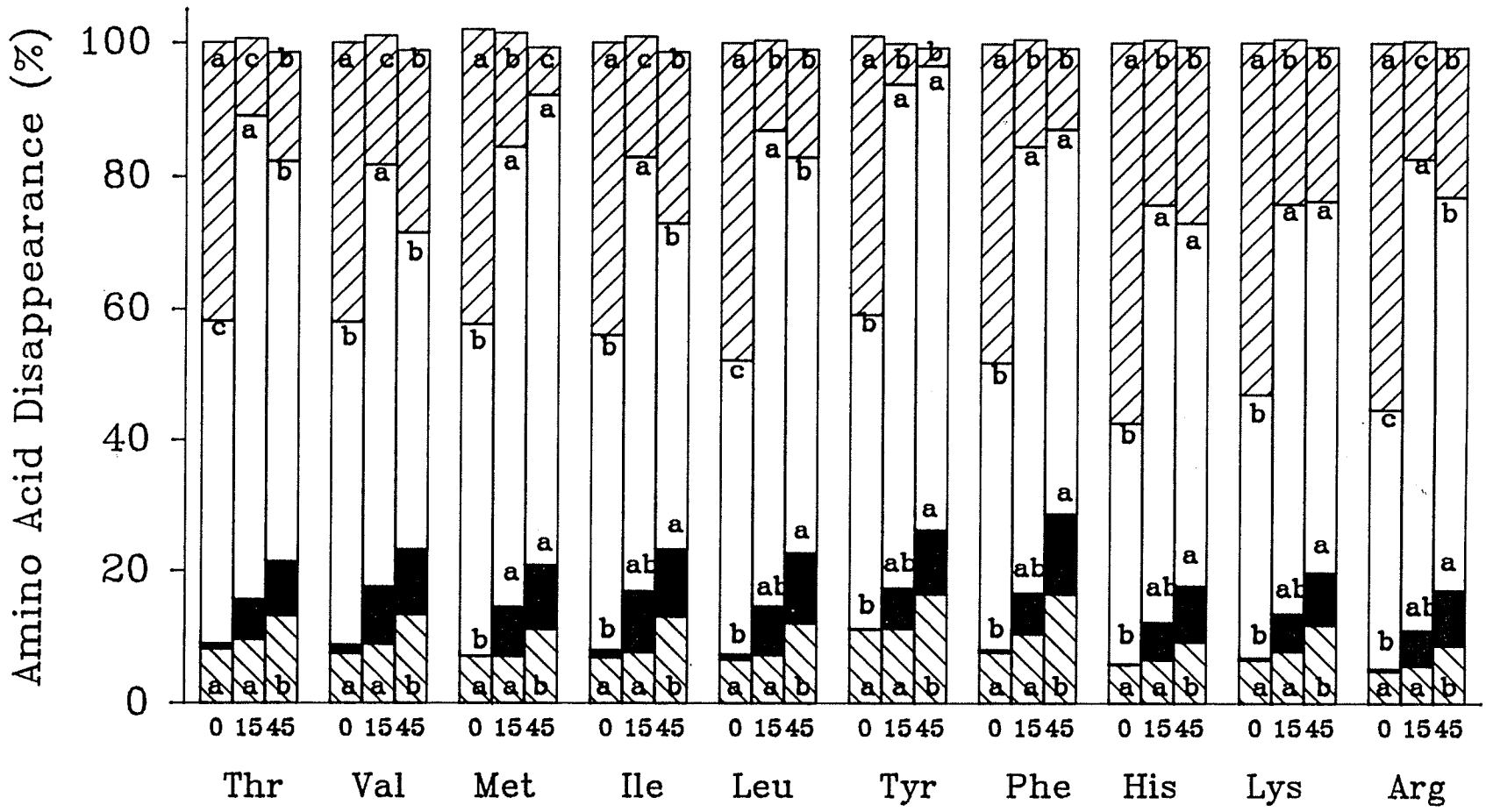

Figure 3. Effect of 0, 15 and 45 min moist heat treatment of canola meal on essential amino acids disappearance in the rumen ▨, small intestine □, large intestine ■ and fecal ▧ content of samples incubated 8 h in the rumen of steers.
a-c means within an amino acid within each segment of digestive tract, not having the same letter are different ($P < .01$). The letters above solid bar indicate the significant difference for the large intestine.

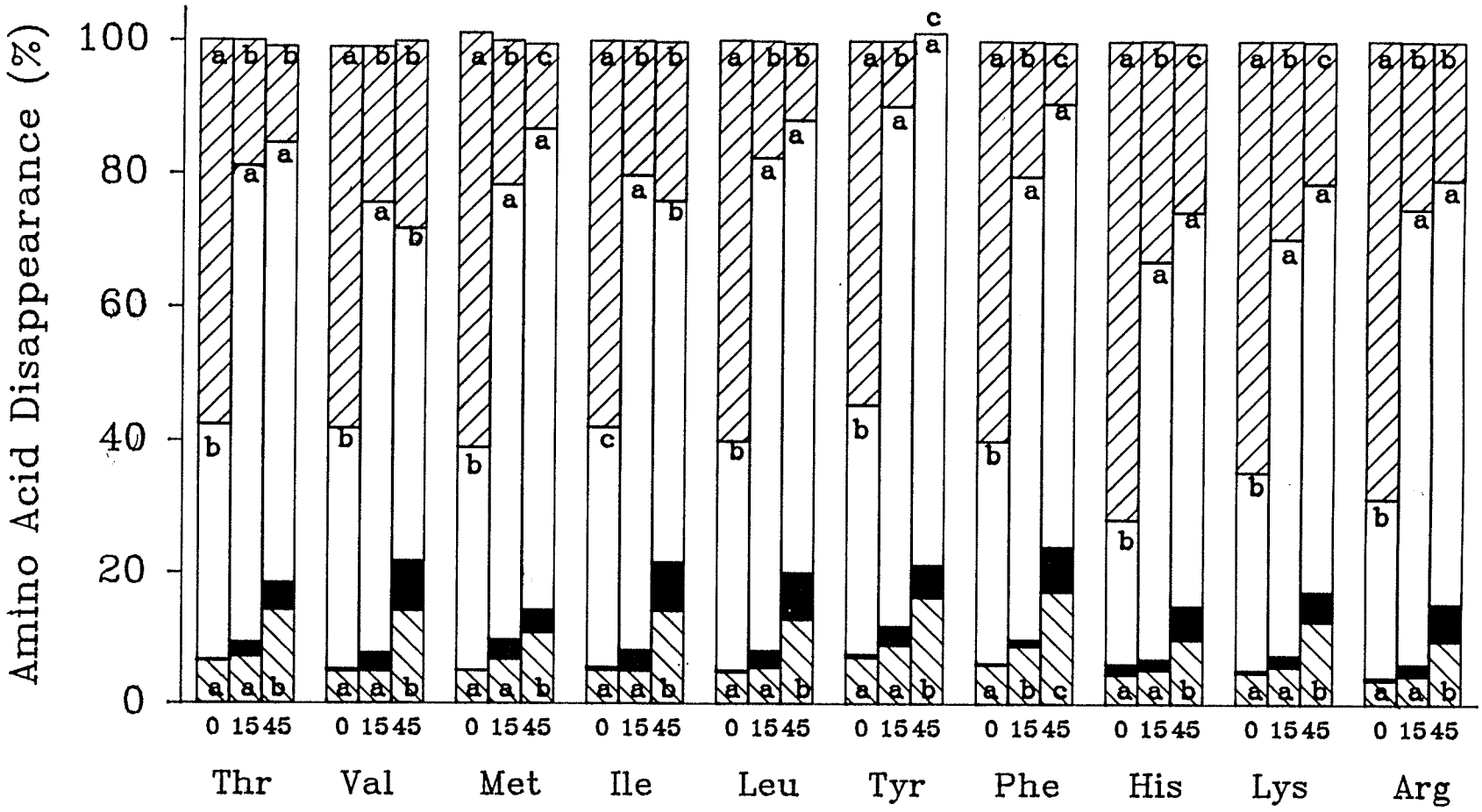

Figure 4. Effect of 0, 15 and 45 min moist heat treatment of canola meal on essential amino acids disappearance in the rumen ▨, small intestine □, large intestine ■ and fecal ▧ content of samples incubated 16 h in the rumen of steers.
a-c means within an amino acid within each segment of digestive tract, not having the same letter are different ($P < .01$).

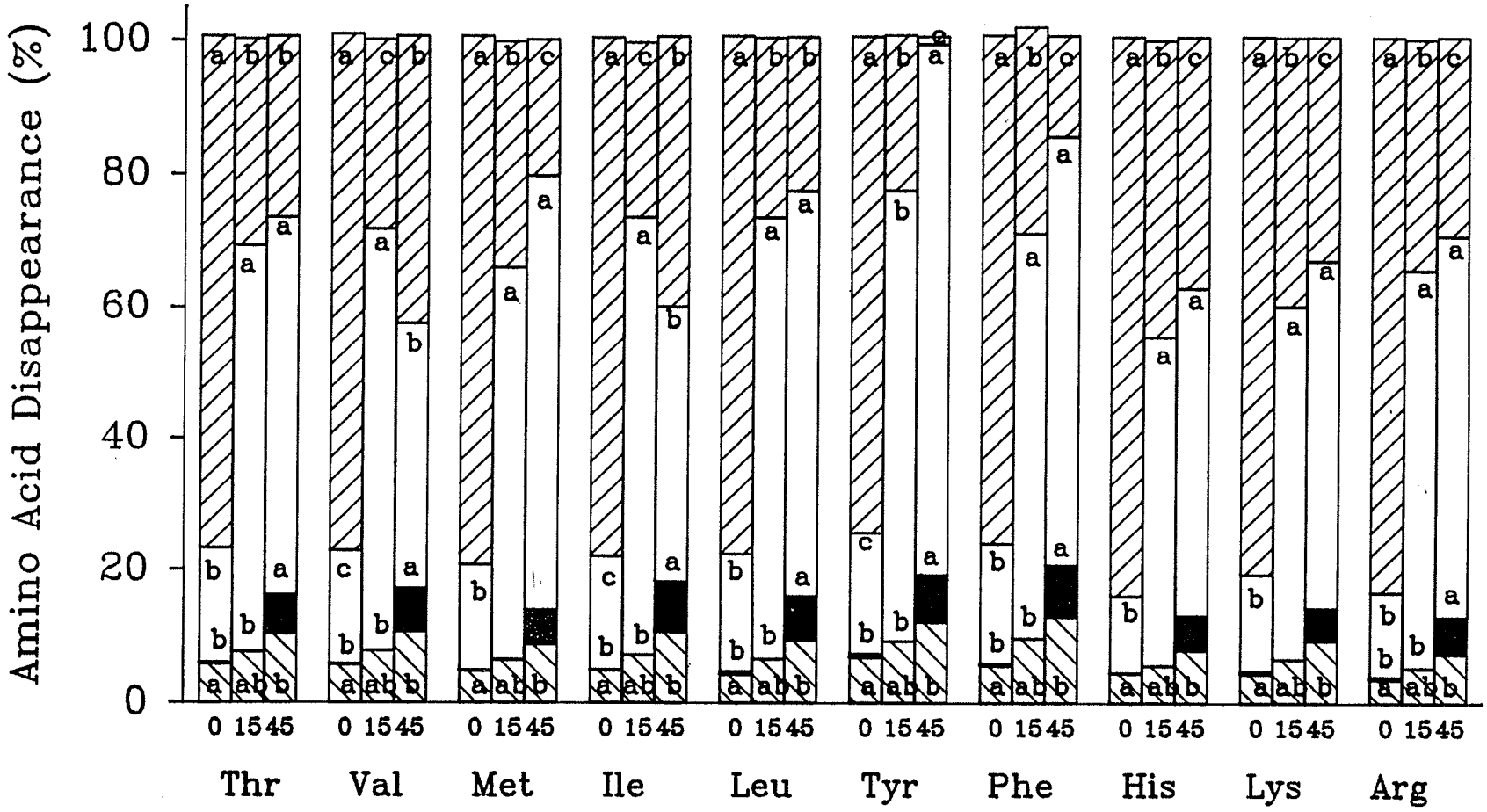

Figure 5. Effect of 0, 15 and 45 min moist heat treatment of canola meal on essential amino acids disappearance in the rumen ▨, small intestine □, large intestine ■ and fecal ▧ content of samples incubated 24 h in the rumen of steers.
a-c means within an amino acid within each segment of digestive tract, not having the same letter are different ($P < .01$). The letters above solid bar indicate the significant difference for the large intestine.

reduction was most marked for tyrosine, methionine, and phenylalanine, especially at 45 min of heat treatment. The degradable nature of tyrosine and methionine in CM has been previously reported (Boila and Ingalls 1992). The degradation of histidine by ruminal microorganisms seems to occur at a faster rate than for other EAA for untreated CM (Figures 3, 4, and 5). The rate of degradation for histidine was higher in CM samples from five different processing plants (Kendall et al. 1991).

The disappearance of individual EAA in the small intestine increased significantly ($P<.01$) by 15 min of heating, especially at 8, 16, and 24 h (Figures 3, 4, and 5). Heat treatment at 45 min also increased disappearance of EAA in the small intestine compared with 0 min of treatment except, for valine and isoleucine at 0.1 and 8 h (Figures 2 and 3). In general, 15 min of moist heat treatment of CM was more effective than 45 min in increasing disappearance of EAA in the small intestine.

Heat treatment tended to increase the disappearance of individual EAA in the large intestine compared with unheated CM at all incubation times. The 45 min heat-treatment, on average, increased disappearance of EAA in the large intestine by five percentage points, except for lysine at 0.1 h (Figure 2).

Apparent total tract disappearance of individual EAA at all incubation times was reduced ($P<.01$) by 45 min of heating compared with 0 and 15 min as indicated by increased fecal residue in Figures 1 to 4. No differences occurred (except for methionine at 0.1 h and phenylalanine at 16 h) between 0 and 15 min of heating, which indicates that heat treatment at 15 min had little effect on digestion of EAA in the total tract of steers.

The disappearance of individual AA in the rumen, small intestine, large

intestine,and total tract after 0.1, 8, 16, and 24 h ruminal incubation is presented in Appendix II.

The disappearances of EAA, nonessential (NEAA), and total amino acids (TAA) in each segment of the digestive tract are presented in Tables 11, 12, and 13, respectively. The rates of disappearance of EAA, NEAA, and TAA in the rumen were most rapid for 0 min, intermediate for 15 min and lowest for 45 min treatment. Moist heat treatment effectively reduced the rate of rumen degradation of CM, thus increasing the dietary AA available for absorption in the small intestine. Similar effects of heat treatment on ruminal degradation of AA of soybean and sunflower have been reported previously (Schingoethe and Ahrar 1979).

Increasing ruminal incubation time from 8 to 24 h linearly decreased disappearance of EAA, NEAA, and TAA in the small intestine for unheated CM. As the disappearance of AA increased in the rumen with increasing incubation time, the disappearance in the small intestine decreased, thus reducing the amount of AA available from CM in the small intestine (Tables 11, 12, and 13). The disappearance of total EAA appears to be similar to that of total NEAA (Tables 11 and 12). However, the rate of decline of AA disappearance in heat-treated CM was slower than the rate of decline in unheated CM with increasing ruminal incubation time. The disappearance of TAA of the unheated CM in the small intestine (Table 13) declined from 63 to 15%, whereas disappearance of TAA of the CM heated for 45 min declined from 64 to 59% for ruminal incubation 0.1 to 24 h. Heat treatments significantly ($P<.01$) increased the disappearance of AA in the small intestine compared with untreated CM at 8, 16, and

Table 11. The effect of moist heat treatment of canola meal on essential amino acids disappearance (% of initial) in the digestive tract of steers.

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	26.3[a]	47.6[a]	61.5[a]	79.3[a]
15	11.0[b]	16.9[b]	22.1[b]	32.0[b]
45	9.3[b]	17.9[b]	16.7[b]	27.1[c]
SEM	0.54	0.90	1.35	0.91
	Small intestine			
0	64.2[b]	45.0[c]	32.9[b]	16.0[b]
15	73.0[a]	68.7[a]	69.3[a]	61.2[a]
45	63.9[b]	59.0[b]	64.1[a]	57.2[a]
SEM	1.68	1.87	2.67	1.69
	Large intestine			
0	2.9	0.2[b]	0.3	0.1[b]
15	6.1	6.8[ab]	2.3	-0.7[b]
45	4.7	9.6[a]	5.2	6.2[a]
SEM	1.23	1.80	1.52	1.35
	Total tract			
0	93.0[a]	92.8[a]	94.7[a]	95.0[a]
15	90.5[a]	91.7[a]	93.7[a]	92.8[ab]
45	80.7[b]	87.4[b]	87.4[b]	90.1[b]
SEM	0.90	0.44	2.76	0.77

[a-c] Values within a column of each variable with different letters differ ($P < .01$).

SEM=standard error of the mean (n=9).

Table 12. The effect of moist heat treatment of canola meal on non-essential amino acids disappearance (% of initial) in the digestive tract of steers.

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	28.8[a]	55.1[a]	66.9[a]	81.6[a]
15	12.5[b]	19.8[b]	28.0[b]	38.9[b]
45	13.2[b]	18.6[b]	18.2[c]	26.2[c]
SEM	0.33	0.82	1.19	0.70
	Small intestine			
0	62.0[b]	37.2[b]	27.4[b]	13.4[b]
15	73.5[a]	62.8[a]	63.8[a]	54.7[b]
45	63.2[b]	61.7[a]	65.2[a]	60.3[a]
SEM	1.55	2.95	2.53	1.34
	Large intestine			
0	0.6	-0.9[a]	-0.8	-0.5
15	3.1	3.5[ab]	0.7	-1.6
45	3.9	5.7[b]	1.9	3.0
SEM	1.15	1.31	1.11	0.98
	Total tract			
0	92.4[a]	92.5[a]	94.2[a]	94.8[a]
15	91.3[a]	92.3[a]	93.8[a]	93.3[ab]
45	84.0[b]	88.9[b]	88.9[b]	91.2[b]
SEM	0.60	0.41	0.25	0.55

[a-c] Values within a column of each variable with different letters differ ($P < .01$).

SEM=standard error of the mean (n=9).

Table 13. The effect of moist heat treatment of canola meal on total amino acids disappearance (% of initial) in the digestive tract of steers.

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	27.5[a]	51.3[a]	64.2[a]	80.5[a]
15	11.7[b]	18.4[b]	25.1[b]	35.4[b]
45	11.2[b]	18.3[b]	17.5[c]	26.7[c]
SEM	0.41	0.86	1.26	0.80
	Small intestine			
0	63.1[b]	41.1[b]	30.1[b]	14.7[b]
15	73.3[a]	68.1[a]	66.5[a]	57.9[a]
45	63.5[b]	60.3[a]	64.6[a]	58.8[a]
SEM	1.60	1.78	2.49	1.51
	Large intestine			
0	1.8	-0.4[a]	-0.3	-0.2
15	4.6	5.2[ab]	1.5	-1.1
45	3.4	7.2[b]	3.6	4.3
SEM	0.97	1.54	1.41	1.20
	Total tract			
0	92.7[a]	92.6[a]	94.4[a]	94.9[a]
15	90.9[a]	92.0[a]	93.7[a]	93.0[ab]
45	82.4[b]	88.1[b]	88.1[b]	90.4[b]
SEM	0.76	0.43	0.26	0.61

[a-c] Values within a column of each variable with different letters differ ($P < .01$).

SEM=standard error of the mean (n=9).

24 h of incubation (Tables 11, 12, and 13). As the per cent disappearance of AA was decreased in the rumen by heat treatment, the per cent disappearance of AA increased in the small intestine with a relatively small increase in fecal loss (Figures 3, 4, and 5). Heat treatment shifted the disappearance of AA from the rumen to the small intestine. Moist heat treatment for 15 and 45 min, on average, increased disappearance of TAA in the small intestine by 118% after 16 h and 297% after 24 h ruminal incubation (Table 13). Others (Cros et al. 1992b; Koeln and Paterson 1986) also have observed increased disappearance of AA from the small intestine with heat-treated soybean meal and horsebean.

Although disappearance of EAA, NEAA, and TAA in the large intestine tended to increase with 15 and 45 min of heating, the difference was significant only ($P < 0.01$) for 8 h of ruminal incubation with 45 min of treatment (Tables 11, 12, and 13). However, regardless of incubation time, the 45 min of heat treatment tended to increase the disappearance of AA in the large intestine by an average of 5 percentage points compared with the control (Figures 2 to 5).

Apparent total tract disappearance of EAA, NEAA, and TAA appears to change very little with increasing ruminal incubation time for untreated CM and CM treated for 15 min (Tables 11, 12, and 13). Total tract disappearance of AA for treatments of 0 and 15 min exceeded 90%, even in 0.1 h of ruminal incubation, however, with 45 min of heating, total tract disappearance of AA apparently increased from 82% for 0.1 h to 87 to 90% for 8 to 24 h of incubation. Although the 15 min of moist heat treatment had no significant detrimental effect on total tract disappearance of AA, the 45 min of heat

treatment resulted in the classic decrease ($P<.01$) in digestibility found with extended heating. However, the total tract decrease of 4 to 5 percentage points for CM treated for 45 min was small compared with the large increase in the disappearance of AA from the small intestine for samples incubated for 8 to 24 h in the rumen.

For each treatment, the amount (g AA per 100 g CM) of individual EAA available for absorption in the small intestine was computed using the disappearance value for an EAA in the small intestine multiplied by the concentration of that EAA in CM after heating (Table 10). The availability (g) of total EAA in the small intestine were lowest ($P<0.01$) for untreated CM (Table 14). By calculating the amounts of total EAA of CM available to the small intestine, approximately 5.6 and 11.5 g of total EAA per 100 g of CM were available in the small intestine with untreated samples and samples treated for 15 min, respectively (Table 14). This availability indicates that moist heat treatment for 15 min increased the intestinal availability of total EAA of CM by 105%. For individual EAA, the increase varied from a low of 75% for lysine to 154% for histidine (Table 14). This effect was due to increased resistance of protein in moist heat-treated CM to ruminal microbial degradation; with only small increases in EAA reached the large intestine.

The 15 and 45 min treatments were not different in individual EAA available for absorption in the small intestine except for valine and isoleucine (Table 14). The amounts of lysine and arginine available for absorption from CM were significantly ($P<0.01$) increased by 15 and 45 min of heat treatments even though the concentration of these two EAA decreased with heat treatment (Table 10). Faldet et al. (1991) also

Table 14. Effect of moist heat treatment on amino acid availability (g per 100 g CM DM) in the small intestine of steers after 16 h rumen incubation.

	Heat treatment (min)			
	0	15	45	SEM
Essential				
Threonine	.61[b]	1.25[a] (105)[1]	1.14[a] (87)[1]	.048
Valine	.61[b]	1.15[a] (88)	.85[b] (39)	.059
Methionine	.31[b]	.61[a] (100)	.64[a] (109)	.019
Isoleucine	.47[b]	.90[a] (91)	.67[b] (42)	.044
Leucine	.99[b]	2.14[a] (91)	1.96[a] (97)	.086
Tyrosine	.41[b]	.85[a] (107)	.87[a] (112)	.029
Phenylalanine	.59[b]	1.24[a] (110)	1.16[a] (95)	.049
Histidine	.25[b]	.63[a] (154)	.63[a] (156)	.019
Lysine	.70[b]	1.23[a] (75)	1.01[a] (44)	.051
Arginine	.65[b]	1.49[a] (131)	1.28[a] (98)	.054
Total essential	5.59[b]	11.49[a] (105)	10.20[a] (83)	.450
Total non-essential	5.63[b]	16.60[a] (195)	17.74[a] (215)	.484
Total amino acid	11.22[b]	28.09[a] (150)	27.94[a] (149)	.922

[a-b] Values within a row of each variable with different letters differs ($P < .01$).

SEM = standard error of the mean (n=9).

[1] Numbers in parentheses indicate per cent increase over 0 min treatment.

found a reduction in lysine content by heat treatment of soybean meal and a corresponding increase in postruminally available lysine.

The relationships of individual EAA in milk protein are compared with that from CM in the small intestine (Table 15) using data from Table 14. The first five limiting EAA of the unincubated CM used in this experiment were tyrosine, isoleucine, valine, lysine, and leucine, which provided 53 to 73% of corresponding EAA in milk protein (Spires et al. 1975), respectively. The relative distribution of EAA of CM available in the small intestine after 16 h of ruminal incubation was slightly different from the unincubated CM, which histidine was added to the first five limiting EAA. Because histidine was the most degradable EAA in the rumen for untreated CM, less histidine was available in the small intestine. Both isoleucine and tyrosine were first-limiting EAA, followed by lysine, valine, and histidine, which provided 20 to 23% of corresponding EAA in milk protein. In our laboratory (Boila and Ingalls 1994, unpublished data) similar values of 16 to 21% for the same EAA with 12 h of ruminal incubation were found.

The sequence of limiting EAA changes some what for 15 and 45 min heat-treated CM. The first four limiting EAA for 15 min treated CM were lysine, isoleucine, tyrosine and valine, which provided 37 to 43% of corresponding EAA in milk protein, respectively. Histidine, the next limiting was relatively less limiting than untreated CM. The order of the first four limiting EAA of 15 and 45 min treated CM was essentially the same (Table 15). Methionine, which has been mentioned as a limiting AA for milk production (Cros et al. 1992a; Susmel et al. 1989), was not included in the first group

Table 15. Effect of moist heat treatment on individual essential amino acid (EAA) availability (g/16 g N) of canola meal (CM) in the small intestine of steers after 16 h rumen incubation relative to milk EAA composition.

			Heat treatment (min)		
	Milk[1]	CM[2]	0	15	45
Threonine	4.6	4.24 (92)[3]	1.51 (33)	3.08 (67)	2.81 (61)
Valine	6.6	4.12 (62)	1.51 (23)	2.83 (43)	2.10 (32)
Methionine	2.6	2.23 (86)	0.77 (30)	1.50 (58)	1.58 (61)
Isoleucine	5.9	3.20 (54)	1.17 (20)	2.22 (38)	1.65 (28)
Leucine	9.7	7.07 (73)	2.46 (25)	5.28 (54)	4.84 (50)
Tyrosine	5.1	2.70 (53)	1.02 (20)	2.10 (41)	2.15 (42)
Phenylalanine	4.9	4.34 (86)	1.46 (30)	3.06 (62)	2.86 (58)
Histidine	2.7	2.78 (103)	0.62 (23)	1.55 (57)	1.56 (58)
Lysine	8.1	5.78 (71)	1.74 (21)	3.03 (37)	2.49 (31)
Arginine	3.5	5.88 (168)	1.61 (46)	3.67 (105)	3.16 (90)

[1] Milk amino acid content (g/16 g N) as reported (Spires et al. 1975).

[2] Untreated, commercial canola meal Animo acid content (S/16 g N) as reported in (table 1)

[3] Numbers in parentheses indicate per cent of EAA in CM relative to that of milk.

of limiting AA for CM or heat-treated CM. However, a somewhat reduced concentration of lysine in heat treated CM reduced its effectiveness to meet the EAA requirement for milk protein. Feeding CM that has been heat-treated for 15 min instead of commercial CM to lactating cows, the amount of AA presented to the small intestine for absorption appeared to almost double and the effectiveness of CM as a source of histidine was improved.

CONCLUSIONS

Moist heat treatment of CM decreased the degradation of AA in the rumen and increased the supply of AA from CM to the small intestine. The degree of heating as practiced in commercial canola seed processing plants during oil extraction and preparation of CM is insufficient to maximize the escape of protein and AA in CM from the rumen. The increased availability of AA for absorption in the small intestine from moist heat treatment of CM could benefit ruminants when increased quantities of EAA are required, if microbial protein synthesis in the rumen is not decreased.

MANUSCRIPT IV:

INFLUENCE OF TIME OF FEEDING THE DIETARY PROTEIN SUPPLEMENT ON MILK PRODUCTION AND COMPOSITION IN LACTATING DAIRY COWS

ABSTRACT

Twelve multiparous and ten primiparous cows in late lactation were used over a 16-wk study to determine the influence of feeding time of a protein supplement (PS) on dry matter (DM) intake, ruminal fermentation, and milk yield and milk composition. One mixed ration consisting of 50% whole crop oat silage, 10% alfalfa silage and 40% grain concentrate was offered twice daily with two thirds at 08:00 h and the remainder at 18:00 h. In addition, a PS was offered in one meal per day at either 08:30 h or 12:30 h at an amount equal to 15% of the mixed ration DM intake. Half of the cows were assigned to each of the two dietary treatments which were designated as DAY (08:30 h) or NIGHT (12:30 h). Eight cows in the production study, previously fitted with large diameter rumen cannulae, were used to study treatment effects on rumen fermentation and ruminal in situ nutrient disappearance. Rate of passage of PS was measured using chromium-mordanted PS. Time of feeding PS had no effect on DM intake, body weight, and condition score. Milk yield and milk composition were not influenced by treatment. Treatment had no effect on in situ DM and crude protein (CP) disappearance or ruminal rate of passage of PS. Feeding PS at 12:30 h increased ruminal concentrations of acetate, propionate, isobutyrate, and isovalerate and decreased butyrate and caproate. All measured VFA concentrations were higher during the period of 24:00-10:00 h for cows fed the PS at night. Time of feeding a PS can alter ruminal fermentation characteristic without influencing animal performance.

Key words: Protein supplement, feeding sequence, lactating dairy cows, milk production, rumen fermentation

INTRODUCTION

Ruminant animals normally prefer to eat during the day (Deswysen et al. 1984) using the hours of darkness to digest the feed and ensure its onward passage in time for intake of fresh feed the next morning. Diurnal cycles in rumen fill and rumination were also observed to be greater at night (Ulyatt et al. 1984). Further, an increase in the muscular activity of the rumen wall during the night has been observed in young cattle offered forage diets (Sissons et al. 1986). The muscular activity of the rumen is maximized at night, possibly resulting in faster passage of newly ingested feedstuffs. If so, then offering a protein supplement (PS) to coincide with this theoretical higher passage rate could be a means of decreasing net ruminal protein degradation by rumen microorganism. Thus, if PS is retained in the rumen for a shorter period of time, less ammonia-N will be lost to peripheral blood and excreted in the urine. Additionally, if dietary PS were to leave the rumen rapidly, more protein would be available for digestion in the intestines to meet metabolic requirements. Robinson et al. (1993) investigated changing the sequence of feeding a PS in relation to a basal mixed ration, and suggested that rates of rumen passage of dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP) were lower in the day feeding cycle (08:00 to 18:00 h) for cows fed a PS at 08:30 h and higher in the evening feeding cycle for cows fed the PS at 12:30 h. Thus if the ruminal rate of passage of the PS can be increased by feeding when overall rate of passage is high, then the undegraded intake protein (UIP) value of the dietary protein should be increased. This in turn should result in more efficient utilization of dietary protein and potentially, increased milk production.

The objective of this study was to examine the influence of time of feeding a dietary PS on milk yield and milk composition, as well as rumen fermentation, in Holstein cows.

MATERIALS AND METHODS

Production Study

COWS AND DIETS. Twelve multiparous and ten primiparous Holstein cows were utilized from approximately 20-34 wks postpartum and were assigned to one of two dietary treatments for a period of 16 wks. They were tethered in tie-stalls and had free access to water. One mixed ration (Table 16) consisting of 50% whole crop oat silage, 10% alfalfa silage and 40% grain concentrate was offered ad libitum to all cows twice daily with two thirds given at 08:00 h and the remainder at 18:00 h. In addition, a pelleted PS was offered in one meal per day at either 08:30 h (DAY) or 12:30 h (NIGHT) at an amount equal to approximately 15% of mixed ration DM intake. Treatments are designated 'DAY' (08:30 h) or 'NIGHT' (12:30 h) respectively. Orts were removed at 24:00 h. After a three-wk adjustment period, during which the PS was fed at 13:00 h, cows were blocked and assigned to treatment based on performance in the adjustment period. Cows were milked daily at 05:00 and 15:00 h.

SAMPLING. Mixed ration offered and refused was recorded daily. Cows were observed to eat the PS rapidly and completely upon offer. Mixed ration and PS were sampled daily and composited on a weekly basis during weeks 4, 8, and 12. Milk

Table 16. Ingredients and chemical composition of mixed diet and protein supplement

	Mixed diet	Protein supplement
Ingredients		
	g kg^{-1} DM	
Oat silage	500	-
Alfalfa silage	100	-
Ground barley	376	305
Canola meal	-	330
Soybean meal	-	330
Salt	2.16	-
Limestone	3.56	-
Vitamine mixture	0.40	-
Trace-mineral salt	3.72	-
Molasses	14	35
Chemical analysis		
	g kg^{-1} DM	
Dry matter	440	863
Organic matter	901	933
Neutral detergent fibre	442	157
Acid detergent fibre	258	85
Cellulose	404	130
Lignin	38	27
Starch	167	203
Nitrogen	1.84	5.39
Ammonia nitrogen	1.56	0.04

weights were recorded daily. Milk samples were taken from consecutive PM and AM milkings on the same day at week for compositional analysis. Feed intake and milk yield and milk composition were calculated weekly. Body weights and condition scores were taken at the beginning of the experiment and at subsequent 28 day intervals.

Rumen Fermentation Study

Eight cows in the production study were previously fitted with large diameter rumen cannulae (10 cm i.d., Bar-Diamond Inc., Parma, Idaho, U.S.A.) and used concurrently to determine treatment effects on ruminal fermentation, rate of passage and in situ disappearance of DM and CP in the mixed diet and PS, and disappearance of NDF in the mixed diet. Housing and management were the same as for the non-cannulated cows. Measurements were taken during the 4th and 8th wk on on test.

IN SITU TECHNIQUE. In situ rumen DM and CP disappearance from PS as well as DM, N, and NDF disappearance from the mixed diet were estimated. The in situ procedure used was similar to that described by de Boer et al. (1987b), but times of rumen incubation were 10 h (08:00-18:00), 14 h (18:00-08:00), and 24 h (08:00-08:00). Percentage disappearance of DM, CP, and NDF from the nylon bags at each incubation time was calculated from their respective amounts remaining after ruminal incubation.

SAMPLING. The feed intake pattern of the ruminally cannulated cows was estimated

by weighing uneaten feed at 08:20, 09:15, 10:15, 13:00, 14:15, 17:50, 19:15, 20:30, 22:15, and 24:00 h. Concentration of various soluble metabolites and pH were measured in rumen fluid samples collected as previously described (Robinson and Sniffen 1985). Rumen fluid was sampled over a 24 h period at 07:50, 08:20, 09:00, 09:30, 10:00, 10:30, 11:00, 14:00, 17:50, 18:30, 19:00, 19:30, 20:30, 22:00, 24:00, 01:00, 01:30, 02:00, 03:00 and 05:00 h. Rumen fluid pH was measured immediately. A 4-mL portion of the sample was frozen (-20°C) in tightly capped tubes for ammonia analysis. A second 4-mL portion was added to a tube containing 2.5 mL of methanol containing 2 mg mL^{-1} malonic acid, and frozen (-20°C) in tightly capped tubes for lactic acid analysis. A third 4-mL portion of rumen fluid, except for 09:30, 10:30, 18:30, 19:30, 01:00 and 02:00 h samples, was added to a tube containing 1 mL of 25% (V/V) orthophosphoric acid and frozen (-20°C) in tightly capped tubes for VFA analysis.

PROTEIN SUPPLEMENT RATE OF PASSAGE. Rate of PS passage from rumen was measured by treatment of PS with sodium dichromate according to the method of Uden et al. (1980). Mordanted material, 75 g, was fed as a single dose in the meals at 12:30 h or 08:30. Fecal samples were collected by rectal grab sampling at 0, 7, 13, 31, 37, 45, 55, 61, 69, 81, 93, and 105 h post dosing. Fecal samples were immediately dried in a forced-air oven at 60° C for 48 h or to constant weight in preparation for analysis.

Analytical Procedures

Dry matter was determined at 105° C and organic matter (OM) was determined as the

weight lost upon ashing at 550°C overnight. Neutral detergent fibre was determined according to the procedure of Goering and Van Soest (1970). Acid detergent fibre (ADF) was determined by the procedures outlined by the Robertson and Van Soest (1981). Residue of ADF was further treated with 72% sulphuric acid and ashed at 550°C to estimate cellulose and lignin, respectively. Starch was determined by the procedure of Aman and Hesselmann (1984). Nitrogen was determined by a Kjeldahl procedure (Bradstreet 1965). Ammonia nitrogen and VFA were determined as described by Robinson et al. (1988). Rumen fluid samples for ammonia and lactic acid analysis were centrifuged at 200 X g for 10 min and ammonia and lactic acid were determined as described by Fawcett and Scott (1960) and by Mamer and Gibbs (1973) respectively. Chromium in feces was determined by atomic absorption spectrophotometry following the procedure described by Williams et al. (1962).

Statistical Analysis

Animal performance parameters, as well as rumen metabolites, were analyzed using SAS version 6.03 (Statistical Analysis System Institute, Inc. 1988) using a repeated measures design (Steel and Torrie 1980).

Diurnal patterns of ruminal pH and metabolites were fitted by polynomial analysis using polynomials up to the seventh degree. In preliminary analysis, it was determined seventh degree polynomials were suitable for most patterns. Fitted lines were used to calculate weighted means at each observation time for each cow and period, range (fitted maximum minus fitted minimum) and total hours less than 6 for pH for each cow and

period. Weighted means for each observation time, for each cow and period, were analyzed using analysis of variance, with a repeated measures design (Steel and Torrie 1980), to determine effects of treatments. Range, and total hours less than 6 for pH were analyzed in a model that included treatment, cow and period.

Rate of passage of PS was calculated from the concentration of chromium in the feces, after transformation to natural logarithms, and a series of linear regressions were then performed on the post-peak values (Hartnell and Satter 1979). The slope with the best fit was then taken as the rate of passage of PS from the rumen.

RESULTS AND DISCUSSION

Feed intake was adjusted daily during the 16-wk of study to assure minimal orts (<500 g). There were no differences in total DM intake between cows fed PS in the DAY or NIGHT (Table 17). While cumulative feed intake patterns of cannulated cows did significantly different ($P<0.05$) between treatments (Figure 6), this was primarily because the cannulated cows on DAY treatment consumed 2.9 kg d^{-1} more than cows on NIGHT treatment. The rate of consumption of mixed diet for cows on NIGHT treatment was similar in the first 75 min of morning feeding (5.4 vs 5.0 kg DM) and then was less, relative to DAY cows, for the balance of the day (Figure 6). However, there is no indication that the pattern of consumption of feed differed between treatments. In general, cows on both treatments did not eat at a constant rate throughout the day (Figure 6). Cows consumed feed at a faster rate for period of approximately two hours immediately after fresh feed was offered at 08:00 and 18:00 h and then consumed the

Table 17. Mean DM intake, body weight, condition score, milk yield and composition as influenced by time of feeding of protein supplement at 08:30 (DAY) or at 12:30 (NIGHT)

	Treatment		
	DAY	NIGHT	SEM
DM intake	——— kg d^{-1} ———		
Total	19.86	19.68	0.820
Mix diet	17.71	17.53	0.706
Supplement	2.16	2.15	0.072
Body weight (kg)	640	641	21.8
Condition score (unit)	2.33	2.51	0.126
Milk yeild	——— kg d^{-1} ———		
Milk (total)	19.88	18.44	1.387
AM milk	11.97	10.59	0.823
PM milk	7.91	7.84	0.578
Fat	0.74	0.68	0.047
Protein	0.64	0.61	0.041
Lactose	0.96	0.89	0.072
Milk composition	——— % ———		
Fat	3.79	3.72	0.144
Protein	3.23	3.31	0.073
Lactose	4.85	4.81	0.064

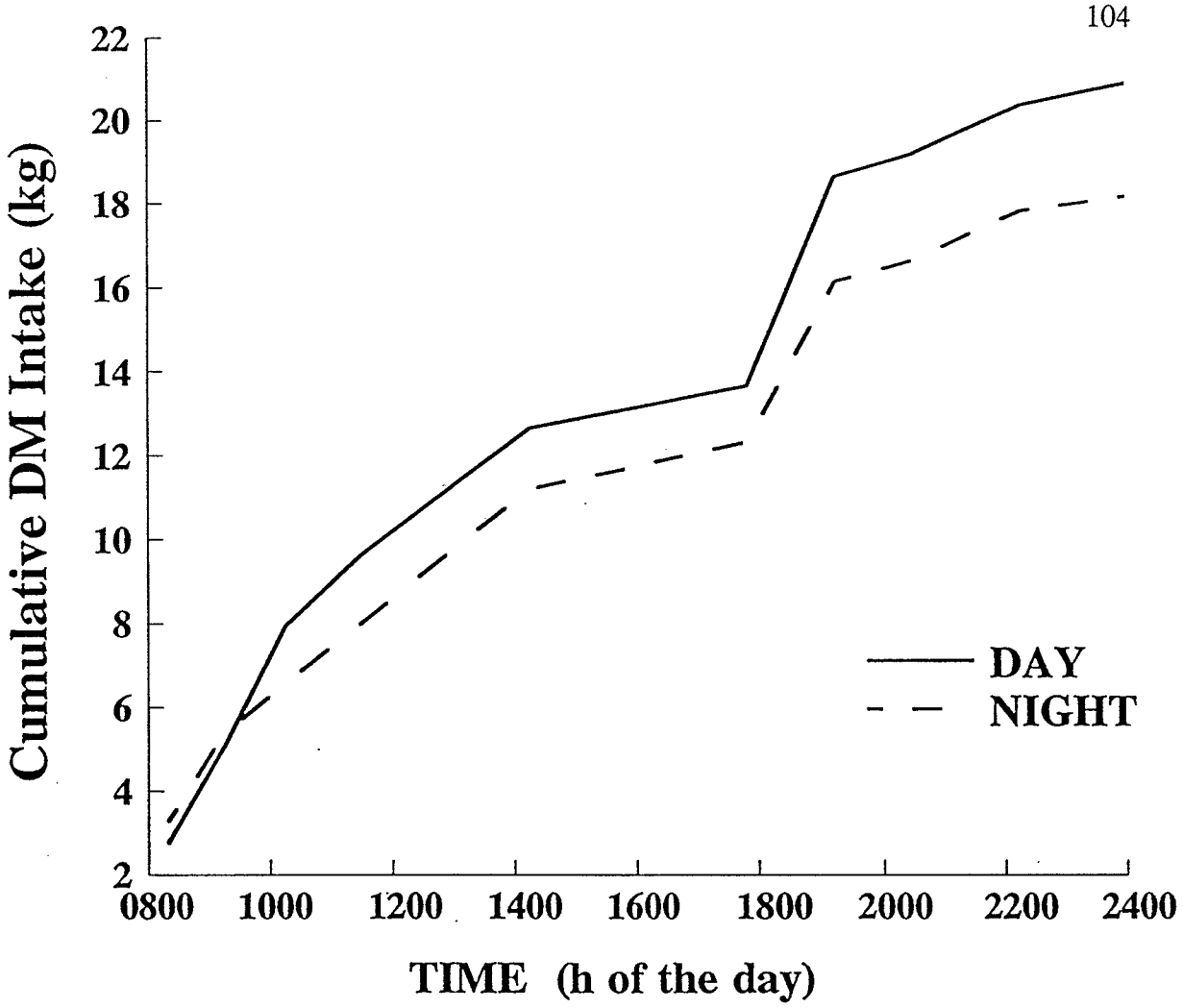

Fig. 6. The cumulative mix diet DM intake of dairy cows offered a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT). The protein supplement is not included in the mix diet.

remainder of the feed in a number of smaller meals.

Time of feeding PS did not ($P>0.05$) influence milk yield (Table 17). However, there was a trend to lower milk production for cows fed the PS at 12:30 h. This trend was almost completely the result of difference in the amount of milk produced in the morning milking. No influence of time of feeding PS on milk composition was occurred. Feed intake, body weight, condition score, milk yield and composition of cannulated cows as well as multiparous and primiparous cows are presented in Appendix III.

Treatment had no effect on in situ DM and CP disappearance from PS or on DM, CP and NDF disappearance from the mixed diet (Table 18). However, there was a trend for DM and CP disappearance to be higher in cows fed the PS at 08:30 h than in cows given their PS at 12:30 h. The trend was most marked for CP disappearance at 14 h (18:00-08:00) of ruminal incubation, which reflect night PS feeding, with a 4.5 percentage point increase for cows fed PS at 08:30 h. Although there was a trend for higher CP disappearance in cows on the DAY treatment, the rate of passage of PS was not influenced ($P>0.05$) by time of feeding PS. Robinson et al. (1993), in a similar experiment found that rate of rumen passage of CP was lower in the day feeding cycle for cows fed PS at 08:30 h and higher in the evening-feeding cycle for cows fed the PS at 12:30 h.

Time of feeding PS had no effect on ruminal pH ($P>0.05$) mean, range and hours below 6.0 during a 24-h feeding cycle (Table 19). The pH values were essentially equal shortly before feeding at 08:00 h. After 08:00 h, however, ruminal pH decreased 0.8 to 0.9 pH unit, over period of 3 h for both treatments, with the greatest total decline

Table 18. Disappearance of dry matter, protein, neutral detergent fibre and rate of passage as influenced by feeding protein supplement at different time of day

	Incubation time	Treatment		SEM
		DAY	NIGHT	
Protein supplement				
DM	10	77.6	75.7	1.87
	14	83.8	81.9	1.86
	24	90.6	89.8	0.69
Cp	10	77.6	74.8	2.71
	14	86.5	82.0	2.29
	24	95.7	93.4	0.94
K(h)		0.058	0.054	0.0027
Mixed diet				
DM	10	55.6	55.4	0.52
	14	58.9	57.6	0.47
	24	63.3	63.3	1.14
Cp	10	84.0	82.1	1.69
	14	85.0	84.5	0.32
	24	87.3	89.0	1.17
NDF	10	20.4	18.8	2.02
	14	24.3	21.7	2.23
	24	31.8	31.3	3.15

for cows on the DAY treatment resulting in a pH of 5.8 at 14:00 h (Figure 7). However, feeding PS at 12:30 h resulted in less fluctuation of rumen pH and fewer the number of hours pH below 6.0 (7.4 vs 9.2) for NIGHT and DAY treatment respectively before returning to prefeeding morning values.

Ruminal ammonia concentrations were higher ($P<0.05$) in cows fed the PS at night (Table 19), particularly between the hours of 02:00 and 10:00 (Figure 7). Rumen lactate concentrations, and ranges during the day, were not influenced by time of feeding PS (Table 19). Lactate concentration increased very rapidly in response to feeding and normally reached the highest value within one hour (data not shown), followed by a rapid decline.

Concentration of acetate, propionate, isobutyrate, and isovalerate were higher when PS was fed at night (Table 19). In contrast, butyrate and caproate concentration declined, and valerate concentrations were not treatment-influenced. VFA ranges during the day were generally not influenced by treatment, with the exception of butyrate and caproate which declined in response to feeding the PS at night. In general, diurnal patterns of VFA were treatment-influenced. Feeding PS at 12:30 h generally increased concentrations of VFA's during the period between 24:00 and 10:00 h except for propionate, butyrate and isobutyrate between 04:00 and 08:00 h (Figures 8 and 9) and for caproate between 08:00 and 12:00 h (Figure 11). In contrast, total VFA concentrations during 10:00 through 24:00 h period were generally lower, with the exception of propionate, which tended to be higher (Figure 8) and isobutyrate and isovalerate were not influenced by time of feeding PS (Figures 9 and 10).

Table 19. Effect of protein supplement feeding time on rumen pH, and concentration (mmol L^{-1}) of ammonia, lactate, and volatile fatty acids of dairy cows

	Treatment		
	DAY	NIGHT	SEM
pH			
Mean	6.2	6.1	0.04
Range	1.2	1.1	0.14
h<6.0	9.2	7.4	1.09
Ammonia			
Mean	3.42b	4.29a	0.089
Range	4.89	5.21	0.414
Lactate			
Mean	3.8	3.6	0.36
Maximum	10.3	10.2	1.93
Acetate			
Mean	68.1b	73.4a	0.78
Range	42.9	35.1	6.71
Propionate			
Mean	23.2b	28.9a	0.54
Range	20.7	17.3	1.42
Isobutyrate			
Mean	0.99b	1.06a	0.015
Range	0.42	0.48	0.059
Butyrate			
Mean	13.5a	12.8b	0.20
Range	12.3a	7.2b	1.45
Isovalerate			
Mean	1.53b	1.70a	0.049
Range	1.00	1.03	0.135
Valerate			
Mean	1.62	1.72	0.065
Range	1.72	1.16	0.204
Caproate			
Mean	0.60a	0.44b	0.026
Range	0.85a	0.43b	0.114
Total VFA	109.5b	120.1a	1.42
Acetate:Propionate	3.05a	2.71b	0.05

a-b means within a row with different letters are significantly (P<0.05) different.

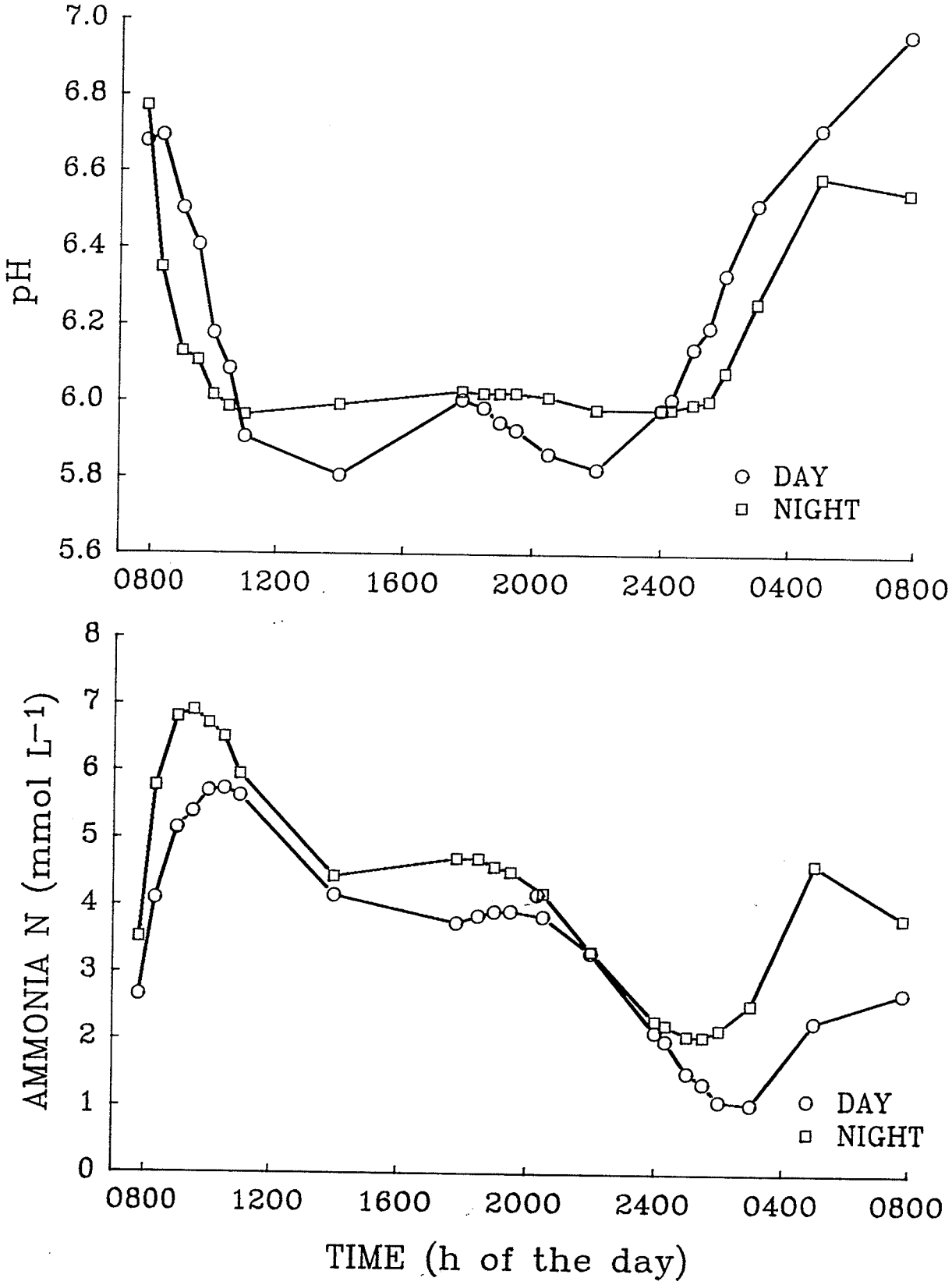

Fig. 7. Diurnal patterns of rumen pH and ammonia N concentration as influenced by time of feeding a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT).

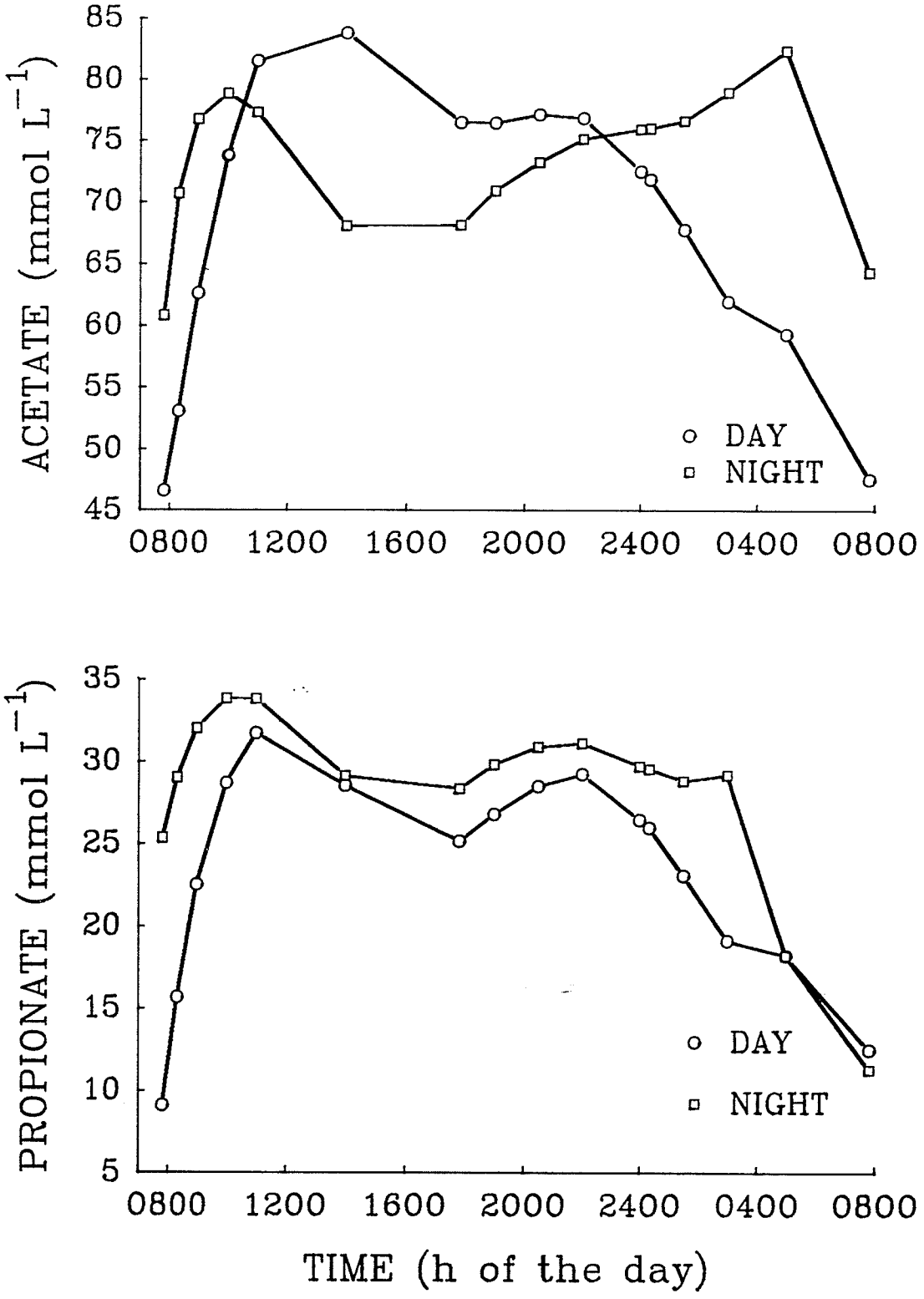

Fig. 8. Diurnal patterns of rumen acetate and propionate concentrations as influenced by time of feeding a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT).

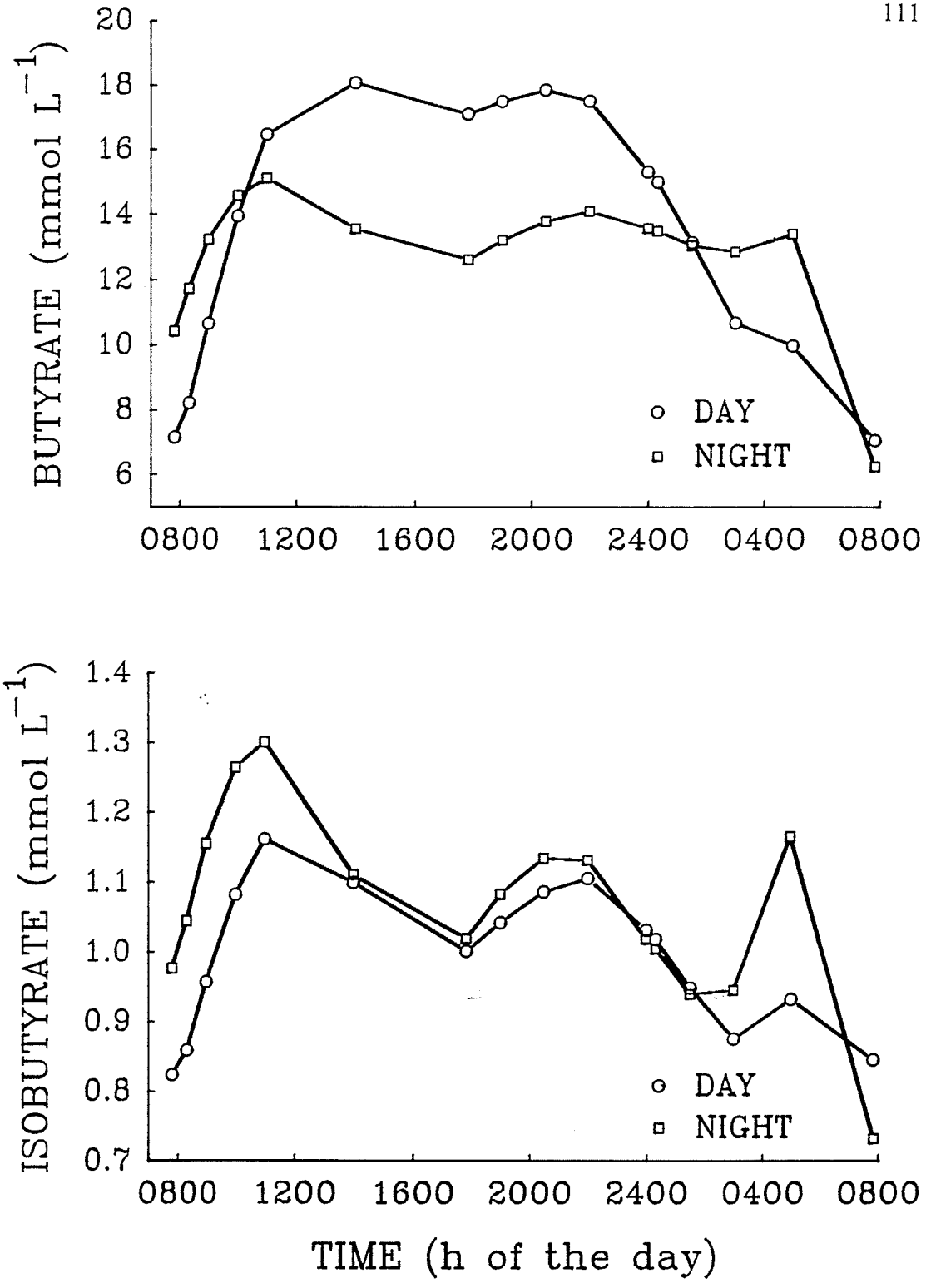

Fig. 9. Diurnal pattern of rumen butyrate and isobutyrate concentrations as influenced by time of feeding a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT).

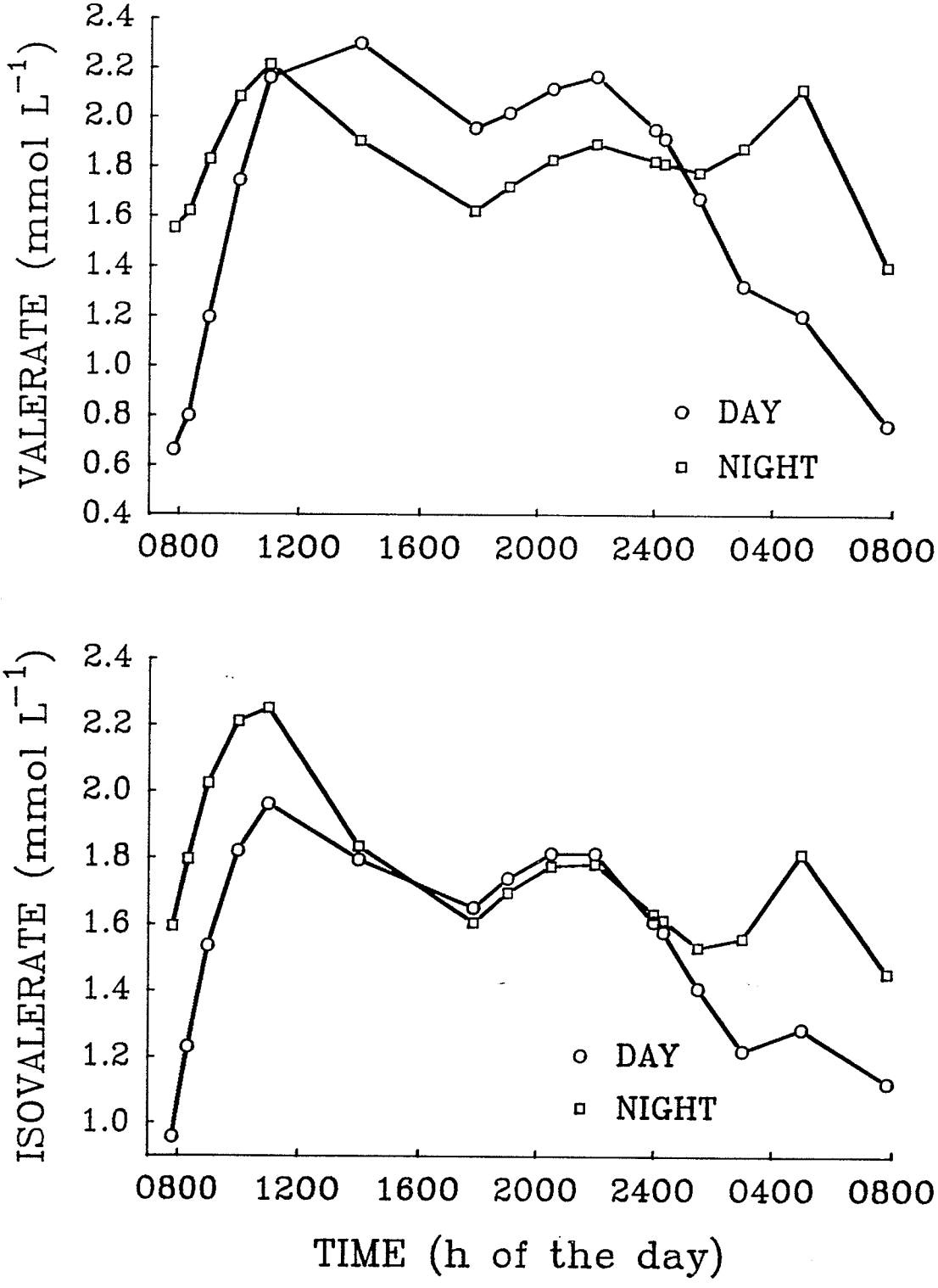

Fig. 10. Diurnal patterns of rumen valerate and isovalerate concentrations as influenced by time of feeding a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT).

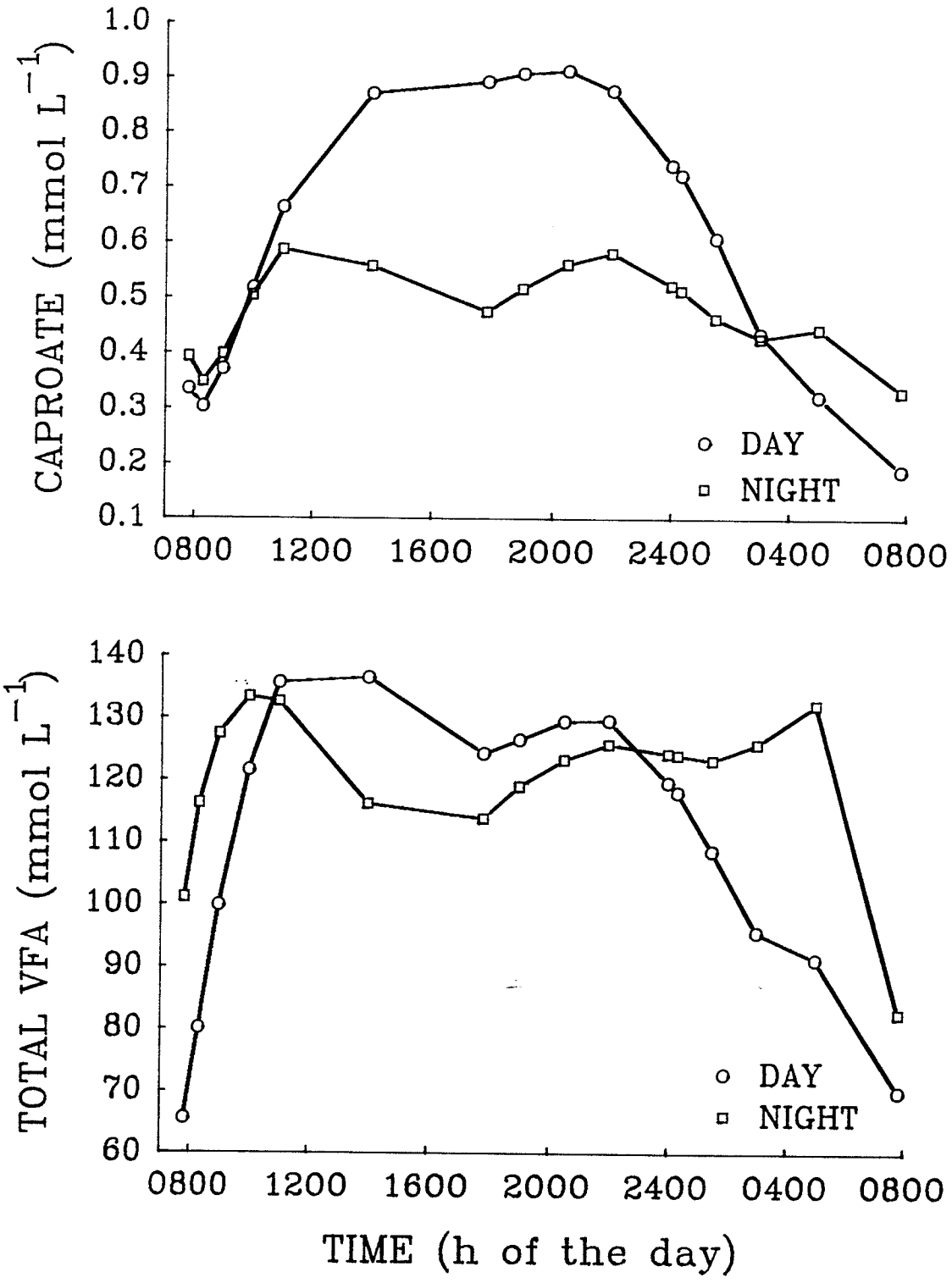

Fig. 11. Diurnal pattern of rumen caproate and total VFA concentrations as influenced by time of feeding a protein supplement either at 08:30 (DAY) or at 12:30 (NIGHT).

Cows in this study did not eat at a constant rate throughout the day and intake tended to follow a pattern of a large meal immediately after feeding with progressively smaller meals and longer times between meals as time from offer of feed increased. It has been suggested that such a meal pattern could contribute to increased rumen rate of passage immediately postfeeding (Chen et al. 1987), probably due to rumen expansion (Palmquist and Ronning 1963) and increased extent of primary rumen contractions (Colvin et al. 1978). Thus, there appears to be a period of time postfeeding that recently ingested feed particles can exit from the rumen with only a small degree of fermentation. It has also been suggested (Sissons et al. 1986) that muscular activity of the rumen wall is maximum at night which would facilitate a more rapid passage rate. Thus, if there is more than one periods of the day with relatively faster rates of passage of digesta from the rumen, one in the morning and another in the evening after fresh feed is offered and yet another at night, then this would probably explain, at least in part, the lack of difference in rate of passage of PS when fed at different times of the day in this study. Results of Robinson et al. (1993) can be interpreted to support this hypothesis as duodenal flow of protein was higher during the night for cows fed PS at 12:30 h whereas cows fed PS at 08:30 h had a higher daytime duodenal protein outflow.

Dry matter intake as well as milk yield and milk composition were not influenced by offering PS at different times of the day. However, feeding PS at 12:30 h vs 08:30 h led to less fluctuation in concentrations of ruminal metabolites. All of these results are consistant with suggestions of Robinson (1989) that productive benefits due to improved efficiencies of rumen fermentation may be quantitatively small compared with production

benefits due to increased intake associated with more frequent feeding.

GENERAL DISCUSSION

In rapidly growing ruminants or dairy cows in early lactation, production may be limited by a low dietary UIP. In these situations microbial protein synthesis is not sufficient to meet the animals AA requirements. Two approaches were taken to improve the dietary UIP. The first approach was to alter the rate of ruminal degradation of CM protein. When CM, a protein supplement that contains up to 40% crude protein, is fed to dairy cows with high protein diets a large part of the protein is degraded in the rumen (de Boer et al. 1987b) to NH_3-N which may be absorbed and excreted in the urine. However, if CM was protected from microbial degradation, the CM protein would be digested in the small intestine to AA which would be absorbed and available to the animal. Heat treatment has been used to decrease the rumen degradability of rapidly degraded protein sources such as CM and SBM (Plegge et al. 1985; Mir et al. 1984; McKinnon et al. 1990).

Although moderate heat may increase the protein flow to the lower digestive tract, excessive heat is detrimental to protein quality (Smith and Zebrowska 1989). Traditionally, heat damage of the protein has been measured as ADIN. However, ADIN is partially digestible (Rogers et al. 1986; Weiss et al. 1989). Moist heat treatment of CM at 127° C for 15 and 45 min in this study reduced the N solubility by 78 and 75% and increased ADIN contents by 37 and 169% as a per cent of total N for 15 and 45 min respectively. The added ADIN due to moist heat treatment of CM was digested by two thirds to three quarters in the total digestive tract of steers, which supports the finding of Arieli et al.(1989) and Pena et al.(1986) that ADIN can be digested.

Moist heat treatment effectively reduced the rate of rumen degradation of DM and N of CM, therefore increasing the amount of dietary DM and N available for digestion in the lower digestive tract. The increase in rumen protein escape may be a result of protein denaturation and reduction in solubility and degradation rate caused by heat-treatment which favours more rumen escape of intact undegraded proteins to the lower digestive tract. Vanhatalo and Aronen (1991) also reported reduced degradation of DM and N in the rumen by increased moist heat treatment of CM. Heat treatment of CM for 15 and 45 min in this study increased the disappearance of DM and N in the small intestine. As the amount of DM and N degradation was decreased in the rumen by heat treatment, the amount of DM and N available for absorption in the small intestine was increased. Therefore, the treatment shifted the disappearance of DM and N from the rumen to the small intestine. In general, there were no significant effects of treatment on disappearance of DM and N in the large intestine.

Although the 15 min treatment had little effect on total tract DM and N disappearance, the 45 min treatment resulted in the classical decrease in apparent digestibility found with prolonged heating of feed stuffs. However, the beneficial effects of increased digestibility of protein in the small intestine even with the excessive heating appeared to be greater than the decreased protein digestibility in total tract caused by limited heat damage. The degree of heating as practiced in a commercial canola seed processing plants during oil extraction and preparation of the meal is insufficient for maximizing the amount of CM protein available for absorption in the small intestine of ruminants.

Heat treatment did not change the individual AA composition of CM greatly other than the concentration of lysine and arginine was reduced by 30% and 9% respectively, with 45 min heat treatment compared to control. The loss may be a result of the Maillard reaction between sugar and amino groups where some of the lysine and arginine nitrogen is no longer accounted for in routine AA analysis. However, the amount of lysine and arginine available for absorption in the small intestine was increased by 44 and 98% respectively. Faldet et al. (1991) also found a reduction in lysine content by heat treatment of soybean meal with a corresponding increase in postruminal available lysine.

Rumen degradation of individual AA was reduced by moist heat treatment. Treatment increased the available AA for absorption in the small intestine compare to untreated CM. Similar to DM and N, treatments shifted the disappearance of AA from rumen to small intestine. Increased disappearance of AA from the small intestine with heat treated soybean meal and horsebean have also been observed (Cros et al. 1992; Kibelolaud et al. 1993). In general, moist heat treatment of CM in this study for 15 min was more effective than 45 min in increasing available AA in the small intestine.

Similar to DM and N disappearance in the large intestine, the 45 min heat treatment tended to increase the AA disappearance in the large intestine by an average of five percentage points compared to unheated CM.

Although the 15 min moist heat treatment did not reduce AA digestibility in the total tract, the 45 min heat treatment reduced total tract digestibility of AA. The decrease of 4-5 percentage points on total tract AA digestibility for 45 min heat treated CM was still small compared to the large increase in the small intestine AA availability.

The relative distribution of essential AA of unheated CM available for absorption in the small intestine are compared to the corresponding AA in milk protein. The first five limiting AA in unheated CM compared to the balance of AA in milk protein were isoleucine, tyrosine, lysine, valine and histidine. The sequence of first limiting essential AA changes somewhat for 15 and 45 min heat treated compare to unheated CM. The first four limiting essential AA for 15 and 45 min heat treated CM were lysine, isoleucine, tyrosine and valine which provided 37 to 43% and 31 to 32% to corresponding essential AA in milk protein, respectively. Histidine, the next limiting AA for untreated meal was relatively less limiting for heat treated CM. The order of the first four limiting essential AA of 15 and 45 min treated CM was essentially the same. Methionine, which has been mentioned as a limiting AA for milk production (Cros et al. 1992; Susmel et al. 1989), was not included in the first group of limiting AA for CM or heat treated CM. However, a somewhat reduced concentration of lysine in heat treated CM reduced its relative effectiveness to meet the essential AA requirement for milk protein. Feeding 15 min heat treated CM instead of commercial CM to lactating cows appeared to almost double the amount of AA presented to the small intestine for absorption and to improve the effectiveness of CM as a source of histidine.

The second approach taken to improve rumen "bypass" protein was feeding dietary protein supplement (PS) at different times of the day. Time of feeding the PS at DAY or NIGHT did not influence the DM, CP disappearance and rate of passage of PS. The feed intake of the cows tended to follow a pattern of a large meal immediately after feeding with progressively smaller meals and longer times between meals. Such a meal

pattern could contribute to increased rumen rate of passage immediately postfeeding (Chen et al. 1987), probably due to rumen expansion (Palmquist and Ronning 1963) and increased extent of primary rumen contractions (Colvin et al. 1978). Thus, there appears to be a period of time postfeeding that recently ingested feed particles can exit from the rumen with only a small degree of fermentation. It has also been suggested (Sissons et al. 1986) that muscular activity of the rumen wall is maximum at night which would facilitate a more rapid passage rate. Thus, if there is more than one period of the day with relatively faster rates of passage of digesta from the rumen, one in the morning and another in the evening after fresh feed is offered and yet another at night, then this would probably explain, at least in part, the lack of difference in rate of passage of PS when fed at different times of the day in this study. Results of Robinson et al. (1993) can be interpreted to support this hypothesis as duodenal flow of protein was higher during the night for cows fed PS at 12:30 h whereas cows fed PS at 08:30 h had a higher daytime duodenal protein outflow.

Dry matter intake and milk yield and composition were not influenced by offering PS at different times. Feeding PS at night led to less fluctuation of ruminal pH, increased ruminal ammonia, acetate, propionate, isobutyrate, isovalerate and decreased butyrate and caproate concentrations. Lactate and valerate concentrations were not treatment influenced. These results are in part confounded by the fact that a part of the diet was taken from the morning feeding and given at night. Robinson (1989), in a recent review has also suggested that productive benefits due to improved efficiencies of rumen fermentation may be quantitatively small compared with production benefits due

to the increased intake associated with more frequent feeding.

CONCLUSIONS

Moist heat treatment of CM at 127° C was effective in increasing the ruminally undegradable protein fraction of CM with corresponding increased digestion in the small intestine. Heating of CM increased the ADIN content of CM and increased the total tract digestibility of ADIN. However, the increase in ADIN was not effective in predicting change in lower digestive tract digestion of N or essential AA. The increased AA available for absorption in the small intestine by moist heat treatment of CM could benefit high producing dairy cows and rapidly growing calves. Rumen metabolite levels were changed by time of feeding protein supplement, with no effect on feed intake, milk yield and milk composition.

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APPENDIX I:

The effect of moist heat treatment of canola meal on acid detergent fibre and acid detergent insoluble nitrogen disappearance (%) in the digestive tract of steers.

Table I-1. The effect of moist heat treatment of canola meal on acid detergent fibre disappearance (% of initial) in the digestive tract of steers

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	-15.0a	-3.6a	1.3a	21.7a
15	-48.2b	-30.1b	-0.4a	13.5b
45	-61.6c	-38.7c	-19.0b	-12.1c
SEM	1.98	1.86	1.72	1.34
	Small intestine			
0	2.3*b*	12.1	22.1	31.5
15	10.8*ab*	18.0	29.7	32.3
45	16.1*a*	26.5	28.8	32.8
SEM	2.66	3.84	2.28	1.59
	Large intestine			
0	7.1	-2.3	-2.5	-1.7
15	0.8	-4.5	-1.9	-3.9
45	6.4	-6.2	2.1	5.47
SEM	3.76	3.36	3.85	3.17
	Total tract			
0	8.0*b*	7.3*c*	19.6*b*	29.8
15	10.5*b*	18.0*b*	27.9*ab*	28.1
45	22.5*a*	25.9*a*	30.6*a*	36.3
SEM	2.06	2.00	2.13	1.88

a-c Values within a column of each variable with different letters differ ($P<0.01$).
SEM=standard error of the mean (n=9).

Table I-2. The effect of moist heat treatment of canola meal on acid detergent insoluble nitrogen disappearance (% of initial) in the digestive tract of steers

Treatment	Rumen incubation time (h)			
	0.1	8	16	24
	Rumen			
0	27.7a	37.4a	46.3a	59.4a
15	-64.3c	-47.3	-25.7c	16.4b
45	-39.6b	-20.7	-5.4b	8.8c
SEM	1.22	3.40	1.67	1.26
	Small intestine			
0	67.2*a*	69.6*a*	71.7*a*	76.2*a*
15	61.6ab	59.0*b*	60.7*b*	72.3*b*
45	58.3*b*	67.4*ab*	72.6*a*	79.2*a*
SEM	1.63	1.84	1.66	0.97
	Large intestine			
0	-3.6*b*	-3.6*b*	1.8*b*	0.7
15	5.4*ab*	13.9*a*	14.2*a*	-0.5
45	13.6*a*	7.0*a*	4.1*ab*	4.3
SEM	2.13	1.92	2.32	1.53
	Total tract			
0	63.3*b*	66.0*b*	73.6	76.9*b*
15	66.2*ab*	72.4*a*	75.3	71.7*c*
45	71.9*a*	75.9*a*	77.3	83.0*a*
SEM	1.73	1.20	1.09	0.94

a-c Values within a column of each variable with different letters differ ($P<0.01$).
SEM=standard error of the mean (n=9).

APPENDIX II:

The effect of moist heat treatment of canola meal on individual amino acids disappearance (%) in the rumen, small intestine, large intestine and total tract after 0.1, 8, 16 and 24 h rumen incubation.

Table II-1. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the rumen after 0.1 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	21.6a	9.8b	10.0b	0.38
Valine	20.5a	21.8a	10.2b	1.17
Methionine	30.3a	0.7b	0.7b	2.15
Isoleucine	23.8a	19.2a	6.2b	1.18
Leucine	25.9a	9.7b	8.3b	0.39
Tyrosine	27.0a	3.9b	6.8b	0.67
Phenylalanine	29.3a	7.5c	10.3b	0.47
Histidine	31.4a	13.8c	17.3b	0.61
Lysine	22.8a	12.7b	11.6b	0.56
Arginine	30.4a	10.9b	11.4b	0.57
Aspartic acid	30.5a	14.0b	14.7b	0.33
Serine	24.9a	9.9b	11.4b	0.36
Glutamic acid	35.1a	26.9b	26.3b	0.30
Proline	28.0a	8.9b	9.2b	1.46
Glycine	28.0a	12.2c	14.7b	0.29
Alanine	26.8a	9.2c	12.4b	0.34
Cystine	28.2a	6.0b	3.7c	0.49
Essential AA	26.3a	11.0b	9.3b	0.54
Non-essential AA	28.8a	12.5b	13.2b	0.32
Total AA	27.5a	11.7b	11.2b	0.41

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-2. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the small intestine after 0.1 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	67.2ab	73.3a	63.1b	1.43
Valine	67.5a	60.3a	61.4a	2.22
Methionine	63.2b	82.3a	74.8b	3.15
Isoleucine	65.0a	63.5a	66.1a	2.22
Leucine	64.8b	74.4a	63.3b	1.95
Tyrosine	61.1b	77.1a	61.9b	1.72
Phenylalanine	60.3b	75.1a	55.1b	1.93
Histidine	61.3b	73.6a	61.1b	1.50
Lysine	68.4ab	73.1a	64.3b	1.48
Arginine	62.9b	77.7a	67.4b	1.80
Aspartic acid	59.7b	70.5a	58.1b	1.79
Serine	63.9b	72.7a	59.2b	1.72
Glutamic acid	62.2a	68.5a	63.2a	1.59
Proline	60.7b	76.6a	67.4b	1.65
Glycine	62.1b	74.6a	62.8b	1.55
Alanine	63.9b	75.4a	61.0b	1.73
Cystine	61.4b	76.3a	70.6a	1.84
Essential AA	64.2b	73.0a	63.9b	1.68
Non-essential AA	62.0b	73.5a	63.2b	1.55
Total AA	63.1b	73.3a	63.5b	1.60

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-3. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the large intestine after 0.1 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	3.4	6.1	5.6	0.92
Valine	5.8	7.7	5.9	0.95
Methionine	0.7b	6.8a	5.5ab	1.04
Isoleucine	5.6	7.9	4.7	1.05
Leucine	3.2	7.1	7.7	1.11
Tyrosine	0.5b	6.8a	4.0ab	1.24
Phenylalanine	2.9	6.9	7.5	1.43
Histidine	1.9	5.4	5.8	0.82
Lysine	3.0	0.8	-5.9	5.71
Arginine	2.1	5.2	5.9	0.78
Aspartic acid	1.7b	5.7ab	8.3a	1.37
Serine	2.6	6.4	9.1	1.36
Glutamic acid	0.9	2.2	5.0	1.33
Proline	2.8	5.0	5.0	1.29
Glycine	2.3	5.1	6.2	1.12
Alanine	-7.2b	-9.2b	-18.3a	0.86
Cystine	0.9b	6.8ab	11.7a	2.14
Essential AA	2.9	6.1	4.7	1.23
Non-essential AA	0.6	3.1	3.9	1.15
Total AA	1.8	4.6	3.4	0.97

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-4. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the total tract after 0.1 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	91.9a	89.2a	80.3b	0.80
Valine	93.5a	89.7a	80.0b	1.06
Methionine	94.1a	89.9a	83.3c	0.78
Isoleucine	94.0a	90.5a	79.4b	1.18
Leucine	93.5a	91.3a	81.6b	0.85
Tyrosine	88.3a	87.5a	74.1b	0.88
Phenylalanine	92.0a	89.5a	74.8b	1.03
Histidine	94.2a	92.9a	86.0b	0.60
Lysine	93.4a	91.2a	82.0b	0.91
Arginine	95.0a	93.7a	86.8b	0.64
Aspartic acid	91.6a	90.1a	81.1b	0.78
Serine	91.1a	89.0a	79.7b	0.80
Glutamic acid	98.1a	97.6a	94.5b	0.23
Proline	91.2a	90.5a	81.6b	0.68
Glycine	92.1a	91.8a	83.7b	0.67
Alanine	92.8a	90.8a	81.7b	0.86
Cystine	90.3a	89.1ab	85.9b	0.81
Essential AA	93.0a	90.5a	80.7b	0.90
Non-essential AA	92.4a	91.3a	84.0b	0.60
Total AA	92.7a	90.9a	82.4b	0.76

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-5. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the rumen after 8 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	41.9a	11.7c	16.3b	0.98
Valine	42.0a	19.4c	27.4b	1.65
Methionine	44.4a	17.2b	7.1c	1.99
Isoleucine	44.0a	18.2c	25.7b	1.63
Leucine	47.8a	13.6b	16.2b	0.97
Tyrosine	41.9a	6.1b	2.7b	1.09
Phenylalanine	48.0a	16.1b	12.2b	0.95
Histidine	57.4a	24.7b	26.4b	0.80
Lysine	53.0a	24.7b	23.0b	0.82
Arginine	55.3a	17.7c	22.3b	0.98
Aspartic acid	45.0a	13.5b	15.5b	0.89
Serine	45.4a	14.9b	13.9b	0.95
Glutamic acid	79.5a	36.7b	31.8c	0.81
Proline	57.6a	14.4b	18.6b	1.22
Glycine	50.6a	19.0b	18.5b	0.89
Alanine	45.9a	14.6b	15.9b	0.96
Cystine	61.6a	25.7b	15.9c	0.80
Essential AA	47.6a	16.9b	17.9b	0.90
Non-essential AA	55.1a	19.8b	18.6b	0.82
Total AA	51.3a	18.4b	18.3b	0.86

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-6. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the small intestine after 8 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	49.2c	73.3a	60.8b	1.76
Valine	49.3b	64.1a	48.2b	2.41
Methionine	50.6b	69.8a	71.3a	3.05
Isoleucine	48.1b	65.9a	49.6b	2.53
Leucine	44.9b	72.2a	60.1b	2.10
Tyrosine	48.0b	76.4a	70.2a	2.02
Phenylalanine	43.8b	67.7a	58.1a	2.48
Histidine	36.7b	63.5a	55.2a	1.69
Lysine	40.2b	62.3a	56.5a	1.62
Arginine	39.5c	71.5a	59.8b	1.68
Aspartic acid	47.0a	63.8a	61.8a	5.07
Serine	45.2a	63.2a	61.5a	4.07
Glutamic acid	18.4b	51.7a	60.5a	4.12
Proline	33.0b	64.2a	61.3a	4.86
Glycine	41.3b	68.5a	62.4a	1.67
Alanine	46.4b	70.5a	61.9a	2.04
Cystine	29.2b	57.7a	62.3a	1.89
Essential AA	45.0c	68.7a	59.0b	1.87
Non-essential AA	37.2b	62.8a	61.7a	2.95
Total AA	41.1b	68.1a	60.3a	1.78

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-7. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the large intestine after 8 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	0.8	6.1	8.2	1.58
Valine	1.2	8.7	9.9	1.82
Methionine	-2.1b	7.5a	9.7a	1.95
Isoleucine	1.1b	9.3ab	10.2a	1.89
Leucine	0.7b	7.4ab	10.7a	2.03
Tyrosine	-1.1b	6.1ab	9.9a	1.88
Phenylalanine	0.4b	6.2ab	12.4a	2.34
Histidine	0.0b	5.7ab	8.5a	1.60
Lysine	0.3b	5.7ab	7.9a	1.51
Arginine	0.3b	5.4ab	8.4a	1.48
Aspartic acid	0.3b	5.6ab	10.2a	1.74
Serine	0.7	14.4	10.9	5.15
Glutamic acid	0.2	12.5	4.3	6.03
Proline	0.5	14.4	7.3	5.38
Glycine	0.6	14.0	8.3	5.42
Alanine	-6.9	3.2	-11.7	6.17
Cystine	-1.8	16.1	10.3	5.15
Essential AA	0.2b	6.8ab	9.6a	1.79
Non-essential AA	-0.9b	3.5ab	5.7a	1.31
Total AA	-0.4b	5.2ab	7.2a	1.54

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-8. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the total tract after 8 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	91.8a	90.4a	86.2b	0.50
Valine	92.5a	91.1a	86.6b	0.56
Methionine	92.9a	92.9a	88.8b	0.51
Isoleucine	93.1a	92.3a	86.9b	0.53
Leucine	93.4a	92.6a	87.9b	0.50
Tyrosine	88.8a	88.7a	83.5b	0.61
Phenylalanine	92.3a	89.5a	83.5b	0.71
Histidine	94.1a	93.4a	90.7b	0.35
Lysine	93.4a	92.1a	88.1b	0.38
Arginine	95.1a	94.3a	91.2b	0.29
Aspartic acid	92.3a	91.2a	87.2b	0.46
Serine	91.3a	90.2a	86.0b	0.56
Glutamic acid	98.1a	97.9a	96.6b	0.14
Proline	91.1a	91.5a	87.1b	0.45
Glycine	92.5a	92.7a	88.9b	0.46
Alanine	93.1a	92.1a	88.0b	0.55
Cystine	89.0a	90.4a	88.6b	0.34
Essential AA	92.8a	91.7a	87.4b	0.44
Non-essential AA	92.5a	92.3a	88.9b	0.41
Total AA	92.6a	92.0a	88.1b	0.43

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-9. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the rumen after 16 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	57.7a	19.0b	14.6b	1.51
Valine	57.2a	23.5b	28.3b	1.77
Methionine	62.3a	21.8b	12.9c	1.41
Isoleucine	58.0a	20.3b	24.0b	1.73
Leucine	60.2a	17.7b	11.7b	1.41
Tyrosine	54.7a	10.0b	-1.6c	1.57
Phenylalanine	60.1a	20.4b	9.3c	1.25
Histidine	71.8a	33.3b	25.5c	0.94
Lysine	64.7a	29.9b	21.4c	1.39
Arginine	68.7a	25.3b	20.8b	1.24
Aspartic acid	57.6a	15.6b	9.0b	1.63
Serine	58.0a	22.2b	12.2c	1.40
Glutamic acid	86.5a	44.9b	31.8c	0.61
Proline	70.7a	30.8b	21.3c	1.19
Glycine	63.8a	25.3b	16.7c	1.28
Alanine	59.1a	20.2b	13.1c	1.51
Cystine	72.5a	37.2b	23.4c	1.04
Essential AA	61.5a	22.1b	16.7b	1.35
Non-essential AA	66.9a	28.0b	18.2c	1.19
Total AA	64.2a	25.1b	17.5c	1.26

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-10. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the small intestine after 16 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	35.8b	71.7a	66.0a	2.77
Valine	36.6b	67.8a	49.8b	3.50
Methionine	33.9b	68.5a	72.3a	2.13
Isoleucine	36.5c	71.5a	54.2b	3.52
Leucine	34.9b	74.2a	67.9a	2.98
Tyrosine	37.8b	78.2a	80.0a	2.69
Phenylalanine	33.8b	69.7a	66.4a	2.78
Histidine	22.1b	59.9a	59.2a	1.76
Lysine	30.2b	62.7a	61.3a	2.55
Arginine	27.3b	68.5a	63.7a	2.49
Aspartic acid	36.2b	75.6a	71.6a	3.01
Serine	35.2b	67.9a	67.0a	2.70
Glutamic acid	12.1c	52.8b	61.9a	1.08
Proline	22.7b	61.0a	62.0a	2.30
Glycine	30.1b	67.0a	67.3a	2.39
Alanine	35.5b	71.3a	68.2a	2.87
Cystine	19.7b	51.2a	58.1a	2.36
Essential AA	32.9b	69.3a	64.1a	2.67
Non-essential AA	27.4b	63.8a	65.2a	2.35
Total AA	30.1b	66.5a	64.6a	2.49

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-11. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the large intestine after 16 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	0.1	2.2	4.3	1.42
Valine	0.3	2.8	7.7	1.80
Methionine	-1.2	3.0	3.5	1.21
Isoleucine	0.4	3.2	7.5	1.76
Leucine	0.2	2.6	7.2	1.81
Tyrosine	0.5	2.9	5.0	1.80
Phenylalanine	0.1	1.0	6.9	1.99
Histidine	1.6	1.6	5.2	1.21
Lysine	0.3	1.7	4.6	1.31
Arginine	0.3	1.8	5.7	1.35
Aspartic acid	0.1	1.7	5.4	1.56
Serine	0.1	2.2	5.7	1.52
Glutamic acid	0.1	0.7	2.4	0.58
Proline	0.7	1.8	3.1	1.15
Glycine	0.7	1.9	4.0	1.27
Alanine	-5.3a	-6.3a	-13.2b	0.56
Cystine	-1.8	3.2	5.7	1.71
Essential AA	0.3	2.3	5.2	1.62
Non-essential AA	-0.8	0.7	1.9	1.11
Total AA	-0.3	1.5	3.6	1.41

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-12. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the total tract after 16 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	93.5a	92.9a	85.8b	0.49
Valine	94.1a	94.1a	85.9b	0.34
Methionine	95.0a	93.3a	89.2b	0.45
Isoleucine	94.9a	95.0a	85.9b	0.42
Leucine	95.2a	94.5a	87.2b	0.37
Tyrosine	93.0a	91.1a	83.8b	0.46
Phenylalanine	94.0a	91.2b	82.9c	0.53
Histidine	95.5a	94.8a	90.2b	0.30
Lysine	95.2a	94.3a	87.4b	0.36
Arginine	96.3a	95.6a	90.3b	0.27
Aspartic acid	93.9a	92.9a	86.7b	0.40
Serine	93.3a	92.3a	85.4b	0.54
Glutamic acid	98.6a	98.4a	96.1b	0.15
Proline	94.1a	93.5a	86.8b	0.35
Glycine	94.6a	94.1a	88.4b	0.45
Alanine	94.8a	93.7a	87.2b	0.55
Cystine	90.4a	91.5a	87.9b	0.45
Essential AA	94.7a	93.7a	87.4b	0.28
Non-essential AA	94.2a	93.8a	88.9b	0.27
Total AA	94.4a	93.7a	88.1b	0.28

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-13. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the rumen after 24 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	77.4a	31.0b	27.1b	0.92
Valine	78.1a	28.4c	43.1b	1.14
Methionine	79.9a	33.9b	20.4c	0.82
Isoleucine	78.4a	26.2c	40.5b	1.13
Leucine	78.3a	26.9b	23.0b	1.06
Tyrosine	75.0a	23.2b	1.2c	1.23
Phenylalanine	76.8a	30.9b	15.1c	0.90
Histidine	84.5a	44.5b	37.6c	0.66
Lysine	81.2a	40.2b	33.4c	0.80
Arginine	83.8a	34.5b	29.7c	0.81
Aspartic acid	77.1a	23.9b	13.7c	1.05
Serine	76.4a	32.4b	19.8c	0.90
Glutamic acid	93.1a	54.8b	41.0c	0.62
Proline	83.4a	45.3b	32.3c	0.58
Glycine	80.0a	36.0b	24.1c	0.78
Alanine	77.2a	30.2b	20.8c	0.92
Cystine	84.0a	49.5b	32.0c	0.54
Essential AA	79.3a	32.0b	27.1c	0.91
Non-essential AA	81.6a	38.9b	26.2c	0.70
Total AA	80.5a	35.4b	26.7c	0.80

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-14. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the small intestine after 24 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	17.3b	61.6a	57.3a	1.64
Valine	17.1c	63.9a	40.4b	1.85
Methionine	15.9b	59.5a	65.8a	1.55
Isoleucine	17.1c	66.3a	41.9b	1.96
Leucine	17.7b	66.9a	61.5a	1.90
Tyrosine	18.3c	68.3b	80.2a	2.10
Phenylalanine	18.2b	61.4a	64.9a	1.96
Histidine	11.5b	49.9a	49.8a	1.32
Lysine	14.6b	53.6a	52.8a	1.47
Arginine	12.7b	60.3a	57.8a	1.48
Aspartic acid	17.5b	69.0a	71.0a	1.79
Serine	17.9b	59.9a	63.9a	1.69
Glutamic acid	5.6c	43.4b	53.9a	0.90
Proline	11.0b	48.2a	52.8a	1.16
Glycine	14.8b	58.0a	62.4a	1.45
Alanine	18.4b	63.5a	64.8a	1.69
Cystine	9.0c	40.7b	53.4a	1.09
Essential AA	16.0b	61.2a	57.2a	1.69
Non-essential AA	13.4b	54.7a	60.3a	1.34
Total AA	14.7b	57.9a	58.8a	1.51

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-15. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the large intestine after 24 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	0.1b	-0.5b	5.8a	1.26
Valine	-0.3b	-0.5b	6.5a	1.34
Methionine	-0.5	0.0	5.2	1.35
Isoleucine	-0.2b	-0.1b	7.6a	1.50
Leucine	0.4ab	-0.9b	6.6a	1.46
Tyrosine	0.5ab	-1.1b	7.1a	1.63
Phenylalanine	0.2ab	-2.6b	7.8a	1.78
Histidine	-0.1	-0.4	5.2	1.18
Lysine	0.3	-0.7	4.8	1.17
Arginine	0.3b	-0.4b	5.5a	1.09
Aspartic acid	0.2ab	-1.1b	5.7a	1.23
Serine	0.4ab	-1.2b	6.2a	1.34
Glutamic acid	0.1b	-0.2b	2.5a	0.46
Proline	0.7b	-0.5b	5.8a	1.08
Glycine	0.9a	-0.9b	5.2a	1.12
Alanine	-4.4a	-7.7ab	-9.0b	0.90
Cystine	-1.0b	0.2ab	4.8a	1.06
Essential AA	0.1b	-0.7b	6.2a	1.35
Non-essential AA	-0.4	-1.6	3.0	1.00
Total AA	-0.1	-1.1	4.3	1.20

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

Table II-16. The effect of moist heat treatment of canola meal on amino acid disappearance (%) in the total tract after 24 h incubation

	Treatment			
Amino acid	0	15	45	SEM
Threonine	94.2a	92.4ab	89.7b	0.70
Valine	94.3a	92.2ab	89.4b	0.86
Methionine	95.2a	93.6ab	91.3b	0.72
Isoleucine	95.1a	92.8ab	89.4b	0.94
Leucine	95.7a	93.4ab	90.6b	0.84
Tyrosine	93.2a	90.7ab	87.9b	0.89
Phenylalanine	94.4a	90.3ab	87.1b	1.01
Histidine	95.5a	94.4ab	92.1b	0.69
Lysine	95.6a	93.4ab	90.6b	0.70
Arginine	96.4a	94.7ab	92.6b	0.59
Aspartic acid	94.3a	92.3ab	89.8b	0.64
Serine	94.0a	91.7ab	89.2b	0.69
Glutamic acid	98.7a	98.0ab	97.2b	0.22
Proline	94.5a	93.4a	90.3b	0.54
Glycine	95.1a	93.5ab	91.2b	0.58
Alanine	95.3a	92.8ab	90.8b	0.72
Cystine	91.6	91.0	89.7	0.51
Essential AA	95.0a	92.8ab	90.1b	0.77
Non-essential AA	94.8a	93.3ab	91.2b	0.55
Total AA	94.9a	93.0ab	90.4b	0.61

a-c Values within a row of each variable with different letters differ ($P<0.01$).
SEM = standard error of the mean (n=9).

APPENDIX III:

Influence of time of feeding a protein supplement on DM intake, body weight, condition score, milk yield and composition of dairy cows during 16 wks.

Table III-1. Intake, body weight, condition score, milk yield and composition of cannulated cow during 16 wks as influenced by time of feeding of protein supplement

	Treatment		
	DAY	NIGHT	SEM
DM intake (kg d^{-1})			
Total	21.86	20.58	1.174
Mix diet	19.51	18.33	1.047
Supplement	2.36	2.24	0.127
Body weight (kg)	648b	739a	24.3
Condition score (unit)	2.10	2.64	0.116
Milk yeild (kg d^{-1})			
Milk (total)	21.94	19.89	2.210
AM milk	13.13	11.37	1.424
PM milk	8.81	8.52	0.803
Fat	0.82	0.73	0.096
Protein	0.70	0.66	0.070
Lactose	1.03	0.93	0.127
Milk composition (%)			
Fat	3.72	3.73	0.277
Protein	3.20	3.37	0.132
Lactose	4.69	4.45	0.154

Table III-2. Intake, body weight, condition score of dairy cow during 16 wks as influenced by time of feeding of protein supplement

	Treatment		
	DAY	NIGHT	SEM
All cows			
DM intake (kg d^{-1})			
Total	19.86	19.68	0.820
Mix diet	17.71	17.53	0.706
Supplement	2.16	2.15	0.072
Body weight (kg)	640	641	21.8
Condition score (unit)	2.33	2.51	0.126
Multiparous cows			
DM intake (kg d^{-1})			
Total	21.55	21.29	1.036
Mix diet	19.29	18.95	0.895
Supplement	2.34	2.32	0.094
Body weight (kg)	666	715	30.7
Condition score (unit)	2.20	2.46	0.180
Primiparous cows			
DM intake (kg d^{-1})			
Total	18.17	17.70	0.996
Mix	16.13	15.79	1.056
Supplement	1.98	1.95	0.102
Body weight (kg)	614	567	30.7
Condition score (unit)	2.47	2.55	0.182

Table III-3. Milk yield and composition of all cows during 16 wks as influenced by time of feeding of protein supplement

	Treatment		
	DAY	NIGHT	SEM
Milk yeild	——— kg d^{-1} ———		
Milk (total)	19.88	18.44	1.387
AM milk	11.97	10.59	0.823
PM milk	7.91	7.84	0.578
Fat	0.74	0.68	0.047
Protein	0.64	0.61	0.041
Lactose	0.96	0.89	0.072
Milk composition	——— % ———		
Fat (total)	3.79	3.72	0.144
AM fat	3.38	3.50	0.158
PM fat	4.32	4.22	0.206
Protein (total)	3.23	3.31	0.073
AM protein	3.21	3.34	0.078
PM protein	3.26	3.29	0.084
Lactose (total)	4.85	4.81	0.064
AM lactose	4.80	4.78	0.073
PM lactose	4.89	4.68	0.076

Table III-4. Milk yield and composition of multiparous cows during 16 wks as influenced by time of feeding of protein supplement

	Treatment		
	DAY	NIGHT	SEM
Milk yeild	——— kg d^{-1} ———		
Milk (total)	22.02	19.96	1.875
AM milk	13.15	11.29	1.113
PM milk	8.86	8.67	0.781
Fat	0.80	0.74	0.070
Protein	0.69	0.66	0.055
Lactose	1.05	0.94	0.098
Milk composition	——— % ———		
Fat (total)	3.63	3.76	0.195
AM fat	3.29	3.51	0.210
PM fat	4.23	4.15	0.275
Protein (total)	3.17	3.36	0.098
AM protein	3.17	3.40	0.103
PM protein	3.19	3.27	0.112
Lactose (total)	4.73	4.66	0.087
AM lactose	4.69	4.61	0.097
PM lactose	4.78	4.48	0.101

Table III-5. Milk yield and composition of primiparous cows during 16 wks as influenced by time of feeding of protein supplement

	Treatment		
	DAY	NIGHT	SEM
Milk yeild	——— kg d^{-1} ———		
Milk (total)	17.74	16.92	2.033
AM milk	10.79	9.90	1.206
PM milk	6.95	7.02	0.847
Fat	0.69	0.62	0.065
Protein	0.58	0.55	0.060
Lactose	0.88	0.83	0.106
Milk composition	——— % ———		
Fat (total)	3.94	3.68	0.213
AM fat	3.47	3.49	0.235
PM fat	4.42	4.30	0.307
Protein (total)	3.29	3.27	0.107
AM protein	3.26	3.27	0.116
PM protein	3.23	3.32	0.125
Lactose (total)	4.97	4.96	0.094
AM lactose	4.91	4.96	0.108
PM lactose	5.01	4.88	0.113