HEAT TREATMENT OF CANOLA MEAL AND SUBSEQUENT AVAILABILITY OF RUMEN ESCAPE PROTEIN AND AMINO ACIDS IN RUMINANTS

A Thesis

Submitted to the Faculty

of

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The University of Manitoba

by

Tobias Atali Onyango

In Partial Fulfilment of the

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of

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TOBIAS ATALI ONYANGO

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

DOCTOR OF PHILOSOPHY

ABSTRACT

As a continuing effort to increase the level of rumen escape protein and post ruminally available amino acids (AA) in high producing ruminants of today such as dairy cows in early lactation, three trials were conducted using three non-lactating Holstein cows fitted with rumen and T-shape proximal duodenal canulae; fifty adults Single Comb White Leghorn cockerels; and thirty two lactating cows. The purpose was to determine the effect of moist heat treatment of canola meal (CM) as a source of rumen escape protein as measured from *in situ* rumen and lower gastro intestinal (GI) tract degradability of: dry matter (DM), fiber, protein, essential (EAA) and non essential (NEAA); availability of EAA, NEAA and true nitrogen-corrected metabolizable energy (TME_n) in precision-fed cockerels; and feed intake, milk production, milk composition and body weight change in dairy cows in early lactation. Four different batches of commercial CM were exposed to moist heating at 110°C for 0 (CM 0), 23 (CM 23), 45 (CM 45) and 60 (CM 60) min through a steam jacketed conveyor and slowly steeped at passage rates of 200, 110 and 90 kg h^{-1} respectively. A bypass protein supplement (Bi) made from a mixture of animal-vegetable products was formulated for comparison with CM 60, based on similar calculated rumen escape EAA.

Trial I was an *in situ* 3 X 3 Latin Square design using a sequence of rumen *in situ* bag incubations, *in vitro* pepsin-HCl incubations and lower GI tract mobile nylon bag technique. Two bag types, monofilament and multifilament were compared and effect of inclusion of similar or different test supplement in cows' diet was tested. Crude protein (CP) content of CM 60 and Bi were similar ($P>0.05$), but heat treatment increased

($P<0.05$) neutral detergent fibre (NDF), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitogen (NDIN) contents. Rumen degradation of protein from CM 0 was higher ($P<0.05$) than other levels of heat treated CM. Type of protein in cows' diet affected ($P<0.05$) rumen protein degradation and subsequent rumen disappearance of nitrogen. Protein and EAA degradation was higher ($P<0.05$) in monofilament bag type than multifilament type. Lower GI tract degradation of protein from both bag types was not affected ($P<0.05$) by removal of pepsin-HCl digestion step. Heat treatment did not ($P<0.05$) change individual EAA and NEAA but tended to decrease ($P<0.05$) concentration of arginine, leucine, lysine, methionine and valine. Disappearance of EAA 12 h post rumen incubation was reduced ($P<0.05$) in the rumen but increased ($P<0.05$) in the lower GI tract with 60 min heat treatment and resulted in increased ($P<0.05$) fecal excretion of, arginine, histidine, isoleucine, lysine, methionine and threonine. These changes resulted in an increased ($P<0.05$) 80-90% available EAA quantity in the small intestine.

Trial II was a randomized block design using five groups each of ten precision-fed cockerels. Test supplements were similar to those in Trial I. Heat treatment did not ($P<0.05$) change TME_n contents of CM but decreased ($P<0.05$) availability of EAA and NEAA [g $(16g\ N)^{-1}$] and as percent of meal fed. The Bi supplement was similar to CM 60 for EAA availability.

Trial III involved fourteen primiparous (PP) and eighteen multiparous (MP) Holstein cows two weeks post-partum arranged in a split-plot design for 12 lactation weeks. Heat treated CM 60 or Bi substituted CM 0 as Iso-nitrogenous and iso-caloric

diets fed as total mixed ration once daily. Dry matter intake (DMI) of CM 0 and CM 60 was higher ($P<0.05$) than Bi. The Bi diet reduced ($P<0.05$) DMI compared with other CM diets but resulted in equal milk yield when averaged over all cows. Milk yield by PP cows was higher in CM 60 than CM 0 and lower ($P<0.05$) in Bi diet than CM 0 and CM 60. Parity affected ($P<0.05$) milk yield with MP cows producing 34% more milk d^{-1} than PP cows. Treatment and parity did not affect milk contents of fat, protein, SNF, body weight and condition score over the experimental time.

Results from these trials indicated decreased ($P<0.05$) rumen degradation of CM with increased heating, resulting in higher levels of protein and AA availability post-ruminally. Although the potential for rumen escape of heat treated CM increased $P<0.05$), milk yield and composition did not differ ($P<0.05$) from the unheated CM> It is suggested that higher temperatures above 110°C or longer heating times than 60 min be adopted with continued use of CM in dairy rations.

DEDICATION

To my wife, Grace and to our children, Arnold, Geraldine, Preston and Michael. Without their support, encouragement, understanding and patience I would not have accomplished this task as far away from home as it was. Their encouragement and that of my parents, Serefina and Naphtali, was indeed a constant source of inspiration to me throughout the time of this programme.

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FORWARD

This thesis is written in a manuscript format and three manuscripts will be submitted for publication. The authors for manuscript I will be T.A. Onyango, J.R. Ingalls and G.H. Crow. The authors for manuscript II and III will be T.A. Onyango and J.R. Ingalls.

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

1. Onyango, T.A. and Ingalls, J.R. 1994. Feed intake and lactation response of cows fed heat treated canola meal and high undegradable protein supplements in early lactation. J. Anim. Sci. 72:, suppl. 1 / J. Dairy Sci. 77:, suppl. 1
2. Onyango, T.A. and Ingalls, J.R. 1994. Heat treated canola meal as a source of rumen escape protein for dairy cows. Manitoba Agri-forum. pp 128.
3. Onyango, T.A., Ingalls, J.R. and Crow, G.H. 1995. Rumen and lower GI tract degradability of heated canola meal and effect of dietary protein, bag type and pepsin-HCl digestion on nitrogen and amino acid degradability. Can. J. Anim. Sci. (abstract).

ABBREVIATIONS USED IN THE TEXT

AA - Amino acid

ADF - Acid detergent fibre

ADIN - Acid detergent insoluble nitrogen

AME - Apparent metabolizable energy

ATP - Adenosine tri-Phospahte

Bi - By-pass protein supplement

CH_4 - Methane

CM - Canola meal

CO_2 - Carbon-dioxide

CP - Crude protein

CSM - Cotton seed meal

DM - Dry matter

DMI - Daily dry matter intake

DIP - Degradable intake protein

EAA - Essential amino acids

ED - Effective degradability

EDDM - Effective degradability of dry matter

EDN - Effective degradability of nitrogen

EDP - Effective degradability of protein

EE - Ether extract

EEL - Endogenous energy loss

GE - Gross energy

GIT - Gastro-intestinal tract

GLM - General linear models procedure of Statistcal Analysis System.

MP - Multiparous cows.

N - Nitrogen

NDF - Neutral detergent fibre

NDIN - Neutral detergent insoluble nitrogen

NEAA - Non-essential amino acids

NH_3 - Ammonia

NH_3N - Ammonia nitrogen

NPN - Non-protein nitrogen

PP - Primiparous cows.

RSM - Rapeseed meal

SBM - Soybean meal

SNF - Solids-not-fat

TME_n - True metabolizable energy (endogenous corrected)

TMR - Total mixed ration

UIP - Undegradable intake protein

Urea-N - Plasma urea nitrogen

VFA - Volatile fatty acids

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INTRODUCTION

The ability of ruminants to digest relatively unlignified plant cell wall materials in the rumen places them in a particular niche in the food chain. The capacity to digest cell wall carbohydrates results from microbial fermentation in the rumen. Justification for continued use of all ruminants for meat, milk, hide and wool or hair production resides in four broad areas: their ability to digest carbohydrate sources not digested by monogastric species; their ability to use non-protein nitrogen (NPN) to supply the host animal with protein through microbial growth in the rumen; their efficient utilization of dietary protein (provided it has an optimum balance of degradable and undegradable protein) and their highly efficient use of dietary lipids for productive purposes.

The digestive tract of ruminants provide an environment capable of supporting a dense population of microorganisms that ferment carbohydrates and other plant materials to produce mainly volatile fatty acids (VFA), methane (CH_4), carbon dioxide (CO_2) and energy (adenosine tri-phosphate, ATP) for the growth of microorgamisms. The rumen microorganisms on the other hand, have the ability to synthesize amino acids (AA) and proteins from ammonia, which provide the major source of protein for the host ruminant animal under regular circumstances, to be post-ruminally digested and finally available as AA through the use of proteolytic enzymes in the lower gastro-intestinal tract (GIT).

With continuing research efforts, debate has arisen as to the adequacy of microbial protein as the sole source of protein and AA for high producing ruminants of today; and more specifically, with high producing dairy cows in early lactation being one major area of focus and concern. It has been suggested from various studies that there

is need to provide some form of *rumen bypass (escape) protein* also referred to as ruminally undegradable intake protein (UIP) to such classes of ruminants in order to provide sufficient levels of essential AA required for high levels of production.

Bypass protein has been defined as any portion of a protein meal which escapes the rumen intact and is presented for digestion in the lower GIT or that part of the protein which on escaping rumen fermentation is digested and absorbed from the small intestine. High requirements for UIP have led to substantial research efforts to improve feedstuff resistance to microbial degradation, naturally, or through chemical, physical and or heat treatment which may modify the protein with a purpose of increasing UIP levels available post-ruminally for high producing dairy cows.

Canola meal (CM) contains 38 to 46% crude protein (CP) with relatively low UIP (Kendall et al 1991; Boila and Ingalls 1992). Research has focused on increasing UIP in CM and its potential as a protein source in the rations of high producing cows. The utilization of CM in dairy cattle rations has received extensive investigation. The review of research results from 1968 to 1979 by Bell (1982) show that up to 346 studies were conducted on CM alone, as a protein source for animals. Other experiments have since shown the value of CM in dairy diets as the main protein source (Thomke et al. 1983)

These reports have shown that CM will either maintain or slightly increase milk yield compared to soybean meal (SBM) based rations. However, in the past 20 years milk production per cow has increased about 100% with some of the top herds producing 50% more than the average. These large increases in milk yield require new strategies to meet the cow's nutrient requirements and require research to apply these strategies to

make efficient and economic use of the available feedstuffs such as CM.

Because of the cow's limited ability to consume feed and coupled with reliance on rumen bacteria to produce AA, an increase in energy and rumen escape of essential amino acids (EAA) is required particularly in early lactation and at peak milk production. Research work has shown that in some cases, increasing the amounts of rumen escape protein will increase milk yield and hence the need for high quality dietary protein supplementation for high producing cows (McKinnon et al. 1990).

The hypothesis for these studies was that untreated CM protein is rapidly degraded in the rumen as is barley and high protein legume forages which form the major part of dairy rations and do not meet NRC (1989) requirements for lactating dairy cows. Heat treatment of CM can improve the value of protein through increased amounts of rumen escape of essential AA available for assimilation and subsequent increased milk yield. Thus the value of canola meal as a source of AA for absorption in the gastro intestinal tract (GIT) could be improved by decreasing the amount of degradation of CM protein in the rumen.

Studies were conducted using CM exposed to moist heat treatment at different temperatures with the objectives of measuring the effect of moist heat treatment of CM on, (1) the rates of dry matter (DM), acid detergent fibre (ADF), neutral detergent fibre (NDF), nitrogen (N), and amino acids' disappearance from the rumen and lower GIT for CM. This was conducted as an *in situ* study using rumen and mobile nylon bags incubated in cannulated lactating dairy cows; (2) energy level and amino acid availability as assayed using precision fed roosters; (3) feed intake, milk production and milk

composition for dairy cows in early lactation. Comparision of milk response from moist heat treated CM with that from a higher cost animal-plant by product rumen escape (Bi) protein supplement formulated to have equal levels of undegradable intake EAA as heat treated CM at 60 min.

(4) Determination was also made of disappearance rates for test supplements from two types of nylon bags of different thread patterns and percentage open surface area, designated as "monofilament" and "multifilament" bags (ANKOM Co., Fairport NY). The effect of post-ruminal pepsin-HCl digestion on the lower GI tract AA availability from test supplements was also investigated. Effect of cows' diets on rumen degradability of DM and N was also measured.

The results of the three experiments that were conducted using cannulated dairy cows, precision fed roosters, and lactating dairy cows are being reported as three manuscripts. Manuscript I contains the results of rumen dry matter (DM), acid detergent fibre (ADF) and neutral detergent fibre (NDF) disappearances of the untreated CM (CM 0), heat treated CM (CM 60) and that of a by-pass protein supplement (Bi), formulated to provide similar levels of AA as those found in heated CM. The measurements were made using in situ techniques with rumen cannulated cows. Results also include rumen, lower GIT disappearances and faecal excretion of DM, ADIN, NDIN, N, and AA as obtained from measurements using the "mobile bag" technique. Manuscript II has results for energy levels and AA availability from heat treated CM assayed by precision fed cockerels. Manuscript III has results for feed intake, milk production and milk

composition of lactating dairy cows fed rations formulated to include the test supplements examined in the first manuscript.

LITERATURE REVIEW

CANOLA MEAL

The first Canadian report on the feeding value of rapeseed meal to poultry appearred (Pettit 1944) soon after first field cultivation of rapeseed took place in 1943. These reports indicated that meal was causing thyroid enlargement due to high glucosinolate levels. Plant breeding research was initiated to make rapeseed meal safe. By 1968 the first low erucic acid rapeseed cultivar was produced in Canada, followed by several others until the first 'double low (zero)' cultivar, 'Tower' (*B napus*), low in both erucic acid and glucosinolates was released in 1974 (Bell 1984). The double low nature of Tower was derived from combined intercrossing 'Turret', selection Liho and the Polish cultivar 'Bronowski' (Bell 1984). The name "*Canola*" was adopted in 1979 Canada to separate these improved cultivars from rapeseed cultivars grown in Europe.

CANOLA MEAL COMPOSITION

Fibre levels of CM vary depending on factors such as the seed variety, enviromental conditions under which the crop is grown and the processing conditions to which the seed is subjected during oil extraction (Clandinin et al. 1959). The crude fibre (CF) in CM has been shown to be of beneficial value to ruminants, however in poultry and swine, a lower fibre level is preferable (Clandinin et al. 1986) to increase energy density. Current efforts being made to reduce the fibre content of CM include dehulling or air classification and breeding canola cultivars which produce seed with thin hulls.

Protein content of CM similarly varies depending on the cultivar from which the meal is produced. On the avarege, CM from the mixture of low glucosinolate cultivars contain 36-38% protein (N X 6.25, Clandinin 1986). The AA content of the protein of CM compares favourably with the protein of soybean meal (SBM). Soybean meal contains more lysine than canola meal but the latter contains more methionine, hence, these two protein supplements tend to compliment each other when used in livestock rations (Clandinin et al. 1986; Boila and Ingalls 1992; Moshtaghi-Nia 1994; appendix table L.1). The balance of AA in canola meal relative to milk is better than most protein supplements after fish meal (McKinnon et al. 1990).

The energy content of CM is somewhat lower than that of SBM mainly due to the high fiber content in the hull portion which makes up about 16% of the seed weight (Appelqvist and Ohlson 1972). Reports with species comparison for energy content (DE kcal/kg meal, as fed) have indicated that energy content for CM is highest for swine, intermediate for cattle and lowest for poultry (Clandinin 1986).

Attempts to obtain information on composition of the CM hull has been difficult because of problems in separating the hull fraction and difficult because there is uncertainty about the amounts of lignin and polyphenols present. Bell (1984) suggested that cellulose forms the bulk of carbohydrates in canola meal and that the remainder of the carbohydrates are pentosans. Efforts are continously being made in Canada and France to produce CM with a metabolizable energy value comparable to that of SBM. In this direction, two approaches have been in use; removal of part of the hull fibre

during or after processing; and developing yellow-seeded cultivars in which the hull is thinner than that of the brown-seeded cultivars (Clandinin et al. 1986).

Canola meal tends to have higher ether extract (EE) than SBM because in Canada gums are added back to canola meal at about 1.5%. The gums as obtained during refining of canola and oil consists of glycolipids, phosholipids and variable amounts of triglycerides, sterols and fatty acids (Clandinin et al. 1986). Addition of gums to canola meal tends to increase its energy conentration. In studies involving dairy (Grieve 1978) and beef (Mathison 1978) cattle, it has beeen noted that addition of gums to CM at levels higher than that already in practice with canola seed processed in Canada have no adverse effects on the feeding value of the meal for these classes of animals.

In general, CM has higher minerals than SBM although it has been shown (Nwokolo and Bragg 1977) that high phytic acid and fibre levels in the meal reduces the availability of calcium (Ca), magnesium (Mg), phosphorous (P) and zinc (Zn) for poultry. Recent studies by Ingalls and Okemo (1994) have indicated that increased amounts of CM in ruminant diets increases plasma P and that availability of P from CM equals that from an inorganic P source. Though CM has lower availability of minerals for poultry compared to SBM, CM contains higher levels of Ca, Fe, Mn, P and selinum (Se) than SBM (Clandinin 1986).

The level of glucosinolates, precursors of thyrotoxic substrates, in current CM is estimated at about one-eighth that of the older Canadian high glucosinolate rapeseed meal or less than one-tenth that of European high glucosinolate rapeseed meals (Clandinin et al. 1986). There is evidence that sufficient heating during oil extraction destroys the

enyzme myrosinase, responsible for conversion of the glucosinalate progoitrin pre-curssor to goitrin; the principal thyroid enlarging factor in rapeseed meal (Clandinin et al. 1986).

Results from canola meal analyses (Jaikaran and Robblee 1986) for glucosinolates averaged 1.27 mg/g glucosinolates (10.7 μmol/g); whereas Bell and Jeffers (1976) reported average values for older Canadian high glucosinolates *B. napus* and high glucosinolate *B. campestris* rapesed meals at 8.5 and 6.3 mg/g (71.5 and 53.0 μmol/g), respectively.

Glucosinolates as goitrgenic compounds in rapeseed meal (RSM) have been shown to interfere with normal thyroid function thus limiting its usefulness as a feed protein (Lo and Bell 1972). Fisher and Walsh (1976) concluded that 1788-RSM (a lower glucosinolate *B. napus)* at levels of 34% in the grain mixture was goitrogenic to lactating cows. Sharma et al. (1977) obtained similar results with 1788-RSM, but not with Tower RSM, another low glucosinolate cultivar. Papas et al. (1978) reported that Tower RSM was not goitrogenic but later observed that Tower RSM did have some goitrogenic activity (Papas et al. 1979). These results were obtained (Laarveld et al. 1981) using relatively insensitive methods of evaluating goitrogenic effects based on the concentrations of thyroxine (T4) and triiodothyronine (T3) in plasma. Later studies were conducted by Laarveld et al. (1981) using the thyrotropin-releasing hormone (TRH) test as adopted from Ormston et al. (1971) and as modified by Hall et al. (1975) and Sawin and Hershman (1976) which considered a sensitivity index of thyroid function allowing for detection of small alterations in thyroid activity. Results from these studies concluded that when Tower RSM made up to 18.9% of the total diet there were no goitrogenic

effect while 'Midas' RSM fed at 13.2 and 18.9% contained sufficient quantities of goitrogens to affect thyroid function as measured by the TRH test (Laarveld et al. 1981).

AMINO ACID DIGESTIBILITY AND AVAILABILITY IN POULTRY

Processes that lead to the final quality of AA available include digestion (actual protein hydrolysis and absorption of the final products) and utilization (retention of absorbed AA). It is, therefore, procedural to describe the AA in terms of their availability from the extent of disappearance from the gastrontestinal tract during digestion, the so called digestibility coefficients. It is possible to characterize circumstances whereby an AA can be digested but eventually not made available for the use by the animal. Certainly the undigested AA (those in the feces) make no obvious contribution to the needs of the animal (McNab 1994). Therefore efforts are being made to describe the proteins in feedstuffs in terms of digestible AA's thus providing information on the actual amounts available to the animal for maintenance and production.

In general, AA's are obtained from results of balance studies measuring the differences between input and output (Sibbald 1987). In this case it is usually an expression of difference between amounts eaten and excreted as feces as a proportion of the amount comsumed or the *digestibility*. There is always some confusion arising as a result of the use of this terminolgy because not all the AA present in the feces has arisen from undigested food residues (McNab 1994). Sibbald (1987) distinguished between what is metabolic fecal component (secretions, abraded cells, mucus, bile) and

endogenous fecal fraction (bacteria and bacterial debris) and this therefore provides a measurement of *true digestibility*.

METHODS FOR DETERMINING AMINO ACID AVAILIABILITY IN POULRTY

Though digestibility is frequently regarded as a property of a diet or feedstuff it is really a characteristic of individual animals to which the feed is provided (McNab 1994). There are three general types of AA balance experiments which have been in frequent use to derive digestibility and or availability coefficients. These methods include:

(a) The traditional assays involve a preliminary adaptation period to establish equilibrium conditions within the digestive tract of the bird (Sibbald 1987). In this method, diffrerences arising from carryover between the start and the end of the experimental period ("end effects") are monitored and controlled to ensure similarity.

(b) The second method, rapid assay, involves the use of starvation before and after provision of known amounts of test diet to control the "end effects", but permits birds free access to the diet. In this assay, in most cases, complete diets must be fed and substitution methods used for ingredients.

(c) The third type of rapid assay (as above) relies on tube-feeding to place the test material into the birds' crops. This method avoids the need to substitute ingredients into a basal diet. The current studies were conducted using the third type of assay.

DETERMINATION OF TRUE METABOLIZABLE ENERGY IN POULTRY

One of the popular methods (Sibbald and Price 1976; Sibbald 1987) in use to determine true metabolizable energy (TME) involves the use of adult roosters housed in

individual cages, starved for 12 h but have free access to water. The birds are weighed and force fed an accurate weighed amount of approximately 25-30 g of the test material. The birds are returned to their cages, plastic trays placed under the cage, and the time recorded.

Birds of similar body weight are selected and returned to their cages without feeding, plastic trays placed under the cage and the time recorded. These steps are repeated three times. Exactly 24 h after placement, the plastic trays are removed, excreta collected quantitatively, frozen, freeze dried and equilibrated to room temp and moisture and then weighed. Samples of the test feedstuff and excreta are ground to pass through a 20 mm-mesh seive and assayed for gross energy (GE).

TME is calculated as:

$$TME\ (Kcal/g\ air\ dry) = (GE_f * X) - (Y_{ef} - Y_{eo})/ X$$

Where:

GE_f is the GE of the test feedstuff (Kcal/g);

Y_{ef} is the energy voided as excreta by the fed bird,

Y_{eo} is the energy voided as excreta by the unfed bird,

X is the weight of test feedstuff fed (g).

This assay was adopted in this study.

CANOLA MEAL AS A PROTEIN SUPPLEMENT FOR RUMINANTS

Rumen degradation potential of untreated canola meal

Ruminants have foregut fermentation capabilities and consequently obtain lower GI tract protein from two major sources. One source being the microbially synthesized

protein which is of good quality relative to levels of EAA (Czerkawski 1976; Storm and Orskov 1983; 1984) and high digestibilty (Armstrong and Hutton 1975; Salter and Smith 1977; Storm et al. 1983; Wallace 1983) and that arising from ruminally degradable feed intake protein (DIP). The second source is the undegraded intake dietary protein (UIP), which escapes microbial degradation in the rumen (NRC 1985). The mixture of microbial protein and UIP are digested in the abomasum and small intestine to yield the amino acid supply for the host animal. The extent to which a protein supplement is ruminally degraded has a significant effect on the manner in which it is utilized by the ruminant animal. Largely, the degraded protein fraction is converted to ammonia (NH_3), AA, peptides, fatty acids and carbon dioxide (CO_2), with part of the ammonia being utilized for microbial protein synthesis (Preston and Leng 1987). The concept of UIP and DIP as components of the digestive process of dietary protein has formed the latest extensive research area with various proposals for protein systems for formulating ruminant diets (Waldo and Glenn 1984).

Lactating dairy cows and rapidly growing ruminant animals cannot satisfy their protein requirements from microbial protein alone (NRC 1989), making it necessary that diets contain less degradable protein with a high potential for rumen escape. With increased use of CM in dairy rations, there has been a need to determine its CM degradability and thus predict quantities of undegraded protein that will be available to the small intestine (Kendall et al. 1991).

Canola meal protein is rapidly degraded in the rumen compared to other protein supplements (Rooke et al. 1983; Kennelly et al. 1986; Kendall et al. 1991) with reported

estimates of its effective degradability of protein (EDP) value at 67.7% compared to SBM at 53.5% as obtained using the in situ nylon bag technique (Ha and Kennelly 1983). Further studies by Ha and Kennelly (1984) using the same techniques reported less N disappearance from SBM than in CM over an incubation range between 4 and 24 h and with higher EDP for CM (65.8%) than SBM (53.6%). These results also suggested initial slower N disappearance rates for SBM than CM up to 12 hours of incubation but disappearance was similar after 24 hours incubation (Ha and Kennelly 1984). At a k value of 0.06, Bailey and Hironaka (1984) reported degradability of total N of untreated CM at 82% which compared close to estimates obtained by English (Rooke et al. 1983) and Swedish (Lindberg et al. 1982) studies with rapeseed meal.

TREATMENT OF CANOLA MEAL TO REDUCE DEGRADATION

Canola meal is more rapidly degraded in the rumen than many other protein supplements (Rooke et al. 1983; Ha and Kennelly 1984; Kennelly et al. 1986; Kendall et al. 1991; Moshtaghi-Nia and Ingalls 1992; Boila and Ingalls 1992), and more rapidly degraded than that required by the dairy cows (NRC 1989). Therefore, treatment of CM to increase rumen escape and to increase lower GI tract absorption has received considerable attention in recent years. The use of both chemical and physical methods to alter CM protein to reduce rumen microbial degradation have been investigated.

Chemical treatment

When high quality protein from supplements such as SBM or CM is fed to ruminants, the protein is subjected to extensive microbial fermentation in the rumen. During fermentation most protein is degraded to peptides, AA and finally to ammonia

(Chapula 1981). When this happens, the advantages of such protein quality in terms of EAA availability and balance are lost. Attempts have been made to maintain the quality and quantity of protein reaching the small intestine by use of chemical agents. Chemical agents form reversible cross linkages with amino and amide groups and hence decrease solubility of protein at certain pH ranges in the rumen. The purpose generally has been to use these agents to protect protein from extensive rumen degradation with subsequent availability of the protein to the ruminant animal in the lower GI tract where the presence of acidic medium facilitates the destruction of the formed linkages and hence releases the AA.

Successful chemical methods have included treatment with formaldehyde (Sharma and Ingalls 1974; Eyre and Rooke 1983; Rae et al. 1983; Rooke et al. 1983; Ha and Kennelly 1984; Mir et al. 1984), sodium hydroxide (Mir et al. 1984) and a number of acids such as acetic, formic, hydrochloric and propionic (Khorasani et al. 1989); xylose, lignosulfonate (McAllister et al. 1993) and with animal byproducts such as whole fresh blood and fish hydrolysate (Mir et al. 1984). Other chemical treatments meant to increase quantities of AA available in the small intestine include tannin materials (Zelter et al. 1970; Nishimuta et al. 1974); alcohol (Van der Aar et al. 1982; Van de Aar et al. 1984) and ammoniation (Tembo 1987; Subiyatno 1989; Bactawar 1991).

Treatment with formaldehyde has received more prominence among these chemical methods as an agent to decrease degradation of protein in the rumen. The extent of degradation of dietary protein in the rumen is largely a factor of its solubility in the rumen and treatment of protein supplements with formaldehyde has been aimed at

decreasing the breakdown of the feed protein. The availability of EAA to the host tissue from a protein supplement such as CM depends on the amount, composition and digestibility of CM and microbial protein which escapes ruminal degradation. Formaldehyde treatment of RSM was shown to reduce production of ammonia in the rumen of steers fed RSM but with little effect on apparent digestibilities of DM, CP or N retention (Sharma and Ingalls 1974: Sharma et al. 1974). Some earlier reports (Ferguson et al. 1967) indicated that formaldehyde treatment of casein prevented its degradation in the rumen, thus allowing more of the protein to be digested in the intestine. In sheep, reports from Reis and Tunks (1969) noted that addition of formaldehyde treated casein into the abomasum resulted in substantial increase in wool growth and N retention.

The use of formaldehyde has received mixed safety concerns and also because its use can lead to overprotection of the very protein required by the host animal (Stanton et al. 1983). Performance results with lactating cows and pepsin insoluble N data, indicated that low level formaldehyde treatment may be more desirable in ruminants supplemented with protein but with low quality roughages (Stanton et al. 1983). Lactating beef cows showed some sensitivity to overprotection of protein with weight gain variation by calves suckling dams fed various formaldehyde treated protein (Stanton et al. 1983). Treatment of SBM with 0.3 g formaldehyde/100 g SBM has been shown to decrease the availability of SBM protein for lactating dairy cows (Crooker et al. 1983). Formaldehyde treatment reduced the solubility of SBM from 22.7 to 2.9%, and also reduced milk protein (from 3.08 to 2.85%) and solids-not-fat (from 8.51 to 8.35%), but did not affect

intake or overall milk yield as reported in the study of Crawford and Hoover (1984). These authors attributed the lack of production response and reduction in milk protein to overprotection of dietary protein. Treatment of SBM with 0.3, 0.6 and 0.9% formaldehyde resulted in AA preservation but caused a decrease in contents of tyrosine and lysine (Rae et al. 1983) which Crooker et al. (1983) attributed to formation of crosslinked products known to be resistant to 6 N HCl hydrolysis.

Crawford and Hoover (1984) suggested that rapid flow of digesta through the abomasum and small intestine would result in shorter exposure to acid hydrolysis, which may reduce the maximum levels at which formaldehyde could be incorporated in feeds without adversely affecting the availability of AA in the small intestine. Formaldehyde treatment of SBM and RSM has also been shown to alter the amount and form of protein presented for postruminal digestion (Nishimuta et al. 1974; Sharma and Ingalls 1974).

To determine the effect of formaldehyde treatment on soy protein quality, many workers have used rat and chick bioassays rather than cattle in the interest of time and economy (Schmidt et al. 1973; Thomas 1979). However results have tended to indicate depressed protein quality, probably due to formation of irreversible crosslinkages between AA that cause resistance to enzymatic attack (Van Soest 1982).

Rate of passage for solids in the rumen at about 14 h were reported (Junkins 1981) in feeds that were formaldehyde treated (0.3% by weight of formaldehyde). A decreased CP digestion by 42% as compared to only 20% decreased at retention times over 24 h also were reported. Junkins (1981) noted that shorter retention times found for high producing dairy cows consuming more than 3% of their body weight required lower

rates of formaldehyde treatement of feeds to protect protein from rumen degradation. Greater amounts of formaldehyde are required for treatment of feeds offered to animals with low production levels, lower intake and longer rumen retention times (Junkin 1981).

Other chemical agents have been studied for their ability to protect protein from extensive rumen degradation. Studies conducted by Mir et al. (1984) involved treatment of CM and SBM with sodium hydroxide (NaOH) with a 50% solution at levels of 1, 2, 3, and 4 NaOH g/100 g DM), whole fresh bovine blood at levels of 0.5, 0.75, 1.5 and 2.0 l/kg DM, fish hydrolysate at similar levels as whole fresh blood and formaldehyde (HCHO) at 0.8 g HCHO/100 g CP. They noted a reduction in degradation of protein in the rumen at all the concentration levels of treatments. These reductions in protein degradability were reflected in both the decrease in rate of disappearance and reduction of the soluble fraction (Mir et al. 1984). Examination of degradability ratios for untreated to treated protein supplements suggested that formaldehyde treatment achieved the best protection of CM protein and sodium hydroxide and formaldehyde preserved or increased EAA contents of SBM, but inclusion of blood significantly increased valine, threonine, histidine, lysine and phenylalanine in SBM (Mir et al. 1984). Protein quality measurements from these data indicated that exposure of untreated CM to rumen microbes resulted in reduced quality of protein escaping digestion.

Treatment with chemicals resulted in more degradabile full fat soybean and canola seeds. It has been suggested that fat in seeds block the pores in nylon bags and prevents normal exchange of rumen fluid rather than exerting a protective effect on protein (Lindberg et al. 1982). Subsequently degradation of protein in vivo from chemically

treated full fat seeds may be higher than estimates obtained from nylon bag measurements. Alkali treatment of proteins result in crosslinkages between AAs, thus protecting proteins from microbial attack. Alkali treatment also results in racemization of AAs such as methionine and lysine (Provansal et al. 1975) and in decomposition of some AAs (Gould and MacGregor 1976).

Treatment of SBM and CM with whole blood resulted in protection of protein from rumen degradation at levels as low as 0.25 litres of blood/kg SBM or peanut meal (Orskov et al. 1980). Though the mode of action of blood on protecting plant protein is not clear, it may be speculated (Mir et al. 1984) that since blood protein is not easily degraded in the rumen (Gonzalez et al. 1979) the coating of plant protein by blood provides a physical protection from rumen microbes. The role of fish hydrolysate as an effective agent in reducing N disappearance of SBM and CM from nylon bags suspended in rumen may be associated with the hydrolysate itself, being rich in protein resistant to ruminal degradation (Chapula 1981) and hence reduction in N disappearance.

Results from treatment of CM with acids such as hydrochloric, acetic, formic or propionic at concentrations of 2.5 and 5% (vol/wt) followed by 105°C drying indicated that all acid treatments decreased CP solubility of CM in borate-phosphate buffer (Khorasani et al. 1989), decreased NH_3 production and reduced CP digestion by protease enzyme (Khorasani et al. 1989). These results also indicated that acid treatment reduced ruminal degradability of canola meal CP and, with the exception of HCl, there was no depression of estimated intestine CP digestibility. Waltz and Loerch reported that treating SBM with acetic or propionic acid, while decreasing CP solubility *in vitro* and *in situ* did

not affect acid detergent insoluble CP or acid pepsin insoluble CP. Vicini et al. (1983) indicated that treatment of protein with acetic, formic and propionic acids decreased CP solubility and degradability, but intestinal digestibility of CP was not depressed contrary to the depression effect observed (Khorasani et al. 1989) with HCl treated CM which they attributed to creation of bonds resistant to postruminal enzymatic digestion.

Results by Windschitl and Stern (1988) showed that treatment with lignosulfonate followed by heating at 90 - 95°C for 45 min decreased rumen degradability of SBM protein. McAllister et al. (1993) treated CM with 5 or 10% lignosulfonate for 1 or 2 h at 100°C which resulted in increased borate-insoluble CP and ADIN and decreased in vitro NH_3 concentration compared with untreated or heated CM. Results from this study (McAllister et al. 1993) also indicated increased ADIN for CM treated with 2% xylose and 10% lignosulfonate and heated for 2 h, but decreased ruminal degradability of CP.

Heat treatment

The quantities of protein degraded in the rumen determines the quantity of rumen escape protein entering the small intestine. In rapidly growing ruminants or dairy cows in early lactation, production can be limited by low rumen escape protein available in the lower GI tract. When plant-derived protein supplements such as CM are fed to high producing ruminants a large part of the protein is degraded (de Boer et al. 1987) in the rumen hence effectively depriving the host animal of the nutritive value of much of the protein it consumes. Methods have therefore been sought that will render CM protein less degradable. Heat treatment (baking, moist heating and roasting) has been used to decrease the rumen degradability of rapidly degraded protein sources such as CM and

SBM (Mir et al. 1984; Plegge et al. 1985; Ahmadi 1988, McKinnon et al. 1990; Moshtaghi-Nia and Ingalls 1992; McAllister et al. 1993); Demjanec et al. 1995; Hussein et al. 1995).

Heat treatment has been used as a method to protect proteins from degradation in the rumen because of its efficacy (Tagari et al. 1962; Plegge et al. 1985; Hussein et al. 1995), cost effectiveness, safety and ease of application. Heat treatment decreases the solubility of proteins by creating crosslinkages both within and among peptide chains and to carbohydrates (Deacon et al. 1988), thus lowering their susceptibility to ruminal degradation. However, high temperatures and excessive heating times renders protein less digestible (Van Soest 1982) through the Maillard reaction between sugar aldehyde groups and free AA groups thus lowering the digestibility of constituent AAs (Khorasani et al. 1989).

Generally the beneficial effects of reduced degradation rate in the rumen would appear to be greater than the reduced whole tract protein digestibility caused by limited heat damage (de Boer et al. 1987). Heat treatment of protein in the dry state at neutral pH causes the Maillard reaction which forms isopeptide bonds between lysine residues and the β or γ-carboxamide group of asparagine and glutamine residues (Belitz and Grosch 1987). Excessive heating and for a longer time generally leads to more extensive denaturation of protein and pH and water seem to be the most important factors, in association with heat, in determining the reaction rate (Van Soest 1982). The Maillard reaction polymers increase with increased pH and breakpoints in polymer formation occur at pH 6 and 5 for 100 and 110°C, respectively (Lee et al. 1984). In the majority

of cases, the damage is small enough that the advantageous effects of reducing protein solubility through denaturation may override the losses in digestible CP (Van Soest 1982).

Heat damage of protein has been associated with increased acid detergent insoluble nitrogen (ADIN) which was for sometime assumed indigestible (Van Soest 1982) and ADIN, therefore, has remained the measure of extent of damage effected on protein (Britton et al. 1987). It has since been established that ADIN is partially digestible (Pena et al. 1986; Rogers et al. 1986; Ariele et al. 1989; Weiss et al. 1989; Moshtaghi-Nia and Ingalls 1992; McAllister et al. 1993; Hussein et al. 1995) and that heat damage may be overestimated (Britton et al. 1987; Weiss et al. 1989; Van Soest and Mason 1991) when ADIN alone is used as a measure of protein damage. Klopfenstein and Britton (1987) were able to support steer gains using distillers dried grains with high levels of ADIN. Recent studies (Hussein et al. 1995) have emphasised the importance of defining the nature of preparation of samples before analysis if ADIN is to be used as an indicator of heating effects. Results of other researchers have suggested ADIN values (percent of total N) in unheated SBM to range from 1.8 (Clark et al. 1987) to 4.6% (Loerch et al. 1983) and this range has been explained (Hussein et al. 1995) by variation in particle size of samples. Moshtaghi-Nia and Ingalls (1992) recorded a range in ADIN (percent of total N) of 5.4% for untreated CM to 41% for 90 min heat treated CM, while McAllister et al. (1993) recorded ADIN (percent) of 6.7 with untreated CM and 22.5% with 10% xylose CM heated for 2 h. Since residual N remaining following acid detergent extraction is an estimate of how much N has become insoluble from Maillard reaction,

ADIN has been suggested as a sensitive assay (Goering and Van Soest 1972) for artifact lignin formed from the non-enzymatic browning reactions between reducing sugars and free AA groups.

Results have indicated that extended heating time of CM (Moshtaghi-Nia and Ingalls 1992) and SBM (Hussein et al.1995) increases ADIN in these protein supplements, and similarly increases ADIN intake, duodenal and ileal ADIN flows, with larger fecal recovery of ADIN from the longest heated supplements. Britton et al. (1987) evaluated ADIN as a measure of indigestible N in protein supplements and indicated that fecal recoveries of ADIN were poor and ranged from 31 to 87%. Van Soest (1989) stated that ADIN remains unchallenged as a simple straight forward method of measuring bound N, but from results of Armentano et al. (1986) and Britton et al. (1987), there is an implication that ADIN as a measure of indigestible N is not adequate for high protein supplements. Moshtaghi-Nia and Ingalls (1992) and Hussein et al.(1995) have since generated results that indicate quantities of ADIN are digestible in the stomach and in the total tract despite increased ADIN concentrations in both CM and SBM, respectively, with extended heating.

The major portions of protein in plant sources such as CM and SBM are in the form of globulins and albumins, with limited amounts of glutelins and prolamines (Hussein et al. 1995). Globulins and albumins are relatively sensitive to heating as prolonged heating causes denaturation and increases the fraction of insoluble N, ADF and NDF as Maillard products are formed. Increases in ADF content as a result of heating

plant proteins can be attributed to production of artifact lignin via enzymatic browning (Goering and Van Soest 1972).

The indigestible protein formed through Maillard reactions is thought to be quantitatively recoverable in ADF as lignin (Van Soest 1982) and the N content of ADF of forages is positively correlated with lignin content and negatively correlated with digestibility (Van Soest 1982). Increase in ADF due to heating CM (Moshtaghi-Nia and Ingalls 1992) and in SBM (Hussein et al. 1995) had no negative effect on digestion of ADF in the total tract. Extended heating time of CM (Moshtaghi-Nia and Ingalls 1992) and SBM (Hussein et al. 1995) increased ADF from 15.8 (untreated CM) to 36% (90 min heated CM) and from 5.8 (untreated SBM) to 22.9% (SBM heated for 210 min), respectively.

Increased heating of plant protein increases NDF content and the residue remaining following NDF extraction contains largely cellulose, lignin and hemicellulose. Less soluble cell wall proteins and heat damaged cytoplasmic and chloroplastic proteins usually contribute to NDF (Van Soest 1982) therefore any increases in NDF content reflects greater quantities of these components availing themselves as part of the NDF fraction as heating time increases. Hussein et al. (1995) noted increased NDF intake and subsequent disappearance, as g per day, in the rumen as a result of a linear increase of NDF with heating time of SBM, whereas disappearance of NDF in the rumen as a % of intake, was not affected by increased heating time and that, disappearance of NDF in the total tract, as % of intake, was linear with increasing time of heating.

Heat treatment of CM resulted in greater increases of NDIN content than ADIN and was up to 736% of the content in the original meal with 90 min heating (Moshtaghi-Nia and Ingalls 1992). McAllister et al. (1993) recorded 60% lower NDIN values compared to those measured in CM heated for 60 min at 127°C (Moshtaghi-Nia and Ingalls 1992). The formation of NDIN involves the polymerization reaction of condensed amino groups from AA with carbonyls from reducing sugars into a lignin-like matrix reflected by an increased NDF fraction and with resulting N products becoming recoverable in the neutral detergent extract (Goering and Van Soest 1972).

METHODS OF STUDYING RUMEN DEGRADATION OF NITROGENOUS COMPOUNDS

There is an important need to establish the amounts of protein necessary for optimal microbial and animal response. Prediction of adequate AAs available in the lower GI tract has lead to a number of techniques *(in situ, in vitro* and *in vivo*) devised to evaluate the protein degradability in the rumen. One area of concern is to have techniques that are reasonably accurate, able to correlate to the actual measured biological response in question (such as degradability) generally measured by in vivo experimentation (Nocek 1988). The procedure should also provide a significant degree of precision, within and between laboratories and should be cost-effective in time and funds involved with the studies.

Most *in vivo* techniques use post ruminal collection of digesta to estimate protein degradation, while *in vitro* techniques include solubility (Krishnamoorthy et al. 1983), artificial enzyme-based reactions (Crooker et al. 1978; Wohlt et al. 1973), use of batch

(Tilley and Terry 1963) and continuous (Abe and Kumeno 1973) cultures. *In situ* techniques (Czerkawaski and Breckenridge 1977; Broderick 1978; Erdman et al. 1987; Nocek 1985) utilize rumen suspended nylon bags; and more recently mobile nylon bags (Sauer et al. 1983; Rae and Smithard 1985; Campbell et al. 1988; Varvikko and Vanhatalo 1990; Frydrych 1992; Moshtaghi-Nia and Ingalls 1992).

IN SITU TECHNIQUE

The technique involves the suspension of feed materials in artificial fibre bags such as nylon or polyester which are totally resistant to microbial degradation in the rumen. This technique also refered to as the *in sacco* technique or artificial fibre bag technique, was first used with silk bags (Quin et al. 1939) which were later replaced by nylon bags (Erwin and Elliston 1959).

The technique provides for intimate contact of the test protein with the rumen environment and hence simulates the rumen environment within a given feeding regime (temperature, pH, buffer substrate and enzymes). The technique has received mixed reactions due the uncertainty as to the period of incubation which would be most appropriate (Orskov and McDonald 1979), that test feed is not subjected to the mastication, rumination and passage processes (Nocek 1985). This technique has been used for several years and forms the basis for prediction of several feeding systems (Chapula 1975; Waldo and Glenn 1984; NRC 1985). However, extensive evaluation and criticisms of the procedure has identified many inherent factors influencing digestion including bag pore size, sample size, sample particle size, microbial contamination and animal diet (Nocek 1985). More recent concerns being evaluated include the interaction

of bags, protein samples, diets, rumen population thus caution is required in interpretation of in situ results.

FACTORS INFLUENCING PROTEIN DEGRADABILITY AS ESTIMATED *IN SITU*

Bag porosity

An appropriate bag porosity is expected to offer a compromise between limiting influx of rumen contents not associated with the test protein and allowing influx of microbial populations to initiate degradation of test the protein while limiting the efflux of undegraded protein particles. With this technique the "mechanical" loss of feed particles from the bag prior to ruminal incubation and the "rapidly" soluble, the *a* part measured at 0.1 h (Mahadevan et al. 1987) are not separated. The real digestibility of this fraction is not quantifiable through in situ technique but is generally assumed as rapidly degradable and readily "available" fraction, which may or may not be precise. Mahadevan et al. (1987) reported a fraction of CM that was soluble but not digested.

Nocek et al. (1983) opted for preruminal incubation (15 min in 39°C water; litres per 250 mg feed N) of SBM which resulted in 15 and 27% loss of N and DM respectively from bags ranging in pore size from 6 to 59 μm. As pore size increased to 80 and 120 μm, N and DM washout increased by 30 and 14%, respectively. Lindberg and Knutsson (1981) observed increased loss of DM as bag porosity increased from 10 to 36 μm after a 6-h soak with agitation. Weakley et al. (1983) showed lower disappearance of DM and N from SBM and distillers grains from 5 μm pore bags, as

compared with that from 52 μm dacron bags, irrespective of ruminal incubation time, and noted that much of the difference was established in the first hour.

Selection of microorganisms depends on bag pore size, and this could modify degradation conditions. A pore size of 3 μm or less was found to strongly inhibit penetration of microorganisms into the bag (Van Hellen and Ellis 1977) or at least protozoal penetration (Michalel-Doreay and Ould-Bah 1992); with a size of up to 3 μm only small-sized protozoa (Entodinium type) were found in the bag, and above this, the number of Holotricha noticeably increased (Lindberg et al. 1984). When Meyer and Mackie (1983) compared total culturable counts in rumen ingesta and in residue samples within nylon bags of different pore sizes (5 to 53 μm), total culturable counts were $<10\%$ in 5 μm and 10 μm bags and about 60% of the amount in rumen digesta in 53 μm bags. These workers suggested that 30 to 53 μm bag pore size was optimal relative to counts of protozoa and bacteria. However it is, difficult to arrive at the limits of bag porosity and it is more likely to depend on sample particle size and nature and type of feedstuff under investigation. Nocek (1985) suggested a porosity of 40 to 60 μm as a good compromise with regard to microbial and content of influx and digested material efflux.

Particle size

Particle size of the test protein is critical because bag contents used for *in situ* digestibility studies are not exposed to mastication or ruminated. Therefore microbial fermentation and detrition by ruminal activity alone is the means by which particle reduction takes place. There is some controversy as to the degree of particle breakdown

associated with microbial digestion (Murphy and Nicoletti 1984; Nocek and Kohn 1988) which has kept the debate as to whether prepared material for in situ study should mimic that which is fed, or mimic that left after mastication and presentation to the rumen. Nocek (1985) investigated the effect of grinding (1, 2 or 5 mm) on DM and N digestion rates for SBM incubated in bags of 59 μm pore size. Grinding changed the relative rates at which N and non-protein DM were digested, particularly for forages, which had increased surface area per unit weight of sample thereby increasing the surface area accessible for microbial attachment (Nocek 1985). It is difficult to establish which particle size is the most appropriate for use in in situ studies, since no studies have been conducted with the specific objectives of developing correlations between *in vivo* and in situ digestion with respect to particle size (Nocek 1985). In general, longer and coarser materials are associated with slower rates of digestion and greater variation, while finely ground materials are subjected to greater mechanical losses from the bags (with occasional unrealistic rapid rates of digestion) but variation is more controlled. A logical approach has been to establish some degree of uniformity in size within major categories of feedstuffs with protein supplements (SBM, CM and fish meal) and byproduct type ingredients (wheat middlings, corn gluten meal and feed) being ground to pass a 2 mm sieve prior to in situ digestion (Nocek 1985).

Sample size to bag surface ratio

(Nocek 1985) described optimum sample size as that which provides enough residue at the end of extended rumen incubation for chemical analysis without overfilling the bag so as to delay bacterial attachment, increased lag time, and underestimate

digestion rates. Sample to bag surface ratio has been used to suggest an appropriate sample size that can be used among laboratories. Van Keuren and Heinemann (1962) observed a decrease in DM digestion with increased sample size over a 24 to 48 h of ruminal incubation of alfalfa, orchard grass and sudan grass. Bullis et al. (1967) reported that increased sample size decreased DM disappearance. Figroid et al. (1972) recorded no difference in DM disappearance of sorghum grain when sample size was increased from 2 to 10 g (1 mm grind, 10.2 X 17.8 cm bags) at 3 and 6 h of ruminal incubation. The same workers reported reduced DM disappearance of barley with narrower bags with 14 g of sample but no further reduction in DM disappearance was noticed with an 18 g sample. In general, as sample size increases in relation to bag surface the test protein sample tends to become more compacted within the micro enviroment of the bag, thus restricting rumen fluid flow and its contact with the protein particles and thereby reducing digestion rate especially in the initial periods of incubation (Nocek 1985). It is recommended that the range in sample size to bag surface area ratio should fall in the range of 10 to 20 g/cm^2 for most forages and concentrate ingredients (Nocek 1985).

Nylon bag incubation procedure

The procedure adopted when incubating bags may influence degradation rates. Some systems involve placement of all bags in the rumen at one time and removal is done at designated time intervals while others introduce bags in reverse sequence and the bags are removed all at the same time. Results comparing these two methods have indicated that the former procedure results in lower variation and slower digestion rates, possibly because the digestion process is interfered with as the rest of the bags intended

for the terminal end of incubation are retrieved and then reinserted into the rumen (Nocek 1985). From these results the author recommends the second procedure of introducing bags at different times and removal of all at the same time. On the other hand, introduction of bags at different times does not expose bags to the same degradation conditions in the rumen, more over, in most *in situ* experiments, diets are offered in two meals per day, and the rumen environment cannot be considered stable. Immediately after the cannulated animals are fed, a rapid change in rumen conditions follows including osmotic shock effects, temperature changes, influx of oxygen, dilution, passage rate changes and diurnal changes in viable bacteria and protozoal number (Dehority and Opin 1988). Also changes occur in enzymatic activity of microbes (Williams et al. 1989). Introduction of all bags at the same time rather than at different times may be the best option for studies on the rate of degradation of rapidly degradable constituents such as CM.

The relative location of bags suspended within the rumen could affect degradation rates. Some methods suspend bags in the rumen fastened along a string weighted with a sinker to ensure they do not float on top of the rumen fluid (Nocek et al. 1979), while others suspend all bags in laundry bags with open 2 X 3 mm mesh and weighted with stones or marbles (Boila and Ingalls 1992). Nocek et al. (1979) used a template (spool-shaped vessel in plexiglass) to allow maximum contact between the test fed and the incubation enviroment, but results from such apparatus have not been compared with those obtained by other procedures.

The appropriate withdrawal time for bags from the rumen is critical because the disappearance rate depends on the shape of the degradation curve with time. It is therefore not possible to propose incubation times for all substrates (Michalet-Doreau and Ould-Bah 1992). In order to make an adequate description of the degradation curve, it is important that the most sensitive part of the curve is well supported by observation, and the asymptote is well described. Most *in situ* N degradability measurements are obtained from 14 to 16 h incubations but typically the first incubation time corresponds to 1 or 2 h (Michalet-Doreau and Ould-Bah 1992). The determination of the asymptote has been made from long incubation times such as 48 h (Nocek 1985) to 12 days (Van Vuuren et al. 1989). Similarly the decision on degradation end-point has an effect on the estimate of kinetics of degradation as underestimation of N degradability may occur for results from short time, for example 48 h, and overestimation of N degradation may occur from too long time digestion (Van Soest 1982).

Dietary effects

The role of diet fed to experimental animals is an important factor because it determines the amount and types of microbial populations in the rumen which may effect the rate and extent of digestion of test feeds in the bags. High concentrate diets with rapidly soluble carbohydrates ferment rapidly and reduce ruminal pH to cause a shift to a more amylolytic population at the expense of cellulolytics and protozoal organisms (Lindberg 1981). Mertens and Loften (1980) showed that rate and extent of cell wall digestion of different forages varied with forage type as starch increased from 0 to 80%

in the diet and digestion rate of alfalfa decreased with starch addition, however, coastal Bermuda grass, fescue and orchard grass were relatively unaffected.

The N and energy concentration of the ration fed to the cannulated cow has also been shown to cause variation on *in situ* digestion results. deFaria and Huber (1984) evaluated *in situ* DM digestion of corn silage, alfalfa silage and grass hay in diets containing three different protein levels (8.1, 11.2 and 13.3%) and three energy concentrations (39, 29.9 and 21% ADF). Neither protein nor energy had a significant effect on DM digestibility for any forages; however specific sampling time by forage interactions were important. In general, protein supplements have higher N and DM disappearances than forages (Vik-Mo and Lindberg 1985).

Animal effects

Different species of animals have been used for the *in situ* digestion technique including cows (Nocek et al. 1979), heifers (Uden and Van Soest 1984), sheep (Prigge et al. 1984), goats (Castle 1956) and horses (Uden and Van Soest 1982), and the limit has only been the extent of surgical creation of a fistulae. Cattle and sheep are the most commonly used species. Comparison of sheep and steers fed similar diets at maintenance levels indicated that sheep had higher ruminal ammonia N than steers, lower VFA, similar ruminal pH and a similar rumen fluid dilution rate (Siddons and Paradine 1983). Though protein (casein) degradation activity was not different between species, rumen degradation of other feedstuffs was. One exception was fish meal which was higher in UIP in sheep. The ranking of feedstuff degradabilities was similar.

Differences within species may arise due to sex and physiological status. Specific experiments have addressed differences in rumen parameters as related to age (Castle 1956), pregnancy (Ehle et al. 1984) and stage of lactation (Hartnell and Satter 1979). The majority of these differences relate to specific dietary type associated with a specific physiological state rather than to what extent ruminal influence has on digestion (Prigge et al. 1984).

Adaptation period on each dietary regime is important. Since the *in situ* technique usually uses few animals, the experimental design commonly used includes a Latin square or switch over design. Under these circumstances it is important to provide adequate time for the animals and the rumen to adapt to dietary changes.

The experimental objectives before should be considered when selections of animal type and diet. In most cases experiments are directed towards a specific set of circumstances such as dairy research with early lactation cows, beef research with finishing cattle. The researcher, therefore, should use the type of animal to which the results will be applied and to subsequently feed a ration that would best meet maximum performance requirements. Details about the animals, physiological status, diets, feeding and management should be documented accordingly.

Microbial colonization

There is intimate contact of the test protein particles with ruminal microbes when bags are suspended in the rumen causing potential contamination that may affect degradation results. Reports have indicated that many morphological types of bacteria become associated with the plant cell wall through the glycoprotein coat as degradation

takes place (Akin and Amos 1975; Akin 1976). Latham et al. (1978) noted that adhesion to plant cell wall increased linearly up to 9 h of incubation with rumen fluid.

The presence and extent of microbial contamination in feed residues after ruminal incubation varies from one experiment to the other. Mehrez and Orskov (1977) reported that concentrate ingredients generally contain little microbial contamination (5 to 10% of residual N), while forage residues tend to have more contamination (Nocek and Grant 1987). Nocek (1988) suggested that for low protein forages and coarse feedstuffs the results should be corrected for microbial contamination using markers such as diaminopimelic acid and RNA. More recently N^{15} is being used to measure N contamination and then using near infrared spectroscopy (NIR) to predict contamination of forage samples (Alexandrov et al. 1995).

Since microorganisms enter the bag to cause feed degradation, it becomes essential that they be eliminated from the bag or accounted for after the desired incubation to avoid underestimation of DM and N degradation. Two objectives of washing bags after rumen incubation include stopping microbial activity and to free feed residue from rumen fluid and rumen microbes (Mehrez and Orskov 1977) without increasing the loss of feed particles through the bag pores. Many mechanical rinsing methods have been suggested for post incubation bags, including hand for approximately 90 sec per bag rinsing until water is clear (Weakley et al. 1983), machine washing for 2 min (Nocek and Grant 1987), three times with 2 min washing (Ould-Bah 1989), 15 min washing (Varvikko and Lindberg 1985), or 10 min initial machine rinsing followed by 5 min machine rinsing (Boila and Ingalls 1992). Cherney et al. (1990) compared different

rinsing methods and indicated that machine rinsing twice for 2 min or hand rinsing of bags gave similar results, but higher DM disappearance and standard error for DM disappearance was noted when bags were machine rinsed twice for 5 min than when bags were either machine rinsed twice for 2 min or hand rinsed. Rinsing of multiple bags such as 50, 100, or 200 bags simultaneously in a machine (68 L of water) had little effect on DM disappearance and standard error (Cherney et al. 1990). Machine rinsing of bags can be an acceptable alternative to hand rinsing, at least when the time involved is not too long. Increased agitation may wash away more small residues or can be too strong to cause stretching in bag pores resulting in increased escape of residue which may lead to inaccurate estimates.

IN VIVO PROCEDURES

Determination of nutrient digestibility post ruminally is achieved through abomasally or duodenally (anterior to the pancreatic and bile ducts) cannulated animals. Two types of cannulae have been used, the duodenal re-entrant and simple "T" cannulas. Re-entrant cannulas (Phillips et al. 1978) offer the advantage of total digesta collections, eliminating the need for digesta phase markers. Also they are adaptable to automation (Zinn et al. 1980). Stop-gate diversion apparatus with "T" cannulas (Komarek 1981) make their use more physiological than the previous techniques, where the digesta flow was diverted outside, then returned inside the animal. The "T" cannulas can be fitted such that disruption of nervous interaction is minimized, and less opportunity exists for intestinal blockage. The use of simple "T" cannulas requires spot sampling and indigestible solid and liquid phase markers (Nocek 1988). *In vivo* procedures require

considerable investment of resources, including intensive management of both the surgically treated ruminants and various types of cannulas mentioned in order to generate reliable results.

Two mathematical methods used to estimate protein degradation from samples collected as digesta are "regression technique" and the "difference technique". The first technique assumes that the proportion of undegraded dietary protein can be estimated from the relationship between duodenal protein flow and protein intake (Stern and Satter 1982). The second technique measures dietary protein intake and the total protein flow to the duodenum. Microbial, endogenous protein and abraded gut wall cells are estimated at the duodenum and the undegraded protein is obtained by difference (Stern and Satter 1982).

The technique requires accurate measurement of the amount and composition of digesta flow because inaccuracies have resulted from problems in areas such as, estimation of flow due to markers, sampling, or cannulation effects, and of differentiation between feed protein, microbial and endogenous origin in digesta (Broderick et al. 1991). Problems of flow determination and sampling has been reduced because cannulation and marker technologies are now well understood (Faichney 1986) and good estimates of AA passage at the duodenum can be obtained (Stern et al. 1985). Inaccurate assignment of the proportion of AAs contributed by feed protein and microbial protein has invalidated many estimates of rumen escape of feed protein (Broderick et al. 1991; Hvelplund 1985).

MOBILE NYLON BAG TECHNIQUE

This technique which was originally developed for pigs (Sauer et al. 1983) has been adopted for ruminant studies as a method for measuring lower GI tract digestion of individual feeds.

To measure the digestibility of protein substrate in the lower GI tract, the protein source is introduced in a porous nylon bag, sealed and introduced into the duodenum (with or without rumen incubation) and the bags recovered from the terminal ileum or from the feces (de Boers et al. 1987; Kendall et al. 1991; Frydrych 1992). Disappearance from the washed bag is regarded as a measure of digestibility in the small intestine. Bags containing samples can be treated with pepsin-HCl or inserted into the duodenum without treatmant. Hvelplund (1985) indicated that the pepsin-HCl treatment had little effect on the results of bags collected at the ileum or in the feaces.

IN VITRO TECHNIQUES

In vitro methods involve a simple physical or chemical laboratory technique that determines degradation characteristics of protein supplements. Solubility has been used for a long time as an estimate of protein degradability (Chalmers and Synge 1954). As long as the test protein has little less soluble protein, a reasonable estimate may be obtained. By its nature, however, such a static determination cannot give the dynamic constants required for a full description of degradation (Broderick et al. 1991).

Other *in vitro* measurements include the use of enzymes (Poos-Floyd et al. 1985) to determine the rate of AA release; incubation with microorganisms (Broderick 1987); addition of starch or fermentable carbohydrates in graded amounts to incubations of

rumen fluid with protein supplement (Raab et al. 1983); labelling proteins with dyes (Mahadevan et al. 1979) or radioisotopes (Wallace 1983).

NUTRIENT FRACTIONATION, DEGRADATION KINETICS AND PROTEIN PASSAGE

Ruminal protein degradation is often described by a first-order disappearance model (Orskov and McDonald 1979) which incorporates two features. The feed proteins must comprise fractions of widely different degradabilities and that disappearance of the protein is a result of two simultaneous activities, degradation and passage. In applying the NRC (1985) notation, data generated from *in situ, in vivo* or enzymatic degradations are generally fitted into the model which divides feed protein into three pools or fractions termed A, B, C, which total up to unity (NRC 1985). Fraction A is the proportion of intake protein degraded very rapidly or already degraded at zero hour (e.g. NPN), B is the fraction of the protein that is potentially degradable and C is the protein fraction completely unavailable in the digestive tract.

In practical feeding conditions, fraction A is rapidly and almost totally converted to ammonia in the rumen since the rate of degradation is ten times faster than the passage of solids from the rumen (NRC 1985). If the ammonia released is not incorporated by rumen microbes into microbial protein, then it passes from the rumen through absorption across the rumen wall and is subjected to at least partial loss as urinary urea or other NPN forms. The B fraction represents the difference between total intake protein and the sum of rapidly degraded (A) plus unavailable intake protein (C) and therefore it is that part of intake protein that can potentially escape degradation in

the rumen and be available for absorption in the intestine. The C fraction is the undegraded, unavailable intake protein that due to either natural conditions, chemical, heat or other reactions during processing, is therefore rendered nutritionally unavailable after any of the ruminant digestive processes and is quantitatively recovered in feces (NRC 1985). Fraction C as defined by NRC (1985) behaves as an inert component in any dynamic description of the digestive process and is normally associated with silages, forages and many chemical and physical treatments and processing of protein supplements.

The proportion of B fraction actually degraded in the rumen is determined by the fractional rate for degradation (K_{dB}) and passage (K_{pB}) for fraction B, and is given by the ratio $K_{dB}/(K_{dB} + K_{pB})$ and the extent of rumen protein degradation is calculated using the equation:

$$\text{Degraded protein} = A + B\,[K_{dB}/(K_{dB} + K_{pB})];$$

with the extent of rumen protein escape given by the complementary equation:

$$\text{Escaped protein} = B\,[K_{pB}/(K_{dB} + K_{pB})(P_i)] + C;$$

where $P_i = e^{-K_p L}$, where P is the portion of protein nutrient remaining at initial digestion ($t = 0$) and L = lag time hour. The lag function is of particular importance in forages (Mertens 1979) since *in vitro* and *in situ* experimentation usually yield data with the lag dependent on the fastest of the components among degradation fractions (Nocek 1988). Though fraction C will escape the rumen degradation, in the NRC (1985) model it is assumed practically indigestible in the lower GI tract and will therefore not contribute to the supply of AAs to the host animal.

The approach used by the ARC (1984) to describe extent of rumen protein degradation and escape is:

Degraded protein = $a + bc/(c + k)$, and

Escaped protein = $bk/(c + k) + [1 - (a + b)]$,

where a is the rapidly degraded fraction or already degraded at zero hour (designated "A" in the NRC model), b is the potentially degraded fraction ("B"), c is the fractional rate of degradation of fraction b ("K_{dB}") and k is the fractional rate of rumen passage from fraction b ("K_{pB}"). The fraction "C" in the NRC model is given by subtracting fraction a and b from the total $[1 - (a + b)]$. In the ARC model, the undegradable protein, $1 - (a + b)$, is ideally the amount of total protein remaining undegraded in situ as time approaches ∞. In practice , incubation times of 12 to 24 h are commonly used , and it is often therefore difficult to distinguish the degradation rates obtained for slowly degraded proteins such as fish meal (ARC 1984) and blood meal (Loerch et al. 1983) from rates close to zero. Thus fish meal and blood meal appear to have large undegradable protein values, $1 - (a + b)$, but still have satisfactory digestibilities of the c in the small intestine (ARC 1984).

Interpretation of *in situ* data

The disparity between the two models described above becomes evident when interpretation is made from in situ degradation data (Broderick et al. 1991). Both approaches arrive at essentially the same estimates for extent of protein degradation and escape, but differ in the interpretation of the subsequent availability of fraction C in the lower GI tract. This is because in situ incubations are often conducted over time periods

approximating the rumen retention time of the protein under study, the point at which there is little practical difference between very slow degradation rates and degradation rates approaching zero (Broderick et al. 1991).

Although fraction A is usually quantified as part of total protein that disappears rapidly during in situ incubation this can be inappropriate because soluble proteins may be resistant to degradation (Broderick et al. 1991). Indigestible protein has been estimated as acid detergent insoluble N (Goering et al. 1972) or by use of active enzyme preparations of *Streptomyces griseus* protease (Krishnamoorthy et al. 1983). Although similar estimates of indigestible fraction C were obtained by both methods, the use of proteolytic enzymes, rather than chemical extraction, provides a biological method (Broderick et al. 1991).

Both NRC (1985) and ARC (1984) note the importance of passage rate in determining the extent of rumen degradation and escape. For any given degradation rate, as passage rate increases, extent of degradation decreases; the magnitude of this effect increases directly with rate of degradation (ARC 1984). Soluble and particulate matter differ greatly in their rates of rumen passage from studies (Owens and Goetsch 1986) with cattle and sheep with dry matter intake ranging from 1.1 to 4% of body weight. The ratio of passage rate for liquids to solids ranged from 1.7 to 2.2 (average 2.0). Therefore any alteration of a protein structure that makes it insoluble, so that it may pass with solids, has important implications for rumen escape (Broderick et al. 1991).

The AA composition in the protein leaving the rumen will ultimately determine its nutritive value since the AA content of microbial protein does not vary appreciably

with diet (Bergen et al. 1986) and therefore undegradable intake protein (UIP) is the main means of manipulating the composition of AAs reaching the lower GI tract. Storm and Orskov (1984) reported that the first most limiting AA in microbial protein was methionine, followed by lysine, arginine and histidine, and that, therefore UIP would be of maximal value if its AA quality were complementary to microbial protein. Protein supplements high in limiting AA provide substantial amounts of UIP of great value since the EAA composition of UIP is likely to be similar (Ganev et al. 1979) to that of the protein fed.

UTILIZATION OF NITROGENOUS COMPOUNDS BY RUMINANTS

The ruminant animal is unique in its N metabolism because the microbial and protozoal population in the reticulo-rumen modifies the composition of the dietary intake protein en route to the lower GI tract. Another factor involved is that the nutrient requirements of the rumen microbes are not similar to that of the host animal and therefore these events result in modified microbial activity and reduced efficiency of the total digestive process with respect to intake protein offered. These processes usually affect the quantity of AA available to the animal and the make-up of the mixture of the AA absorbed compared to that in the diet. Any improvement in the utilization of a given N-compound by the ruminant ultimately starts with keen dietary formulation, knowledge of dietary composition with respective to N, energy and other nutrients and knowledge about the expected behaviour of the diet in the digestive tract of the animal.

In the past, protein allocation to ruminants was indicated as amounts of CP protein over a 24 h period (NRC 1985). Now new concepts give specific address to N

fractions or metabolic dynamics that affect utilization (NRC 1989) in which all values are in protein (N X 6.25) equivalents. Intake protein is described in this new concept relative to digestive physiology of the ruminant and protein is expressed in three major fractions: A (rapidly soluble), B (potentially degradable) and C (undegradable) as measured by in situ procedure.

Practical ration formulation for ruminants requires knowledge on UIP and DIP. The DIP estimates the amounts of N nutrients available to the microbial population, and the UIP is required to estimate the amount known as "bypass protein". Methods for assessing rates of degradation of protein sources have already been discussed. Whichever method is used to assess the rates at which different dietary intake protein sources are degraded in the rumen, it is also necessary to consider the rate of turnover in the rumen due to flow of digesta through the rumen if their passage to the abomasum is to be calculated (Smith 1989). Prediction of turnover rates of proteins in the rumen is difficult and is influenced by a number of factors including dietary composition, physiological state of the animals and climate.

Feeding of non protein nitrogen (NPN)

The most abundant source of NPN naturally occurring in ruminant feeds is provided by the nucleic acids that make up 5-25% of total N (McAllan 1982). Some small amounts may survive passage through the rumen in conjunction with resistant plant structures but the great majority are rapidly degraded by a sequence of microbial enzymes to yield purines and pyrimidines which themselves soon disappear and are presumably further degraded to ammonia (Smith 1975).

The real concern with NPN compounds in ruminant nutrition relates mainly to supplements added to diets to provide a source of ammonia as a microbial nutrient. Some NPN supplements provide ammonia rapidly (such as urea and uric acid) which may lead to potential toxicity, inhibition of feed intake, muscle tremors, tetany and if in excess death may occur (Bartley et al. 1976). The possibility of such an accident can be avoided by providing readily available carbohydrate, using an NPN supplement such as biuret, isobutylidene diurea or lactosyl urea that is degraded relatively slowly, and also allowing the animals an adaptation period with use of these products (Smith 1975).

Availability of microbial protein

Rumen microbial protein synthesis and passage of microflora into the lower GI tract results in the availability of this type of protein to the host animal. Microbial protein synthesis is complex because the microbial population is very varied in composition and is unevenly distributed (Demeyer 1981). In terms of microbial protein synthesis, protozoa are generally seen as predators which reduce the overall energetic efficiency of synthesis by increasing turnover of bacterial protein (Viera 1986).

As long as other nutrients (such as, sulphur, phosphorous or nitrogen) are not limiting, the amount of microbial protein synthesized in the rumen is primarily dependent on the energy in the form of high-energy phosphate bonds (essentially ATP) provided by anaerobic fermentation of dietary organic matter (Smith 1989). Therefore, microbial protein quantities finally available in the lower GI tract of the host animal will depend on the rate of rumen bacterial turnover (Nolan and Stachiw 1979) and bacterial growth rate .

Degradation of protected protein

Most protein resources offered to ruminants have inherent properties that affect their susceptibility to degradation in rumen. Rumen degradation of proteins can be reduced by processing or treatment of protein, as well as association with other constituents of digesta such as condensed tannins (Zelter et al. 1970); formaldehyde treatment (Sharma and Ingalls 1974), reaction with NaOH (Mir et al. 1984), treatment with whole fresh blood (Mir et al. 1984), treatment with fish hydrolysate (Mir et al. 1984) and heat treatment (de Boer et al. 1987; McKinnon et al. 1990; Moshtaghi-Nia and Ingalls 1992; McAllister et al. 1993; Demjanec et al. 1995; Hussein et al. 1995). These treatments form complexes that are largely resistant to rumen degradation but at extreme levels some of them may provide complexes that are also resistant to digestion in the lower GI tract and are therefore nutritionally of little or no value. N compounds associated with ADF fall in this category, however some complexes containing proteins that are resistant to rumen degradation (Moshtaghi-Nia and Ingalls 1991) are digestible in the lower GI tract and therefore add to the UIP (B fraction) content of the diets.

Provided that a treated protein has not been overprotected it should, in theory, be useful as a supplement under conditions where UIP limitation has been identified. This is more likely to be the case for high yielding ruminants and benefits have sometimes been demonstrated experimentally (Mir et al. 1984; Hussein et al. 1995; Demjanec et al. 1995) under carefully chosen conditions for both chemically treated and heat treated protein supplements where limiting AA were increased by the supplements.

Nitrogen recycling in ruminants as a protein utilization process

Urea formation is an end-product of N metabolism in the liver of ruminants. Functionally it is thought (Smith 1989) to leave the body fluid pool by four routes: urine, the rumen, abomasum and the small intestines. Urea entering the urine is related to plasma urea concentrations and represents iversible loss and is also negatively influenced by glomerular filtration rate (Cocimano and Leng 1967). Tubular reabsorption of urea appears to be controlled metabolically and, therefore, N loss into the urine is low in ruminants consuming low N-diets relative to monogastric animals under similar conditions (Leng et al. 1985).

Nitrogen conservation is proposed to be due to reduced glomerular filtration rate (Eriksson and Valtonen 1982) and partly through tubular reabsorption of urea (Harmeyer and Martens 1980). Compensatory changes in urea clearance by the kidney with changes in plasma urea concentration are not restricted to the ruminants (Leng et al. 1985) and it seems possible that the very low urine urea excretion often observed in ruminants fed low N-diets is a *result* of high recycling to the rumen and lower GI tract rather than its cause.

Even with normal N intakes, endogenous urea levels entering the rumen are frequently more than 40% of production and it can be as much as 90% in sheep, cattle, goats and other ruminants fed low N-diets (Cocimano and Leng 1967). Whatever the mechanism, this demonstrates the potential existing for a high degree of recycling in ruminants, in parallel with, a low degree of loss in the urine.

Digestion of nitrogen for fibre digestion

In order to maximally digest the main dietary fibre components such as cellulose, hemicellulose and pectins, rumen microbes require an adequate supply of available N compounds as well as other essential nutrients such as sulphur and phosphorous. Dietary N deficiency leads to depressed fibre digestion in the rumen (Campling et al. 1962) and this may be true for a diet apparently adequate in total N compounds but in which these compounds are of low degradability (Smith 1989). There is need to provide an adequate amount of rumen degradable intake nitrogen to maximize microbial activity and protein synthesis. Recent N-formulation schemes (NRC 1989) involve a calculation in which rumen degradable intake N is related to some index of energy available to the microbes.

Uptake of nitrogen in the omasum

Reports indicate that urea enters the omasum directly from the blood stream (Siddons et al. 1985) and urease activity, presumably of microbial origin occurs both in the wall and contents of the omasum (Boda et al. 1976). Ammonia would, therefore, be added to omasal digesta from degradation of endogenous urea as well as from the microbial degradation of residual N sources entering the rumen. At the same time, as the ammonia is absorbed, there may be a net disappearance of ammonia from the digesta passing through the omasum (Engelhardt and Hauffe 1975). These processes have not been well studied and factors governing them are not understood (Smith 1989).

Absorption of nitrogen in the abomasum

The process of absorbing N compounds from the abomasum is probably quantitatively not important (Stangassinger and Gieseck 1980) because absorption of ammonia at this site is presumably prevented by the low pH. If a favourable

concentration gradient exists, intact urea might diffuse out but this would be expected only if a dietary component, such as isobutylidene diurea, were offered allowing escape from rumen degradation but release of urea under abomasal conditions (Stangassinger and Gieseck 1980).

Large quantities of endogenous N enter the abomasum either as urea or as digestive proteinous secretions. Total N entering the abomasum of sheep in this way was estimated by Harrop (1974) to be about 0.5 - 2.8 g per day of which 65 - 90% was protein, much in the form of proteolytic enzymes.

Digestion of microbial, endogenous and residual dietary intake proteins is initiated through the abomasal enzymes similar to monogastric animals, and degradation of protein mainly to peptides form the bulk of the AA yielding N compounds entering the proximal duodenum.

Amino acid composition in the duodenum

Ruminant animals depend on satisfactory N nutrition both as to the total quantity and the quality of AA presented in the duodenum. Practical ration formulation schemes would aim at determining requirements at this site for individual AAs, and especially EAAs, and formulation efforts must relate these AAs to dietary requirements (NRC 1989). There is a general recognition for the variation in AA composition ultimately available in the abomasum, but under practical feeding conditions it is difficult to consider individual transactions in the ruminant (Smith 1989).

The group term EAAs discussed in the manuscripts to follow include: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, valine and threonine

while NEAAs include alanine, aspartic acid, cyst(e)ine, glutamic acid, glycine, proline, serine and tyrosine.

Protein supplements used in ruminant feeding can differ in the proportion of individual AAs in the feed vs EAAs in UIP at the duodenum as was recorded from feeding corn gluten meal (Smith 1984). No EAAs quality differences were noted duodenally when fish meal and protected casein were fed, though total AA flows were different (Sharma et al. 1974). Total AA flows at the duodenum were greater with diets containing fresh alfalfa but the EAA quality to total AA ratio were similar (Flores et al. 1986).

It is evident that digesta entering the ruminant duodenum, consisting of varying mixtures of microbial proteins, residual UIP and endogenous proteins includes enzymes can result in AA composition variation.

USE OF CANOLA MEAL IN DAIRY RATIONS

Prior to the release of canola, there had been continued expansion of rapeseed production in Canada in the 1960's with greater quantities of rapeseed meal (RSM) becoming available for domestic and foreign use by the livestock industry. RSM was considerably less expensive per unit protein than other plant protein supplements and hence had an advantage in dairy cattle rations (Waldern 1973). Early work by Asplund (1962) indicated that inclusion of RSM, at 20% in the dairy ration lowered both total dry matter (DM) intake and milk production, whereas at 10% there was no significant effect. Subsequent studies by Ingalls et al. (1968) to determine effects of RSM on *ad libitum* consumption of grain rations indicated that addition of RSM up to 12-13% of dietary DM

significantly decreased grain consumption with no effect on milk production composition. Ingalls and Seale (1971) later determined the effect of RSM inclusion levels at 13.7, 6.8 and 0% of concentrate DM on growth rate and performance of dairy calves from birth to the end of their first lactation. Results from this study suggested that though difference in first lactation were not statistically significant ($P>0.05$), heifers raised on grain rations containing 13.7% RSM produced 16.8% less milk with 9.6% less fat than those fed SBM as a protein supplemnent.

When Burgess and Nicholson (1971) compared levels of up to 1.75% urea and 15% SBM with 22.5% RSM as the only supplemental protein source in the ration for lactating cows there was difference in milk production and composition, but cows receiving RSM produced 9.6% less milk than those fed SBM. Waldern (1973) showed some decline in milk yield for cows fed upto 27% of concentrate RSM compared to SBM during a 112-day trial that measured total ration digestibility, nitrogen utilization and performance of lactating cows fed corn silage as the only roughage source.

As plant breeders continued to develop low levels of glucosinates in rapeseed, animal studies with the resultant RSM continued. Ingalls and Sharma (1975) conducted a study to determine the perfomance of dairy cows on Bronowski RSM (a high erucic acid, low glucosinolate cultivar) as compared to commercial RSM (high glucosinolates) that was either "flavoured", pelleted or with molasses addition. The results from this study indicted that inclusion of commercial RSM up to 14% of grain mixture reduced consumption compared to the Bronowski containing mixture with no effect on milk production; and that inclusion of Bronowski up to 24% of grain mixture in place of SBM

did not affect feed intake, milk composition, ration digestibility and N-retention (Ingalls and Sharma 1975). From the same results, pelleting or addition of molasses or flavouring did not improve *ad libitum* intake of rations with commercial RSM. Plant breeders released double low (0 - 0, < 2% erucic acid and < 30 μg/g glucosinolates) rapeseed (1788 and 940 or Tower) cultivars in 1974 in which contents of glucosinolates in the meal and erucic acid in the oil were relatively low compared to other varieties of rapeseed commonly in use. Animal studies (Sharma et al. 1977) were conducted to determine the effects of high levels (25%) of dietary DM (0 - 0) RSM in dairy rations on grain consumption and animal performance as compared with SBM or commercial RSM. The results indicated no difference in feed intake, milk yield and fat content but protein content was higher for cows fed commercial RSM and SBM diets as compared to those fed the 1788-RSM diet. With present day knowledge the lower protein content was probably due to the increased fat levels.

Fisher and Walsh (1976) concluded that 1788-RSM (a low glucosinolate *Brassica napus* cultivar) fed at a level of 34% in the grain mixture displayed a significant linear depression in milk yield as the proportion of CM in the concentrate increased. The negative response may have been related to the quality of CM used, since the workers (Fisher and Walsh 1976) reported high residual oil contamination containing erucic acid and hexane as a consequence of processing problems. Papas et al. (1978) incorporated upto 30% of 1821 RSM or 26% Tower RSM in dairy rations with results showing increased milk yield on 1821 RSM compared to SBM or Tower RSM but with no other treatment effects on feed intake, milk yield and contents or plasma thyroxine levels.

The conclusions drawn by Fisher and Walsh (1976) after a 28-day experimental period resulted in further studies by Sanchez and Claypool (1983) to analyze a longer experimental period including phases of the lactation curve, feed utilization and health. Sanchez and Claypool (1983) argued that over short experimental periods the ability of the lactating cow to adapt to short periods of stress by using body tissues could mask any nutritional unbalance. Sanchez and Claypool (1983) fed 15% CP diets containing either CM, SBM or cotton seed meal (CSM) as the protein supplement to high producing cows during the first 12 weeks of lactation. Milk yield was not significantly affected by protein supplement, but cows receiving CM produced 3.2 and 1.2 kg more milk daily than cows receiving SBM and CSM respectively. Later results from DePeters and Bath (1986) from four trials evaluating the effects of substituting CM for CSM in dairy diets indicated no effects on milk yields, milk components and feed intake with respect to different protein supplements. McLean and Laarveld (1991) conducted studies to determine the effects of level (0.5, 10 or 15% of total ration DM) of CM on performance of dairy cows and concluded that levels had no effect on feed intake, milk yield or on body weight of cows during the experimental period; suggesting that CM can make up as much as 15% of total ration DM or 30% of a corn based portion of the concentrate without affecting performance of dairy cows in lactation.

All these studies with low glucosinalate, low euric acid CM support the potential for incorporating CM in dairy rations up to the level of 25% with no adverse effects on the performance of lactating dairy cattle and that CM can be substituted for SBM in dairy

rations. One of the limitations for including CM in dairy rations is its relatively high DIP level.

MANUSCRIPT I:

EFFECT OF HEAT TREATMENT ON *IN SITU* DEGRADATION AND LOWER GASRTO INTESTINAL TRACT DISAPPEARANCE OF CANOLA MEAL.

ABSTRACT

In order to study the potential to decrease rumen degradation of canola meal (CM) and increase post-ruminal availability of amino acids in early lactating dairy cows, four batches of commercial CM were exposed to moist heating (110°C) for 0 (CM 0), 23 (CM 23), 45 (CM 45), and 60 (CM 60) minutes through a steam jacketed and steeped to represent four treatments. A fifth supplement (Bi) made from a mixture of animal-vegetable protein was formulated in order to compare with CM 60. Three non-lactating Holstein dairy cows with rumen and proximal duodenal T-shape canulae were used in a 3 x 3 Latin square design to conduct an *in situ* degradation trial with incubation times of 0.1, 2, 4, 8, 12, 16, 24, and 72 h. Duplicate 5 g samples of each supplement were incubated in large (7 cm X 5.5 cm) multifilament nylon bags and duplicate 1 g samples of supplement in monofilament and multifilament small (3.5 cm X 3.5 cm) nylon bags. Rumen disappearance of dry matter (DM), nitrogen (N), acid detergent fibre and neutral detergent fibre were measured from the large bags and lower gastro intestinal (GI) tract disappearances of DM, N, acid detergent insoluble nitrogen, neutral detergent insoluble nitrogen, essential amino acids (EAA) and non-essential (NEAA) were measured from small bags digested in vitro with or without pepsin-HCl. Effect of protein type in the cows' diets on degradation of DM and protein at 12, 16 and 24 h was measured for supplements CM 0, CM 60 and Bi. The EAA profile of CM 60 and Bi were similar ($P<0.05$). Rumen degradation of nitrogen for CM 0, CM 23, CM 45, CM 60 and Bi at 68, 60, 52, 44 and 38%, respectively were different ($P<0.05$) from each other. Increased heat treatment of CM decreased ($P<0.05$) rumen nitrogen degradation but

increased ($P<0.05$) post-ruminal nitrogen disappearance. There was a decrease ($P<0.05$) in mean effective degradability of DM (43%) and N (36%) with increased heating of CM with the exception of N (41%) with 45 min heating. Type of protein supplement in the cows' diets affected ($P<0.05$) DM and protein degradation. Bag type influenced ($P<0.05$) degradation and disappearance of both DM and N. Monofilament bag type was higher ($P<0.05$) than the multifilament type with one exception. Degradation of DM and N for both bag types were not affected by removal of the pepsin-HCl digestion step. Heat treatment decreased lower GI tract concentration of, arginine, leucine, methionine, and valine in the CM. Rumen and lower GI tract disappearance of individual EAA decreased ($P<0.05$) with heating but fecal excretion of arginine, histidine, isoleucine and threonine increased ($P<0.05$). There were increased ($P<0.05$) quantities of EAA digested in the lower GI tract with increased heating. Heat treatment reduced ($P<0.05$) mean rumen N disappearance of CM 0 (14%), CM 60 (31%) and Bi (28%) from multifilament bag digested in pepsin-HCl more than monofilament bag for CM 0 (20%), CM 60 (35%) and Bi (38%) respectively. Results suggest a potential for use of heat treatment to reduce rumen degradation of CM as a protein supplement in dairy rations.

KEY WORDS: canola meal, moist heating, ruminal degradation, bag type, pepsin-HCl, lower GI tract EAA, fecal excretion.

INTRODUCTION

Lactating dairy cattle depend on both microbial protein and rumen escape feed protein digestion in the lower gastro intestinal tract (GIT) in order to satisfy their amino acids (AA) requirements. Much of the dietary feed protein that enters the rumen is, however, degraded to peptides and AA which are ultimately deaminated (Kendall et al. 1991). The extent to which dietary feed protein is degraded in the rumen depends on the length of time in the rumen and also on its physical and chemical nature (Cotta and Hespell 1984). Although undegraded feed protein may be digested in the lower GIT, it is difficult to quantitate the dietary protein escaping rumen degradation. Therefore, the true value of protein in the feedstuff can become dependent on the extent of rumen degradability of individual dietary AA by the rumen microorganisms (Susmel et al. 1989).

Canola meal (CM) has a high level of protein which is rapidly degraded in the rumen (de Boer et al. 1984). Research efforts have been focused on decreasing the rumen degradability of CM protein through various treatment methods including heat (Mir et al. 1984; Ahmadi 1988; McKinnon et al. 1990; Moshtaghi-Nia and Ingalls 1992; and McAllister et al. 1993).

Heat treatment of feedstuff protein has been associated with increasing the amounts of rumen escape protein available in the lower GIT of ruminants (Nishimuta et al. 1974; Mir et al. 1984; Ahmadi 1988; McKinnon et al. 1990; Moshtaghi-Nia and Ingalls 1992). Heat treatment of protein supplement results in a decrease in the solubility of the proteins by creating cross-linkages both within and among peptide chains and to

carbohydrates (Robinson et al. 1986), thus lowering their susceptibility to ruminal degradation which favours more escape of dietary protein to the lower GIT. Very high temperatures and extended heating times may render protein less digestible through the Maillard reaction between sugar aldehyde groups and free AA groups, thus lowering digestibility of constituent AA (Deacon et al. 1988).

In situ incubation of feedstuff in the rumen provides data on the potential disappearance of the constituents, and this may be used to estimate the size of the soluble and potentially degradable fraction of a nutrient and the rate of degradation of the nutrient fraction (Satter 1986; Nocek 1988; Boila and Ingalls 1992; Moshtaghi-Nia and Ingalls 1992). Previous studies using the *in situ* technique with protein supplements have emphasised the extent of dry matter (DM) and Nitrogen (N) degradabilities (Nocek 1988). Other studies have focused on CM as a feed protein supplement in ruminant nutrition, and the investigations have similarly looked at the degradability of DM and N (Ha and Kennelly 1984; de Boer et al. 1987). More recent studies have investigated rumen escape potential of individual AA from CM heat treated in the laboratory (Kendall et al. 1991; Boila and Ingalls 1992, Moshtaghi-Nia 1994).

Moist heat is an integral part of the CM extraction process, however heating under these conditions is kept to a minimum to maintain a high biological value of the CM for monogastric animals. Additional moist heat treatment of CM at the plant level and holding of the meal for various lengths of time on steeping trays may be a simple method to improve its value for ruminants.

The objective of this study was to measure the rumen degradability and lower GI tract availability of CM that had been exposed to moist heat treatment (110°C) for various time periods under extraction plant conditions and to compare these results with a by-pass protein supplement formulated to provide similar levels of essential amino acids (EAAs) as those expected in heat treated CM. An associated objective was to measure rumen *in situ* disappearance rates using two types of nylon bags, the effect of post ruminal pepsin-HCl digestion on the lower GI tract availability of AA and to determine the effect of cow dietary protein supplement on rumen DM and N disappearance of test protein supplements.

MATERIALS AND METHODS

Experimental protocol

Canola meal batches used in this study were exposed to moist heat (110°C) for 0, 23, 45, and 60 minutes through a steam jacketed conveyor set at slow speed and then held in steeping trays at passage rates of 200, 110, and 90 kg h^{-1} at the Protein Oil Starch Pilot plant in Saskatoon, Saskatchewan (for average retention times of 23, 45, and 60 min respectively). The samples were thereafter identified as CM 0, CM 23, CM 45 and CM 60 representing time of heating. A fifth sample (Bi) was formulated to supply similar levels of rumen escape essential AA as CM 60 using a mixture of commercial CM, corn gluten meal, fish meal and meat meal. The Bi supplement contained (on DM basis) 16% fish meal, 8% meat meal, 22% corn gluten meal and 54% commercial canola meal.(table 1.1).

Three non-lactating Holstein dairy cows weighing approximately 646 kg were fitted surgically with large 4" diameter rumen canula (Bar Diamond, Parma, ID) and proximal duodenal T-shape canula made from plastisol (F. H. and Sons Manufacturing Ltd., Rexdale, ON). During the experiment cows were fed a barley concentrate mixture formulated for early lactating cows (NRC 1989) with protein supplement from either CM 0, CM 60 or Bi. Rations were fed twice daily (08:00 and 15:00) as a TMR (Table 1.1 and 1.2) containing concentrate mixture (57%), corn silage (20%) and alfalfa silage (10%), with long hay (13%) fed separately.

The experiment was conducted as 3 x 3 Latin square. The cows were adapted to test diets for 2 weeks prior to supplement *in situ* degradation studies. The cows were housed indoors in the Animal Science Research Unit and were kept individually on bedded concrete floor pens except during the week of data collection when they were housed in individual stalls. Water was available at all times. All cannulated cows were managed in accordance with the Canadian Council on Animal care requirements.

RUMINAL INCUBATIONS

During each period, duplicate large (7 cm X 5.5 cm) multifilament nylon bags of porosity 50 μm (ANKOM Co., Fairport, NY), prepared by heat sealing, with one open end, were filled with a 5 g sample of each test protein supplement, closed by heat sealing and incubated in the rumen at time intervals of 0.1, 2, 4, 8, 12, 16, 24, and 72 hours. Twelve nylon bags for each test supplement were incubated per cow as a result of 2 (duplicate) bags per cow X 3 cows X 2 runs within a period. Nylon bags containing no sample were incubated as blanks, 1 bag per cow for each incubation run of a trial period

for a total of 6 blanks per trial. The blank bags were used to correct for DM, and N contributed by microbes and feed particles adhering to the bags in the rumen. Within each incubation run, sample-filled bags plus blanks were placed in the rumen in reverse order of incubation, 72 h first and 0.1 h last, such that, all bags were removed from the rumen at the same time. The test samples CM 0, CM 60 and Bi incubated through to 72 h were incubated in the rumen of cows receiving diets formulated with protein supplement similar to the test samples. All protein samples used in the trials were studied as received from the processing plant and were not ground prior to being placed in the nylon bags.

A similar number of large (7 cm X 5.5 cm) nylon bags as set out in the protocol above were filled with CM 0, CM 60 or Bi and incubated at 0.1, 12, 16, and 24 h in the rumen of cows receiving each of the three dietary protein combinations to determine the effect of the cows' diet on *in situ* degradation of DM and protein. Test supplements CM 23 and CM 45 plus blanks for similar time intervals (0.1, 12, 16 and 24 h) were also incubated in the rumen of cows receiving diets composed of protein from CM 60 only.

Mobile nylon bag technique

Two types of small bags (3.5 cm X 3.5 cm) were prepared by heat sealing nylon (ANKOM Co., Fairport, NY) and leaving one open end. The two bag types were monofilament of porosity 50 μm and multifilament 50 μm which differed in the pattern of thread weaving (figure 1.7) and extent of open surface area for particle in and outflow. Both bags types are made from polyester (ANKOM CO., Fairport, NY) but had

different thread weaving and percentage openings. The monofilament bag type had thread bands (figure 1.7) woven and "welded" on top of each other, providing a more fixed and more open surface area. Multifilament type had interlocking threads woven in and out in succession on top and under each other and had less open surface area due to this pattern of weaving. These bags were used to determine the lower GIT disappearance of DM, N, acid detergent insoluble nitrogen (ADIN), neutral detergent insoluble nitrogen (NDIN), EAA and non-essential amino acids (NEAAs) for the test protein supplements. Each small bag of both types were filled with 1 g of test protein sample and closed by heat sealing the open end. Twelve bags for each supplement together with two blanks for each bag type were incubated in the rumen for a 12 h period as a result of 2 bags per cow X 3 cows X 2 incubation runs per period. A ruminal incubation time of 12 h was chosen to approximate a ruminal outflow which may be typical of feed particles such as CM in high-yielding dairy cows consuming mixed diets and fed energy greater than twice maintenance (ARC 1984). All the small bag samples were incubated in the rumen of cows receiving diets whose protein base were similar to that of the respective test supplement. Sample filled small bags and blanks were placed in the rumen at same time for the 12 h rumen *in situ* bags, such that all bags (large and small) were removed from the rumen at the same time.

Both large and small bags were placed in large mesh (2 mm X 3 mm) laundry bags and weighted down in the rumen with sand-filled plastic bottles. After the 12 h incubation, bags were removed from the rumen and frozen separately unwashed. All the bags were thawed and two of each small bag type (total of 6 bags per protein

supplement) together with one blank for each bag type without the pepsin-HCl digestion were inserted randomly into the duodenum of the same host cow (for rumen incubation) at the rate of two bags per h. This rate was used to minimize chances of blockage in the lower GIT. The other 6 bags were digested in pepsin-HCl solution (1 g pepsin per litre of 0.01 N HCl) in separate beakers for each test supplement at 39°C for 3 h as suggested in earlier reports (Sauer et al 1983; Kirkpatrick and Kennelly 1984; and Kendall et al 1991) to simulate abomasal digestion. After 3 h of pepsin-HCl digestion, the bags were removed and placed on ice cubes at 4°C until it was time to insert them into the duodenum of the original host cow at the rate of 2 bags per h. Bags were recovered in the feces after an average passage time of 16 h and were separated from the feces by placing in a wire mesh box and washing with a garden hose.

The small bags recovered from the feces and large rumen in situ bags were washed for 10 min in cold tap water while held in a perforated plastic container attached to the agitator of a wringer-type washing machine. The first wash water was drained and the bags were washed for an additional 5 min in clean tap water. All the bags were then dried to constant weight in a forced-air oven at about 60°C for 48 hour for DM determination and further analyses.

Sample compositing

Both large and small nylon bags containing samples from the ruminal incubation and lower GI tract digestion were hand opened and sample residues from two bags for each incubation run were composited into separate plastic bottles, providing a total of two

samples for each cow per period. The samples and blanks were marked accordingly and stored in bottles at room temperature until time for other analyses.

Chemical analysis

Samples from marked bottles were analyzed for total N content of the residual DM as Kjehldal N using a micro-Kjeldahl analyzer (Tecator 1030 analyzer, method 47.023, Association of the official analytical chemists, AOAC 1984). Dry matter of sample residues was analyzed using method 7.013 of Official Methods of Analysis of AOAC 19844). Acid detergent fibre (ADF) was determined by method 7.076 (AOAC 1984) and neutral detergent fibre (NDF) by the procedure of Goering and Van Soest (1970). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) analyses were completed on respective ADF and NDF residues using micro-Kjeldahl method 47.023 (AOAC 1984).

Composited samples drawn from plastic storage bottles were analyzed for amino acids following a 24 h hydrolysis in 6N HCl (Andrews and Baldar 1985) at 110°C. Cystine and methionine were hydrolysed and analyzed after 20 h of oxidation with performic acid (Andrews and Baldar 1985). All AA were determined on a LKB Biochrom 4151 Alpha plus (Biochrom, Science Parks, Cambridge, UK).

Calculations

The percentage of DM, N, ADIN and NDIN recovered in residues was calculated after the nylon bags were corrected for the DM and N in respective blank bags. Percentage disappearance of DM, N, fibre-N and individual AA was calculated as 100 minus the percent recovered in residues. Degradation parameters for DM, N and

individual AA were estimated using the equation of Orskov and McDonald (1979) which incorporated a lag time, *d*. The formula $P = a + b (1 - e^{-ct})$ was used to estimate *P*, the amount degraded at time (*t*), *a* (rapidly soluble fraction), *b* (slowly degradable fraction) and *c* (fractional rate constant at which *b* is degraded), with the constraint that $a + b \leq 100$, by an iterative least-square procedure applying PROC NLIN of SAS (1989). In the discussion to follow the *a* fraction is identified as a soluble fraction, with recognition that it may also represent a rapidly degradable fraction (Satter 1986). The constants *b*, *c* and *d* were estimated using disappearance data for DM and N obtained at incubation times of 0.1, 2, 4, 8, 12, 16, 24 and 72 for test supplements that were incubated in the rumen of cows fed diets whose dietary supplementary proteins were similar to the test supplement. The effective degradabilities (ED) for DM and N were estimated for each test supplement using the equation of Orskov and McDonald (1979) modified to include lag time:

where:

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

ED is the effective degradability of a nutrient, *a*, *b*, *c*, and *d* are as defined above; and *k*, is the outflow rate for feed particles from the rumen. Effective degradabilities were estimated at a rumen particulate outflow rate of 0.05 h^{-1} (Agricultural Research Council 1984), the reciprocal of turnover time 20 h. As lag time approaches turnover time, the estimate of ED approaches *a*, the size of the soluble fraction, while the fraction escaping rumen digestion approaches *b*, the size of the potentially degradable fraction. Since lag

time differed among DM and N, estimates of lag time were included in the calculation to estimate an ED as suggested by Boila and Ingalls (1992).

AAs were classified as metabolically essential or non-essential based on criteria established by Black et al (1957), Downes (1961) and Clark et al (1978).

Statistical analysis

Data were analyzed as a 3 X 3 Latin square with period, cow and test supplement in the model. Analysis of variance for estimated parameters' estabalished from disappearance curves for test supplements incubated (0.1, 2, 4, 8, 12, 16, 24, and 72 h) in rumen of cows receiving similar diets to test supplements, was completed by the GLM procedure with the repeated-measure option of SAS (1989) using the model:

$D_{ijk} = \mu + C_i + P_j + T_{(k)} + e_{ij(k)}$ where:

D = Dependent variable,

μ = The overall mean,

C = Cow,

P = Period,

T = Test supplement, and

e = error.

Test of hypothesis included test supplement, cow, period and used cow by period by cow diet as the error term.

Analysis of estimates of disappearance parameters for test supplements rume incubated for 0.1, 12, 16 and 24 h, bag type and pepsin-HCl digestion and cows receiving different

diets from the test supplements was completed by the GLM procedure with repeated measurements option of SAS (1989) using the model:

$D_{ijkl} = \mu + C_i + P_j + T_k + e_{ijk} + S_l + TS_{kl} + \epsilon_{ijkl}$ where:

D = Dependent variable,

μ = The overall mean,

C = Cow,

P = Period,

T = Test supplement,

e = Error for main plot,

S = Cow diet,

TS = Test supplement by cow diet interaction, and

ϵ = error for sub-plot.

Test of hypothesis included test supplement, cow and period with test supplement by cow by period interaction used as the error term. Contrasts were made where Lsmeans for cow diet and test supplement interactions were significant. The Student Newman-Keuls' test was used to determine the difference between means.

RESULTS AND DISCUSSION

The composition of CM supplement was affected by heat treatment (table 1.3). There was no ($P>0.05$) effect on N content as a result of heat treatment. The values for CP and ADF contents of all CM samples and Bi supplement were similar but NDF, ADIN and NDIN contents were higher ($P<0.05$) for CM 60 and Bi than those of CM 0, CM 23 and CM 45.

Fibre and fibre-N

Heat treatment of CM resulted in a non-significant ($P > 0.05$) increase in ADF and a significant ($P < 0.05$) increase in NDF among the CM samples. There was a 13% and 18% increase in ADF and NDF contents respectively for the CM heated for 60 min. Previous reports (Lindberg et al. 1982; Moshtaghi-Nia and Ingalls 1992) have indicated similar increasing trends in ADF and NDF values in rapeseed meal and CM due to heating. Increased heating can cause denaturation of proteins, which combined with the less soluble proteins become recoverable in the NDF fraction, thereby exhibiting higher NDF values. Nitrogen content associated with NDF increases more with heating than increases in N associated with the ADF fraction. The Maillard reaction and condensed protein-carbohydrate complexes are required in order to get appreciably increased N in the ADF fraction.

The ADF and NDF contents of the Bi supplement were similar ($P < 0.05$) to CM 60 (table 1.3). The ADIN and NDIN content of CM increased ($P < 0.05$) with length of heating time. The ADIN contents of CM 23, CM 45 and CM 60 increased by 224, 290 and 386% respectively (table 1.3), relative to the unheated CM sample. Increased exposure to heat of CM from 23 to 45 min and from 45 to 60 min resulted in 20 and 25% increase for ADIN. The higher ADIN and NDIN contents in heated CM 60 relative to unheated CM 0 is thought to be a result of less soluble proteins and condensed protein-carbohydrate complexes that are recovered in the fibre fractions of the supplement.

The increasing level of ADIN in CM with heating is in agreement with the results of Moshtaghi-Nia and Ingalls (1992) whose study incorporated autoclave heating of CM

at 127°C for 15, 30, 45, 60 and 90 min. Moshtaghi-Nia and Ingalls (1992) indicated that autoclave heating (127°C) of CM for 45, 60 and 90 min increased ADIN content by 265, 396 and 669% respectively. The increase in both ADIN and NDIN contents of protein supplements due to increased heating is in agreement with previous studies (Plegge et al. 1985; Pena et al. 1986; Ahmadi 1988; Arieli et al. 1989). There was a greater increase ($P<0.05$) in NDIN content than ADIN with NDIN content increasing by 708% with 60 min heating of CM relative to unheated CM 0. This observation was in agreement with previous results of Moshtaghi-Nia and Ingalls (1992), and McAllister et al. (1993) which recorded NDIN increase in CM due to heating at values of 736% and 185%, from each respective study, relative to unheated CM 0.

Ruminal escape and degradation of DM and N

There was an inverse relationship between rumen DM and N disappearances from CM samples from the nylon bags with increased heat treatment (appendix figure I.1 and appendix table I.3). There was more ($P<0.05$) rapid disappearance of N from unheated CM 0 compared to all other heated CM samples after 4 h of rumen incubation which indicated that heating reduced rates of rumen degradation of protein from CM therefore increasing the potentially available amounts in the lower GI tract. DM disappearance appeared to follow a similar trend.

The decreasing ($P<0.05$) trend in disappearance of N as heating of CM increased was associated with increasing concentrations of ADIN (table 1.3). Similar degradation effects on DM and N of CM due to moist heating have been reported in other studies (Satter 1986; Vanhatalo and Aronen 1991; Moshtaghi-Nia and Ingalls 1992; Boila and

Ingalls 1994). The N disappearance of the bypass protein supplement (appendix figure I.1) was similar to CM 60 suggesting that our estimated value for CM 60 and the calculated rumen degradation value for the combined plant-animal protein ingredients taken from NRC (1989) were similar. Intermediate heating of CM at 23 and 45 min provided relatively less rumen escape of DM and N potential compared to 60 min of heating.

Moist heating reduced ($P<0.05$) on average, DM and N rumen disappearances (tables 1.4 and 1.5) of CM 60 compared with untreated CM 0, therefore shifting digestion of DM and N from the rumen to the lower GI tract. At 12 h rumen incubation, heat treatment reduced rumen DM and N disappearances of CM 60 relative to untreated CM 0 by 29 and 30% while at 16 h the reduction was by 28 and 17% respectively.

The values obtained in this study were consistent with observations of reduced DM and N rumen disappearances from moist heated CM in earlier studies (Mir et al. 1984; Ahmadi 1988; Moshtaghi-Nia and Ingalls 1992; McAllister et al. 1993). Mir et al. (1984) reported a reduction of protein degradability of CM from 47.6 to 41.0% due to a 20 min heating at 120°C whereas Ahmadi (1988) reported reduction in rumen disappearance from 79.6 to 61.4% for a 16 h rumen incubation with dry heating (128°C) of CM. Moshtaghi-Nia and Ingalls (1992) reported that N disappearance in the rumen decreased from 74.4 to 18.9% with 45 min heating of CM at 127°C while McAllister et al. (1993) recorded a reduction from 72.3 to 54.0% for CM treated with lignosulfate and heated for 2 h at 100°C.

Effective degradability

The effective degradability of DM and N (EDDM and EDN) measured at an outflow rate of 0.05 h^{-1} (table 1.6) indicate a ($P<0.05$) decrease in EDN with increased heating of CM. There were no ($P>0.05$) difference in values for the rapidly soluble fraction (*a*), potentially degradable fraction (*b*) and fractional-rate constant (*c*) for heat treatment of CM at 0, 23 and 45 min for both DM and N with the exception of *a* for N with 45 min heating. Heat treatment of CM for 60 min significantly ($P<0.05$) reduced all the *a* and *b* fractions for both DM and N. Bypass protein supplement had a lower ($P<0.05$) *a*, *b* and *c* fraction than CM 0, CM 23 and CM 45 samples for both DM and N measurements. Heat treated CM 60 and Bi supplement were similar to each other ($P<0.05$) but had lower EDDM and EDN values than CM 0, CM 23 and CM 45; suggesting increased potential for availability of N in the lower GI tract. The EDDM and EDN for CM 60 in this study were higher than those recorded by Moshtaghi-Nia and Ingalls (1992) for CM heated at 45 min. Results for CM 0 were similar to that reported by Kirkpatrick and Kennelly (1984) for untreated CM. The EDDM for untreated CM in this study was similar to those of Kendall et al. (1991) but was higher than that reported by Ha and Kennelly (1984) and Boila and Ingalls (1992) whereas, EDN was similar ($p<0.05$) to the reports of Ha and Kennelly (1984) but was lower than those reported by Kendall et al. (1991) and Boila and Ingalls (1992).

Other factors that influence the estimates of degradability include differences among manufacturing processes of CM (Kendall et al. 1991), the level of concentrate (Barrio et al. 1986; Ariele et al. 1989) and of CP (Kirkpatrick and Kennelly 1984) in the

diets fed to cannulated cows used for *in situ* incubations. Previous results by de Boer et al. (1987) indicated lower rumen escape values which may have been due to the diet. Cannulated cattle used in *in situ* incubations by de Boer et al. (1987) received a 50% concentrate diet , as compared to 65% (Kendall et al. 1991) and 60% (Ha and Kennelly 1984).

Cow diet effect

Dry matter and nitrogen

Effect of protein type in the cows' diet on in situ degradation of DM and N at 12, 16 and 24 h was studied for the protein supplements, CM 0, CM 60 and Bi. Cow diet influenced ($P<0.05$) effect on *in situ* DM degradation at incubation times of 12, 16 and 24 h (table 1.7). Rumen incubation for 12, 16 and 24 h of CM 0 in cows receiving diets with CM 0 resulted in greater ($P<0.05$) degradation than when cows received CM 60 which was greater ($P<0.05$) than when cows received Bi supplement. For CM 60 rumen DM degradation was greater ($P<0.05$) when the cows received CM 60 and Bi rather than CM 0. For Bi supplement rumen DM degradation was highest at 16 and 24 h incubation when the cows received Bi in the diet with similar degradation of Bi for cows receiving the CM 0 and CM 60 diets with the exception of the 12 h incubation.

There was a significant ($P<0.05$) effect on N degradation for all protein supplements due to cows' diets at incubation times of 12, 16 and 24 h (table 1.8). Incubation for 12, 16 and 24 h of CM 0 in cows receiving diets with CM 0 recorded the highest ($P<0.05$) degradation compared to receiving diets with CM 60 which resulted in greater ($P<0.05$) degradation than for cows receiving Bi with the exception of the 12

h incubation. Incubation for 16 or 24 h of CM 60 or Bi supplement in cows' diet with CM 60 had higher ($p < 0.05$) degradation of N than with diet CM 0 which was higher ($p < 0.05$) than for diet Bi (table 1.8) at 12 h incubation. Protein degradation of CM 60 and Bi supplement for cows receiving diets with CM 60 or Bi supplement was lower ($p < 0.05$) than those recorded for CM 0 (table 1.8).

Contrasts ruminal degradation of DM and N

Since there was a significant interaction effect between cows' diets and test supplements incubated in situ, contrasts of interaction Lsmeans for degradation of test protein supplements in a given cows' diet for both DM and N were compared (appendix tables I.1 and I.2). Graphical illustration of contrasts of interaction Lsmeans for the DM and N degradation of protein supplements incubated in a given cows' diet are shown in figure 1.1. Individual contrasts of interaction for the four Lsmeans at 0.1, 12, 16 and 24 h incubation times for DM and N are shown in figures 1.3, 1.4, 1.5, and 1.6 with significant differences shown in appendix tables I.1 and I.2. The influence of protein supplement in the cow's diet on the degradation of both DM and N was of importance to measure the *in situ* degradation of protein and thus the protein available for rumen escape.

Faster rates of *in situ* degradability of DM and N have been observed for high roughage than for high concentrate diets (Ganev et al. 1979; Weakley et al. 1983) possibly due to changes of N solubility with varied ruminal pH, or shifts in microbial types in response to pH or chemical or physical characteristics of the ruminal medium. The cow diets in this study contained on average 57% concentrate.

Solubility of N and *in situ* degradability of N for mixed supplements has been correlated more closely to disappearance at 1 h of rumen incubation than at longer incubation times (Weakley 1983), but solubility characteristics in this study were estimated at 0.1 h rumen incubation (table 1.6, figure 1.2). At 0.1 h incubation CM 0 had the highest ($P<0.05$) rapidly soluble *a* fraction for DM and N when test supplement CM 0 was incubated in cow diet CM 0 followed by test supplements CM 60 and Bi incubated in their respective cows' diets, CM 60 and Bi (table 1.6). The disappearance of *a*, the rapidly soluble fraction for DM and N at 0.1 h incubation can be related to their solubility.

Heating of CM for 60 min reduced the solubility of DM and more so the N proportion of CM 60 as compared to unheated CM 0 with the results as shown in figure 1.2 and table 1.6. Addition of Bi supplement which contained corn gluten meal, fish meal and meat meal (three feed substrates known for their low rumen degradability and high bypass potential), to cows' diets appeared to reduce degradation of the supplements CM 0 and CM 60 incubated at 0.1 h (table 1.5; figure 1.2). The reason for this is not apparent as incubation time is only 6 min. At 0.1 h incubation, solubility becomes more critical than degradability *per se* (Weakley 1983). Solubility of feed substrates from CM 60 supplement at 0.1 h incubation, was lower than CM 0, which is consistent with results of Mahadevan et al. (1980).

Marked high relationships between EDDM and EDN has been associated with feeds processed as homogenous materials as compared to heterogenous protein material (Barrio et al. 1986). The CM 0 and CM 60 supplements used in this study were of the

same origin, expected to be of homogenous type, only that heat treatment caused the difference. On the other hand the Bi supplement had a heterogenous vegetable-animal protein mix which may explain the relatively lower degradability rates recorded with this supplement relative to the CM supplements.

Dry matter, nitrogen and "bypass" potential.

At 12 h incubation (figure 1.3; tables 1.7 and 1.8) DM and N degradation of CM 0 incubated in cows' CM 0 diets was greater ($P<0.05$) than for either CM 60 or Bi incubated in CM 0. Degradation of DM and N for CM 60 incubated in either cows' diets CM 60 or Bi was not different ($P>0.05$) from Bi supplement incubated in cows' diets with either CM 60 or Bi as protein supplements (figure 1.3; tables 1.7 and 1.8). This suggested a reduced microbial susceptibility of both CM 60 and Bi at only 12 h of incubation due to the "bypass" nature of the two supplements as compared to CM. CM 0 was more degradable with CM 0 in the diet compared with CM 60 or Bi in the diet (appendix table I.2. figure 1.3).

Barrio et al. (1986) suggested that in most protein supplements, 100% minus the residue remaining in the nylon bag after about 24 h rumen incubation matches and represents the in vivo estimates of bypass potential. Cows' diets which had Bi as a supplement with Bi as a test supplement (in bag) formed a combination which had the highest ($p<0.05$) "bypass" potential at 12, 16 and 24 h (table 1.5) with the least degradation of the three possible cows' diets/test supplement combination. Conversely a combination of cows' diets which had CM 0 as a supplement in cow's diet and CM 0 as a test supplement in the bag had the least ($p<0.05$) "bypass" potential and the

highest degradation of the three possible cows' diets/test supplement combination. The presence of corn gluten meal in Bi could have inhibited passage through bag pores due to its glutenous nature. At 16 and 24 h incubation (figure 1.4 and 1.5) degradation of protein from CM 0 incubated in cow diet CM 0 was greater ($p<0.05$) than when incubated in cows receiving CM 60 or Bi. Degradation of protein from CM 60 incubated in cows fed diet CM 60 was more than when incubated cows receiving CM 0 diet which was more degradable than when incubated in cows receiving the Bi diet. The same relationships held for Bi supplement incubated in cows receiving the CM 0, CM 60 and Bi diets. The results from this study suggest that inclusion of high bypass protein supplements (achieved through heating or mixing individual high bypass ingredients) may lead to a different rumen microbial population or a different rumen environment and result in different degradation profiles of a given protein supplement. It is not clear from the results presented here if reduced degradation could be attributed to change in N solubility and variation in rumen pH in response to extent of heating or amounts of bypass ingredients in the supplement as suggested by Ganev et al. (1979) and Weakley et al. (1983). Rumen pH and ammonia levels were not shown to be different (manuscript III).

Bag comparison

Rumen degradation of DM and protein (table 1.9) for CM 0, CM 60 and Bi was not different ($P>0.05$) for bag type at 0.1 h incubation with the exception of N for Bi. Playne et al. (1972) noted that small particles could be lost and gained through the undefined woven terylene material used while Uden et al. (1974) reported influx of

forage fibre particles into a 35 μm porosity container. The two bag types in this study had different percent open surface area which seemed to have contributed little difference in DM and N disappearance at 0.1 h for CM 0 and CM 60, but there was for N with the Bi supplement. Waldo and Goering (1979) suggested that loss of particles was a factor of N solubility measured in various solvents and 0.1 h rumen disappearance from nylon bags. There was a mixture of animal and vegetable products in the bypass protein supplement and may have had different particle size than the CM.

At 12, 16 and 24 h incubation, disappearance (table 1.9) of both DM and N, with one exception was higher ($P<0.05$) for samples incubated in the monofilament multifilament bag type. At 16 h incubation, 10, 14 and 45% more N disappeared from monofilament than multifilament bags for CM 0, CM 60 and Bi respectively. The suggestion could be made that the monofilament resulted in greater degradation due to greater percentage open area, which may have allowed higher rates of microbial influx and efflux of enzymatically digested material from the bag. In the multifilament bag type there was less open surface area for microbial influx, great potential for pore clogging due to gluten and subsequently, lesser efflux of digested material. Also, movement of the threads for the multifilament bag could have reduced opening size. Movement could have also increased opening size.

These studies indicate that bag type did have an effect on degradation of DM and protein. It is not possible to determine if that difference was due to percent open area or change in size of openings in the bag weave for the multifilament bag type.

Pepsin-HCl digestion

Lower GI tract DM and N degradation from both bag types after a 12 h rumen incubation was not affected ($P>0.05$) by removing the pepsin-HCl digestion step (table 1.10). Ingalls and Okemo (1994) suggested differences in lower GI tract disappearances of phosphorous from CM and SBM samples pre-digested with pepsin-HCl. Deacon et al. (1988) did not include the pepsin-HCl digestion step in their study but obtained DM and CP disappearance values of over 90% for the protein sources studied.

These results suggest that under the conditions of this trial the pepsin-HCl digestion had no effect on the lower GI tract disappearance of DM or N. It is assumed the pepsin enzyme was active but no test was made to ensure an active digestion was obtained.

Rumen and lower GI tract digestion of ADF and NDF

Results for *in situ* disappearance of ADF and NDF from large rumen bags incubated at different times are presented in appendix figure I.2 and appendix table I.4. Heat treatment in general decreased ($P<0.05$) both ADF and NDF disappearances in the rumen with increased heating time for the 12, 16 and 24 h incubations. Reduced rumen ADF and NDF degradation due to heat treatment would be expected to decrease availability of these fibre fractions in the total tract. These results follow similar trends as those reported by Moshtaghi-Nia and Ingalls (1992), in which there was decreased rumen disappearance of ADF for heated (127°C) CM incubated at 0.1, 8, 16 and 24 h.

Rumen disappearance of NDF (appendix figure 1.2 and appendix table I.4) increased ($P<0.05$) progressively through 24 h *in situ* incubation and the values for 60

min heated CM was very close to that of Bi supplement, both of which had lower disappearance relative to CM 0 and CM 23, CM 45. Digestibility of NDF has been noted to be erratic and generally negative after 12 h *in situ* incubation (Van Hellen and Ellis 1977) due to influx of NDF from rumen digesta which exceeds the extent of digestion and efflux of sample NDF in the bag. In this study there were no negative NDF values with increased incubation time.

Compared with CM 0 rumen disappearance of NDF decreased ($P<0.05$) at all incubation times except at 0.1 h due to 60 min heat treatment, with 60 min heating of CM showing equal ($p>0.05$) disappearance rate to that of Bi (appendix table I.4.). Disappearance rates of ADF from CM 60 and Bi were similar except at 16 and 24 h incubations. There was a decrease ($P<0.05$) in the *a* fraction of ADF (table 1.11) with increased heating and no ($P<0.05$) difference in the *b* fraction for ADF except for all CM's vs Bi. Although the numbers indicate the same trend the *a* value differences were not significant ($P>0.05$) for NDF among the CM samples (table 1.11). Effective degradabilities of ADF and NDF decreased ($P<0.05$) for the CM 60 vs CM 0. Effective degradablity of ADF for Bi was lower ($P<0.05$) than CM 60 but was similar for NDF (table 1.11).

Results for N, ADIN and NDIN disappearance and or remainder in each segment of the digestive tract and fecal recovery as measured with mobile nylon bags initially incubated *in situ* for 12 h, are shown in appendix table I.5 and figure 1.7. Heat treatment of CM for 60 min decreased ($P<0.05$) rumen disappearance of N, ADIN and NDIN (appendix table I.5) by about 20, 15 and 10 percentage points, respectively, but as a

percent of total N in the original sample, the ADIN and NDIN values were increased ($P<0.05$) by 19 and 52 percentage points respectively, due to heating (table 1.3). Heat treatment of CM increased ($P<0.05$) amounts of N, ADIN and NDIN recovered in the feces by about 5, 15 and 9 percentage points respectively (table I.5). Heat treatment of CM did not ($P>0.05$) change percent lower GI tract disappearance for ADIN and NDIN ($P>0.05$) but increased ($P<0.05$) N disappearance by 15 - 70 percentage points.

Rumen disappearance values for ADIN and NDIN values (table I.5) obtained in this study were 8 and 2 percentage points, respectively, higher than those measured in CM heated for 2 h at 100°C (McAllister et al. 1993) but were 15 and 52 percentage points, respectively, lower than those measured in CM heated for 1 h at 127°C (Moshtaghi-Nia and Ingalls 1992). However, they questioned their results for rumen disappearance. It is common to associate ADIN with heat-damaged protein and hence assume that ADIN represents unavailable N in the lower GI tract (Van Soest 1982). Increased heating of CM may have increased ADIN through denaturation of CM protein and also formation of Maillard products which is expected to decrease intestinal digestibility of these products associated with ADIN. The increased amounts of ADIN and NDIN (table I.5) in this trial were 6.3 g and 12.8 g respctively per 100 g of N for the CM 0 versus CM 60. The digestibility (based on disappearance in the total tract) of the increased ADIN and NDIN due to heat treatment was 72 and 88% respectively (table 1.3 and appendix table I.5). This observation supports other findings suggesting that ADIN can be digested (Pena et al. 1986; Arieli et al. 1989). Steer gains from distillers

dried grains which had various levels of ADIN support the utilization of ADIN (Klopfenstein and Britton 1987).

The biological significance of NDIN has not been demonstrated clearly (Krishnamoorthy et al. 1982) but chemical analysis indicate that N content of the NDF fraction varies from sample to sample. The percentage of ADIN (% total N) measured in feces from this trial was higher ($P<0.05$) than those of NDIN (% total N) for the test supplements studied (table appendix I.5). This may be due to denaturation of terminal free AA ends which complex with carbohydrates due to heating and, hence, increased the ADIN rather value than precipitation of some neutral detergent soluble components by the detergent solution that cause interference resulting in the lower NDIN measured.

The NDIN content formed a significant fraction of total (table 1.3) N in both CM 60 and the Bi supplement used in this study. Pichard (1977) reported a positive correlation between the slowly solubilizable pool of N and NDIN in forage samples. There may be a possibility of using NDIN as an index of the slowly degradable N pool in heat treated CM but more data would be required. McAllister et al. (1993) reported increased NDIN with heating (100°C for 2 h) of CM with lignosulfonate or treating CM with xylose. Moshatghi-Nia and Ingalls (1992) also observed a greater increase in NDIN content than ADIN with increased heat treatment of CM but it was not examined relative to rumen and lower GI tract disappearances.

Moist heating decreased ruminally degraded ADF and NDF (table 1.11), possibly due to increased ADIN and NDIN. It was not possible because of sample size to establish

whether reduced ruminal degradation of ADF and NDF resulted in increased degradation in the lower GI tract as was found for N (figure 1.7.).

Amino Acids

Composition and disappearance

Heat treatment did not ($P>0.05$) change the AA composition (table 1.12) but tended to reduce the concentration of arginine and lysine by 32 and 29%, respectively. The apparent loss of AAs may be a result of Maillard reaction with increased heating causing the N associated with these AA to be unaccounted for in routine AA analysis. The results from this study seem to agree with those by Schingoethe and Ahrar (1979) which recorded no major changes in AA composition of soybean and sunflower meals. Craig and Broderick (1981) found a decreased concentration of lysine with increased heating time of cotton meal, while Moshtaghi-Nia (1994) observed a decrease in lysine and arginine composition with increased autoclave heating up to 45 min. Heat treatment effect though significant ($P<0.05$) for aspartic acid and proline indicated a similar trend for reduced levels of NEAA with 60 min heating.

The disappearance of individual EAA in the rumen decreased (table 1.13) with 60 min heating and increased ($P<0.05$) within the lower GI tract with 12 h incubation (table 1.15). At 12 h rumen incubation, increased heat treatment of CM from 0 to 60 min resulted in decreased ($P<0.05$) ruminal degradation (table 1.14 and figure 1.11) of 73.7, 59.3, 69.9, 62.0, 60.7, 66.1, 53.7, 70.1 and 80.7%, respectively for arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine (table 1.14 and figure 1.11). At 16 h rumen incubation, similar heat treatment resulted in decreased

($p<0.05$) ruminal degradation (table 1.14 and figure 1.14) of the respective EAA as above of 69.2, 52.6, 52.6, 54.0, 48.6, 53.6, 51.6, 61.0 and 77.5%, while at 24 h incubation for the similar treatment, the decreased ruminal degradation for respective EAA were as above of 69.3, 38.3, 53.4, 53.0, 33.2, 38.1, 60.3, 54.5 and 73.4%. The ruminal degradation of AAs from Bi supplement was greater ($P<0.05$) than that of CM 60 for the same AAs except for leucine, methionine and phenylalanine at 12 h incubation, isoleucine, leucine, lysine, methionine and phenylalanine at 16 h incubation and histidine, isoleucine, leucine, lysine, methioinine and phenylalanine at 24 h incubation.

In general there were significant increases in EAAs' rumen degradation from 12 to 24 h (table 1.14). For CM 0, phenylalanine and valine did not ($P<0.05$) change, between 12 and 16 h incubation times. All other AA were degraded more ($P<0.05$) at 24 h incubation except lysine (table 1.14). For CM 60 there was no ($P<0.05$) difference in percentage rumen escape of arginine and lysine (table 1.14) between 12 and 16 h incubation times. Phenylalanine did not ($P<0.05$) change between 16 and 24 h incubation times. The disappearance of EAA in Bi supplement with the same exceptions increased ($P<0.05$) with incubation times (table 1.14). The implication of these changes are that any increased rumen retention time would reduce the amount of AA escaping rumen degradation, such that the final quantities and composition reaching the lower GI tract would be changed relative to the original quality offered in the test supplement as part of the UIP.

The 45 and 60 min heat treatment significantly decreased ($P<0.05$) the rumen disappearance of EAA after 12 h incubation (tables 1.15 and figure 1.8) with an increased disappearance in the lower GI tract. The 60 min heat treatment of CM increased ($P<0.05$) fecal excretion of arginine, histidine, isoleucine, lysine, methionine and threonine (table 1.15). Fecal excretion of essential AA was higher ($P<0.05$) from the Bi supplement in comparision to CM 60 except for threonine (table 1.15). Among the heated CM samples, 60 min heating decreased ($P<0.05$) rumen disappearance and increased disappearance of all EAA in the lower GI tract (figure 1.8). The quantity of original AA available in the lower GI tract are indicated in table 1.16.

The disappearance in the rumen (table 1.13) and passage to lower GI tract of EAA from CM heated at 0, 23, 45 and 60 min incubated for 16 and 24 h in the rumen are presented in figures 1.9 and 1.10 respectively. Heat treatment of 45 and 60 min in most cases decreased ($P<0.05$) rumen disappearance of EAAs and increased passage to lower GI tract for the 16 h incubation with a similar trend for the 24 h incubation. From these figures and table 1.15 it is apparent of the need for the increased heating for 60 min to increase rumen escape and lower GI tract disappearance of EAAs. Heat treatment tended to shift the disappearance of AA from the rumen to the lower GI tract. This observation is in agreement with other results (Cros et al. 1992; Koeln and Paterson 1986) that recorded increased disappearance of AA from the small intestine with heat treated soybean meal and horsebean. Heat treatment did not ($P>0.05$) change the g (16 g $N)^{-1}$ of EAA in the rumen digesta after incubation for 12, 16 and 24 h (table 1.18). CM heated for 60 min was deficient in all the EAA relative to milk AA except arginine

and threonine at 12 h incubation and arginine, threonine and phynlalanine at 16 and 24 h incubations (table 1.18). Moshtaghi-Nia (1994) observed that the first limiting EAA for a 15 min heat treated CM incubated for 16 h were lysine, isoleucine and valine which provided 37 to 43% of corresponding EAA in milk protein. Methionine has been noted (Cros et al. 1992; Susmel et al. 1989) as a limiting AA for milk production.

In the present study methionine, lysine and isoleucine respectively in CM 60 rumen escape protein were the first limitimg AA relative to milk AA (table 1.18) for 12 h incubation with the same three AA's being in the first limiting group for the 16 and 24 h incubations. The level of lysine and isoleucine as a percent of milk protein was rather constant at 55 to 67% and 60 to 63% respectively for the 12, 16 and 24 h incubation (table 1.18). The levels for methionine varied from 50 to 71% which may be an indication of more variability for methionine analysis. Moshtaghi-Nia (1994) observed that isoleucine and tyrosine (not usually considered an EAA) were the first limiting EAA providing 20 to 23% of corresponding EAA in milk protein, followed by lysine, valine and histidine. Results from Boila and Ingalls (1994) suggest similar values of about 16 to 21% for isoleucine and tyrosine at 12 h rumen incubation.

Research has suggested that lysine should make up 15% of the EAAs and methionine should be 5.1 - 5.8% (Rulquin et al. 1995). Rumen escape protein of CM 60 after 12 h incubation contained lysine and methionine as 11 and 3% of EAAs respectively with 12 and 5% and 11 and 4% for the 16 and 24 h incubation respectively.

The percentage digestive tract availabilities of AA from CM samples and Bi incubated for 12 h and digested in pepsin-HCl solution are presented in table 1.19. In

general, heat treatment increased ($P<0.05$) lower GI tract availability of EAAs from CM samples. The availability of EAA from Bi supplement (table 1.18) was higher ($P<0.05$) than from CM 60. Total tract availability of EAA was similar ($P<0.05$) for both CM 0 and CM 60, but was lower ($P<0.05$) in Bi supplement by 4 percentage points compared with CM 60 (table 1.19). The lower GI tract and total tract availability of NEAAs followed a similar pattern as was reported for EAA. The CM heat treated for 60 min resulted in a greater percentage of EAAs available in the lower GI tract than the Bi supplement. The disappearances of NEEA from the rumen and passage to lower GI tract for heated CM 60 and Bi supplement incubated at 16 and 24 h are presented in appendix tables I.6 and I.7 respectively. Heat treatment of CM up to 60 min decreased ($P<0.05$) the rumen disappearances of NEEAs at 16 and 24 h of incubation.

Effect of bag type

The type of bag had an effect on total tract disappearance of DM and N (table 1.10). In all cases N disappearance for the total tract was greater ($p<0.05$) from monofilament vs multifilament bag type. Bag type to a lesser degree affected lower GI tract dispaearnces of both DM and N in comparison to the effect on rumen degradation.

Heat treatment of CM for 60 min reduced ($P<0.05$) rumen disappearance of EAAs incubated for 12 h in multifilament bag type and digested in pepsin-HCl solution (figure 1.11). Phenylalanine and methionine had greater disappearances from CM 60 than Bi incubated in multifilament bag type and digested in pepsin-HCl (figure 1.11). The CM 0 supplement showed highest ($P<0.05$) rumen degradation and the lowest disappearance by EAAs to the lower GI tract as compared to CM 60 min and Bi for 12

h incubation in multifilament bag type with or without pepsin-HCl (figures 1.11 and 1.12). Rumen disappearance for lysine increased for all the test supplements with no pepsin-HCl digestion in comparison to pepsin-HCl digestion. Incubation of test supplements in monofilament bag type and digesting in pepsin-HCl solution resulted in increased ($P<0.05$) rumen disappearance for all EAAs (figure 1.13) and also increased ($P<0.05$) the fecal excretion compared to multifilament bag type. It is not clear if the relatively higher percentage fixed and open surface area of monofilament bag type could have contributed to the high fecal excretion of EAAs from these bags. There was a significant difference ($P<0.05$) between the test supplements due to the two bag types (figures 1.11, 1.12 and 1.13). The disappearances in the rumen and passage to lower GI tract of EAAs from CM 0, CM 60 and Bi incubated in multifilament bag type at 16 and 24 h and not digested in pepsin-HCl solution are shown in figures 1.14 and 1.15. Heat treatment decreased ($P<0.05$) rumen disappearance of EAAs and increased passage to lower GI tract for all the test supplements incubated in mutlifilament bag type for 16 and 24 h.

The individual AA concentration of the concentrate grain mixes used in formulating diets for the experimental cows, relative to milk AA is presented in table 1.17. Considering the percentages of rumen degradation of test proteins (table 1.15) it may be suggested that the first limiting five EAAs in the grain mixes, relative to milk protein would be isoleucine, leucine, lysine, threonine and valine.

CONCLUSIONS

Increased heat treatment of CM decreased rumen degradation of protein with an increased potential for rumen escape protein in the lower GI tract. There was increased lower GI tract availability (80-90%) of both EAA and NEAA from heat treated CM supplements after 12 h rumen incubation. Heat treatment increased fecal excretion of arginine, histidine, isoleucine, lysine, methionine and threionine. Exclusion of pepsin-HCl digestion step did not affect lower GI tract degradation of protein. Protein and AA degradation from monofilament bag type was higher than multifilament bag type. Type of protein in cows' diet affected rumen protein degradability and subsequent rumen disappearance of nitrogen.

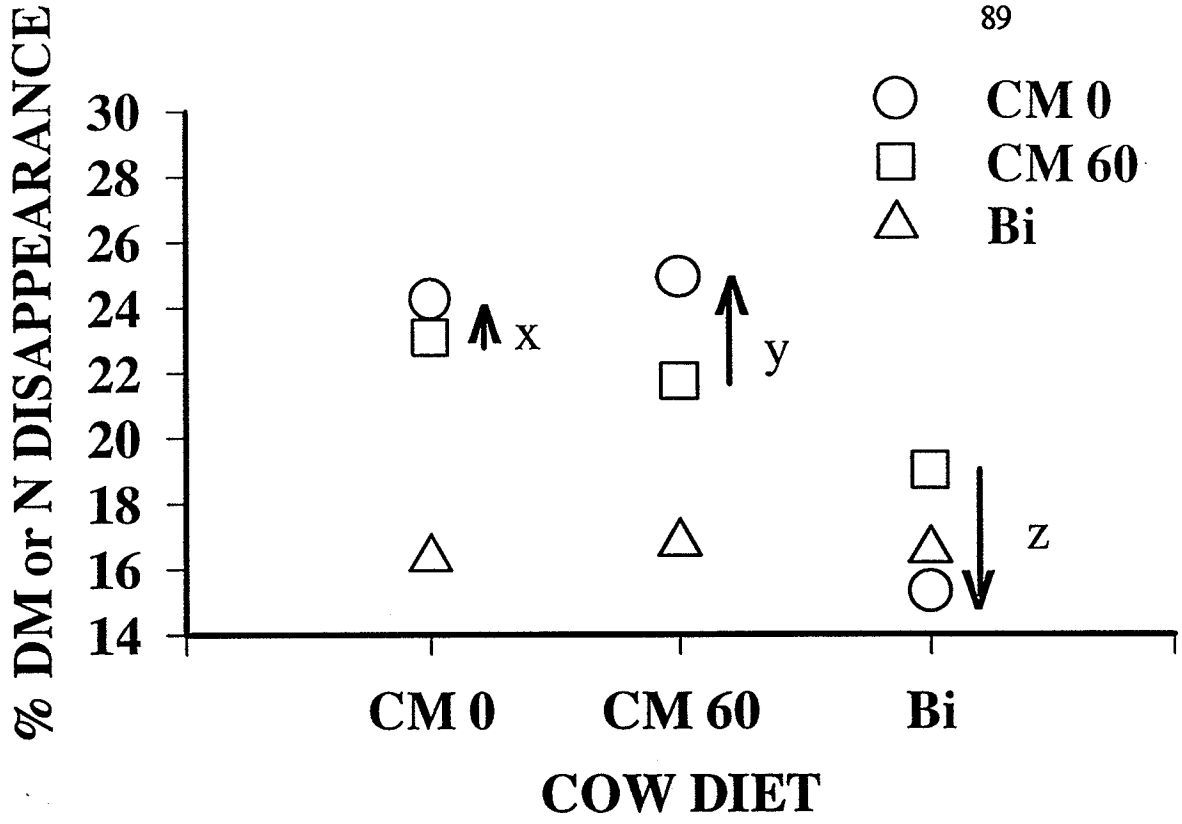


Figure 1.1. Illustration of some of the contrasts of interaction Lsmeans as tested in appendix tables I.1 and I.2.

The contrasts in table	Tests whether	Meaning
CM 60 vs CM 0 in CM 60 vs CM 0	y = x	Is the difference between test supplements, CM 60 and CM 0, the same for cows whose diet is CM 60 as it is for cows whose diet is CM 0 ?
CM 60 vs CM 0 in Bi vs CM 0	z=x	Is the difference between supplements, CM 60 and CM 0, the same for cows whose diet is Bi as it is for cows whose diet is CM 0 ? (Note direction of arrows indicating change in rank of test supplement.)

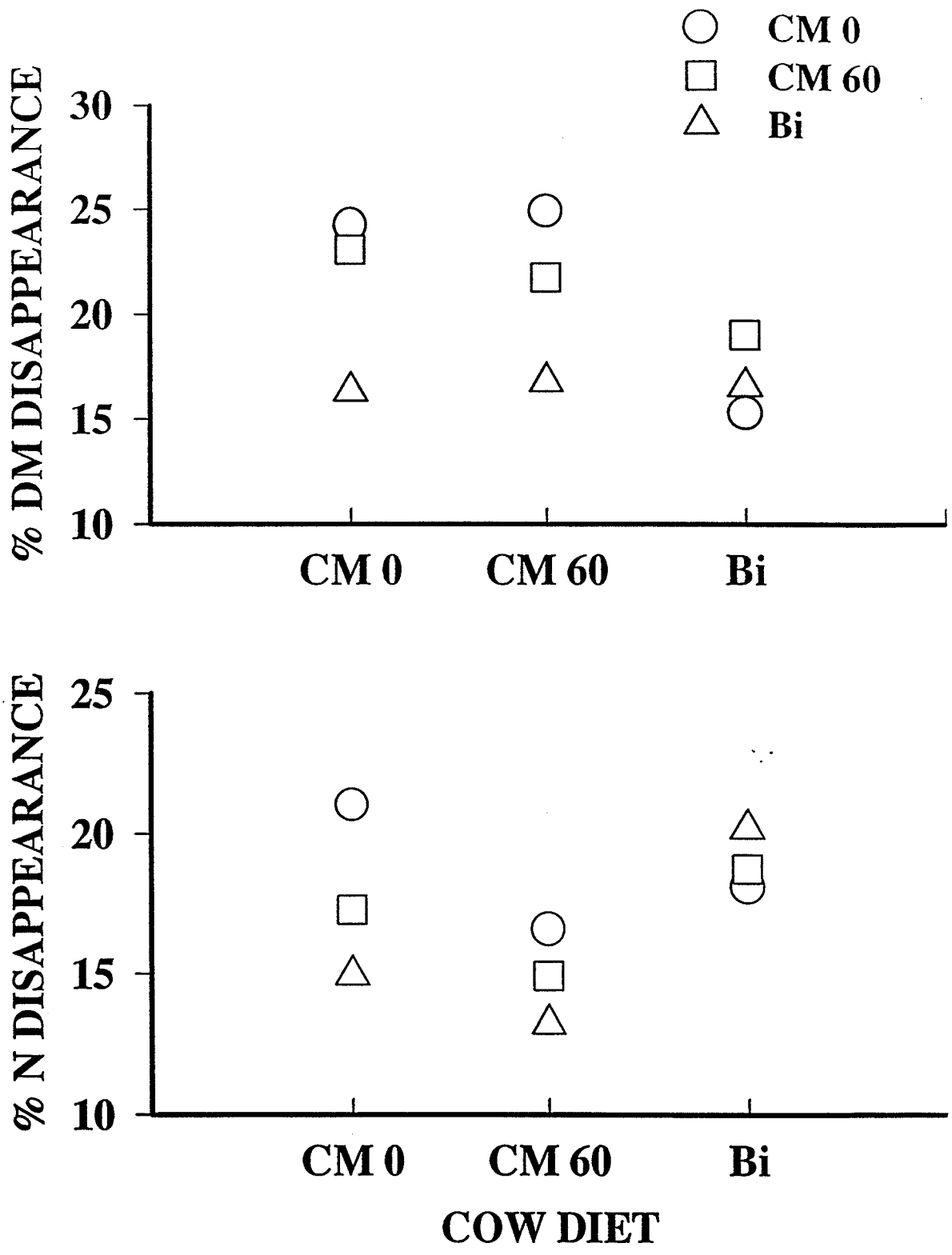


Figure 1.2. Contrasts of interaction Lsmeans for dry matter and nitrogen at 0.1 h incubation.

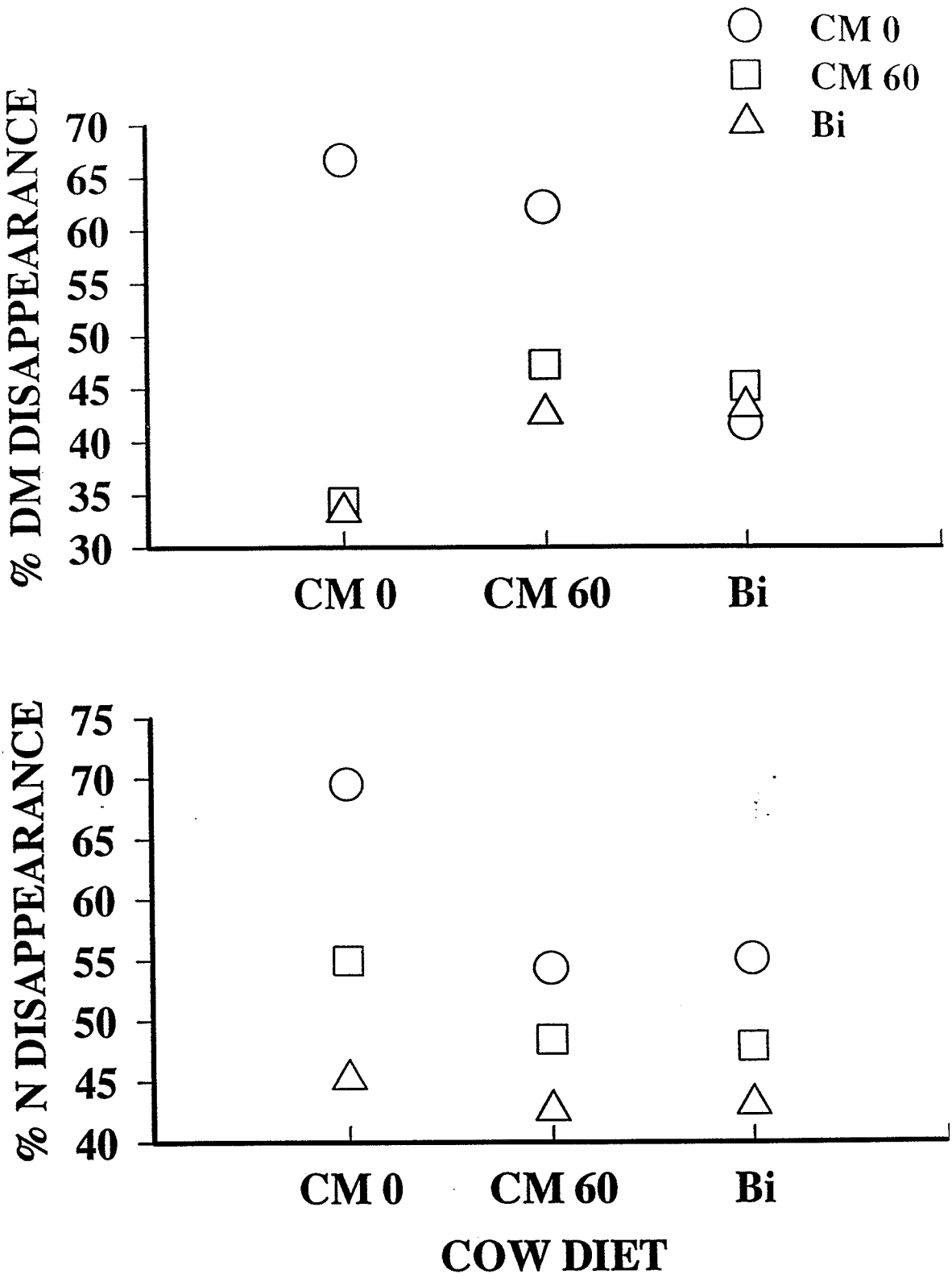


Figure 1.3. Contrasts of interaction Lsmeans for dry matter and nitrogen at 12 h incubation.

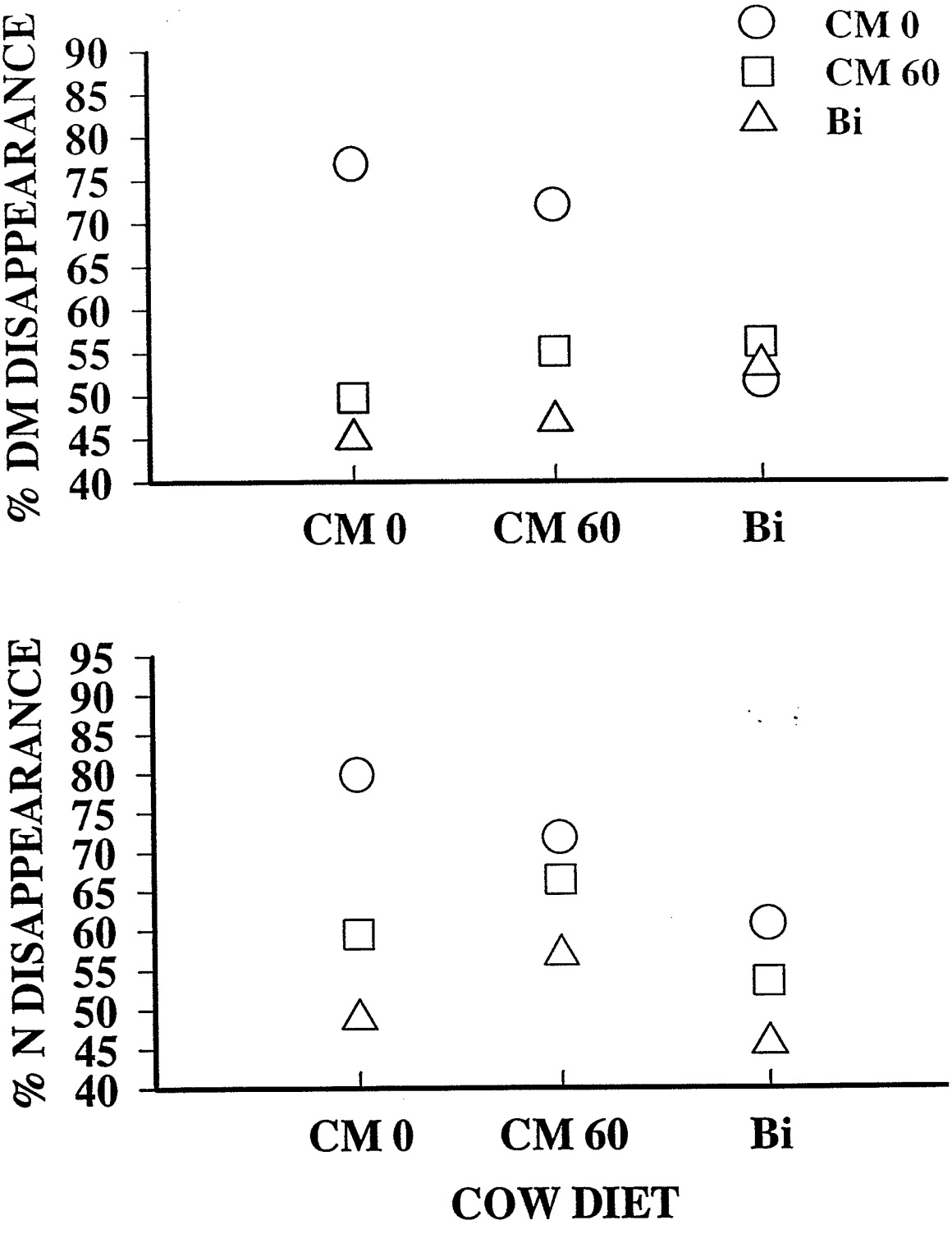


Figure 1.4. Contrasts of interaction Lsmeans for dry matter and nitrogen at 16 h incubation.

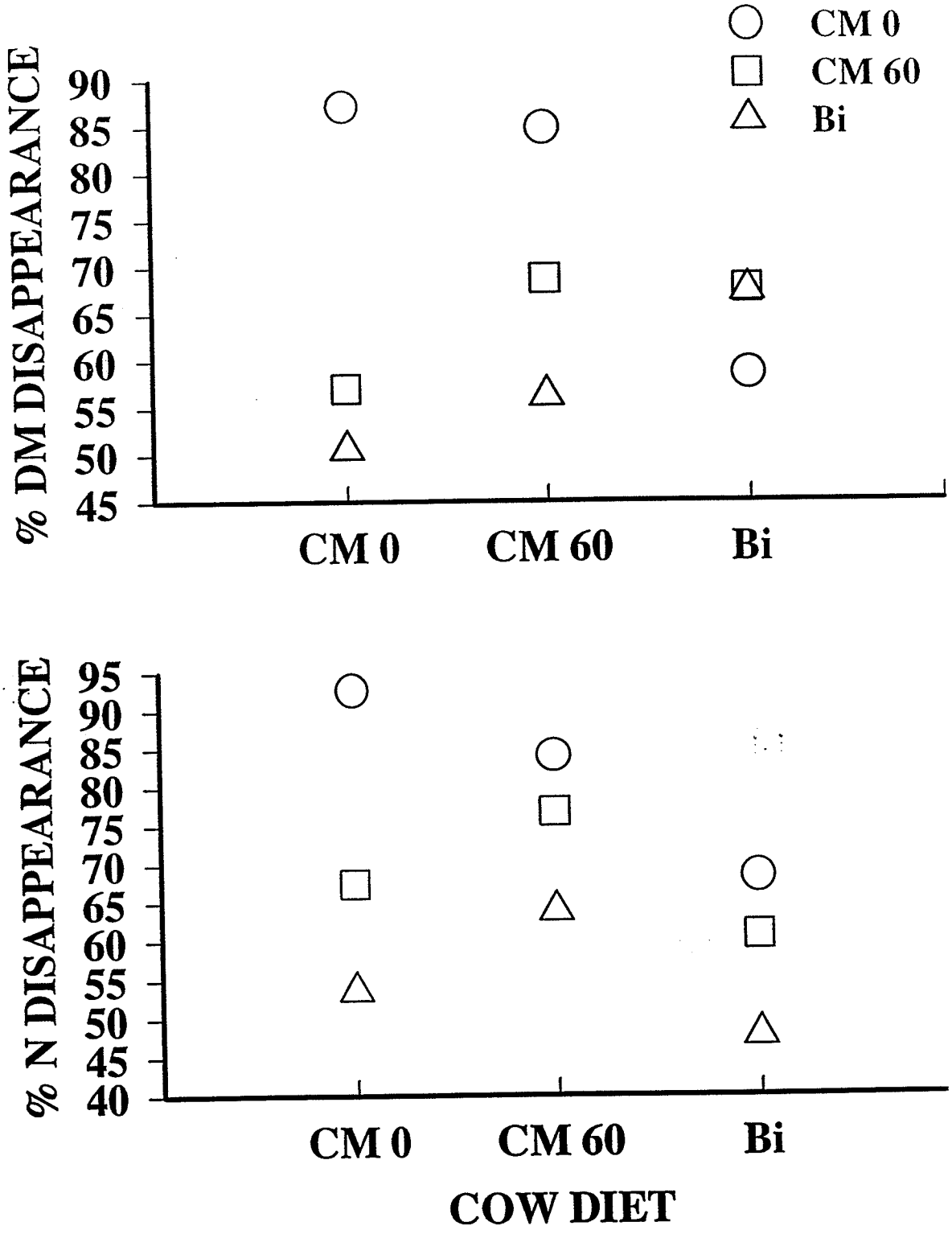
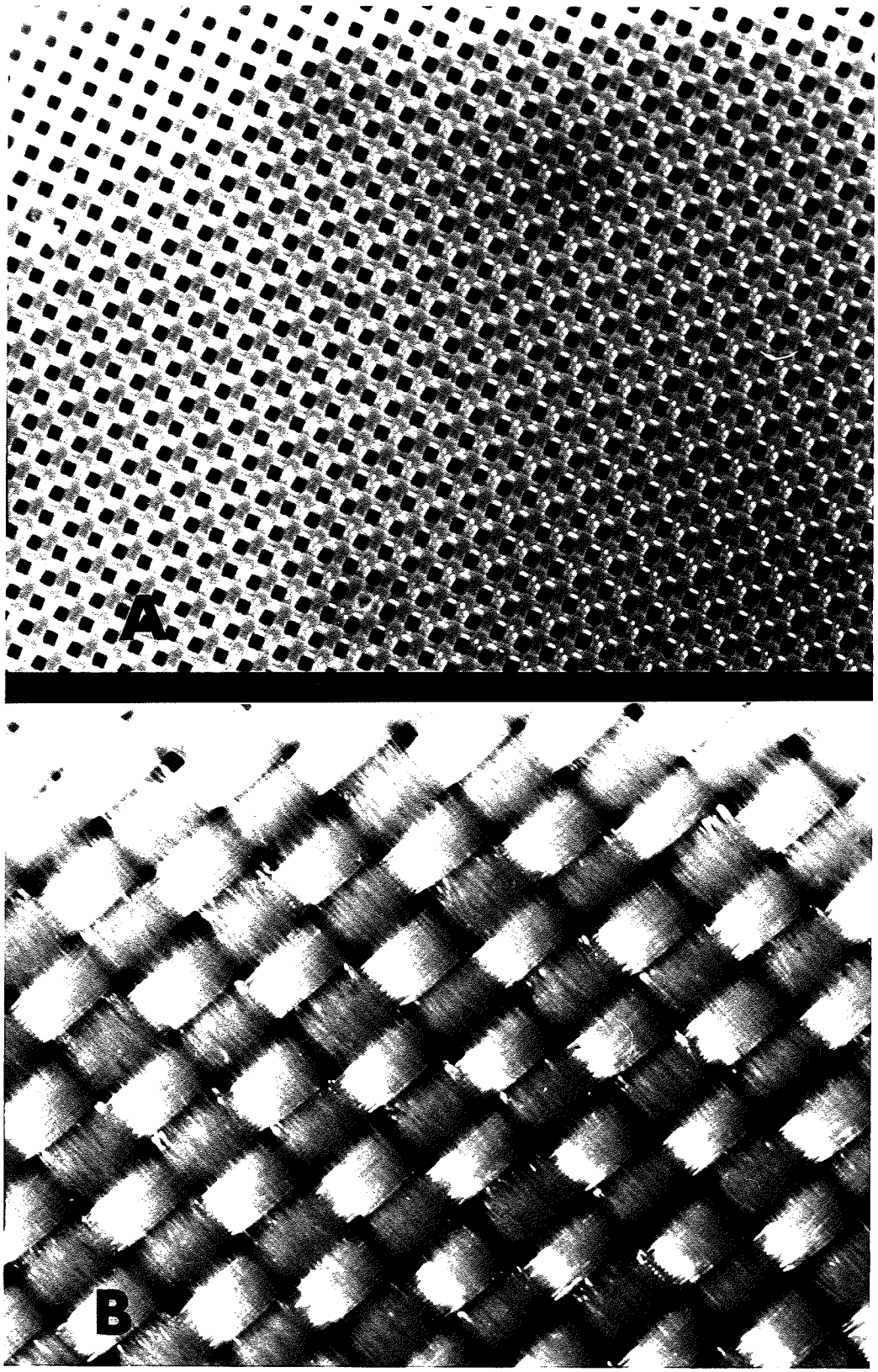


Figure 1.5. Contrasts of interaction Lsmeans for dry matter and nitrogen at 24 h incubation.

Figure 1.6. Pictorial representation of A: Monofilament and B: Multifilament nylon bag (ANKOM Co. Fairport, NY) types used for in situ and lower GI tract incubation of test supplements.


A
B

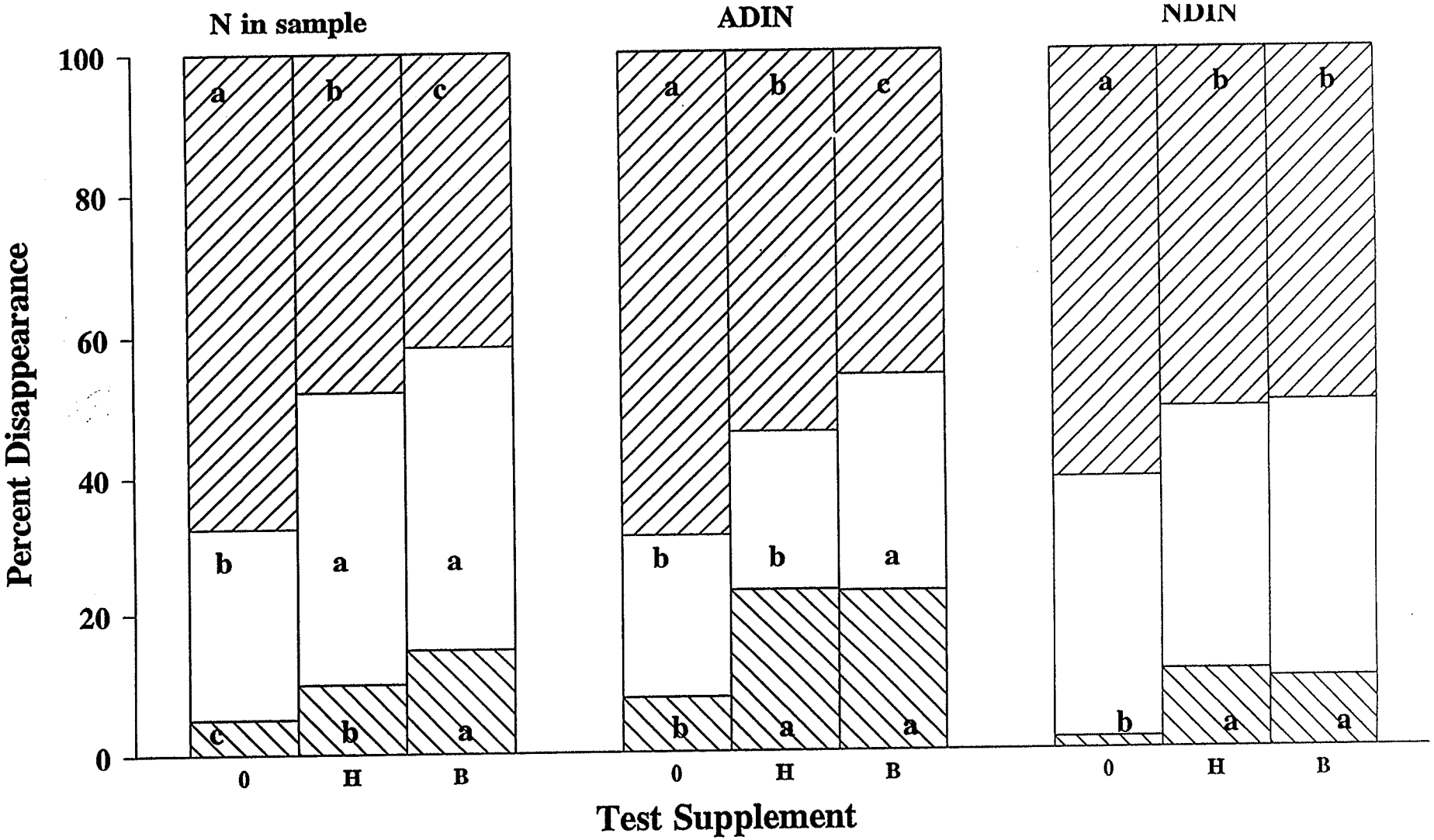


Figure 1.7. Rumen ▨ and lower gastro-intestinal tract □, disappearances, and fecal ▧ recovery of nitrogen (N), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and a by-pass protein supplement (B) using 12 h rumen in situ incubation and digested in pepsin-HCl solution.

a-c, LSmeans for each feedstuff component within each segment of the digestive tract, having different letters differ ($P < 0.05$)

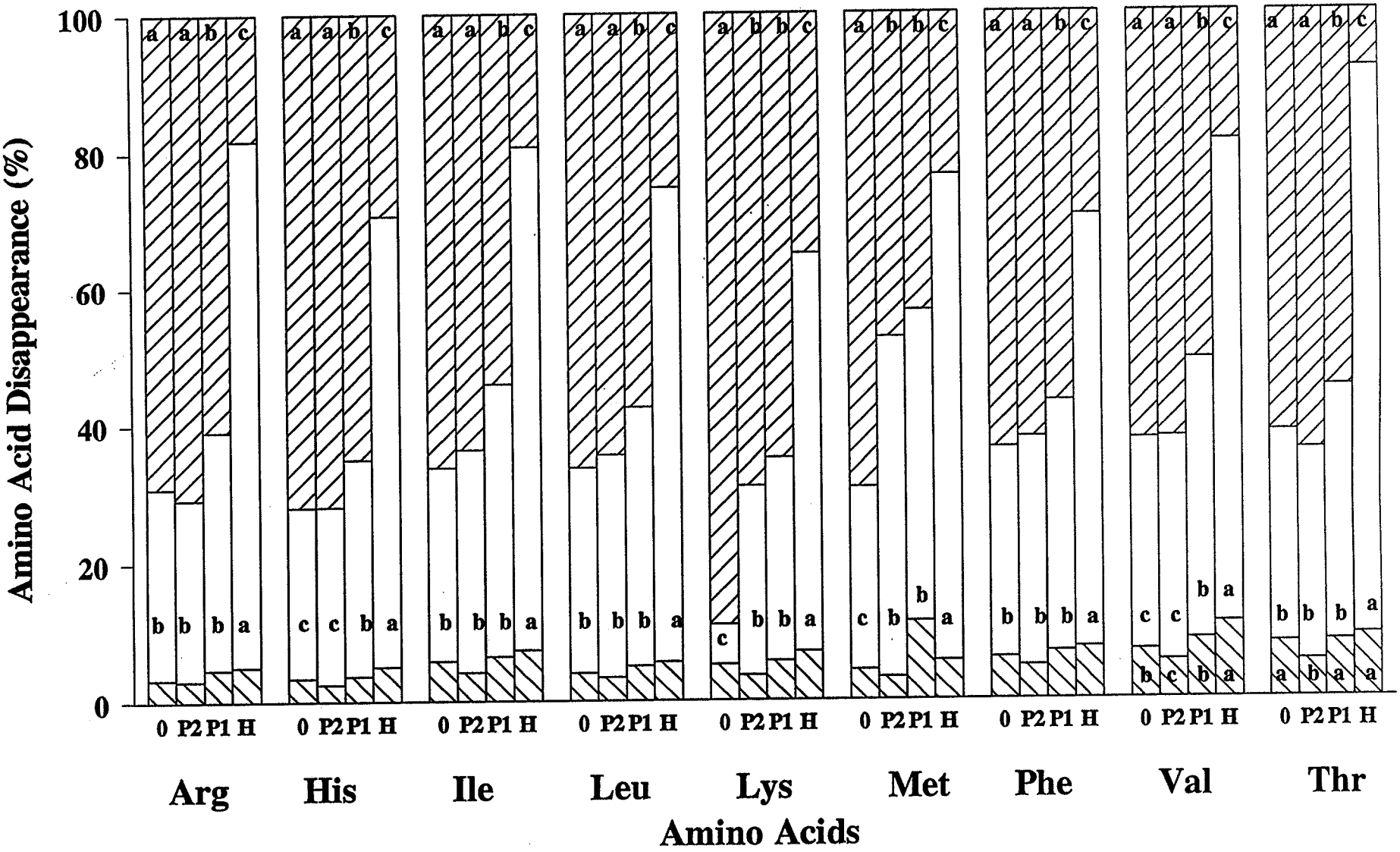


Figure 1.8. The disappearance in the rumen ▨ and passage to lower gastro-intestinal tract □, and fecal ▧ excretion of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 23 (P2), 45 (P1) and 60 (H) min incubated for 12 h in the rumen with pepsin-HCl digestion in the multifilament bags.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

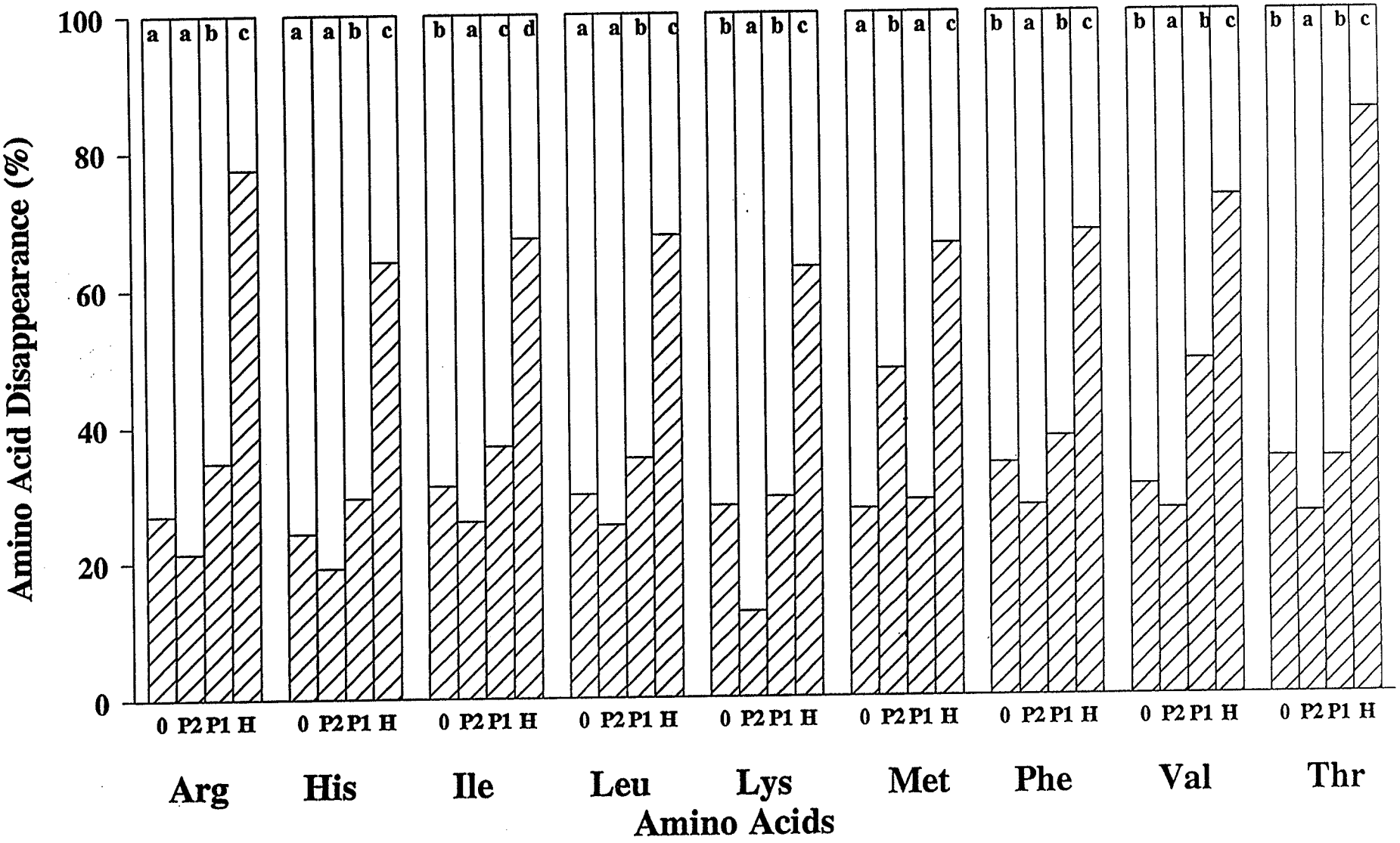


Figure 1.9. The disappearance in the rumen ☐ and passage to lower gastro-intestinal tract ▨ of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 23 (P2), 45 (P1) and 60 (H) min incubated for 16 h in the rumen with no pepsin-HCl digestion in multifilament bags.

a-d LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

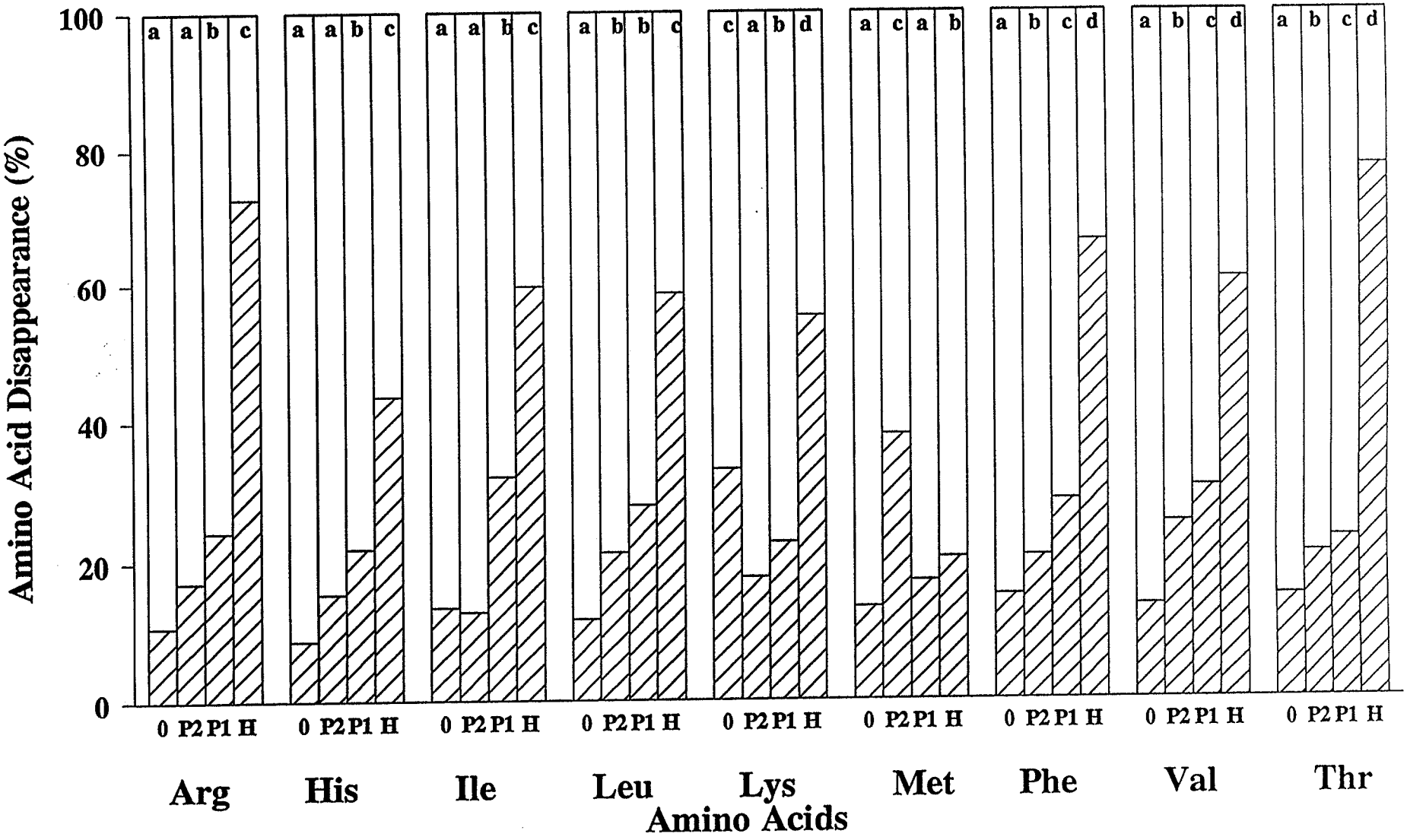


Figure 1.10. The disappearance in the rumen ☐ and passage to lower gastro-intestinal tract ▨ of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 23 (P2), 45 (P1) and 60 (H) min incubated for 24 h in the rumen with no pepsin-HCl digestion in monofilament bags.

a-d, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

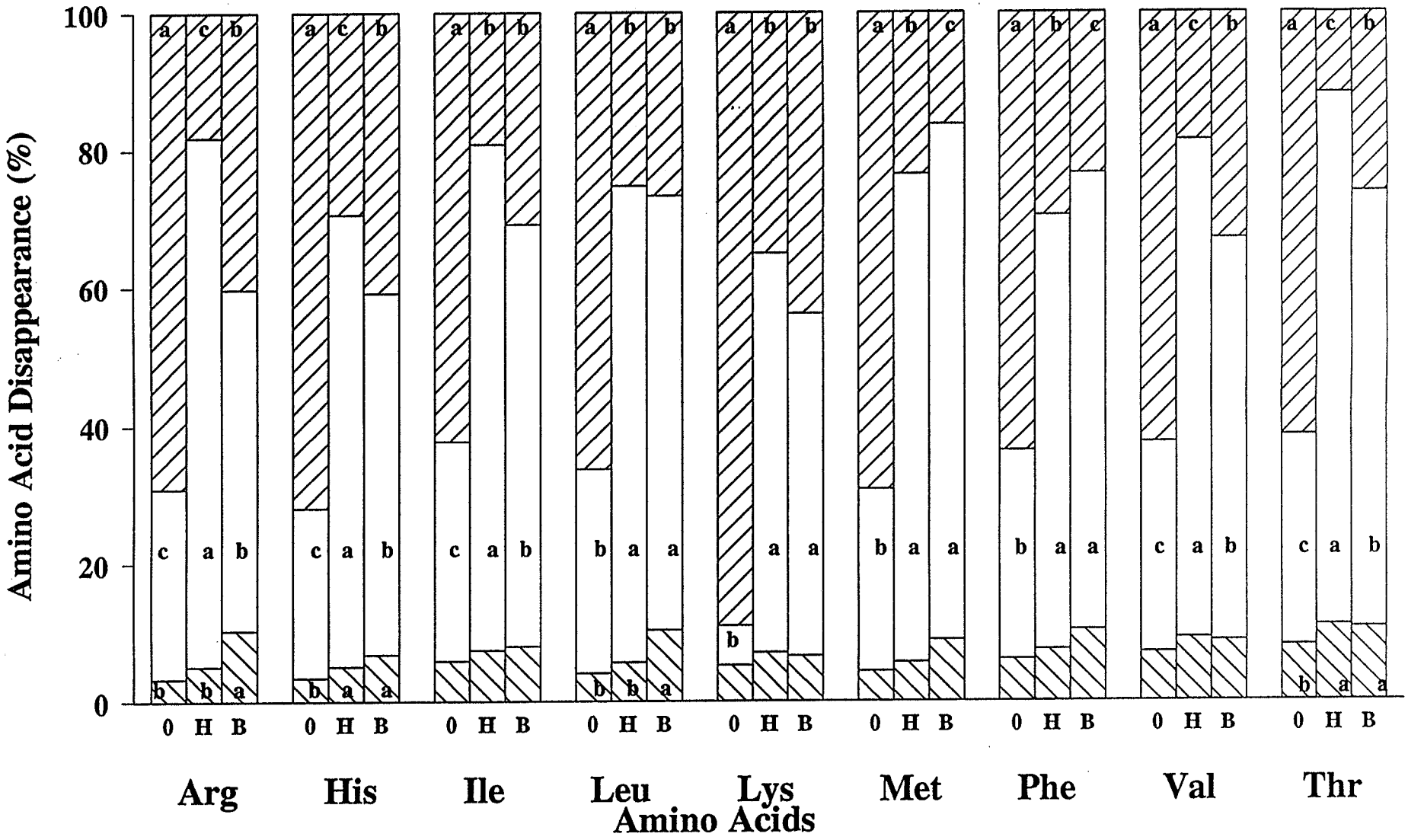


Figure 1.11. The disappearance in the rumen ▨ and passage to lower gastro-intestinal tract □, and fecal ▧ excretion of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and by-pass protein supplement (B) incubated in multifilament bag type for 12 h in the rumen and digested in pepsin-HCl solution.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

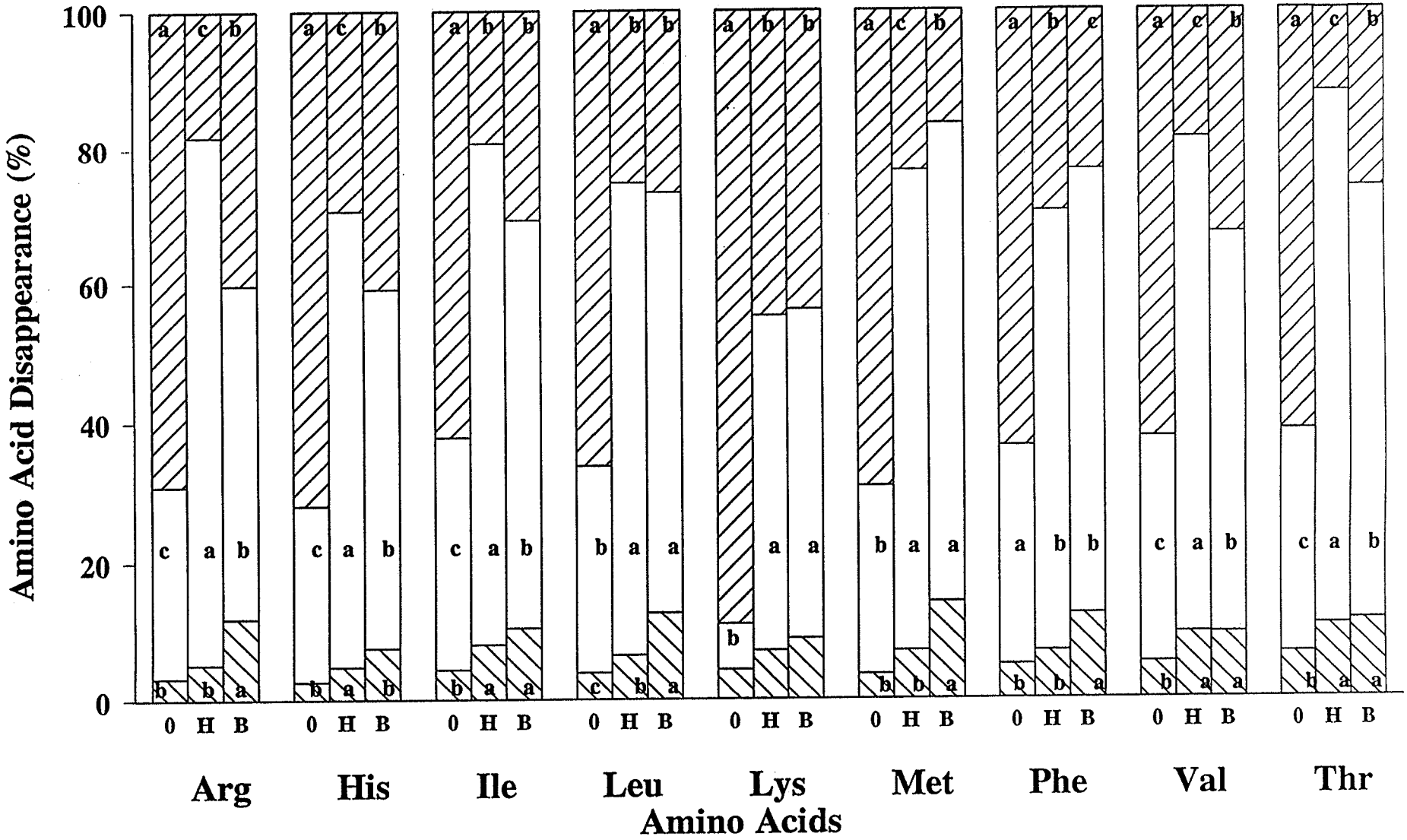


Figure 1.12. The disappearance in the rumen ▨ and passage to lower gastro-intestinal tract □, and fecal ▧ excretion of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and by-pass protein supplement (B) incubated in multifilament bag type for 12 h in the rumen and not digested in pepsin-HCl solution.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

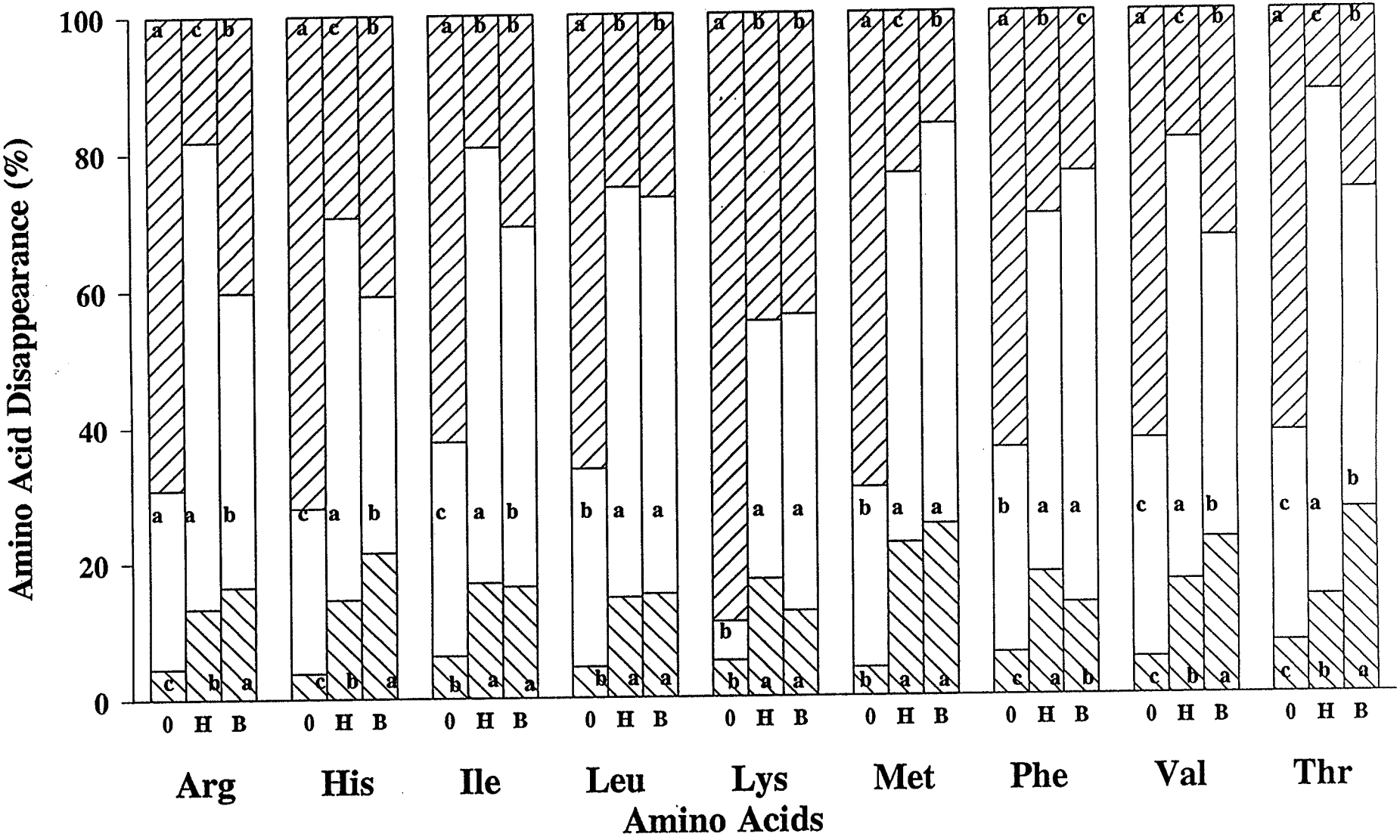


Figure 1.13. The disappearance in the rumen ▨ and passage to lower gastro-intestinal tract □, and fecal ▧ excretion of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and by-pass protein supplement (B) incubated in monofilament bag type for 12 h in the rumen and digested in pepsin-HCl solution.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

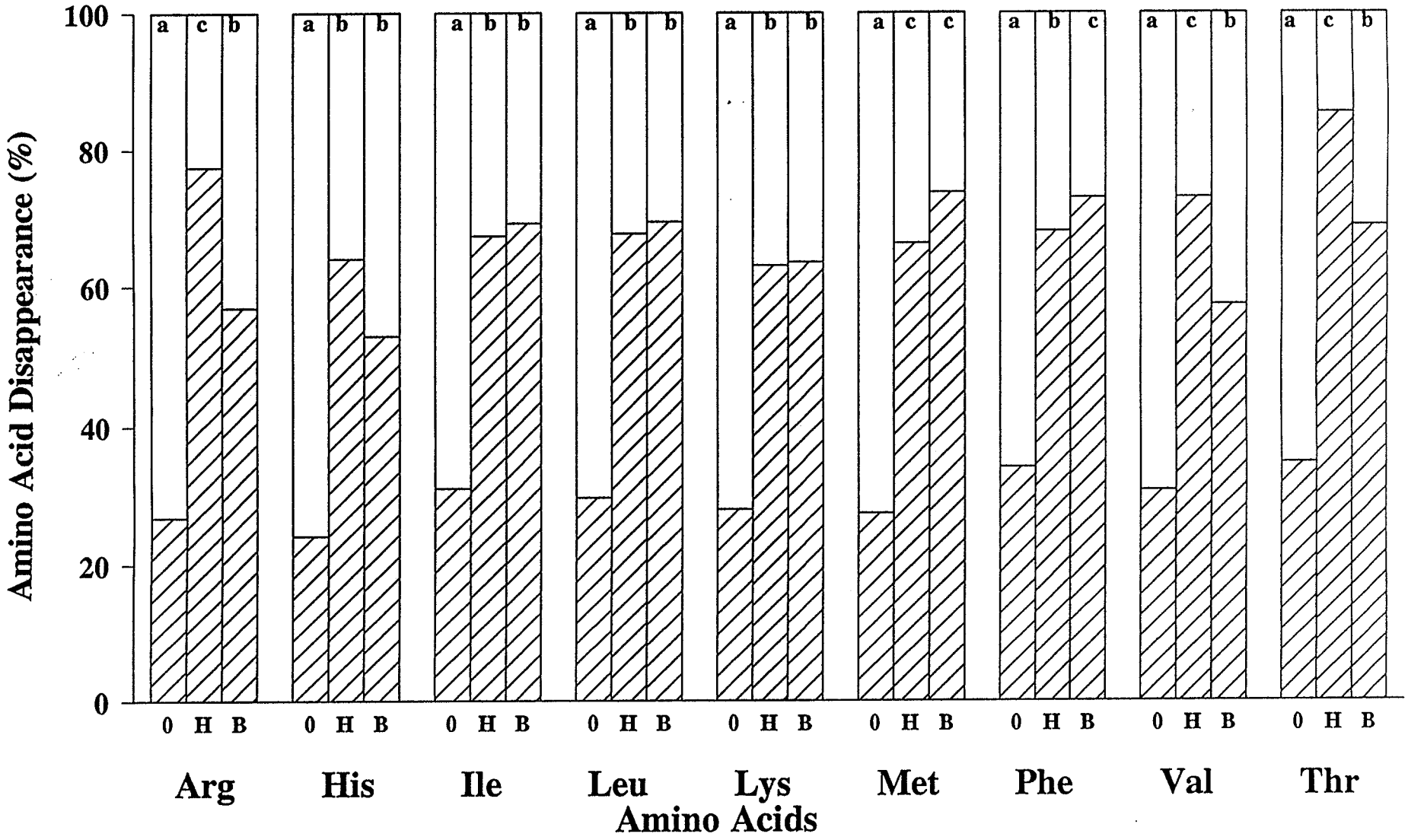


Figure 1.14. The disappearance in the rumen ☐ and passage to lower gastro-intestinal tract ▨ of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and by-pass protein supplement (B) incubated in multifilament bag type for 16 h in the rumen and not digested in pepsin-HCl solution.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

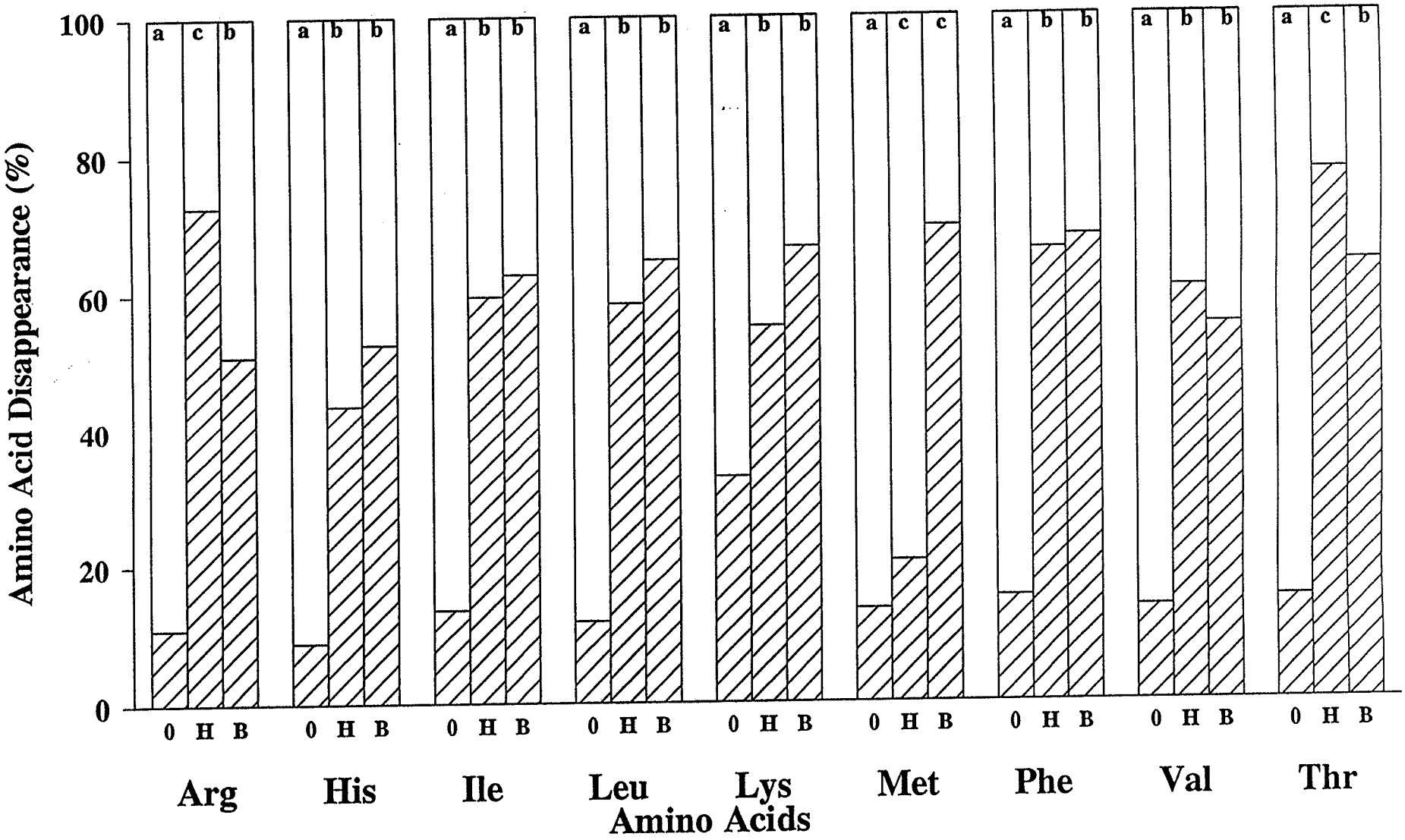


Figure 1.15. The disappearance in the rumen ☐ and passage to lower gastro-intestinal tract ▨ of essential amino acids (AA) from canola meal exposed to moist heat, 110 C for 0, 60 (H) min and by-pass protein supplement (B) incubated in multifilament bag type for 24 h in the rumen and not digested in pepsin-HCl solution.

a-c, LSmeans for each AA within each segment of digestive tract, having different letters differ ($P < 0.05$)

Table 1.1. Ingredient composition (% DM basis) of total mixed ration fed to cannulated cows.

Ingredient	Total mixed ration		
	CM 0[U]	CM 60[V]	Bi[W]
Concentrates			
Barley grain	33.62	34.35	37.68
Canola meal[Y]	20.24	18.53	6.67
Corn gluten meal	-	-	2.75
Animal fat, hydro	2.12	2.43	2.64
Fish meal	-	-	1.94
Meat meal, rendered	-	-	1.22
Molasses liquid	-	1.65	-
Limestone	1.23	1.34	1.12
Salt (CO-I)	0.33	0.31	-
Vitamin-Mineral[Z]	0.71	0.73	0.74
Urea	0.23	0.24	0.21
Potassium Bicarbonate	0.13	0.14	0.12
Dicalcium Phosphate (19%, P)	-	-	0.23
Roughages			
Corn silage	20.14	19.89	19.94
Alfalfa silage	10.13	9.92	10.04
Long hay	11.84	11.01	15.25
Roughage:Concentrate ratio	42:58	41:59	45:55

[Y]Canola meal (CM): [U]Unheated CM 0; [V]CM 60 = CM heated for 60 min; [W]Bi = CM from commercial source).

[Z] Premix provided (per kg diet): 5800 IU vit A, 458 IU vit D_3, 6 IU vit E, 6 mg [illegible] 20 mg zinc, 20.4 mg manganese, 0.2 mg selenium and 328.5 mg magnesium

Table 1.2. Nutrient composition of total mixed ration (DM-basis) fed to cannulated cows and lactating dairy cows.

Nutrient	Total mixed ration			SE[U]
	CM 0	CM 60	Bi	
Dry matter (%)	93.79	94.56	95.34	0.56
Crude protein (%)	17.75	17.38	17.84	0.22
ADF (%)[V]	18.87	18.82	18.42	0.89
NDF (%)[W]	30.82	31.25	31.17	0.82
N composition (% total N)				
ADIN (%)[X]	8.04c	11.57b	14.03a	0.23
NDIN (%)[Y]	19.60c	36.34b	63.74a	0.76
Energy, NE_L, Mcal kg^{-1} [Z]	1.76	1.74	1.76	0.02

[U] Standard error of Lsmeans (n=6, two samples/diet/period).
[V] Acid detergent fibre.
[W] Neutral detergent fibre.
[X] Acid detergent insoluble nitrogen.
[Y] Neutral detergent insoluble nitrogen.
[Z] Calculated.
a-c, Lsmeans within row having different letters differ ($P<0.05$)

Table 1.3. Effect of moist heat treatment on nutrient composition of canola meal relative to a bypass protein (Bi) supplement (on DM-basis).

	Canola meal (min, at 110°C)					
Nutrient (%)	0	23	45	60	Bi	SE[V]
Dry matter	91.31b	96.83a	98.05a	90.29b	96.67a	0.45
Protein (N X 6.25)	40.44	39.67	39.61	39.63	38.22	0.13
ADF[W]	16.07	16.21	17.43	18.09	18.30	0.14
NDF[X]	21.27b	21.34b	22.19b	25.14a	26.07a	0.58
ADIN[Y]	1.94c	6.12b	6.84b	8.21a	7.90a	0.82
NDIN[Z]	2.24c	10.84b	12.85b	14.99a	14.49a	0.39
N composition (% total N)						
ADIN	4.82d	15.62c	18.80b	23.43a	23.62a	0.42
NDIN	7.36d	36.43c	44.98b	59.44a	61.72a	0.47

[V] Standard error of Lsmeans (n=6, two samples per treatment per period).
[W] Acid detergent fibre.
[X] Neutral detergent fibre.
[Y] Acid detergent insoluble nitrogen as a % of ADF.
[Z] Neutral detergent insoluble nitrogen as a % of NDF.
a-c, Lsmeans within nutrient with different letters differ ($p<0.05$).

Table 1.4. In situ percent dry matter disappearance of unheated (CM 0), heated (CM 60) canola meal and of bypass (Bi) protein supplement of cows fed similar or different supplement.

Diet[R]	0			60			Bi			
TS[S]	0	60	Bi	0	60	Bi	0	60	Bypass	SE[T]
Hour[U]										
0.1	24.25a	23.08a	16.33b	24.91a	21.75b	16.79c	15.30b	19.03a	16.57b	0.93
2	32.75	-[V]	-	-	26.02	-	-	-	20.58	0.51
4	35.31	-	-	-	32.28	-	-	-	29.08	1.14
8	50.42	-	-	-	42.07	-	-	-	38.64	0.52
12	66.68a	34.31b	33.35b	62.16a	47.26b	42.60c	41.59b	45.20a	43.17b	0.43
16	76.87a	49.81b	44.98c	72.01a	55.06b	46.95c	51.52b	56.08ab	53.37b	1.31
24	87.20a	57.08b	50.62ch	84.80a	68.75b	56.27c	58.57b	67.69a	67.37a	0.53
72	89.12	-	-	-	87.12	-	-	-	74.56	0.88

[R] Cow diet: 0 = unheated CM, 60 = heated CM at 60 minutes and Bi = formulated animal-vegetable protein supplement.
[T] Standard error of Lsmeans for individual incubation time (n=3).
[S] Test supplement.
[U] Incubation time.
[V] No data was collected.
a-c, Lsmeans in each row within cow diet with different letters differ ($P<0.05$).

Table 1.5. In situ percent nitrogen disappearance of unheated (CM 0), heated (CM 60)canola meal and of bypass protein (Bi) supplement of cows fed similar or different supplement.

Diet[R]	0			60			Bi			
TS[S]	0	60	Bi	0	60	Bi	0	60	Bi	SE[T]
Hour[U]										
0.1	21.03a	17.28b	14.96c	16.59a	14.92b	13.22b	18.09b	18.73b	20.18a	0.78
2	36.25	-[V]	-	-	20.14	-	-	-	21.38	0.90
4	44.51	-	-	-	28.04	-	-	-	31.06	1.08
8	54.15	-	-	-	44.57	-	-	-	40.42	0.69
12	69.50a	54.97b	45.21c	54.29a	48.43b	42.61c	55.06a	47.88b	43.07c	0.32
16	79.83a	59.52b	48.78c	71.76a	66.41b	56.82c	60.80a	53.54b	45.53c	1.07
24	92.63a	67.42b	53.60c	84.03a	76.82b	63.84c	68.32a	60.74b	47.75c	0.40
72	95.29	-	-	-	92.92	-	-	-	65.65	0.86

[R] Cow diet: 0 = control canola untreated meal, 60 = heat treated canola meal at 60 minutes and by-pass = formulated animal-vegetable protein supplement.

[T] Standard error of Lsmeans for individual incubation time (n=3)

[S] Test supplement.

[U] Incubation time.

[V] No data was collected.

a-c, Lsmeans in each row within cow diet with different letters differ ($P<0.05$).

Table 1.6. Disappearance parameters[W] and effective degradabilities of dry matter and nitrogen in heat treated canola meal and bypass (Bi) protein supplement.

	Heat treatment (min at 110°C)					
Parameter	0	23	45	60	BI	SE[X]
Dry matter						
a, %	24.22a	22.66b	20.67c	19.00d	16.77e	0.45
b, %	64.61a	62.39b	60.34c	50.77d	48.03e	0.29
c, $10^{-3}h^{-1}$	56.08ab	59.42a	57.37ab	51.17b	41.30c	2.15
d, h	0.38b	0.54b	0.61b	0.90a	0.96a	0.07
ED, %	49.94a	48.58a	45.14b	37.92c	32.27d	0.99
Nitrogen						
a, %	21.66a	18.33b	16.56c	14.89d	13.55e	0.16
b, %	75.72a	75.83a	73.56b	70.77c	67.91d	0.29
c, $10^{-3}h^{-1}$	68.93a	66.68b	66.70b	60.54c	52.62d	0.41
d, h	0.37c	0.54bc	0.59bc	0.73ab	0.87a	0.07
ED, %	56.15a	51.96b	49.13c	44.35d	39.33e	0.21

[W] Disappearance parameters: *a*, rapidly soluble fraction; *b*, potentially degradable fraction; *c*, the fractional-rate constant at which fraction described by *b* is degraded per h, and *d* is the lag time.
ED, Effective degradability estimates at rumen outflow rate, k=0.08.
[X] Standard error of Lsmeans (n=3).
[Y] Acid detergent fibre .
[Y] Neutral detergent fibre.
a-e, Lsmeans within a row followed by different letters differs ($p<0.05$).

Table 1.7. Effect of cow diet (CD) on percent rumen in situ dry matter disappearance of unheated (CM 0), heated (CM 60) canola meal and of bypass (Bi) protein supplement.

CD	0	60	Bi	SE[S]	0	60	Bi	SE	0	60	Bi	SE
TS[T]	CM 0				CM 60				Bi			
Hour[U]												
0.1	24.25a	24.91a	15.30b	0.93	23.08	21.75	19.03	0.14	16.33	16.79	16.57	0.51
12	66.68a	62.16b	41.59c	0.99	34.31b	47.26a	45.20a	0.52	33.35b	42.60a	43.17a	0.89
16	76.87a	72.01b	51.52c	1.71	49.81b	55.06a	56.08a	1.44	44.98b	46.95b	53.37a	1.34
24	87.20a	84.80b	58.57c	0.89	57.08b	68.75a	67.69a	0.97	50.62b	56.27b	67.37a	0.87

CD: Cow diet 0 = unheated CM , 60 = heated CM at 60 mins, and Bi = formulated animal-vegetable protein supplement.
[S] Standard error of Lsmeans for analyses conducted for each incubation time within cow diet (n=3).
[T] Test supplement.
[U] Incubation time (h).
a-c Lsmeans in each row within in situ protein supplement with different letters differ ($P<0.05$).

Table 1.8. Effect of cow diet (CD) on percent rumen in situ nitrogen disappearance of unheated (CM 0), heated (CM 60) canola meal and of bypass (Bi) protein supplement.

CD	0	60	Bi	SE[S]	0	60	Bi	SE	0	60	Bi	SE
TS[T]	CM 0				CM 60				Bi			
Hour[U]												
0.1	21.03a	16.59b	18.09b	0.57	17.28a	14.92b	18.73a	0.68	14.96b	13.22b	20.18a	0.70
12	69.50a	54.29b	55.06b	0.33	54.97a	48.43b	47.88b	0.45	45.21a	42.61b	43.07b	0.24
16	79.83a	71.76b	60.80c	0.47	59.52b	64.41a	53.54c	0.62	48.78b	56.82a	45.53c	0.53
24	92.63a	84.03b	68.32c	0.88	67.42b	76.82a	60.74c	0.92	53.60b	63.84a	47.75c	0.67

CD: Cow diet 0 = unheated CM, 60 = heated CM 60 mins, and Bi = formulated animal-vegetable protein supplement.
[S] Standard error of Lsmeans for analyses conducted for each incubation time within cow diet (n=3).
[T] Test supplement.
[U] Incubation time (h).
a-c Lsmeans in each row within protein supplement with different letters differ ($P<0.05$).

Table 1.9. Percent rumen in situ dry matter (DM) and nitrogen (N) disappearance of unheated (CM 0), heated (CM 60) canola meal and bypass protein (Bi) from multifilament and monofilament bag types.

Bag type		Multifilament				Monofilament			
Test Protein[Y]		CM 0	CM 60	Bi	SE[Z]	CM 0	CM 60	Bi	SE
Time (h)									
0.1	DM	27.7a	22.3b	16.7c	0.5	28.1a	23.3b	16.7c	1.0
	N	25.8a	21.8b	14.2d	0.4	24.6a	22.7b	17.3c	1.1
12	DM	57.7b	50.9c	42.4e	0.1	67.4a	52.5c	46.9d	0.9
	N	50.6b	45.9d	42.3e	0.5	64.9a	51.9b	48.2c	1.1
16	DM	64.6b	53.3c	45.2d	0.9	70.9a	60.7b	51.9c	1.9
	N	63.8c	55.2d	44.9e	0.3	69.9a	62.8c	65.2b	0.2
24	DM	80.7b	67.9d	46.7f	1.2	87.3a	73.3c	52.2e	1.5
	N	66.8c	60.5d	52.3e	0.4	78.6a	69.1b	68.9b	1.3

[Y] Cow diet contained test protein.
[Z] Standard error of Lsmeans (n=3).
a-f, Lsmeans in each row with different letters, differ ($P<0.05$).

Table 1.10. Total tract and lower GI tract % disappearance of dry matter (DM) and nitrogen (N) from unheated (CM 0), heated (CM 60) and bypass protein (Bi) with bag type, without and with pepsin-HCl digestion.

Bag type	Multifilament			Monofilament			
Test protein	CM 0	CM 60	Bi	CM 0	CM 60	Bi	SE
DM Digestion							
Total tract							
Pepsin	82.5d	85.4c	77.6e	90.4a	88.8b	77.8e	0.2
No-pepsin[Y]	81.6d	85.3c	79.1e	91.4a	87.7b	77.7e	0.2
Lower GI tract[Z]							
Pepsin	15.8e	38.1b	34.4c	23.7d	41.5a	34.6c	1.0
No-pepsin[Y]	14.9d	11.0e	35.9b	24.7c	40.4a	33.5b	1.2
N digestion							
Total tract							
Pepsin[X]	83.6b	79.6c	70.9d	89.9a	82.9b	81.2bc	0.9
No-pepsin[Y]	83.2b	80.9c	71.9d	90.2a	83.0b	82.5b	0.1
Lower GI tract[Z]							
Pepsin[X]	14.1e	31.2c	27.8c	20.4d	34.5b	38.1a	0.9
No-pepsin[Y]	13.7e	32.5b	28.8c	20.7db	34.6b	39.4a	1.0

[W] Standard error of Lsmeans (n=3).
a-e Lsmeans in each row with different letters differ ($p<0.05$).
[X, Y] Non significant difference for pepsin vs no-pepsin digestion ($p>0.05$).

Table 1.11. Disappearance parameters[W] and effective degradabilities of acid detergent fibre (ADF) and neutral detergent fibre (NDF) in heat treated canola meal and bypass (Bi) protein supplement.

	Canola meal (min at 110°C)					
Parameter	0	23	45	60	Bi	SE[X]
ADF[Y]						
a, %	29.37a	25.51b	24.34c	20.00d	19.60d	0.48
b, %	63.15a	61.19ab	58.61bc	53.97bd	55.44cd	0.93
c, $10^{-3}h^{-1}$	65.23a	58.90a	62.77a	50.77ab	41.83b	3.80
d, h	0.44b	0.45b	0.46b	0.53a	0.48ab	0.013
ED, %	57.17a	52.91ab	49.38b	40.41c	38.16c	1.36
NDF[Z]						
a, %	32.37	31.17	28.00	26.34	24.60	0.21
b, %	67.05a	63.52b	58.61c	51.31e	56.31d	0.50
c, $10^{-3}h^{-1}$	48.17	52.57	52.76	46.10	47.67	5.09
d, h	0.45a	0.47b	0.50c	0.58d	0.63e	0.008
ED, %	56.37d	55.78c	50.73c	44.54b	44.95a	1.97

[W] Disappearance parameters: *a*, rapidly soluble fraction; *b*, potentially degradable fraction; *c*, the fractional-rate constant at which fraction described by *b* is degraded per h, and *d* is the lag time.
ED, Effective degradability estimates at rumen outflow rate, k=0.08.
[X] Standard error of Lsmeans (n=3).
[Y] Acid detergent fibre .
[Y] Neutral detergent fibre.
a-e, Lsmeans within a row followed by different letters differs ($p<0.05$).

Table 1.12 Amino acid (AA) concentration [g(16g N)$^{-1}$] of unheated, heated and of bypass protein (Bi) supplement.

	Heat treatment (minutes at 110°C)					
	0	23	45	60	Bi	SE[x]
Dry matter %	97.50	96.83	95.05	98.29	96.67	1.02
Essential AA[w],						
Arginine	6.75a	6.17a	5.36ab	4.46b	5.45b	0.19
Histidine	2.91a	2.75a	3.46a	2.21b	2.12b	0.71
Isoleucine	3.56a	3.08a	2.67c	2.87c	3.07b	0.83
Leucine	7.90a	6.98b	6.33b	6.61b	9.62a	0.92
Lysine	6.13a	5.58a	4.74b	4.37b	4.71b	0.15
Methionine	2.52	1.09	1.07	1.86	1.87	0.03
Phenylalanine	4.94	4.71	4.44	4.48	4.93	0.38
Threonine	5.10	5.56	4.72	4.04	4.06	0.18
Valine	4.57	3.86	3.40	3.66	4.09	0.21
Non essential AA,						
Tyrosine	**2.86**	**3.25**	**3.12**	**2.65**	**3.26**	**0.13**
Alanine	**5.16**	**4.95**	**4.59**	**4.36**	**7.44**	**0.19**
Aspartic acid	**9.37a**	**7.49b**	**7.18b**	**7.86b**	**8.77b**	**0.82**
Cystine	**2.72**	**1.33**	**0.97**	**2.10**	**1.69**	**0.14**
Glutamic acid	**18.86**	**19.24**	**17.28**	**16.93**	**17.33**	**1.02**
Glycine	**5.52**	**5.61**	**5.19**	**4.67**	**6.49**	**0.14**
Proline	**7.22a**	**6.68b**	**6.48b**	**6.62b**	**8.19a**	**0.19**
Serine	**5.41**	**5.40**	**4.83**	**4.51**	**5.39**	**0.45**
Ammonia	**1.98**	**1.95**	**1.77**	**1.73**	**1.71**	**0.03**
Total N, % DM	**6.29b**	**6.19b**	**6.23b**	**5.96b**	**8.33a**	**0.78**

[w] values represent duplicate analyses of each supplement sample (DM-basis).
[x] Standard error of Lsmeans (n=3).
a-c, Lsmeans in each row with different letters differ (P<0.05).

Table 1.13. The percent ruminal disappearance of essential AA from CM moist heated at CM 0, CM 23, CM 45, CM 60 mins and of bypass protein (Bi) supplement after rumen incubation for 12, 16 and 24 h.

	Heat treatment, minutes at 110°C					
Essential AA	0	23	45	60	Bi	SE[w]
			12 h rumen incubation			
Arginine	69.12a	70.79a	60.90b	18.19d	40.26c	0.37
Histidine	71.91a	71.81a	64.96b	29.29d	40.82c	0.24
Isoleucine	62.18a	63.62a	54.07b	19.17d	30.75c	0.89
Leucine	66.22a	64.41a	57.45b	25.17c	26.61c	0.67
Lysine	88.95a	68.99b	64.86c	34.99e	43.82d	0.61
Methionine	69.21a	47.35b	43.38b	23.47c	16.35d	0.86
Phenylalanine	63.52a	62.03a	56.73b	29.41c	23.34d	0.66
Threonine	62.33a	62.12a	50.69b	18.62d	32.77c	0.61
Valine	61.37a	63.95a	54.83b	11.85c	26.06c	0.53
			16 h rumen incubation			
Arginine	73.09a	78.60a	65.25b	22.54d	43.04c	1.27
Histidine	75.78a	80.79a	70.65b	35.95d	46.99c	2.79
Isoleucine	68.88b	74.09a	62.90c	32.67d	30.82d	1.75
Leucine	70.18a	74.71a	64.74b	32.30c	30.63c	1.16
Lysine	71.93b	78.45a	70.70b	36.95c	36.49c	1.44
Methionine	72.51a	51.86b	71.24a	33.68c	26.29d	1.47
Phenylalanine	65.84b	72.15a	61.90b	31.90c	27.08c	1.54
Threonine	69.17a	72.79a	50.69b	26.97d	42.57c	1.91
Valine	65.19b	73.43a	65.21b	14.64d	31.04c	1.41
			24 h rumen incubation			
Arginine	89.20a	82.78a	75.76b	27.35d	48.93c	0.90
Histidine	91.22a	84.51b	78.14c	56.32d	47.21e	2.77
Isoleucine	86.49a	87.11a	68.03b	40.35c	37.15c	0.95
Leucine	88.21a	78.68b	72.14b	41.42c	35.16c	1.08
Lysine	67.02c	82.27a	77.13b	44.78d	33.25e	0.82
Methionine	86.59a	62.05c	82.80a	79.57b	30.40d	1.44
Phenylalanine	84.84a	79.30b	71.49c	33.69d	31.77d	0.63
Threonine	86.39a	74.76b	69.73c	39.31e	44.71d	1.56
Valine	85.07a	79.02b	76.90c	22.67e	35.76d	0.69

[w] Standard error of Lsmeans (n=3).

a-e Lsmeans within each row with different letters differ ($p<0.05$).

Table 1.14. The percent ruminal disappearance of essential AA from unheated (CM 0), heated (CM 60) canola meals and bypass protein (Bi) supplement after 12, 16, 24 h ruminal incubation, compared for changes over incubation times and among supplements.

	Test supplement			
Essential AA	CM 0	CM 60	Bi	SE[w]
	12 h rumen icubation			
Arginine	69.12a	18.19c	40.26b	0.35
Histidine	71.91a	29.29c	40.82b	0.24
Isoleucine	62.18a	19.17c	30.75b	0.78
Leucine	66.22a	25.17b	26.61b	0.56
Lysine	88.95a	34.99c	43.82b	0.56
Methionine	69.21a	23.47b	16.35c	0.98
Phenylalanine	63.52a	29.41b	23.34c	0.67
Threonine	62.33a	18.62c	32.77b	0.56
Valine	61.37a	11.85c	26.06b	0.67
	16 h rumen incubation			
Arginine	73.09a	22.54c	43.04b	1.23
Histidine	75.78a	35.95c	46.99b	1.34
Isoleucine	68.88a	32.67b	30.82b	1.67
Leucine	70.18a	32.30b	30.63b	1.67
Lysine	71.93a	36.95b	36.49b	1.22
Methionine	72.51a	33.68b	26.29c	1.25
Phenylalanine	65.84a	31.90b	27.08b	1.43
Threonine	69.17a	26.97c	42.57b	1.12
Valine	65.19a	14.64c	31.04b	1.17
	24 h rumen incubation			
Arginine	89.20a	27.35c	48.93b	0.89
Histidine	91.22a	56.32b	47.21c	2.52
Isoleucine	86.49a	40.35b	37.15b	0.87
Leucine	88.21a	41.42b	35.16c	1.06
Lysine	67.02a	44.78b	33.25c	0.67
Methionine	86.59a	79.57b	30.40c	1.23
Phenylalanine	84.84a	33.69b	31.77b	0.67
Threonine	86.39a	39.31c	44.71b	1.45
Valine	85.07	22.67cd	35.76b	0.67

[w] Standard error of Lsmeans (n=3).

a-c, Lsmeans within a row with different letters differ ($P<0.05$).

Table 1.15. The percent ruminal, lower gastro-intestinal disappearance and fecal excretion of essential amino acids (EAA) from canola meal moist heated at 0, 23, 45, 60 min and of bypass protein (Bi) supplement incubated for 12 h in multifilament bag type.

	Heat treatment, minutes at 110°C					
EAA	0	23	45	60	Bi	SE[w]
	Percent ruminal disappearance					
Arginine	69.12a	70.79a	60.90b	18.19d	40.26c	0.37
Histidine	71.91a	71.81a	64.96b	29.29d	40.82c	0.24
Isoleucine	62.18a	63.62a	54.07b	19.17d	30.75c	0.89
Leucine	66.22a	64.41a	57.45b	25.17c	26.61c	0.67
Lysine	88.95a	68.99b	64.86c	34.99e	43.82d	0.61
Methionine	66.21a	47.35b	43.38b	23.47c	16.35d	0.86
Phenylalanine	63.52a	62.03a	56.73b	29.41c	23.34d	0.66
Threonine	62.33a	62.12a	50.69b	18.62d	32.77c	0.61
Valine	61.37a	63.95a	54.83b	11.85d	26.06c	0.53
	Percent disappearance in lower GIT					
Arginine	27.55d	26.41d	34.39c	76.75a	47.83b	1.27
Histidine	24.62d	25.70d	31.30c	65.65a	51.58b	1.79
Isoleucine	31.96d	32.17d	39.44c	73.41a	58.75b	1.75
Leucine	29.68d	32.14d	37.46c	69.19a	60.74b	1.16
Lysine	15.83d	27.37c	29.42c	57.97a	47.29b	1.44
Methionine	26.42d	49.36b	45.23b	70.89a	69.46a	1.47
Phenylalanine	30.41c	33.11c	36.38b	63.13a	64.24a	1.54
Threonine	30.60d	32.41d	40.70c	70.33a	57.71b	1.91
Valine	30.54c	30.61c	36.90b	78.98a	62.50b	1.41
	Percent fecal excretion					
Arginine	3.33c	3.07c	4.71c	5.06b	11.91a	0.90
Histidine	3.47c	2.49c	3.74c	5.06b	7.60a	0.77
Isoleucine	5.86c	4.21d	6.49c	7.42b	10.50a	0.95
Leucine	4.10b	3.45b	5.09b	5.64b	12.65a	1.08
Lysine	5.22b	3.64c	5.72b	7.04a	8.89a	0.82
Methionine	4.37d	3.29d	11.39b	5.64c	14.19a	1.44
Phenylalanine	6.07b	4.86c	6.89b	7.46b	12.42a	0.63
Threonine	7.07b	5.47c	8.61b	11.05a	9.52a	1.56
Valine	8.09b	5.44c	8.26b	9.17b	11.44a	0.69

[w] Standard error of Lsmeans (n=3).

a-e, Lsmeans on the same row with different letters differ ($P<0.05$).

Table 1.16. Lower GI tract availability [g(16g of original N)$^{-1}$] of essential AA from unheated, heated and bypass protein (Bi) supplement after 12 h rumen incubation (% in original supplement).

	Heat treatment (minutes at 110°C)					
	0	23	45	60	Bi	SE[Y]
Essential AA						
Arginine	1.81[z]	1.63	1.84	3.42	2.61	0.03
Histidine	0.72	0.71	1.08	1.45	1.09	0.12
Isoleucine	1.14	0.99	1.05	2.11	1.80	0.05
Leucine	2.34b	2.24b	2.37b	4.57a	5.84a	0.44
Lysine	0.97b	1.53b	1.39b	2.53a	2.23a	0.14
Methionine	0.67	0.54	0.48	1.32	1.29	0.23
Phenylalanine	1.50	1.56	1.62	2.83	3.17	0.42
Threonine	1.56	1.80	1.92	2.84	2.34	0.13
Valine	1.39	1.18	1.25	2.89	2.56	0.24

[Y] Values represent duplicate analyses of each supplement sample.
a-b, Lsmeans on the same row with different letters differ ($P<0.05$).
[z] Animo acid level in original sample x percent disappearance in lower GI tract = available amino acid in GI tract.

Table 1.17. Amino acid concentration [g(16g N)$^{-1}$] of canola meal-based concentrate grain mixes as used in formulating diets for the experimental cows, relative to milk amino acids.

Essential AA,	Control	Heated	Bypass	Milk[W]
Arginine	7.84[X](224)[Y]	4.94(141)	4.13(118)	3.5
Histidine	3.24(120)	1.84(68)	1.53(57)	2.7
Isoleucine	4.26(72)	2.81(48)	2.22(38)	5.9
Leucine	9.06(93)	6.14(63)	5.74(59)	9.7
Lysine	6.86(85)	3.72(46)	2.84(35)	8.1
Methionine	5.15(198)	7.33(282)	1.32(51)	2.6
Phenylalanine	5.95(121)	4.59(94)	4.04(82)	4.9
Threonine	6.54(142)	4.39(95)	3.24(70)	4.6
Valine	5.42(82)	3.63(55)	3.01(46)	6.6
Non Essential,				
Alanine	6.08	4.22	3.89	
Aspartic acid	9.42	6.16	4.77	
Cystine	4.57	0.86	3.69	
Glutamic acid	24.74	20.18	17.58	
Glycine	6.66	4.43	4.02	
Proline	8.93	8.98	8.85	
Serine	6.63	4.76	3.98	
Tyrosine	3.66	2.86	2.41	
Ammonia	2.54	2.27	1.90	

[W] Milk amino acid values, [g(16g N)$^{-1}$] as reported by Spires et al. (1975).
[X] Values represent duplicate analyses of each mixed concentrate grain samples.
[Y] Values in parenthesis denote % of respective essential AA in each protein supplement relative to that of milk.

Table 1.18. The concentration [g(16g N)$^{-1}$] of essential amino acids in rumen undegradable intake protein from heat treated camola meal and of bypass protein (Bi) supplement after 12, 16, 24 h rumen incubation and milk amino acids [g (16g N)$^{-1}$].

	Heat treatment, minutes at 110°C					
Essential AA	0	23	45	60	Bi	Milk[W]
			12 h rumen incubation			
Arginine	5.65[X]	5.77	6.16	5.79	4.85	3.5
Histidine	2.24	2.48	2.51	2.38	1.83	2.7
Isoleucine	3.82	1.35	3.58	3.66	2.95	5.9
Leucine	7.31	7.67	7.88	7.49	9.79	9.7
Lysine	5.51	5.55	4.89	4.58	3.93	8.1
Methionine	1.84	2.10	1.86	1.31	2.08	2.6
Phenylalanine	5.09	5.45	5.62	4.97	5.40	4.9
Threonine	6.35	6.17	6.23	5.72	4.29	4.6
Valine	5.16	4.49	4.59	4.53	3.70	6.6
			16 h rumen incubation			
Arginine	6.09	6.14	4.9	5.65	4.66	3.5
Histidine	2.50	2.46	1.87	2.36	1.77	2.7
Isoleucine	4.00	4.08	3.56	3.72	2.99	5.9
Leucine	7.75	8.60	6.60	7.36	9.50	9.7
Lysine	6.12	5.74	4.13	4.45	3.86	8.1
Methionine	1.69	2.39	1.59	1.84	2.41	2.6
Phenylalanine	5.24	6.08	4.47	4.90	5.32	4.9
Threonine	6.78	6.72	4.34	5.73	4.24	4.6
Valine	5.40	5.20	4.42	4.62	3.70	6.6
			24 h rumen incubation			
Arginine	5.43	5.16	5.81	5.84	4.51	3.5
Histidine	2.26	2.15	2.38	2.41	1.91	2.7
Isoleucine	4.20	3.07	3.81	3.52	2.99	5.9
Leucine	7.16	7.41	7.88	8.08	10.18	9.7
Lycine	5.54	4.92	4.79	4.60	3.63	8.1
Methionine	1.91	2.50	2.00	1.71	2.47	2.6
Phenylalanine	5.49	5.55	5.64	5.86	5.79	4.9
Threonine	6.54	5.64	4.87	5.19	4.33	4.6
Valine	5.41	4.04	4.59	4.33	3.76	6.6

[W] Milk amino acids, [g(16g N)$^{-1}$] as reported by Spires et al (1975).

[X] Values represent duplicate analyses of each sample.

Table 1.19. The percent availability of amino acids in CM samples moist heated at CM 0, CM 23, CM 45, CM 60 min and bypass protein supplement incubated for 12 h and digested in pepsin-HCl solution.

Item	Heat treatment, minutes 110°C					
	0	23	45	60	Bi	SE[Y]
Essential AA[U], Lower GIT availability[V]	28.15d	32.11d	36.80c	69.59a	57.79b	1.26
Total tract availability[W]	96.91a	96.01a	93.23a	92.94a	88.98b	0.89
Non-essential AA[X],						
Lower GIT availability	26.40d	32.10c	36.91c	64.72a	54.92b	1.80
Total tract availability	93.93a	95.83a	93.95a	92.33a	87.97b	1.10

[U] Essential AA determined as Lsmean values for Arginine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine and Valine.

[V] Lower GIT availability = (% ruminal escape - % fecal excretion)

[W] Total tract availability = (% rumen disappearance + % lower GI tract disappearance)

[X] Non-essential AA determined as LSmean values from Alanine, Aspartic acid, Cystine, Glutamic acid, Glycine, Proline, Serine and Tyrosine

[Y] Standard error of Lsmeans, n=3 for EAA's and n=3 for NEAA's.

MANUSCRIPT II

ENERGY LEVELS AND AMINO ACID AVAILABILITY FROM UNHEATED AND HEAT TREATED CANOLA MEAL AND BYPASS PROTEIN SUPPLEMENT AS ASSAYED BY PRECISION-FED COCKERELS.

ABSTRACT

This study was conducted to measure amino acid (AA) bioavailability from heat treated canola meal (CM) precision-fed to adult cockerels and to compare these results with those AA post-ruminally available in cannulated cows fed similar CM as measured by the "mobile nylon bag" technique. Four batches of CM samples exposed to moist heating at about 110°C for 0 (CM 0), 23 (CM 23), 45 (CM 45) and 60 (CM 60) min and a fifth supplement (Bi) formulated with a mixture of animal-vegetable protein were precision-fed to five groups of ten adult cockerels using the technique described by Sibbald (1986) as modified by Zhang et al. (1994) to measure AA bioavailability and true metabolizable energy corrected for nitrogen (TME_n). Heat treatment did not ($P>0.05$) change TME_n, protein and acid detergent fibre levels but increased ($P<0.05$) neutral detergent content of CM. Increased heating tended to reduce bioavailability of all AA except methionine. True AA digestibility decreased ($P<0.05$) for all AA with 60 and 45 min heating except hisitidine and leucine. With 60 min heating lysine digestibility decreased by 25%. Results of cockerel and previously conducted *in situ* cow studies indicated that heat treatment of CM decreased ($P<0.05$) bioavailability of all AA except methionine for cockerels. In cows, since heat treatment decreased ($P<0.05$) rumen degradability, there was increased ($P<0.05$) disappearance of AA in the lower gastro intestinal (GI) tract. Lysine digestibility in cockerels was depressed ($P<0.05$) with increased heating, but heating increased ($P<0.05$) rumen escape of lysine with increased ($P<0.05$) lower GI tract lysine availability in cows. Heat treatment increased ($P<0.05$) availability of AA in lower GI tract of cows and but also increased ($P<0.05$) fecal

excretion of arginine, histidine, isoleucine and threonine. Heat treatment of CM did not ($P < 0.05$) affect energy levels in cockerels as expected for the ruminants higher heating temperatures are suggested for future research.

KEY WORDS: Canola meal, heat treatment, cockerels, nitrogen corrected true metabolizable energy, true amino acid availability.

INTRODUCTION

The contribution of dietary protein to the nutritional needs of an animal depends not only on its amino acid (AA) composition but also on how efficiently these AA are utilized (McNab 1994). Recent advances in technology such as ion-exchange and high performance liquid chromatography have made determination of AAs in foods relatively routine. These results have been published in data banks (Degussa 1990; NRC 1994) which give only the AA content of the protein (McNab 1994). For sometime, it has been recognized that for almost all food resources, the contribution made by the dietary protein and hence AA to the animal's requirement falls significantly short of the total requirement (McNab 1991). One reason for this shortfall is the fact that not all AAs supplied by the diet become available to the animal during digestion and metabolism. This has led to the concept of "bioavailability" (Sibbald 1987).

Bioavailability has been described as the proportion of ingested nutrient which can be used for normal metabolic functions (Sibbald 1987). In a recent review McNab (1994) has suggested the use of "available" as a simpler adjective to indicate the "same quantity". Available AAs have hence been described as those which are actually supplied at sites of protein synthesis (McNab 1991; 1994). Despite many attempts to devise methods capable of measuring what proportions of the AA in the ingested protein reaches these sites, very limited quantitative data applicable to diet formulation exists (Moughan and Rutherford 1990, McNab 1991; 1994). Carpenter (1973) holds the general view that responses in animal performance are the only direct way to test the extent to which dietary protein is broken down and absorbed into the portal blood during digestion and

incorporated into body protein or metabolized for some other essential function. The inability of nutritionists to devise an effective and valid assay with wide application has presented a formidable barrier to progress in this area since the latest reviews of this topic (Engster 1986; McNab 1979a, b; Papadopoulos 1985; Sibbald 1987; Whiteacre and Tanner 1988). McNab (1994) suggested that a method should be devised to determine the amounts of each dietary AA becoming available to the animal in order to assist in diet formulation and to maximize the output from poultry and livestock in general.

Heat treatment of feedstuffs may alter the total AA availability and some specific AAs such as lysine in canola meal (CM) have been decreased with increased duration of heating regadless of form of heating (autoclave vs oven) and initial level of moisture of the meal (Goh et al. 1979). Sibbald (1980) has noted that excessive heating of soybean flakes not only reduces the availability of AA but also impairs energy availability.

An experiment was conducted using precision fed cockerels to achieve the following objectives:

(a) To measure essential amino acid and non-essential amino acid availability from untreated and heat treated canola meal samples and a formulated by-pass animal-plant protein supplement.

(b) To measure the levels of true metabolizable energy corrected for endogenous nitrogen sources (TME_n) for the same samples.

This information was compared with data on availabilities of these AA's to the lower GIT of ruminants as determined by the "mobile bag" method (Manuscript I).

MATERIALS AND METHODS

Experimental protocol

Canola meal samples used in this study were moist heated (110°C) for 23, 45, and 60 minutes in a steam jacketed conveyor set at slow speed and then held in steeping trays at passage rates of 200, 110 and 90 kg per hour at the Protein Oil Starch (POS) pilot plant in Saskatoon. The samples were thereafter identified as CM 0 (unheated), CM 23, CM 45 and CM 60 representing time of heating. A fifth sample , bypass protein (Bi) was formulated with a mixture of commercial CM, corn gluten meal, fish meal and meat meal (table 1.1). The latter supplement was calculated to provide similar quantities of essential AA as the CM sample identified as CM 60.

Fifty adult Single Comb White Leghorn cockerels divided into five groups were randomly assigned to individual metabolism cages (62.2 cm X 34.3 cm X 43.3 cm) in an environmentally controlled room (21 $\pm$ 2°C) with 16 hours of light and free access to fresh water throughout the experiment.

After the birds were starved for 28 hours, they were randomly precision-fed 30 g each of the five meal samples using the bioassay method of Sibbald (1986) with minor modifications (Zhang et al. 1994) to determine the true nitrogen-corrected metabolizable energy (TME_n) and availability of EAAs and NEAAs.

Four birds regurgitated CM 0 and a second precision-feeding was repeated with success. The test feeds were fed in meal form as received from the POS plant without any further preparation. There were no problems with the samples adhering to the funnel. Excreta was collected over a 48 hour period using smooth surfaced excreta trays

extending beyond the cage in all the directions. During excreta collection care was taken to remove feathers and scales that fell in the excreta tray.

In order to determine an estimate of endogenous energy and nitrogen excretion, excreta from two groups of 14 cockerels each, was collected for 48 hours using smooth surfaced excreta trays following 28 hours of fasting. In each case, the assumption was that the GIT of birds was empty prior to and following the 48 h collection period. The collected samples of excreta was frozen, freeze dried and weighed after equilibrating to ambient temperature over a 24 h period. The "mobile bag" method was used to measure loss of AA (potentially available) in the lower GI tract of ruminants (Manuscript I).

ANALYSES AND CALCULATIONS

Collected excreta samples from each treatment were ground through a 1-mm screen and analyzed for nitrogen-corrected true metabolizable energy (TME_n). The TME_n values for samples measured in duplicate were calculated for each meal treatment using the endogenous loss (EEL) and endogenous nitrogen values for all unfed birds throughout the experiment adopting the methods of Zhang et al. (1994).

Feed samples offered to the roosters and ground excreta samples were analyzed chemically in duplicate for moisture at 105°C (Method 7.013 of official methods of analysis of Association of the Official Analytical Chemists (AOAC) 1984) and total nitrogen content using a micro-Kjeldahl analyzer (Tecator 1030 analyzer, method 47.023 (AOAC 1984). Acid detergent fibre (ADF) was determined by method 7.076 (AOAC 1984), neutral detergent fiber by assay of Goering and Van Soest (1970). Acid detergent insoluble nitrogen (ADIN), and neutral detergent insoluble nitrogen (NDIN) analyses

were completed on respective ADF and NDF residues using micro-Kjeldahl method 47.023 (AOAC 1984).

Feed samples and excreta were analyzed for both essential and non-essential amino acids following a 24 h hydrolysis in 6 N HCl (Andrews and Baldar 1985) at 110°C. Samples for cystine and methionine analysis were hydrolysed and analyzed after 20 h of oxidation with performic acid (Andrew and Baldar 1985). All AA were determined on LKB Biochrom 4151 Alpha plus (Biochrom, Science Parks, Cambridge, UK).

The true amino acid availability (TAAA) for each bird in each treatment was calculated from the equation of Likuski and Dorrrell (1978) as follows:

$$\%TAAA = \frac{[\ AA\ consumed\ -\ fecal\ \ AA + Endogenous\ \ fecal\ \ AA\ ]}{AA\ consumed}\ x\ 100$$

STATISTICS

Resulting data were analyzed as a completely randomized design (Steel and Torrie 1980) using General Linear Models Procedures (GLM) of Statistical Analysis System , Inc. (1989). Statistical differences between treatments were tested using the Newman-Keuls Test (Snedecor and Cochran, 1980).

Animal care

All cockerels were managed in accordance with the guidance of the Local Animal Care and Use committee, which follows the Canadian Council on Animal care requirements.

RESULTS AND DISCUSSION

The chemical composition of the treatment samples is shown in table 2.1. Dry matter (DM) values of the meals are the result of varying processing, drying conditions and final exposure during transportation from the processing plant and subsequent storage conditions. Moist heating of CM did not change ($P>0.05$) protein or ADF contents. Protein and ADF values (table 2.1) for Bi supplement were similar to CM samples. These values were comparable to those for the same supplements (table 1.3) used in the ruminant studies. Heat treatment increased ($P<0.05$) NDF contents of CM 60 (table 2.1) in the pattern consistent with the observations for the same supplements in ruminant studies (table 1.3). Heat treatment had no significant ($P>0.05$) effect on total N content of CM samples (table 1.3) which agrees with earlier results (Moshtaghi-Nia and Ingalls 1992; Moshtaghi-Nia 1994) that reported heat treatment reduced N solubility with no change in N content.

The TME_n content of heat treated CM samples did not ($P>0.05$) change with heating and remained at a mean value of 9.57 MJ kg^{-1} (Table 2.1) and was also similar ($P>0.05$) to Bi supplement. TME_n values for CM are similar to the average value of 9.71 MJ Kg^{-1} reported by Zhang and Campbell (in press), Mutzar and Slinger (1982) and Simbaya (1992). Lipids are considered major contributing components to the metabolizable energy content of meals coupled with the drying effect of meal to remove solvents. In this study CM samples were steam heated and no physical activity such as pelleting was conducted during meal processing. The increases noted in NDF, ADIN and NDIN with increased heating of CM did not ($P>0.05$) affect the TME_n value of the

samples, though it would be expected that increased heating would decrease the energy values of the samples.

True AA availability as [g(16 g $N)^{-1}$] for the samples as assayed with cockerels (table 2.2) were not ($P>0.05$) different for the supplements. True AA availability of both EAA and NEAA (table 2.2) assayed with cockerels was on average about 30% lower than indicated by chemical analysis (table 1.15). These differences can be attributed to digestibility of AA and the unknown extent of microbial activity in the intestinal tract and digestibility of AA. Such microbial activity has been suggested (Robins et al. 1980) to produce artifacts, including absorption of non-nutritive derivatives of amino acids and cause disappearance (and specious absorption) of non-digestible AA derivatives from heat treated proteins of CM. Studies with cockerels to evaluate plant feedstuffs such as CM (Slominski and Campbell 1990), unlike feedstuffs of animal origin (Parsons 1986), have shown that there is little intestinal bacterial effect on AA bioavailability. Other areas of concern have been the unknown contribution of microbial AA in the excreta (Parsons 1986) and whether the metabolic fecal and endogenous urinary AA contribution to the excreta is the same for fasted controls as it is for birds fed the test ingredients (McNab 1979a). Although there were general decreases in true amino acid availability [g (16 g $N)^{-1}$] of EAAs with increased heating, the differences were not significant ($P>0.05$). In general, CM 60 had relatively lower true availability (86-96%) levels (table 2.2) of EAA than Bi protein supplement which may be due to the presence of fish and meat meals in Bi, since effects of intestinal bacteria of cockerels on animal protein is relatively low (Parsons 1986). The content of EAA was similar

between the 60 min heat treated CM and Bi supplement suggesting that calculated values of the Bi supplement were provided quantities of AA close to those of CM 60.

Increased heating time tended to reduce bioavailability of all NEAA and glutamic acid and serine showed ($P<0.05$) decreases with increased heating beyond 45 min (table 2.2). Results from other studies measuring protein denaturation of rapeseed meal following heat treatments suggested that gentle heating may increase (Goh et al. 1979) AA bioavailability while severe heating may decrease (Sibbald 1980) true AA bioavailability (Undi et al. 1992). Data from the in situ study (manuscript I) indicated that heat treatment for 45 and 60 significantly ($P<0.05$) increased the disappearance of all EAA in the lower GI tract (table 1.16 and figure 1.10) and 60 min heating increased ($P<0.05$) fecal excretion with 12 h rumen incubation. The 60 min heat treatment of CM increased ($P<0.05$) fecal excretion of arginine, histidine, isoleucine and threonine (table 1.18). Fecal excretion of EAA was higher ($p<0.05$) from the Bi supplement compared to CM 60 except for lysine and threonine.

The true AA digestibility values as measured with cockerels ranged from 52 for lysine in CM 60 to 90% for methionine in CM 0 (table 2.3.). The digestibility result for methionine was in general agreement with results of Zuprizal et al. (1991a) who reported methionine as the most available AA (93.5 to 95.6%) in commercial CM. Similar results were reported by Salmon (1994). The digestibility of cystine and lysine is usually compared in rooster studies because they tend to have the lowest availability (Zuprizal et al. 1991b). Of the essential AA lysine was one of the least digestible for CM 60 and Bi. With 60 min heating lysine digestibility was decreased ($P<0.05$) by 25%, relative

to CM 0. Zuprizal et al. (1991b) reported true digestibilities of lysine in whole rapeseed meal of 76.9%, and 81.4% in dehulled rapeseed meal. Lysine digestibility values in this study were 70% for CM 0, 52.4% for CM 60 and 54.9% for Bi protein (table 2.3). The difference between our results for CM 0 and those of Zuprizal et al. (1991a) may be attributed to moistened feed that was fed to birds in their experiment as opposed to ours, in which birds were fed CM as received from the source. Lysine is sensitive to thermal processing and its availability can vary with treatment (Zuprizal et al. 1991b). Miller (1967) showed that a chick bioassay has a low precision for measuring lysine availability, but is still the standard method to which all other technologies must be compared. The dry-force feeding technique of Sibbald (1976) has been shown (Wehner and Harrold 1982) to produce more stress, as shown by the post-feeding behaviour, than the moist-feeding (slurry) method.

True digestibility values of cystine were 72.1, 64.3 and 65.6% for CM 0, CM 60 and Bi supplement, respectively (table 2.3). Zuprizal et al. (1991a) reported true cystine digestibilities of 73.4, 78.1 and 80.5% for whole rapeseed meal, dehulled rapeseed meal and SBM respectively. The value recorded in this study (table 2.3) for CM 0 of 72.1% was similar to the above with a decrease with the longer heating times. In situ data with cows (Manuscript I) suggested that increased heat treatment increased the disappearance of cystine in the lower GI tract (table 1.16) but fecal excretion was higher ($p<0.05$) for lysine and not cystine.

Bioavailabilities of AA in heat treated CM for poultry and ruminants may not be directly comparable, but in general the lower GI tract of a ruminant may be emulated by the digestive tract of cockerels. In this rooster study, heat treatment decreased ($P<0.05$) the bioavailability of all EAA except methionine (table 2.2). "True digestibility" of EAA that escaped rumen degradation (table 2.4) as measured by the mobile nylon bags were higher ($P<0.05$) for most AAs in CM 60 than in CM that had milder heating. True digestibility of EAA in Bi supplement was comparable, in most cases, to those of CM 60 (table 2.4). True digestibility for lysine (table 2.3) was lower ($P<0.05$) than some previous results recorded with cockerels (Zuprizal et al. 1991b) but since its availability is known to vary with treatment (Wenhner and Harrod 1982) it was possible to assume that heat treatment during commercial processing may have affected its digestibility by the cockerels but increased heat treatment increased rumen escape of lysine and increased quantity available in the lower GI tract. In this cockerel study, heat treatment decreased AA bioavailability of some the AAs that are sensitive to thermal changes such as lysine and cystine.

CONCLUSIONS

This study was expected to show clear energy differences between treatments due to increased heating which chemically had indicated increased NDF levels. However there were no differences. It is suggested that higher treatment temperatures be used in future. Comparison of cockerels' digestive behaviour and that of ruminants may not be accurate due different lengths of time the feed spends in each digestive system. It is suggested that samples from rumen digesta from "mobile bags" incubated *in situ* in cows

be precision-fed to cockerels in order to arrive, hopefully, at a close "true digestibility" of the desired nutrients from "feed" presented to the lower gastro intestinal tract of the ruminant.

Table 2.1. True nitrogen-corrected metabolizable energy (TMEn) values and chemical composition of unheated and heat treated canola meal and of a bypass protein supplement (Bi) from the precision-fed cockerel trial.

Item	Heat treatment, min at 110°C				Bi	SE[U]
	0	23	45	60		
Dry matter %	97.50	96.83	95.05	94.67	96.67	0.73
Total N %	6.29	6.19	6.23	6.33	6.15	0.18
ADF (%)[V]	16.77	16.37	16.12	17.50	16.87	1.02
NDF (%)[W]	22.60b	22.68b	22.42b	26.02a	26.81a	0.88
N composition (% of total N)						
ADIN[X]	4.39d	14.03c	17.65b	22.97a	22.50a	0.59
NDIN[Y]	7.10d	35.94c	37.49c	58.22b	63.92a	0.77
TME_n(MJ kg^{-1})	9.66	9.49	9.47	9.46	9.82	1.01

[U] Standard error of Lsmeans (n=3).
[V] Acid detergent fibre.
[W] Neutral detergent fibre.
[X] Acid detergent insoluble nitrogen.
[Y] Neutral detergent insoluble nitrogen.
a-d Lsmeans in each row with different letters differ ($P<0.05$).

Table 2.2. True amino acid (AA) availability values [g(16g N)$^{-1}$] of unheated CM(0) and heat treated canola meal and of a bypass (Bi) protein supplement (on DM-basis) as assayed in precision-fed cockerels.

	Heat treatment, min at 110°C					
	0	23	45	60	Bi[U]	SE[W]
Dry matter	97.50	96.83	95.05	94.67	96.67	0.73
Essential AA,						
Arginine	5.07	4.43	4.18	3.30	3.44	0.37
Histidine	2.43	2.19	2.81	1.65	1.87	0.43
Isoleucine	3.09	2.24	1.87	1.85	2.09	0.31
Leucine	6.93	5.61	5.04	5.11	5.55	0.97
Lysine	4.75	3.58	2.50	2.42	2.70	0.38
Methionine	0.68	0.37	0.48	1.13	1.18	0.18
Phenylalanine	3.33	2.91	2.75	2.79	2.95	0.27
Threonine	4.78	4.12	2.88	2.60	2.78	0.19
Valine	3.09	2.72	2.35	2.40	2.54	0.14
Non essential AA						
Alanine	4.16	3.78	3.51	3.31	3.15	0.29
Aspartic acid	6.88	5.44	5.82	5.57	5.88	0.15
Cystine	1.25	0.93	0.66	0.57	0.99	0.12
Glutamic acid	17.88a	16.33a	14.41b	13.90b	14.43b	0.98
Glycine	4.54	4.05	3.68	3.01	3.98	0.25
Proline	5.08	4.47	4.49	4.57	4.68	0.23
Serine	5.89a	4.84a	3.35b	2.90b	3.09b	0.22
Tyrosine	2.67	2.28	2.18	1.69	1.92	0.23

[U] By-pass protein supplement.

[W] Standard error of Lsmeans (n=3).
a-b, Lsmeans in each row with different letters differ ($P<0.05$).

Table 2.3. True amino acid (AA) digestibility values (% as fed) of unheated and heat treated canola meals and of a bypass protein (Bi) supplement in precision-fed cockerels.

	Heat treatment, min at 110°C					
	0	23	45	60	Bi[U]	SE[W]
Dry matter	97.50	96.83	95.05	94.67	96.67	0.73
Essential AA,						
Arginine	78.07a	77.80a	71.99b	73.99b	74.87b	0.14
Histidine	80.90a	79.64a	81.21a	74.66b	80.67a	0.87
Isoleucine	75.57a	72.73a	70.03b	67.94c	65.14c	1.02
Leucine	81.89a	80.37a	79.62a	77.31b	79.47a	0.41
Lysine	69.88a	64.16b	55.77c	52.38d	54.98c	0.97
Methionine	89.90a	88.94a	84.86b	80.75c	80.84c	0.43
Phenylalanine	65.45a	61.78b	61.94b	60.28b	62.48b	0.17
Threonine	80.90a	74.10b	61.02d	64.36c	63.78c	0.87
Valine	83.42a	79.47b	79.12b	65.57c	66.89c	1.21
Non essential AA,						
Alanine	77.89a	76.36a	76.47a	71.79b	70.80b	0.98
Aspartic acid	81.14a	72.62b	71.06b	70.87b	72.14b	0.19
Cystine	72.14a	69.92b	68.04b	64.29c	65.57c	0.88
Glutamic acid	86.88a	84.88b	83.39b	82.10b	83.37b	0.67
Glycine	74.45a	72.19b	70.91c	64.45d	65.54d	1.04
Proline	70.14a	66.92c	69.29b	69.03b	71.44a	0.74
Serine	78.11a	71.11b	69.36b	65.63c	63.67d	0.81
Tyrosine	71.45a	70.15a	69.87a	63.77b	65.88b	1.07

[U] By-pass protein supplement.
[W] Standard error of Lsmeans (n=3).
a-d Lsmeans in each row within each amino acid with different letters differ ($P<0.05$).

Table 2.4 "True digestibility" of amino acids that escaped rumen degradation[U] as measured by the mobile nylon bag.

	Heat treatment (Minutes at 110°C)					
	0	23	45	60	Bi	SE[W]
Amino acid						
Arginine	89.22b	89.59b	87.95b	93.81a	80.01c	1.13
Histidine	87.65b	91.17a	89.33b	92.84a	87.16	1.32
Isoleucine	84.51c	88.43b	85.87bc	90.82a	84.84c	1.29
Leucine	87.86b	90.31a	88.04b	92.46a	82.76c	1.19
Lysine	75.20c	88.26a	83.72b	89.17a	84.18b	1.25
Methionine	85.81b	93.75a	79.88d	92.63a	83.30c	1.07
Phenylalanine	83.36b	87.20a	84.08b	89.43a	83.80b	1.23
Threonine	81.23b	85.56a	82.53b	86.42a	82.29b	1.14
Valine	79.06c	84.91b	81.71c	89.60a	84.53b	1.95

[U] Data from in situ study (manuscript I).
[W] Standard error of Lsmeans (n=3).
a-c, Lsmeans in a row with different letters differ (P<0.05)

MANUSCRIPT III

HEAT TREATED CANOLA MEAL AS A SOURCE OF RUMEN ESCAPE PROTEIN FOR LACTATING DAIRY COWS.

ABSTRACT

This study was conducted in an effort to decrease rumen degradation of canola meal (CM) through moist heat treatment. Eighteen multiparous (MP) and fourteen primiparous (PP) lactating Holstein cows two weeks post-partum were used in a split-plot design for 12 weeks. Heated canola meal (CM 60) or a bypass protein mixture (Bi) substituted unheated CM (CM 0) in the rations containing corn silage (20%), alfalfa silage (10%), long hay (13%) and barley concentrate (57%). The Bi was formulated with vegetable and animal rumen escape ingredients to provide equal calculated rumen escape of essential AA to that of CM 60. The purpose was to determine the effect of change in rumen degradability of protein on milk production. Diets, formulated to be isonitrogenous and isocaloric, were fed once daily as TMR. Records were taken on DMI, milk production and composition, rumen fluid parameters and blood urea-N concentration. Dry matter intake of CM 0 and CM 60 diets by PP and MP cows was higher ($P<0.05$) than that of the Bi diet. Milk yield by PP cows was lower ($P<0.05$) for cows receiving Bi than CM 0 which was lower ($P<0.05$) than those receiving CM 60. Milk yield by MP cows was higher ($P<0.05$) for cows receiving the Bi diet rather than CM 0 or CM 60. Parity affected ($P<0.05$) milk yield with MP cows producing 34% more milk d^{-1} than PP cows. Treatment and parity did not ($P>0.05$) affect body weight and condition score or milk content of fat, protein and SNF over the experimental period. Rumen acetate:propionate ratio was higher ($P<0.05$) for cows receiving CM 60 than CM 0 or Bi at 0 and 4 h post-feeding. Blood urea-N was higher ($P<0.05$) for cows receiving CM 0 vs CM 60 or Bi diets. Rumen ammonia levels were

similar ($P<0.05$) for all diets. Total rumen molar VFA were higher for cows receiving diets containing CM 0 than CM 60 or Bi at 0, 4 and 6 but not at 2 h post-feeding. Heat treatment of CM to increase rumen escape protein as measured by 16 h in situ data resulted in no ($P>0.05$) effect on milk yield and DMI in comparision with unheated commercial CM. Though results indicated increased ($P<0.05$) rumen escape protein with decreased rumen degradation of heated CM 60, the milk yield and milk composition, particularly protein did not ($P>0.05$) change in this study. The Bi diet reduced DMI compared with the other diets but resulted in equal milk production.

Key words: Canola meal, rumen escape protein, heat treatment, milk yield.

INTRODUCTION

In early lactation, high producing dairy cows rely on both microbial protein and rumen undegraded feed protein digested in the lower gastro intestinal (GI) tract to satisfy their amino acid (AA) requirements. Research suggests that microbial protein synthesis in the rumen plus protein supplements that are rapidly degraded in the rumen may not satisfy the protein requirements. High producing dairy cows cannot, in some cases, meet their protein requirements from microbial protein alone (Rooke et al. 1983; ARC 1984; NRC 1988; Kennelly and Khorasani 1993). The NRC (1989) established requirements for both degradable intake protein (DIP) to meet microbial requirement in the rumen and undegradable intake protein (UIP) to meet bypass requirements. Feed resources used in dairy diets vary in degradability, and attempts have been made to formulate rations based on their degradability potential. The NRC (1989) suggests that about 37% of protein should be undegradable. Barley, canola meal and alfalfa silage diets may contain about 26% UIP. Researchers have attempted to increase proportions of feed protein that can escape rumen degradation with minimum disturbance to the microbial protein yield.

Canola meal which contains up to 40% crude protein (CP) on a dry matter (DM) basis, has been reported to contain largely rumen degradable nitrogen, N (Ha and Kennelly 1984; de Boer et al. 1987) and essential AA (Kendall et al. 1991; Boila and Ingalls 1992). The value of CM as a source of essential AA for absorption in the lower GI tract could be improved by decreasing the amount of degradation taking place in the rumen. Previous research efforts to reduce rumen degradation of CM protein through treatment have included use of formaldehyde (Sharma and Ingalls 1974; Eyre and Rooke

1983; Rae et al. 1983; Rooke et al. 1983; Ha and Kennelly 1984). Though formaldehyde can reduce degradability of canola meal N, there have been concerns related to its use coupled with the difficulty of over protection. Moderate heat treatment (roasting, baking and steeping) offers an alternative method of reducing protein degradability in the rumen. The significant reduction in DM and N degradation of CM in the rumen with heat treatment in the autoclave has been demonstrated to provide a large increase in digestibility of N in the lower GI tract (Moshtaghi Nia and Ingalls 1992). The heating process has been suggested to cause a combined reduction in protein solubility, degradation and denaturation which subsequently increases rumen escape of intact undegraded proteins that are available in the lower GI tract (Van Soest 1982).

At the processing plant heat treatment is an integral part of the CM extraction process, however heating under these conditions has been kept at a minimum to maintain a high biological value of the meal for monogastric animals. Moist heat treatment at the plant level could be a simple method to improve the value of CM for high producing dairy cows and other ruminants that require additional rumen escape of essential AA.

The objectives of this study were as follows:

(a) To measure the effect of moist heat treatment of CM, as processed under conditions similar to that available in processing plants, on rumen degradability of DM and N, and on lactation response of cows.

(b) Secondly the study provides a comparision of the lactation response of cows fed heat treated CM with those fed a ration formulated with a combination of vegetable and

animal rumen escape protein sources having a similar calculated rumen escape essential amino acid profile.

MATERIALS AND METHODS

Feed samples and heat treatment

The CM samples used in the study were heat treated at the Protein Oil Starch (POS) pilot plant based in Saskatoon, Saskatchewan, Canada and shipped to Manitoba. Heat treatment involved passing CM through a steam jacketed conveyor set at the lowest speed with 5 % sparge steam addition (water added at 45 kg hr^{-1}) and at maximum steam pressure of 130 psi. The meal was then held on hot steeping trays at an average temperature of 110°C for different lengths of time. Three batches of CM samples were treated in this way with varying conveyor feeding and water addition rates. The CM samples for each treatment batch were fed into the steam jacket at 90 kg/hr (75 mL min^{-1} water, held on steeping trays for 60 min), 110 kg/hr (92 mL min^{-1} water, held on steeping trays for 45 min) and 200 kg/hr (167 mL min^{-1} water, held on steeping trays for 23 min) and were hence identified as CM 60, CM 45 and CM 23 respectively. The other batch of CM that was not heat treated (CM 0) was shipped together with treated samples. CM 0 and CM 60 were used in the lactation study while CM 45 and CM 23 were included in in the situ study (Manuscript I).

Animals and protocol

Thirty-two Holstein cows two weeks post-partum were used to determine effect of heat treatment on production parameters. The cows were allocated to diet such that five primiparous (PP) and six multiparous (MP) cows were assigned to each of CM 0 and

CM 60 and four PP and six MP cows were assigned to Bi. The third group consisted of four PP and six MP cows. The cows were assigned treatments in a split-plot design with repeated measurements over a 12-week period. Measurements were made of daily feed intake, daily milk production (recorded daily at both A.M and P.M milkings), concentrations of rumen ammonia (NH_3), rumen volatile fatty acids (VFA) and plasma urea-N levels and on body weight and condition score over the experimental period.

The rations were formulated (table 1.1) with protein supplements made up of unheated canola meal (CM 0), heat treated canola meal (CM 60) and vegetable/animal product mixture (Bi). The Bi protein supplement was calculated to provide a similar quantity of essential AA at the small intestine as CM 60. The nutrient composition of roughages, protein supplements and grain mixes used for total mixed ration (TMR) formulation are presented in appendix tables III.1, III.2, III.3, respectively. Rations were formulated to be iso-nitrogenous (18% CP) and iso-caloric (1.7 Mcal/kg, NE_L) with similar ADF (18%), NDF (33%) and soluble carbohydrate (37%) levels. Roughage consisted of 20% corn silage, 10% alfalfa silage and 13% long hay on DM-basis . Diets CM 0, CM 60 and Bi were formulated to meet the requirements for 45.0 L of 3.5% milk and provided calculated 29, 38 and 39 % of UIP respectively. The diets were fed once daily on an ad libitum basis as a TMR. Feed intake and milk production measurements (recorded at both A.M and P.M milkings) were taken daily while weight changes and condition scores were taken every two weeks.

Rumen fluid samples were sampled from cows on weeks 9, 10, 11 and 12 of the trial using an oesophageal tube (Ingalls et al. 1980). These samples were taken at 0, 2, 4 and

6 hours post feeding and the rumen fluid pH recorded at the time of sampling using a model 598S-50 pH metre from the Cole-Parmer Instrument Company. Only one rumen sample was taken from a given cow on any one day time post feeding as randomized over the four week data collection period.

Heparinized tail blood samples were drawn from the cows at similar times as for rumen samples. Both sets of samples were centrifuged at 10,000 x g for 10 minutes and extracted supernatants stored at -20°C for later analysis.

Samples and chemical analyses

Dry matter contents of concentrates, corn and grass silages were determined weekly and adjustments made accordingly for the TMR mixture. All samples were dried for 3 days in a forced air oven at 60°C, ground and saved for further analysis. Feed samples were analyzed for N-content by Micro-Kjeldahl analyzer (Tecator 1030 analyzer). Acid detergent fibre (ADF) and neutral detergent fibre (NDF) were analyzed by the procedure of Goering and Van Soest (1970). Acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) analyses were performed on ADF and NDF residues using micro-Kjeldahl method 47.023. Nutrient composition of the total mixed rations (DM-basis) are given in table 1.2.

Weekly milk samples from an A.M and P.M milking were analyzed for percentage of fat, protein and solids-not-fat (SNF) at Manitoba Milk Recording Corporation using a Foss MS300 infra-red Spectroscopy Analyzer (Milk-O-Scan Model 203B Type 17920, Fosselectric Cornwall, Ontario).

Rumen fluid samples were sampled from cows on weeks 9, 10, 11 and 12 of the trial using an oesophageal tube (Ingalls et al. 1980). These samples were taken at 0, 2, 4 and 6 hours post feeding and the rumen fluid pH recorded at the time of sampling using a model 598S-50 pH metre from the Cole-Parmer Instrument Company. Only one rumen sample was taken from a given cow on any one day time post feeding was randomized over the four week data collection period.

Volatile fatty acids (VFA) in the rumen fluid were determined by gas chromatography as suggested by Erwin at al. (1961) and rumen NH_3 was determined by an ammonia electrode (Model 95-10, Orion Research, Cambridge, MA). Plasma Urea-N was determined using a diagnostic quantitative colorimetric method using procedure No. 535 (Sigma Chemical Company, St. Louis, MO, 1990).

Statistical analyses

The trial was analyzed as a split plot design with repeated measurements for feed intake, milk production and composition. The model contained the factors: Calving season as a block (calblk), dietary treatment, parity of the cow, week on trial, and a two-way interaction of diets with parity. The trial was divided into "calving seasons": Jan/Feb (calblk 1), Mar (calblk 2), Apr/May/Jun (calblk 3) and July/Aug (calblk 4). Analysis for this part of the trial was completed using the General Linear Model (GLM) procedure (SAS Institute Inc. 1989) using the model:

$$Y_{ijklmn} = \mu + T_i + P_j + B_k + (TP)_{ij} + C\,(PTB)_{ijkl} + W_m + (WT)_{im} + WTP_{ijm} + AE_n + (AET)_{in} + e_{ijklmn}$$

Where:

Y = Dependent variable,

μ = The overall mean,

T = Dietary treatment,

P = Parity,

TP = Treatment by parity interaction,

B = Calblk, calving block

C (TPB) = Cow within treatment, parity and block,

W = Week of lactation by treatment,

WT = Week of lactation by treatment interaction,

WTP = Week of lactation by treatment by parity interaction,

AE = a.m and p.m milk sampling,

AET = a.m and p.m milk sampling by treatment interaction, and

e = error.

Test of hypothesis included treatments, parity, calving block .

Cow within treatment, parity and block was used as the error term for testing effects of dietary treatment, parity and calving block.

Statistical difference between dietary treatment were tested using Newman- Keuls test (Snedecor and Cochran 1980).

Data for rumen pH, VFA, NH_3 and plasma urea-N were analyzed as a completely randomized design. The analys model contained dietary treatment, parity and a two-way interaction of dietary treatment and parity. The samples for these parameters were analyzed as duplicate observations.

RESULTS AND DISCUSSION

Both the ingredient and nutrient composition (DM-basis) of the total mixed rations fed had similar energy, protein and ADF levels (tables 1.1 and 1.2). Dry matter intake (DMI) for treatment diets CM 0 and CM 60 at 20.9 and 20.1 kg d-*1*, respectively were higher ($P<0.05$) than for the Bi diet at 16.01 kg d-*1* (figure 3.1, table 3.1). Dry matter intake by PP cows was on average lower ($P<0.05$) than those of the MP cows (table 3.1). Parity affected DMI with PP cows (16.58 kg/d) consuming less feed per day compared to MP cows (21.41 kg/d) and this could be attributed to difference in rumen capacity. The DMI for CM 0 and CM 60 diets by PP cows was greater ($P<0.05$) than that of Bi. Intake by MP cows did not differ ($P>0.05$) among the diets. It is not clear why the average intake for Bi diet was less ($P<0.05$) than those of the CM diets with PP cows in particular, consuming 37.8% less of the Bi diet than MP cows (table 3.1). Dry matter intake for combined PP and MP cows (figure 3.1 and table 3.1) for Bi diet was 23.3 and 20.4 % less ($P<0.05$) than for CM 0 and CM 60 diets respectively. However, there was an interaction ($P<0.05$) between treatment and parity with the very low intake of Bi diet by the PP cows. Calving season did not affect ($P>0.05$) DMI in by both groups of cows.

The NDF level of feeds has been shown to be one major component associated with intake potential of feeds (Mertens 1985; Van Soest 1989). The level of NDF affects rumen fill and only when it is digested or passed out is its effect on rumen fill eliminated. In this study, percent NDF levels (table 1.3) for Bi supplement at 26.1 of

P.M was higher ($P<0.05$) than those for CM 0 and similar to CM 60 supplement at 25.1 and 26.7 respectively.

The NDIN content (table 1.2.) has also been noted to increase with increased heat treatment of CM (Lindberg et al. 1982; Moshtaghi-Nia and Ingalls 1992; McAllister et al. 1993) because less soluble and heat denatured protein become recoverable in the NDF fraction. In this present study 60 min heat treatment of CM increased the content of NDIN as a % of total N by 85.4% (table 1.2) for the CM 60 diet relative to the unheated CM 0 diet. The NDIN content of Bi diet was the highest of the three diets at 63.7% of total N (table 1.2). The relatively low DMI of Bi diet may possibly have been influenced by higher NDIN contents compared to the two CM diets (table 1.2). With the relatively high rumen NH_3 levels (table 3.5) for all treatments, rumen available NH_3 for bacteria should have been adequate.

Heat treatment increased ($P<0.05$) the ADIN content of CM 60 diet by about 4 percentage points above the CM 0 diet. The heat treatment was not severe enough to cause an ($P>0.05$) increase in ADF (table 1.3). Heat treated CM 60 diet was 2.5 percentage points below ($P<0.05$) the Bi diet. These results were in agreement with those of other workers (Plegge et al. 1985; Pena et al. 1986; Ahmadi 1988; Arieli et al. 1989; Moshtaghi-Nia and Ingalls 1991; McAllister et al. 1993) which suggested that heat treatment of CM resulted in increased ADIN contents. In general the increase in ADIN content in general suggests an increased resistance of CM protein to rumen microbial degradation as proposed by Van Soest (1982), through denaturation and formation of primarily Maillard products. This resistance to microbial degradation due to increased

ADIN contents has also been associated with formation of intestinally indigestible terminal Maillard products and condensation of free AA ends with the carbohydrate moiety, the fraction which is recoverable in ADF fraction (Van Soest 1982; McAllister et al. 1993). The results of the present study suggest that even though there exists a negative relationship between increased ADIN content and reduced rumen degradation of protein as would be expected for heat treated CM 60 diet, intake *per se* did not differ ($P>0.05$) between CM 60 and the CM 0 diet (table 3.1).

In situ DM and N degradation measurements (appendix figure I.1.) on CM 60 indicated a 68 % increase of rumen escape CM protein at 16 hours of incubation. Though moist heat treatment of CM ($P<0.05$) reduced DM and N rumen degradability there was no ($P>0.05$) difference in DMI between CM 0 and CM 60 diets. This could be due to a similarity in the microbial fermentation pattern.

There was no significant ($p>0.05$) change in pH value as hours increased post feeding (table 3.5) but there were trends (figure 3.2) of higher pH values at the start of feeding (0 h) than later after feeding. Rumen pH levels ranged from 6.34 to 7.13. Contamination of samples with some saliva with the sampling method cannot be ruled out. Grumpelt (1897) and Mabon (1988) reported no effect on rumen pH with extruded canola seed diets. Murphy et al. (1984) and Kennelly (1983) noted increased rumen pH of 11.7% and 7.2%, respectively with addition of ground canola seed to diets. Rumen pH can affect protein degradation by altering microbial activity and changing protein solubility (Tamminga 1979).

Rumen ammonia levels were not influenced ($P>0.05$) by heat treatment (table 3.5). Tamminga et al. (1983) and Kowalcyzk et al. (1977) observed decreased levels of rumen ammonia when tallow was added to the cow diets, however Sharma et al. (1978) and Kennelly (1983) using 7.2% crushed canola meal, found no change in ammonia levels. Proteolysis and deamination is affected by pH and it has been suggested (Tamminga 1979) that the optimum pH for both proteolysis and deamination is between 6 and 7. In this trial, rumen ammonia concentration (table 3.5) did not differ significantly ($P>0.05$) among the diets and was generally lower at 0 h and increased by the 2nd h (figure 3.6) and dropped at 4 and 6 h post-feeding. Broderick (1992) reported higher plasma urea-N and rumen ammonia levels from fish meal diets compared with SBM diets. The results of this study suggest a possible lower rumen degradation of CM 60 and Bi diets than for CM 0 diet due to heat treatment which may have reduced deamination of protein substrate from CM 60 and Bi diets with time (figure 3.6). The rumen microbial populations require ammonia for efficient synthesis of microbial AAs and microbial protein. Low levels of ammonia in the rumen fluid reactions that fix ammonia into microbial AA require ATP, whereas, when low levels are above a certain optimum, the ammonia is incorporated into microbial AA without an ATP requirement (Preston and Leng 1987). In the present study, the level of ammonia seemed to indicate less ruminal degradability of CM 60 and Bi diets, compared to the CM 0 diet which had relatively higher rumen degradable protein.

Plasma urea-N (table 3.5) levels were higher ($P<0.05$) in CM 0 diet than CM 60 or Bi. After 2 h post-feeding plasma urea-N declined ($P<0.05$) in CM 0 diet only

(figure 3.6) but increased in CM 60 and Bi at 4 h and dropped for the same diets at 6 h post-feeding. Zimmerman et al. (1991) recorded high blood urea-N concentration as a result of rumen protected protein meals and they suggested that this was an indication of less available protein for rumen degradation with subsequent increased amounts for digestion and absorption in the lower GI tract.

Assuming random dilution of rumen fluid with saliva contamination, concentrations (mmol L^{-1}) of rumen fluid acetate (table 3.4) for the CM 0 diet were higher ($P<0.05$) than those of CM 60 and Bi diets at 0 and 4 h and not different ($P<0.05$) at 2 and 6 h post feeding. Acetate concentration for cows receiving CM 0 and CM 60 diets were higher ($P<0.05$) than that of Bi diet at all the sampling hours (table 3.4). Molar percentage (molar %) of acetate (table 3.3) for CM 0 and bypass diets were lower ($P<0.05$) than that of CM 60 at 0 and 4 h but were not different ($P>0.05$) at 2 and 6 post-feeding (figure 3.3).

Propionate concentration (mmol L^{-1}) was higher ($P<0.05$) in CM 0 diet (table 3.4) than in other diets at all the sampling except at 2 h when it was similar ($P<0.05$) to CM 60 diet. Molar % value for propionate (table 3.3) was significantly higher ($P<0.05$) in CM 0 diet than CM 60 and Bi protein diets at 0 and 4 h, but the latter two diets did not differ ($P>0.05$) at 4 h post feeding (figure 3.3). Broderick (1992) noted that feeding 60% fish meal a less degradable protein vs 37% SBM to lactating cows lowered rumen propionate.

Rumen iso-butyrate (table 3.4.) concentration and molar % value of iso-butyrate (table 3.3, table 3.4 and figure 3.4) did not differ ($P>0.05$) among diets. Butyrate

concentration in CM 0 diet was higher ($P<0.05$) than in CM 60 or Bi diets only at 4 h post-feeding. Molar % of butyrate for CM 0 and Bi diets was higher ($P<0.05$) than that of CM 60 at 2 h post-feeding (table 3.3 and figure 3.4) while at 6 h the level was higher for cows receiving the Bi diet. Concentrations of iso-valerate and valerate (table 3.4) and their molar % did not differ ($P>0.05$) among the diets for all the sampling hours post-feeding (figure 3.5). The levels of these branch-chained VFA (iso-butyrate and iso-valerate) are generally expected to increase in rumen fluid with more degradable dietary true protein in diets (Zimmerman et al. 1992). Although not significant ($P>0.05$), our results with CM 0 and CM 60 follow this trend. Isobutyrate and isovalerate are in general degradation products of AAs, valine and leucine respectively (Zimmerman et al. 1992) and may have been a reflection in the reduced concentrations of these AAs (table 1.15) measured in our diets. The present results are supported by the results of Zimmerman et al. (1991) who reported similar levels of isobutyrate and isovalerate from diets containing protected vs unprotected SBM fed to early lactation dairy cows.

Total VFA (table 3.5) was higher ($P<0.05$) in the CM 0 diet than CM 60 or Bi diets for all the sampling times except at 2 h post feeding when CM 0 diet had similar value to that of CM 60 diet. These results suggest relatively less rumen degradation of CM 60 and Bi protein diets than CM 0 diet, with subsequent increased potential availability of the protein substrate of CM 60 and Bi diets in the lower GI tract. Acetate:propionate (A:P) ratio was lower ($P<0.05$) in CM 0 and Bi diets than in CM 60 diet at 0 and 4 h post feeding (table 3.5 and figure 3.2). There was no difference

($P > 0.05$) in the A:P ratio for across diets at sampling times 2 and 6 h post feeding. The A:P ratio has been used as one way to determine (Klopfenstein et al. 1981) loss of energy in the rumen during fermentation and these authors suggested that A:P ratios of rumen VFA of 2.6 to 3.2 were normal. The A:P ratio in the CM 0 and Bi diets in this study were within this range but not with the CM 60 diet at 4 h post-feeding (table 3.5) which was above the suggested maximum value. The high A:P ratio in CM 60 diet is supported by the results of Borderick (1992) who reported high A:P ratio with fish meal diets (69%) vs SBM (35%) fed to lactating cows. Zimmerman et al. (1992) also suggested that increased A:P ratio in the rumen fluid is an indication of increased levels of rumen undegradable protein, which in our case may have been demonstrated more by CM 60 diet, relative to CM 0 or Bi although in situ degradation rate tends to be similar for Bi and CM 60.

The milk yields recorded in this study were average for the general herd at the time of the study. Previous results (manuscript I) show a 68% decrease in rumen degradation of CM protein and a 58% increase in lysine available in the lower GI tract (table 1.19) with increases of 63 to 79% for the other essential AA with 60 min heat treatment, the improvement in milk production over CM 0 was not large ($p > 0.05$) but was consistent, suggesting a modest AA deficiency in CM 0 diet relative to CM 60 (table 3.2). The slight increase in milk protein concentration (table 3.2) with the CM 60 diet which provided a calculated 38% UIP compared with 29% for the CM 0, is indicative of more efficient utilization of protein in CM 60 diet than CM 0 diet. These results agree with those of Zimmerman et al. (1992) which suggested that increasing feed protein level

from 14.3 to 18.7% CP by enhancing SBM with rumen undegradable protein did not affect statistically the milk yield or milk protein of MP cows. Broderick (1992) however noted that feeding fish meal diets which provided 60% UIP versus SBM providing 35% UIP statistically increased milk yield and milk protein content.

Milk production and milk protein response from the present study seemed low despite increased rumen escape protein as indicated by in situ data (manuscript I). Other reports (Wohlt et al. 1991) in the past have obtained greater improvement in milk production but not milk protein from feeding rumen escape protein supplements such as fish meal compared to SBM or corn gluten meal to cows receiving 50% corn silage. Results by Khorasani et al. (1992) similarly detected no significant increase in milk yield resulting from acid treatment of CM despite decreased degradation potential for rumen escape CM protein. A review of published reports spanning 1984-1994 on increased dietary undegradable protein indicted very few papers that showed a response in milk production (Santos and Huber 1995).

In this study, there was a non-significant ($p > 0.05$) increase in milk yield of 0.6 and 0.9 kg/d for heated CM 60 and Bi diets respectively. This suggested a response to reduced rumen degradation of escape protein for these protein supplements compared to the untreated CM 0 diet. *In situ* results indicated reduced rumen degradation of CM protein, however, this increased ruminal escape of CM protein did not change the concentration of milk fat, protein and SNF (figure 3.2.). In studies conducted by Broderick (1992), increasing fish meal from 2.9 to 6.4% of DM in diets containing 56 to 87% alfalfa silage did not result in any change in milk fat content. Results from this

study seem to agree with those of Khorasani et al. (1992) who observed no change in milk constituents from treated CM with high potential for rumen escape protein. There was no ($P>0.05$) change in milk constituents as a result of parity or calving season.

The body weight and condition score (table 3.1) did not ($P>0.05$) change over the experimental period. Broderick (1992) recorded no change in body weight and condition score with early lactating cows fed fish meal verses SBM.

CONCLUSION

In situ data for diets used in this study suggested decreased degradation of rumen escape protein and AAs for heat treated CM 60 and the vegetable/animal protein supplements (bypass protein). These data indicate an increased potential for rumen escape of dietary protein and EAA available for lower GI tract digestion. The bypass protein supplement which included fish meal and meat meal resulted in lower intake, especially with the PP cows. The untreated CM diet and heat treated CM diet resulted in similar intake. Although there was increased potential for rumen escape of heat treated CM, the milk yield and milk composition were not changed. The bypass protein supplement diet reduced feed intake compared to other diets but resulted in equal milk production. There was no treatment nor parity effect on milk composition.

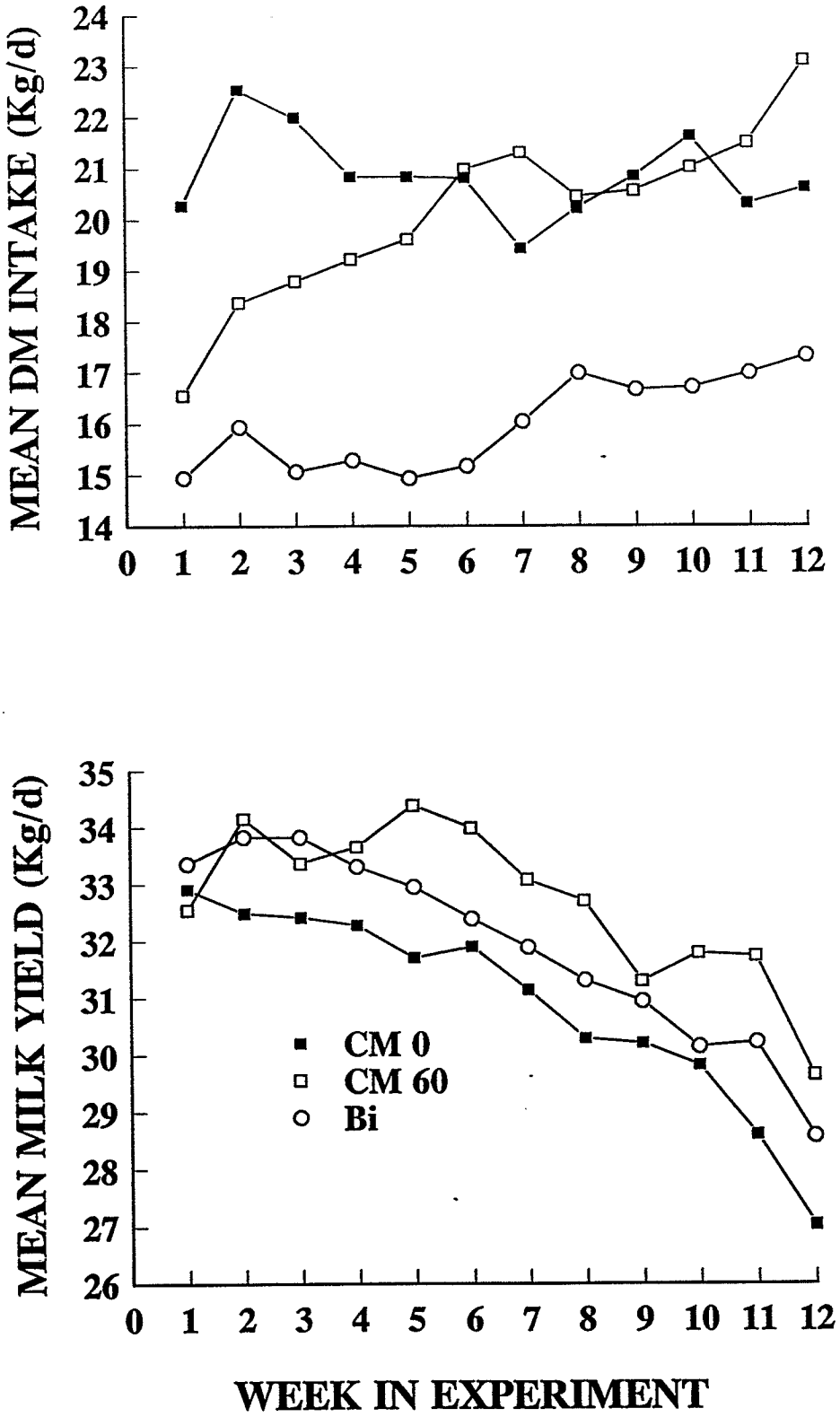


Figure 3.1. Dry matter (DM) intake and milk response by cows fed unheated canola meal (CM 0), heated canola meal (CM 60) and bypass protein supplement (Bi) in early lactation.

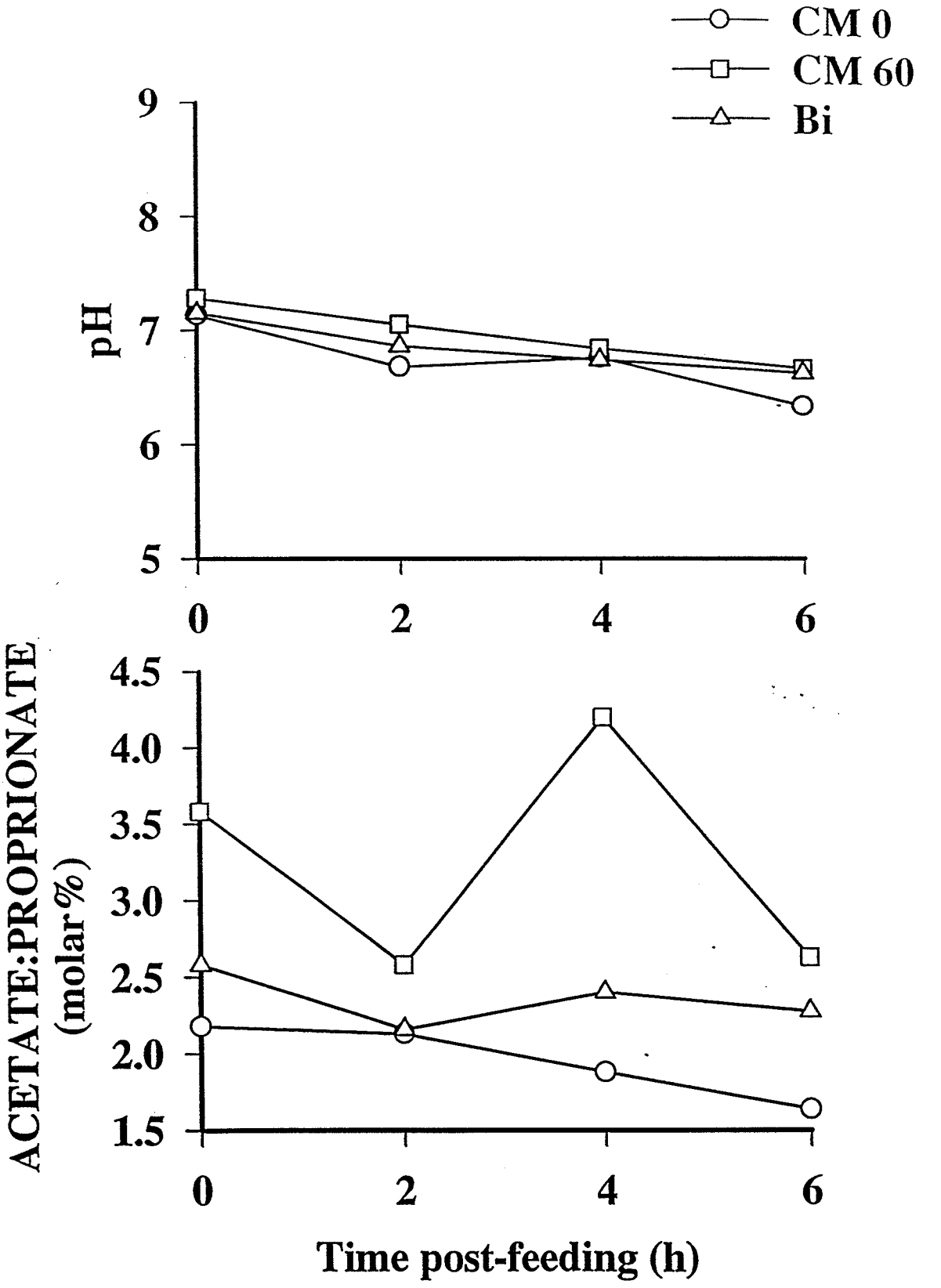


Fig 3.2. Effect of unheated CM O, heated CM 60 and bypass (Bi) protein diets on Lsmeans of pH and acetate: propionate ratio (molar%) for cows in early lactation.

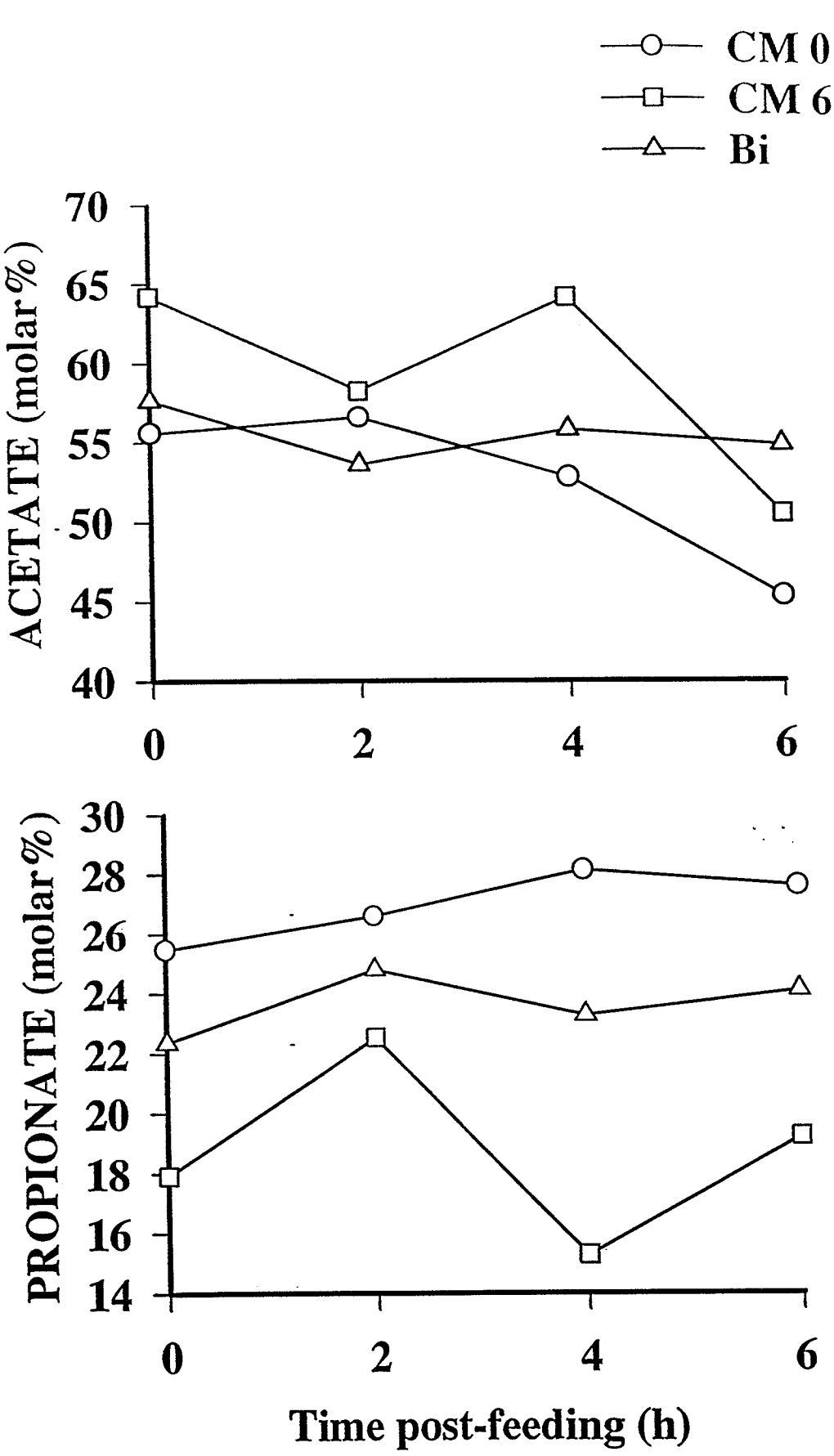


Fig 3.3. Effect of unheated CM O, heated CM 60 and bypass (Bi) protein diets on Lsmeans of acetate and propionate concentration for cows in early lactatio

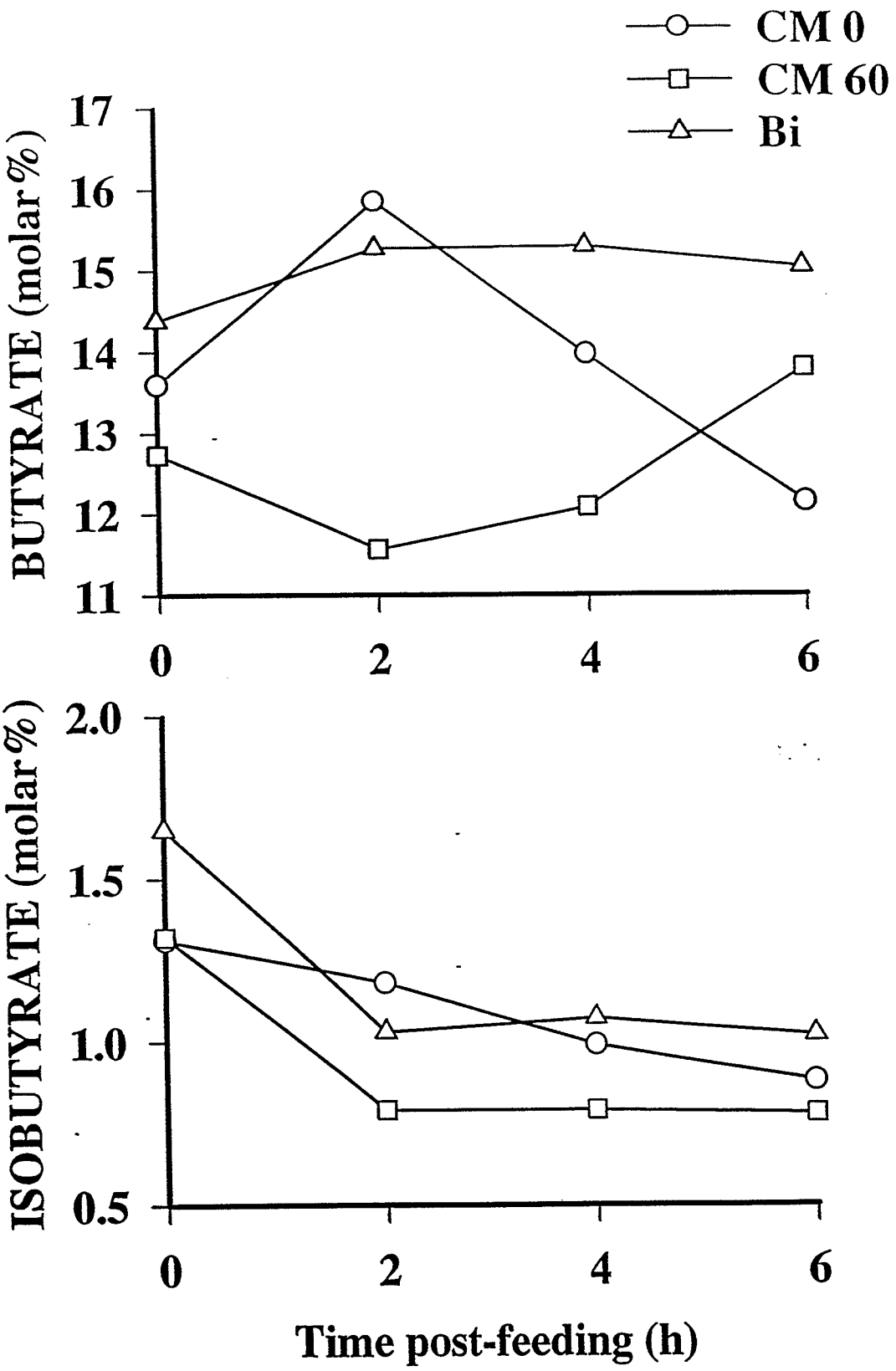


Fig 3.4. Effect of unheated CM O, heated CM 60 and bypass (Bi) protein diets on Lsmeans of butyrate and isobutyrate concentration for cows in early lactation.

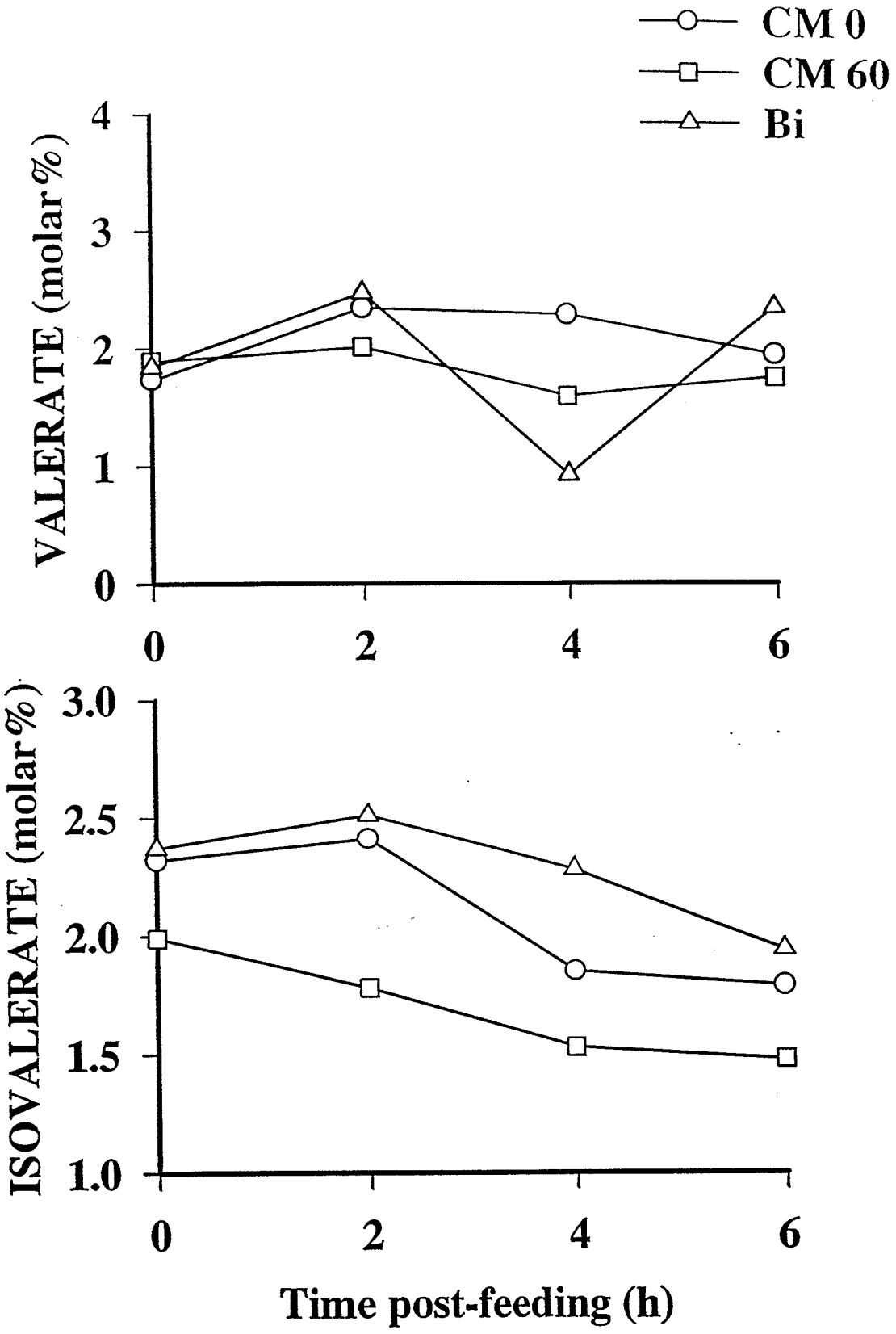


Fig 3.5. Effect of unheated CM O, heated CM 60 and bypass (Bi) protein diets on Lsmeans of valerate and isovalerate concentration for cows in early lactation.

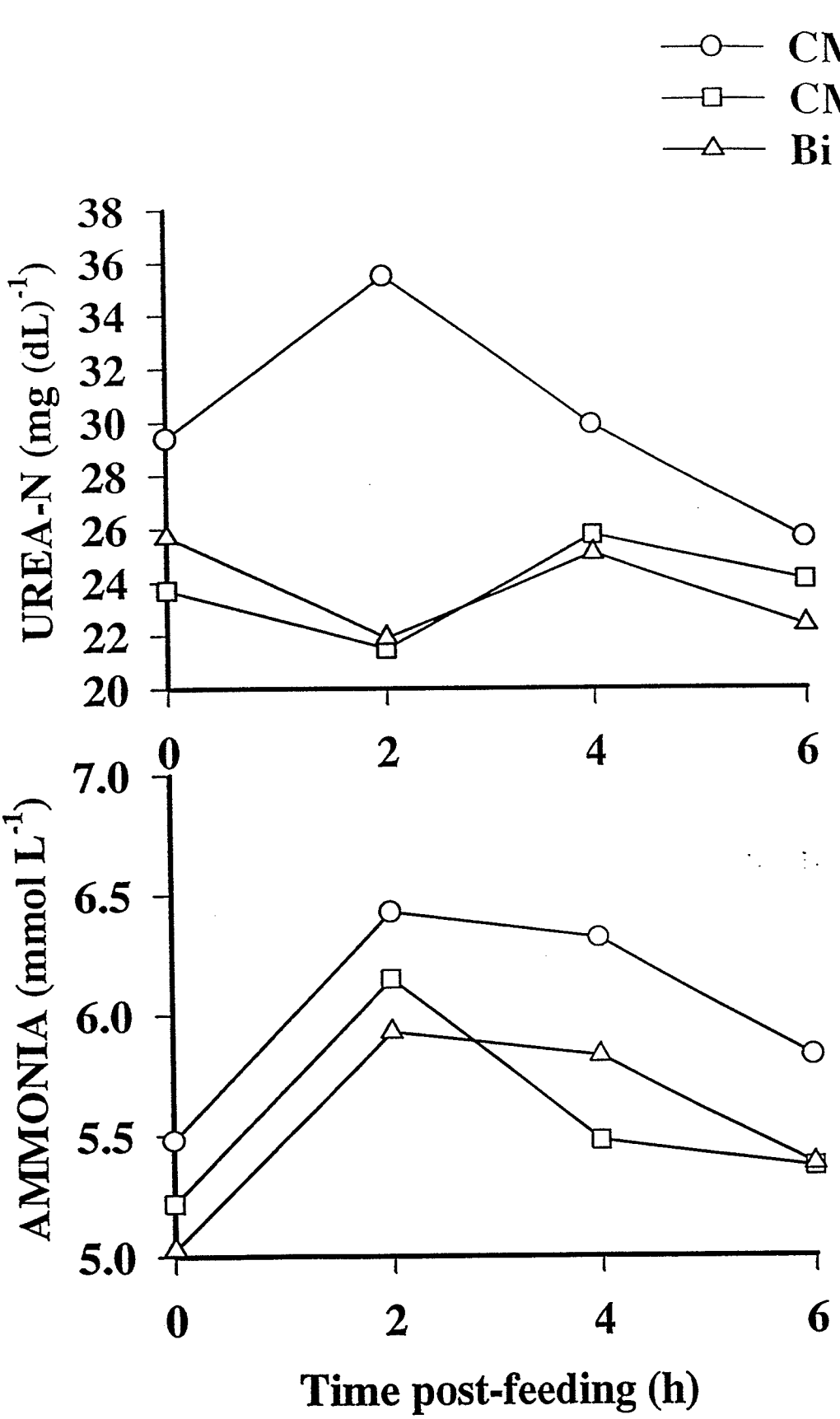


Fig 3.6. Effect of unheated CM O, heated CM 60 and bypass (Bi) protein diets on Lsmeans of plasma urea-N and rumen fluid ammonia concentration for cows in early lactation.

Table 3.1. Effect of unheated canola meal (CM 0), heated canola meal (CM 60) and of bypass (Bi) diets on dry matter (DM) intake, milk yield, mean experimental weight and condition score change for primiparous (PP) and multiparous (MP) cows in early lactation.

	Diet			
Parameter	CM 0	CM 60	Bi	SE[v]
DM intake, kg/d[w z]				
PP cows	19.12a	18.35a	12.28b	1.34
MP cows	22.61	21.90	19.73	1.22
Combined[x]	20.86a	20.12a	16.01b	0.96
Milk yield, kg/d[w z]				
PP cows	27.30b	29.25a	24.97c	2.70
MP cows	34.71b	36.02b	38.68a	2.46
Combined[x]	31.01	32.63	31.92	1.82
Body weight change, kg/84 d [z]				
PP cows	15.50	13.68	13.74	1.91
MP cows	11.36	9.61	12.92	1.20
Combined[x]	12.92	10.93	11.82	1.86
Condition score change, experimental Lsmeans[z]				
PP cows	0.26	0.25	0.23	0.01
MP cows	0.22	0.23	0.25	0.04
Combined[x]	0.24	0.28	0.23	0.09

a-c Lsmeans within rows with different letters differ ($P<0.05$).
[w] Designates a treatment by parity interaction effect ($P<0.05$).
[v] Standard error for Lsmeans based on 5, 5 and 4 PP cows, respectively; and on 6 MP cows per treatment. SE reported here are averaged across treatments.
[x] Lsmeans for both combined primiparous and multiparous data.
[z] Denotes difference ($P<0.05$) between primiparous and multiparous cows.

Table 3.2. Effect of unheated canola meal (CM 0), heated canola meal (CM 60) and of bypass (Bi) protein diets on milk composition for primiparous (PP) and multiparous (MP) cows in early lactation.

Parameter	Diets			SE[v]
	CM 0	CM 60	Bi	
Milk fat[w], %				
PP cows	3.20	3.46	3.67	0.24
MP cows	3.41	3.37	3.01	0.22
Combined[x]	3.30	3.42	3.34	0.16
Milk protein[w], %				
PP cows	2.98	3.08	2.90	0.07
MP cows	2.74	2.87	2.94	0.08
Combined[x]	2.91	2.93	2.92	0.05
Milk Protein yield, kg d^{-1}				
PP cows	0.81	0.90	0.92	0.02
MP cows	0.99	1.11	1.14	0.07
Combined[x]	0.91	0.95	0.93	0.05
Solid-Not-fat, %				
PP cows	8.81	8.74	8.67	0.16
MP cows	8.53	8.35	8.49	0.13
Combined[x]	8.67	8.54	8.58	0.11

[w] Designates a treatment by parity interaction effect ($P<0.05$).

[v] Standard Error for Lsmeans based on 5, 5 and 4 PP cows, respectively; and on 6 MP cows per treatment. SE reported here are averaged across treatments.

[x]Lsmeans for both combined primiparous and multiparous data.

Table 3.3. Effect of unheated (CM 0), heated (CM 60) canola meal and bypass (Bi) protein diets on VFA levels (molar%) in rumen fluid of dairy cows (combined Lsmeans for primiparous and multiparous cows) in early lactation.

Volatile fatty acid	Hours post-feeding	Diet			SE[w]
		CM 0	CM 60	Bi	
Acetate	0 h	55.57b	64.16a	57.63b	0.78
	2 h	56.56	58.21	53.60	0.30
	4 h	52.79b	64.10a	55.76b	0.88
	6 h	45.31	50.52	54.83	0.45
Propionate	0 h	25.46a	17.92c	22.36b	0.33
	2 h	26.58	22.52	24.78	0.21
	4 h	28.13c	15.28b	23.26b	0.67
	6 h	27.59	19.23	24.10	0.56
Isobutyrate	0 h	1.31	1.32	1.65	0.03
	2 h	1.18	0.79	1.03	0.01
	4 h	0.99	0.79	1.07	0.11
	6 h	0.88	0.78	1.02	0.23
Butyrate	0 h	13.59	12.73	14.38	0.34
	2 h	15.85a	11.56b	15.27a	0.34
	4 h	13.96	12.08	15.29	0.56
	6 h	12.14b	13.80b	15.04a	0.43
Isovalerate	0 h	2.32	1.99	2.37	0.24
	2 h	2.41	1.78	2.51	0.25
	4 h	1.85	1.53	2.28	0.05
	6 h	1.79	1.48	1.94	0.08
Valerate	0 h	1.73	1.89	1.82	0.04
	2 h	2.34	2.01	2.47	0.03
	4 h	2.28	1.59	0.92	0.06
	6 h	1.94	1.75	2.34	0.23

[w] Standard error of Lsmeans based on 5, 5, and 4 PP cows/treatment, respectively; and on 6 MP cows/treatment. SE reported are averaged across treatments.
a-b, Lsmeans within rows with different letters differ ($P<0.05$).

Table 3.4. Effect of unheated (CM 0), heated (CM 60) canola meal and bypass protein (Bi) diets on concentration (mmol L^{-1}) of volatile fatty acids (VFA) of dairy cows (combined Lsmeans for primiparous and multiparous cows) in early lactation.

VFA	Hours post-feeding	Diet			SE[w]
		CM 0	CM 60	Bi	
Acetate	0 h	33.04a	27.71b	20.59c	0.91
	2 h	34.58a	36.22a	23.45b	0.76
	4 h	39.11a	34.43b	22.94c	0.66
	6 h	32.91a	33.24a	23.18b	0.93
Propionate	0 h	15.14a	7.74b	7.99b	1.02
	2 h	16.25a	14.01a	10.84b	0.23
	4 h	20.84a	9.51b	9.57b	1.11
	6 h	20.04a	12.65b	10.19b	0.99
Isobutyrate	0 h	0.78	0.57	0.59	0.12
	2 h	0.72	0.49	0.45	0.21
	4 h	0.73	0.49	0.44	0.15
	6 h	0.64	0.45	0.43	0.14
Butyrate	0 h	8.08	5.50	5.14	0.97
	2 h	9.69	6.94	6.68	0.42
	4 h	10.34a	6.37b	6.29b	0.45
	6 h	8.82	9.08	6.36	0.22
Isovalerate	0 h	1.38	0.86	0.85	0.14
	2 h	1.47	1.11	1.10	0.10
	4 h	1.37	0.95	0.94	0.08
	6 h	1.16	0.85	0.82	0.02
Valerate	0 h	1.03	0.82	0.65	0.01
	2 h	1.43	1.25	1.08	0.09
	4 h	1.69	0.99	0.96	0.13
	6 h	1.41	1.15	0.99	0.15

a-c, Lsmeans within rows with different letters differ ($p<0.05$).

[w] Standard error of Lsmeans based on 5, 5, and 4 PP cows/treatment, respectively; and on 6 MP cows/treatment. SE reported are averaged across treatments.

Table 3.5. Effect of unheated canola meal (CM 0), heated canola meal (CM 60) and of bypass (Bi) protein diets on pH, concentration of rumen ammonia [mg(dL)$^{-1}$], total VFA (molar %), plasma urea-N [mg(dL)$^{-1}$] and on rumen fluid acetate:propionate ratio (molar %) of dairy cows (combined Lsmeans for primiparous and multiparous cows) in early lactation.

Parameter	Hours post-feeding	Diet			SE[W]
		CM 0	CM 60	Bi	
pH	0 h	7.13	7.28	7.15	0.11
	2 h	6.68	6.84	6.86	0.01
	4 h	6.76	7.05	6.74	0.19
	6 h	6.34	6.67	6.63	0.02
Ammonia	0 h	27.40	26.10	25.15	0.09
	2 h	32.15	30.75	29.65	0.99
	4 h	31.60	27.40	29.15	0.81
	6 h	29.15	26.85	26.90	0.13
Plasma urea-nitrogen	0 h	29.38a	23.70b	25.70b	0.24
	2 h	35.51a	21.52b	21.91b	0.42
	4 h	29.89a	25.76b	25.06b	0.15
	6 h	25.67a	24.09b	22.38b	0.52
Total VFA	0 h	59.45a	43.19b	35.81c	0.43
	2 h	61.14a	60.02a	43.60b	0.93
	4 h	74.08a	52.74b	41.14c	1.07
	6 h	64.98a	57.42b	41.97c	1.03
Acetate:Propionate	0 h	2.18b	3.58a	2.58b	0.44
	2 h	2.13	2.58	2.16	0.97
	4 h	1.88b	4.20a	2.40b	0.67
	6 h	1.64	2.63	2.28	0.78

[W] Standard error of Lsmeans based on 5, 5, and 4 PP cows/treatment, respectively; and on 6 MP cows/treatment. SE reported are averaged across treatments.
a-b, Lsmeans within rows with different letters differ ($P<0.05$).

GENERAL DISCUSSION

The contribution of dietary protein to the nutritional requirements of rapidly growing ruminants or high producing dairy cows in early lactation depend not only on AA composition but also on how efficiently the AAs are utilized by bacteria to synthesize protein and what is left for digestion in the lower GI tract. Research suggests that microbial protein synthesis in the rumen plus protein supplements that are rapidly degraded in the rumen may not satisfy the requirements. Feed resources used in cattle grower rations and dairy diets vary in rumen degradability of protein and subsequent availability of UIP and microbial protein in the lower GI tract of ruminants. NRC requirements for dairy cattle (1989) suggest that about 37% of protein provided should be undegradable. Barley, CM and alfalfa silage diets would be calculated to provide about 26% UIP.

Researchers have attempted to increase proportions of feed protein that can escape rumen degradation with minimum disturbance to the microbial protein yield. Previous studies conducted in our laboratory (Moshtaghi-Nia and Ingalls 1992) demonstrated that when commercially available CM is moist heated in the autoclave at different temperatures and for different lengths of time, there is a significant reduction in rumen degradation of DM, N and AAs, with subsequent increased availability and digestibility of these components in the lower GI tract. In the present study, commercial CM was moist heated at an experimental oil extraction pilot plant at a fixed temperature and steeped for various lengths in hopes of achieving results similar to laboratory results obtained by Moshtaghi-Nia and Ingalls (1992). The purpose was to increase the dietary

UIP of CM through moist heating with the objective of increasing milk yield. Measurements of the extent of reduced rumen degradation of the heated canola meals was done using three mature dry Holstein cows fitted with rumen and proximal duodenal T-shape canulae in the first trial, fifty Single Comb White Leghorn cockerels to measure available AA for monogastrics and thirty two lactating dairy cows in the third trial to measure milk response.

Heat treatment increases (Smith and Zebrowska 1989) the flow of protein to the lower digestive tract but excessive heating may lead to increased levels of ADIN in CM (Moshtaghi-Nia and Ingalls 1992) which was formerly assumed to be indigestible (Van Soest 1982). It has since been established that ADIN is partially digestible (Pena et al. 1986; Rogers et al. 1986; Arieli et al. 1989; Weiss et al. 1989; Moshtaghi-Nia and Ingalls 1992; Hussein et al. 1995) and that heat damage may be overestimated (Brittton et al. 1987; Weiss et al. 1989; Van Soest and Mason 1991) when ADIN is used as the only measure of protein damage. Heat treatment of CM in the current study resulted in significant increases in ADIN contents of CM 23, CM 45, CM 60 by 224, 290 and 386% respectively, relative to the CM 0. Increasing heat treatment from 23 to 45 min and from 45 to 60 min resulted in 20 and 25% increases for ADIN, respectively. The higher contents of heated CM 60 relative to CM 0 is thought to be a result of less soluble proteins and condensed protein-carbohydrate complexes that are recovered in the fiber fractions of the supplements. Extended heating of CM (Moshtaghi-Nia and Ingalls 1992) and SBM (Hussein et al. 1995) have resulted in increases of ADIN in these protein supplements, and with similar increases in ADIN intake, duodenal and ileal ADIN flows

and with larger fecal recovery of ADIN from the longest heated supplements. These results suggested that ADIN contents are digestible in the rumen and in the total tract despite increased ADIN concentrations for both CM and SBM with extended heating. The increased ADIN due to heating in the present study was 72% digestible as measured by the mobile bag method.

There was a greater increase in NDIN content than for ADIN, with NDIN increasing by 708% with 60 min heating of CM relative to CM 0. NDIN formation involves polymerization reaction of condensed amino groups from AA with carbonyls from reducing sugars into a lignin-like matrix reflected by increased NDF fraction and with resulting N products recoverable in neutral detergent extract (Goering and Van Soest 1972). Results from this study are in agreement with previous one of Moshtaghi-Nia and Ingalls (1992) and those of McAllister et al. (1993) which noted NDIN increase in CM due heating at values of 736% and 185%, in each respective study, relative to unheated CM.

Increased moist heat treatment of CM in this study decreased rumen DM and N disappearances from nylon bags. There was more rapid disappearance of N from CM 0 compared to all other heated CM samples suggesting that heating reduced rates of rumen degradation of protein of CM, therefore increasing the potentially available amounts in the lower GI tract. The decreasing trend in disappearance of N as heating increased was associated with increasing concentrations of ADIN. The N disappearance of Bi was similar to that of CM 60 but intermediate heating of CM at 23 and 45 min provided relatively less degradable DM and protein compared to CM 60. There was a significant

effect of protein type in the cows' diets on in situ degradation of DM and N of the protein supplements. In situ DM and N degradation of CM 0 incubated in the cow diet with heated CM 60 supplement was highest at all incubation hours compared to CM 60 and Bi. The influence of protein supplement in the cows diet on degradation of both DM and N was important in measuring the in situ degradation of protein and thus the protein available for rumen escape.

Rumen degradation of DM and protein for CM 0 and CM 60 was not different for bag type at 0.1 h incubation but was lower for Bi. At 12, 16 and 24 incubation, disappearance of both DM and N with one exception was higher in monofilament bag type than multifilament type. It has been noted that small particles can be lost and gained through the undefined woven terlyene material (Playne et al. 1972) however, in the present experiment there was greater loss of DM and N with the fixed monofilament bags. These two bag types (multifilament and monofilament) have similar pore size (50 $\pm$ 3 μm) but multifilament material has less surface area associated with pores which seemed to have contributed little difference in supplement disappearance at 0.1 h for CM 0 and CM 60 but there was a difference for Bi.

Lower GI tract degradation of DM and protein in both bag types was not affected by removing the pepsin-HCl digestion step. Ingalls and Okemo (1994) suggested differences in lower GI tract disappearances of phosphorous from CM and SBM samples pre-digested with pepsin-HCl. Deacon et al. (1988) did not include the pepsin-HCl digestion step in their study but obtained DM and CP disappearance values of over 90% for the protein sources studied. Results from Kirkpatrick and Kennelly (1984) indicated

that including a pepsin-HCl digestion step resulted in a similar DM disappearance of the supplements when a comparison was made between conventional methods and their modified nylon bag technique, both methods included pepsin-HCl step.

Disappearance of DM and N was higher in monofilament than multifilament bag type. The suggestion could be made that the monofilament bag resulted in greater degradation due to greater percentage open area, which may have allowed higher rates of microbial influx and efflux of enzymatically digested material from the bag. In the multifilament bag type there was lesser open surface area for microbial influx and subsequently, lesser efflux of digested material. Also movement of the threads for the multifilament bag could have reduced opening size.

These studies indicate that bag type did have an effect on degradation of DM and protein. It is not possible to determine if that difference was due to percent open area or change in size of openings in the bag weave for the multifilament bag type. It is assumed the pepsin enzyme was active but no test was made to ensure an active digestion before the enzyme was used up.

Heat treatment did not change ($P<0.05$) the individual composition of AA in canola meal but tended to reduce the concentration of arginine and lysine and to a lesser extent methionine and valine. The apparent loss of AAs may be a result of Maillard reaction with increased heating causing the N associated with these AAs to be unaccounted for in routine AA analysis. The results from the present study seem to agree with those by Schingoethe and Ahrar (1979) that recorded no major changes in AA composition of soybean and sunflower meals. Craig and Broderick (1981) found a

decreased availability of lysine with increased heating time of cotton meal, while Moshtaghi-Nia (1994) observed a decrease in lysine and arginine composition with increased autoclave heating up to 45 min. Heat treatment did not ($p>0.05$) affect the composition of NEAA.

Heat treatment of CM for 60 min reduced rumen disappearance of EAAs form multifilament bag type which was digested in pepsin-HCl solution. Phenylalanine and methionine had greater disappearances from heat treated CM 60 than Bi for all the supplements. Unheated CM 0 had the highest rumen disappearance and the lowest passage of EAAs to the lower GI tract as compared to CM 60 or Bi in multifilament bag type with or without pepsin-HCl. Heat treatment shifted the disappearance of AA from the rumen to the lower GI tract. This observation is in agreement with other results (Cros et al. 1992; Koeln and Paterson 1986) that recorded increased disappearance from the small intestine with heat treated SBM and horsebean meal.

A comparison of individual AA concentration of the grain mixes used in diet formulation relative to milk AA indicated that the first limiting EAAs in the grain mixes were isoleucine, leucine, lysine, threonine and valine. Results by Moshtaghi-Nia (1994) suggested that isoleucine and tyrosine (often not considered an EAA) were the first limiting EAAs providing 20 to 23% of corresponding EAA in milk protein, followed by lysine, valine and histidine. Results from Boila and Ingalls (1994) suggested similar values of about 16 to 21% for isoleucine and tyrosine at 12 h rumen incubation. The levels of EAAs ruminally available from CM 0, CM 23 and CM 45 incubated at 12, 16

and 24 h were on average lower than those in milk AA and it seemed therefore that heat treatment of CM past 45 min led to increased ruminal availability of EAA.

Heat treatment did not change either TME_n nor N content of CM's but decreased availability of both EAA and NEAA, expressed both as [g $(16g\ N)^{-1}$] and as percentage fed to cockerels. "True digestibility" of EAA that escaped rumen degradation as measured by mobile nylon bag were higher for most AAs in CM 60 than in CM that had milder heating. True digestibility of EAA in Bi supplement was comparable, in most cases, to those of CM 60. True digestibility for lysine was lower than some previous results recorded with cockerels (Zuprizal et al. 1991). However, since its availability is known to vary with treatment (Wehner and Harrold 1982), it was possible to assume that heat treatment during commercial processing may have affected its digestibility by the cockerels. Also, increased heat treatment increased rumen escape of lysine and increased the quantity available in the lower GI tract. In the cockerel study heat treatment decreased AA bioavailability of some the AAs that are sensitive to thermal changes such as lysine and cystine. True AA availability and digestibility results by cockerels appeared to be lower than values for lower GI tract availability of AA in ruminants as measured by the in situ mobile bag methods. It is not possible to determine the influence of rumen microbes presence on the values measured in cockerels.

Daily dry matter intake (DMI) of CM 0 and CM 60 was higher than Bi for both PP cows and all cows. The difference was not significant for MP cows. There is a reason for the low intake of the Bi diet since fish meal and meat meal are known to be not as palatable as other feedstuffs. Parity affected DMI with PP cows consuming less feed per

day than multiparous cows. It is not clear why on average, intake of the Bi diet was more severely depressed for PP than MP cows.

Milk yield by MP cows was higher on the Bi diet than for cows receiving CM 0 and CM 60 diets while it was lower for PP cows. These data would suggest that the MP cows consumed relatively similar amounts of the diets and the increased escape of AA with the Bi diet resulted in higher milk production. A similar trend was true for cows on the CM 60 but the difference was not significant. The major difference (although not significant , $p>0.05$) in EAA make up between Bi and CM 60 supplement of the lower GI tract available AA [g $(16g\ N)^{-1}$] was leucine and phenylalanine levels (table 1.19). Undegradable intake AA [g $(16g\ N)^{-1}$] followed a similar trend for leucine, methionine and phenylalanine (table 1.21). True AA availability [g $(16g\ N)^{-1}$] as measured by fed cockerels show an advantage, though not significant ($p>0.05$), for Bi supplement with higher levels of individual EAAs (table 2.2). The larger changes (9 to 13%) were for histidine, isoleucine, lysine and leucine.

Parity affected milk yield with MP cows producing 34% more milk/day than PP cows. Treatment and parity did not affect milk contents of fat, protein, SNF, body weight and condition score over the experimental period. Rumen acetate:propionate ratio was higher in CM 60 than CM 0 and Bi at 0 and 4 h post-feeding. This may have been a reflection of the reduced rate of degradation of the canola meal with heat treatment. Heat treatment of CM to increase rumen escape protein resulted in an increase in milk production by PP cows with no significant effect on DMI. The Bi diet resulted in a large reduction in intake by PP cows and increased milk yield by MP cows.

GENERAL CONCLUSIONS

In situ data for the diets used in this study suggested decreased degradation of rumen escape protein for heat treated CM 60 and the vegetable/animal protein supplements (bypass protein). This indicated an increased potential for rumen escape of dietary protein available for lower GI tract digestion. There was a more rapid rumen degradation of DM and protein of CM 0 than other CM samples.

There was a significant decrease in effective degradability of DM and N with increased heating of CM with the exception of N with 45 min heating. Type of protein supplement in the cows' diets significantly affected DM and protein degradation. Rumen degradation of DM and protein of CM 0 and CM 60 was not different for bag type at 0.1 h incubation but was for Bi. Disappearance of both DM and N with one exception was higher in monofilament bag type than multifilament type. Degradation of DM and protein in both bag types were not affected by removal of pepsin-HCl digestion step. Heat treatment decreased concentration of arginine, leucine, methionine, and valine. Rumen and lower GI tract disappearance of individual EAA decreased with heating but increased fecal excretion of arginine, histidine, isoleucine and threonine. Fecal excretion of EAA was higher for Bi compared to CM 60 except for lysine and threonine. Heat treatment reduced rumen disappearance from multifilament bag digested in pepsin-HCl more than monofilament bag.

Heat treatment did not change TME_n, protein and ADF levels but increased NDF content of CM. The CM samples had lower true bioavailability of AA than Bi

supplement. Increased heating reduced bioavailability of all AA except methionine. True AA digestibility decreased with increased heat treatment, with 60 min heating decreasing lysine digestibility by 25%. Results of cockerel and in situ cows studies indicated that heat treatment of CM decreased bioavailability of all AA except methionine with the cockerel, but increased disappearance of EAA in the lower GI tract of cows. Lysine digestibility in cockerels was depressed with increased heating but this increased rumen escape of lysine and increased lower GI tract lysine availability for cows. Heat treatment increased availability of EAA in lower GI tract of cows but increased fecal excretion of arginine, histidine, isoleucine and threonine.

The bypass protein supplement which included fish meal and meat meal resulted in lower intake, especially with the PP cows. The untreated CM diet and heat treated CM diet resulted in similar intake. Increased potential for rumen escape of CM protein yield for PP but not but MP cows with no change in concentrations of milk fat, protein or SNF. The bypass protein supplement diet reduced feed intake compared to other diets but resulted in equal milk production. There was no treatment/parity effect for milk yield nor milk contents.

SCOPE FOR FURTHER RESEARCH

Some suggested areas for further research in improving rumen escape potential of CM as a protein supplement in ruminants should include:

1. Since moist heating CM for 60 min at plant level did not seem to cause adequate change in UIP of CM relative to CM 0, it is proposed that longer heating time be used on CM to provide, presumably greater protection from rumen degradation.

2. Exclusion of pepsin-HCl digestion step did not seem to affect the protein supplements studied. It is suggested that digestion activity of the enzyme used should be examined with a wider range of other protected protein supplements.

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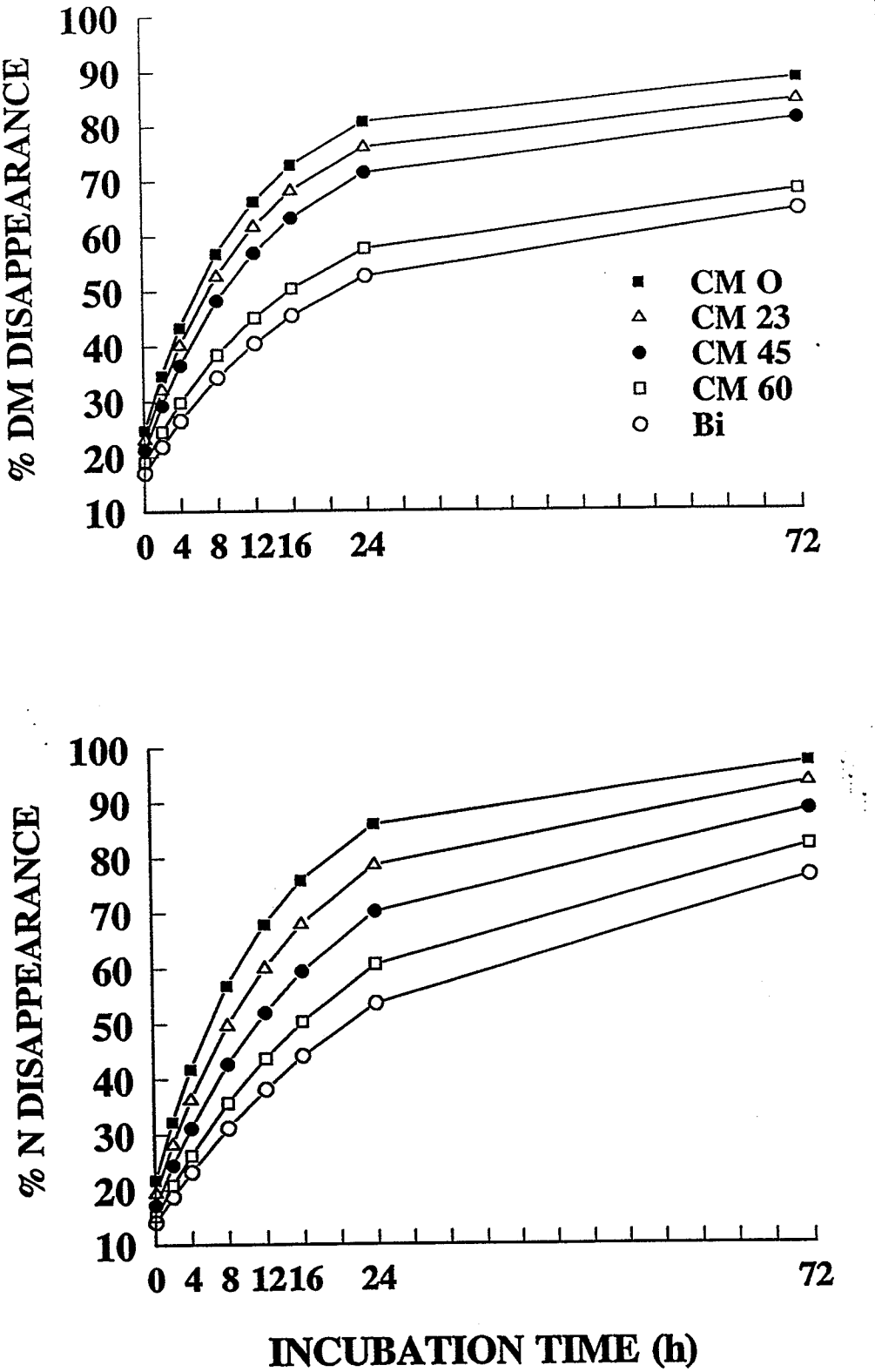
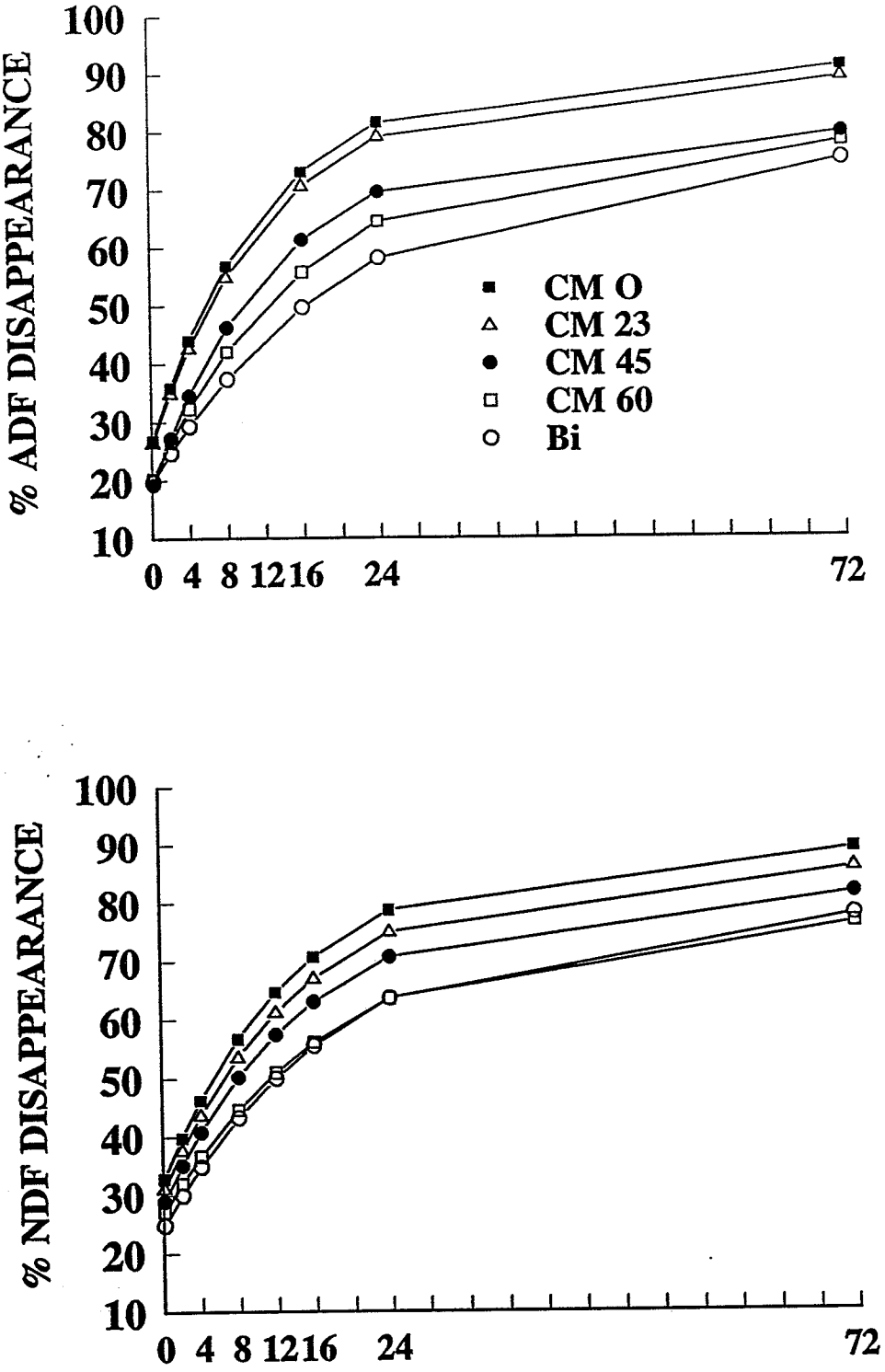
APPENDICES

APPENDIX FIGURES



Appendix figure I.1. In situ dry matter (DM) and nitrogen (N) disappearances from nylon bags incubated in the rumen of cows.



Appendix figure I.2. In situ disappearances for acid detergent fibre (ADF) and neutral detergent fiber (NDF) from nylon bags incubated in the rumen of cows.

APPENDIX TABLE I

Appendix table I.1. Effects of cow diet (CD), test supplement (TS) and their interaction on in situ dry matter disappearance, and contrasts of interaction Lsmeans.

	Factors and interactions			Contrasts of interaction means			
Time (h)[W]	CD	TS	CD*TS	B vs A[X] in B vs A	B vs A in A vs C	C vs B in B vs A	C vs A in C vs B
0.1	*[Y]	*	*	NS[Z]	*	*	NS
12	*	*	*	*	*	NS	NS
16	*	*	*	*	*	NS	NS
24	*	*	*	*	*	NS	NS

[W] Incubation time (h).
[X] A = Unheated CM 0, B = Heated CM 60 and C = Bi, formulated bypass animal-vegetable protein supplement. Contrasts are set up to test, for example, test supplement B versus A in cow diet B versus A (see figure 1.1 for interpretation).
[Y] Significant ($P<0.05$), n=3.
[Z] Not significant ($P>0.05$), n=3.

Appendix table I.2. Effects of cow diet (CD), test supplement (TS) and their interaction on in situ nitrogen disappearance, and contrasts of interaction Lsmeans.

	Factors and interactions			Contrasts of interaction Lsmeans			
Time (h)[W]	CD	TS	CD*TS	B vs A[X] in B vs A	B vs A in A vs C	C vs B in B vs A	C vs A in C vs B
0.1	*[Y]	*	*	NS[Z]	*	NS	NS
12	*	*	*	*	NS	NS	*
16	*	*	*	*	*	*	*
24	*	*	*	*	*	NS	*

[W] Incubation time (h).
[X] A = Unheated CM 0, B = Heated CM 60 and C = Bi, formulated bypass animal-vegetable protein supplement. Contrasts are set up to test, for example, test supplement B versus A in cow diets B versus A (see figure 1.1 for interpretation).
[Y] Significant ($P<0.05$), n=27.
[Z] Not significant ($P>0.05$), n=27.

Appendix table I.3. In situ disappearance of dry matter and nitrogen of unheated canola meal, heated canola meal and bypass protein (Bi) supplement.

	Dry matter disappearance (%)						Nitrogen disappearance (%)					
TS[v]	0	23	45	60	Bi	SE[u]	0	23	45	60	Bi	SE
CD	0	60	60	60	Bi		0	60	60	60	Bi	
Hou[w]												
0.1	24.81	23.14	21.20	18.94	17.01	0.12	21.71	19.41	17.24	15.23	14.14	0.34
2	34.64a	32.12a	29.25b	24.55c	21.88c	0.45	32.27a	28.21a	24.39b	20.82c	18.71c	0.54
4	43.36a	40.15a	36.57a	29.77b	26.48b	0.23	41.80a	36.36b	31.18c	26.23d	23.20d	0.78
8	56.81a	52.73a	48.28a	38.44b	34.27b	0.98	56.89a	49.76b	42.71c	35.72d	31.22e	0.99
12	66.26a	61.76b	56.93b	45.17c	40.50d	1.12	67.90a	60.05b	51.98c	43.69d	38.13d	1.23
16	72.91a	68.24a	63.32b	50.41c	45.49c	1.21	75.93a	67.97b	59.44c	50.37d	44.09e	1.17
24	80.86a	76.22a	71.54b	57.64c	52.67d	1.34	86.06a	78.72b	70.27c	60.68d	53.64e	1.02
72	88.56a	84.55b	81.17b	68.17c	64.55c	1.47	97.30a	93.57a	88.65a	82.24b	76.55b	0.9

CD: Cow diet in which test supplement is incubated.
Cow diet 0 = unheated CM, 60 = heated CM at 60 min and Bi = bypass protein supplement.
[v] Test supplement: 0 = unheated CM 23 = CM heated for 23 min, 45 = CM heated for 45 min, 60 = CM heated for 60 min and Bi = bypass protein supplement.
[u] Standard error of Lsmeans (n=3).
[w] Incubation time (h).
a-e, Lsmeans within incubation time differ ($p<0.05$).

Appendix table I.4. In situ disappearance of acid detergent fibre and neutral detergent fibre of unheated canola meal, heated canola meal and bypass protein (Bi) supplement.

	Acid detergent fibre disappearance (%)						Neutral detergent fibre disappearance (%)					
CD	0	23	45	60	Bi	SE[u]	0	23	45	60	Bi	SE
TS[v]	0	60	60	60	Bi		0	60	60	60	Bi	
Hour[w]												
0.1	26.88a	26.48a	19.35b	20.34b	19.86b	0.32	32.82	31.12	28.96	27.13	24.90	0.14
2	35.88a	32.12a	27.32c	26.51c	24.69c	0.25	39.80a	37.62a	35.09a	32.03b	30.04b	0.64
4	44.01a	34.94b	34.59b	32.31b	29.34b	0.73	46.22a	43.65a	40.78a	36.68b	34.20b	0.98
8	56.86a	42.62b	46.25b	42.05b	37.42b	1.02	56.69a	53.55a	50.18b	44.59c	43.27c	1.09
12	73.08a	54.90b	58.03b	47.87c	47.71c	1.21	64.64a	61.16b	57.45c	50.97d	50.04d	1.23
16	81.71a	70.66b	61.34c	55.86d	49.49e	1.01	70.68a	67.01b	63.06c	56.12d	55.53d	1.17
24	80.86a	79.24a	69.67b	64.51c	58.19d	1.43	78.74a	74.97b	70.75c	63.61d	63.62d	1.02
72	91.29a	89.27a	79.65b	78.13b	75.01b	1.09	89.30a	85.94a	81.64a	76.48b	77.89b	0.97

CD: Cow diet in which test supplement is incubated.
Cow diet 0 = unheated CM, 60 = heated CM at 60 min and Bi = bypass protein supplement.
[v] Test supplement: 0 = unheated CM, 23 = CM heated for 23 min, 45 = CM heated for 45 min, 60 = CM heated for 60 min and Bi = bypass protein supplement.
[u] Standard error of Lsmeans (n= 3).
[w] Incubation time (h).
a-e, Lsmeans within incubation time differ ($p<0.05$).

Appendix table I.5. Rumen and lower GI tract disappearance, and fecal recovery of nitrogen (N), acid detergent insoluble nitrogen (ADIN) and neutral detergent insoluble nitrogen (NDIN) from unheated (CM 0), heated (CM 60) and bypass protein (Bi) supplement initially incubated in situ for 12 h.

Digestive tract	Rumen disappearance			SE[w]	Lower GI tract disappearance			SE	Fecal recovery			SE
Test protein	CM 0	CM 60	Bi		CM 0	CM 60	Bi		CM 0	CM 60	Bi	
N[x]	67.6a	47.8b	41.5c	0.9	27.5b	42.5a	43.8a	0.2	5.0c	9.7b	14.7a	0.1
ADIN[x]	68.8a	53.8b	45.9c	1.2	23.6b	23.2b	31.4a	0.5	7.6b	22.9a	22.8a	1.1
NDIN[x]	60.7a	50.7b	49.9b	0.9	37.7	38.5	40.2	0.1	1.6b	10.9a	9.8a	1.2

[w] Standard error of Lsmeans (n=3)
[x] % of total original N
a-c, Lsmeans within row within each segment of digestive tract with different letters differ ($P<0.05$).

Appendix table I.6. The disappearance in the rumen and passage to lower gastrointestinal tract of non-essential amino acids (NEAA) from canola meal moist heated at 0, 23, 45, 60 min and of bypass protein supplement incubated for 16 h.

NEAA	0	23	45	60	Bi	SE[W]
Alanine	69.26b	75.01a	65.51c	31.05d	33.08d	1.93
Aspartic acid	71.82a	70.55a	62.32b	36.80d	44.26c	1.74
Cystine	78.27a	53.28b	44.12c	47.71c	45.85c	3.98
Glutamic acid	79.96a	81.76a	72.10b	45.37c	38.94d	1.66
Glysine	70.12b	76.83a	68.01b	32.86d	45.67c	1.76
Proline	74.36a	76.38a	73.53a	42.80b	41.52b	1.41
Serine	64.77b	72.96a	66.61b	27.03d	31.94c	1.64
Tyrosine	55.94c	69.36a	63.56b	16.91d	13.51e	1.61

[W] Standard error of Lsmeans (n=3)
a-e, Lsmeans within an amino acid with different letters differ significantly ($P<0.05$).

Appendix table I.7. The disappearance in the rumen and passage to lower gastrointestinal tract of non-essential amino acid (NEAA) from canola meal moist heated at 0, 23, 45, 60 min and of bypass protein supplement incubated for 24 h.

NEAA	0	23	45	60	Bypass	SE[w]
Alanine	87.73a	80.22b	75.12c	34.60d	35.73d	1.93
Aspartic acid	89.06a	78.16b	71.94c	45.26c	47.63c	1.74
Cystine	88.13a	77.15b	51.72d	55.59c	51.54d	3.98
Glutamic acid	91.87a	85.58b	81.04c	47.73d	42.94e	1.66
Glysine	87.08a	82.07b	77.09c	34.87e	53.65d	1.76
Proline	87.65a	81.16b	79.94b	48.89c	42.95d	1.41
Serine	85.31a	79.80b	76.26b	29.05d	36.31c	1.64
Tyrosine	80.86a	80.19a	71.57b	18.61c	17.03c	1.61

[w] Standard error of Lsmeans (n=3)
a-e, Ls means within an amino acid with different letters differ significantly ($P<0.05$).

APPENDIX TABLE III

Appendix table III.1. Nutrient composition of roughages (on DM-basis) fed to lactating and cannulated dairy cows.

Nutrient %	Roughage			SE[X]
	Alfalfa silage	Corn silage	Long hay	
Crude protein	19.11a	9.29b	17.06a	1.29
Acid detergent fiber	35.14a	29.19b	28.17b	0.89
Neutral detergent fiber	49.17b	58.51a	47.07b	0.98
ADIN (% total N)[Y]	0.19b	0.41b	4.22a	0.23
NDIN (% total N)[Z]	1.55	1.18	1.48	0.05

[X] Standard error of Lsmeans (n=8, duplicates for four experimental sub-periods.
[Y] Acid detergent insoluble fiber.
[Z] Neutral detergent insoluble fibre.
a-b, Lsmeans within row with different letters differ ($p<0.05$).

Appendix table III.2. Nutrient composition of protein supplements (on DM-basis) used in formulating diets fed to lactating and cannulated dairy cows.

	Protein supplement			SE[W]
Nutrient %	CM 0	CM 60	Bi	
Crude protein	37.65	38.57	38.00	0.23
Acid detergent fiber	14.95	16.34	17.68	0.45
Neutral detergent fiber	24.62	22.70	23.88	0.34
ADIN (% total N)[Y]	1.77b	7.41a	7.63a	0.98
NDIN (% total N)[Z]	1.99b	9.01a	11.14a	1.03

[W] Standard error of Lsmeans (n=8, duplicates for four experimental sub-periods.
[Y] Acid detergent insoluble nitrogen.
[Z] Neutral detergent insoluble nitrogen.
a-b, Lsmeans within row with different letters differ ($p<0.05$).

Appendxi table III.3. Nutrient composition of grain mixtures (on DM-basis) used in formulating diets fed to lactating and cannulated dairy cows.

	Grain mixture			SE[W]
Nutrient %	CM 0	CM 60	Bi	
Crude protein	20.56	20.01	20.94	0.03
Acid detergent fiber	9.11	10.96	8.86	0.45
Neutral detergent fiber	22.61	23.60	19.91	0.06
ADIN (% total N)[Y]	1.05	2.41	2.62	0.57
NDIN (% total N)[Z]	1.43	3.25	3.48	0.98

[W] Standard error of Lsmeans (n=8, duplicates for four experimental sub-periods.
[Y] Acid detergent insoluble nitrogen.
[Z] Neutral detergent insoluble nitrogen.

Table III.4. Lower GI tract percent availability of essential AA from unheated, heated and of bypass protein (Bi) supplement after 12 rumen incubation, relative to milk[W] amino acids.

	Heat treatment (Minutes at 110°C)					
	0	23	45	60	Bi	SE[Y]
Amino acid						
Arginine	51.71c	46.57c	52.57c	97.71a	74.57b	2.24
Histidine	26.67c	26.30c	40.00b	53.70a	40.37b	1.14
Isoleucine	19.32b	16.78b	17.80b	35.76a	18.31b	0.89
Leucine	24.12c	23.09c	24.43c	47.11b	60.21a	1.27
Lysine	11.98c	18.89b	17.16b	31.23a	27.53a	1.02
Methionine	25.77b	20.77c	18.46c	50.76a	49.62a	1.08
Phenylalanine	30.61c	31.84c	33.06c	57.76b	64.70a	1.13
Threonine	33.91d	39.13c	41.74c	61.74a	50.84b	1.19
Valine	27.06b	17.88c	18.94c	43.79a	38.79a	1.24

[W] Milk amino acid values as reported by Spires et al. (1975).
[X] Standard error of Lsmeans (n=9).

Table L.1. Essential amino acid (EAA) composition of canola meal (CM) and soybean meal (SBM) from previous studies.

Feedstuff	CM[1]	SBM[1]	CM[2]	CM[3]
EAA Composition				
Arginine	6.08	6.71	6.18	5.88
Histidine	2.79	2.48	2.90	2.78
Isoleucine	3.44	4.03	3.21	3.20
Leucine	6.92	7.44	7.29	7.07
Lysine	5.95	6.24	5.96	5.78
Methionine	2.10	1.59	2.99	2.23
Phenylalanine	1.45	2.13	4.59	4.34
Threonine	4.52	3.85	4.54	4.24
Valine	4.42	4.18	4.24	4.12

Source: [1]Clandinin et al. 1986.
[2]Boila and Ingalls 1992.
[3]Moshtaghi-Nia 1994.