


Insacco Rumen Degradation, and Digestibility in
the Lower Digestive Tract of Ruminants, of
Canola Meal and Soybean Meal

by

 Elaine M. Kendall

A thesis presented to the
University of Manitoba in partial fulfillment
of the requirements for the degree
of Master of Science

in

The Department of Animal Science

Winnipeg, Manitoba

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ELAINE M. KENDALL

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ABSTRACT

The "In Sacco" technique and the Mobile Nylon Bag Technique were used to compare canola meal (CM) and soybean meal (SBM) with respect to rumen escape and lower digestive tract digestibility of dry matter, protein, energy, and essential amino acids (EAA).

A sample of CM was obtained from five different processors and designated A, B, C, D, and E. A SBM sample was obtained from a processor in Altona, Manitoba, and designated F. Two rumen cannulated Holstein steers and three duodenally cannulated Holstein steers were used. Small nylon bags, 3 cm x 5 cm, containing .5 g of sample, were incubated in the rumen for various time intervals, then removed. Half of the bags removed were analyzed for rumen effects, the other half were then incubated in pepsin-HCl solution for 3 hrs at 39° C, to simulate abomasal digestion. The bags were then passed through the lower digestive tract of the duodenally cannulated steers, subsequently collected in the feces, and analyzed. Six trials were carried out with the rumen incubation periods as follows: 0 h; 4 h; 8 h; 12 h; 16 h; 30 h.

The data obtained showed that the N escape values of SBM in the rumen fall within the range of values obtained for CM. The data obtained showed that the DM escape of SBM is less than CM at 30 h, but at the other time intervals it falls within the range of values obtained for CM. The data obtained showed that energy escape from the rumen for SBM is higher than that of CM at 4 and 16 h, but at 8, 12, and 30 h, it is similar to the values obtained from some of the CM samples. The data

obtained showed that EAA escape of SBM falls in the range of values obtained for CM at all time intervals, except for: His at 4 h; Met at 12 h; Met at 16 h.

The digestibility data obtained showed that N, DM, and energy digestibility from SBM is greater than from CM. These data suggest that all EAA had digestibilities that were greater from SBM than from CM in the lower digestive tract, except: Met at 0 h; Met at 4 h; Lys His Val Thr Ile Leu and Met at 8 h; Lys His Phe and Met at 12 h; all EAA at 16 h.

In general these data suggest that CM and SBM samples were not different with respect to rumen escape of dry matter, protein, energy and essential amino acids. However, these data suggest that lower digestive tract nutrient digestibility from SBM is greater than CM.

FOREWORD

The format followed in this thesis is that of the Canadian Journal of Animal Science. Manuscript I and Manuscript II will be submitted for publication. Manuscript I, In Sacco Rumen Degradation of Five Different Canola Meal Samples, Compared to Soybean Meal, with Steers Receiving a Diet Formulated for High Producing Cows, was written by E. M. Kendall. Manuscript II, The Digestibility of Five Different Canola Meal Samples, Compared to Soybean Meal, In the Lower Digestive Tract of Ruminants, was written by E. M. Kendall.

LITERATURE REVIEW

INTRODUCTION

The ruminant animal is unique with respect to its digestive physiology and nutrition. The vast microbial population present in the rumen is essential to the ruminant. The microorganisms ferment the fibrous constituents of feedstuffs, which would otherwise be unavailable to the animal, and yield the volatile fatty acids that provide the ruminant animal with most of the energy to meet its requirements.

Rumen microorganisms are also highly proteolytic. They use dietary protein as a source of energy, and in so doing break it down into peptides, amino acids and ammonia. These components are then utilized by the microorganisms to synthesize microbial protein. Therefore, most of the amino acids that reach the small intestine of ruminants, and that are ultimately utilized by the animal, are of microbial origin. There is considerable debate as to whether microbial protein can supply the high producing ruminant animals of today, such as dairy cows, with all of the essential amino acids they require. This makes the measurement of protein degradability in the rumen very important, as it is the amount of protein that escapes degradability and the amount of microbial protein synthesized, that determines the amino acid supply to the small intestine.

The interest in rumen bypass protein, and the ability to accurately measure protein degradability in the rumen, have been at the forefront of ruminant nutrition research for many years. This interest has led to the development of many techniques to measure protein degradability (Ørskov 1982). It has led to a lot of research on feedstuffs that are

naturally resistant to microbial degradation, and on chemical and heat treatments that make feedstuffs resistant to microbial degradation. It has ultimately led to a better understanding of the relationship between rumen microorganisms and the host ruminant animal, and should lead to the improved protein nutrition of the highly productive ruminant animals of today.

RUMEN DEGRADATION

The Rumen Microbes

The environment within the rumen contains a large microbial biomass made up of a great variety of microbial species. The rumen only permits the growth of microorganisms for which the substrate and ruminal pH is optimal, and usually only microorganisms that have a high rate of cell division (Ørskov 1982). The microbial population ferment feed particles that enter the rumen to obtain the energy they need to grow. They also require an adequate supply of nitrogen and major minerals such as sulphur and phosphorus. This microbial action is essential to a ruminant in that plant cell walls, which would otherwise be indigestible, can be digested and then used by the animal. The volatile fatty acids (VFA), acetic, propionic and butyric acids, produced by microbial fermentation, supply the ruminant with up to 65% of its total energy yielding nutrients. According to Van Soest (1982) up to 90% of the digestible fibrous constituents of feedstuffs can be fermented in the rumen.

Rumen microorganisms can be subdivided into three populations by location: microbes that float freely in the liquid content, microbes that adhere to feed particles, and microbes associated with the rumen wall (Ørskov 1982). Protozoa usually move freely through the liquid content or cluster around feed particles. Rumen microorganisms are classified according to substrate specificity, products and nutritional requirements, a system developed by Hungate (1966).

The first classification is the cellulolytic bacteria. These bacteria allow ruminants to efficiently utilize feeds that would be

unsuitable to most monogastric animals. The primary cellulolytic microorganisms are Bacteroides succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens (Baldwin and Allison 1983). These microorganisms are sensitive to a pH of less than 6.2 which inhibits their growth. They are strict anaerobes, and most require nitrogen in the form of ammonia. They also require B-vitamins and branched chain fatty acids such as isobutyrate and isovalerate (Ørskov 1982). These other nutrients are often supplied by other rumen microorganisms.

The amylolytic and dextrinolytic microbial species vary most in numbers because starch varies a great deal with the diet. These bacteria are less sensitive to changes in rumen pH than cellulolytic bacteria. In an experiment by Mould and Ørskov (1981) the rate of digestion of starch in the rumen of sheep, consuming barley as their sole feed, was unaltered by increasing the pH from 5.6 to 7.0. The proportion of VFA's produced are also not affected by a change in rumen pH (Ørskov 1982). The rate at which starch is attacked and fermented in the rumen depends both on type of starch as well as the method of processing of the feedstuff involved. Barley ferments much more rapidly than corn and flaked corn ferments more rapidly than ground corn (Ørskov 1982).

There are only a few major strains of bacteria that are obligatorily proteolytic. One of the principal proteolytic microbes is Bacteroides amylophilus (Mahadevan et al. 1980). The strains that have so far been isolated appear to use other bacteria as their substrate source (Hungate 1966). Nugent and Mangan (1981) suggest that soluble proteins, amino acids and peptides, are degraded rapidly because they

become attached to bacterial cell walls very quickly. The less soluble protein, and particulate matter containing a high proportion of protein, is degraded at various rates. These differential rates are thought to be related to the chemical properties of protein, such as the number of disulfide bridges present and tertiary structures (Ørskov 1982).

Protozoa are assumed to be of less importance than bacteria, mainly because rumen fermentation proceeds normally without them. In fact, cattle and sheep only acquire ciliate protozoa after exposure to other faunated animals (Veira 1986). Protozoa are usually found to be less in number in the rumen than bacteria, however, protozoa are much larger in size. Protozoa do attack major feed components and this indicates that they may serve a more important role in rumen fermentation than was previously thought. However, protozoal nitrogen (N) found to arrive at the abomasum is considerably less than would be expected from their contribution to the microbial biomass (Ørskov 1982). Bauchop and Clarke (1976) suggest that this is because protozoa attach themselves to large feed particles and this actually prevents them from leaving the rumen in the liquid phase. This probably aids in their survival in the rumen since it increases their rumen retention time. Harrison and McAllan (1980) found that the mean division time of protozoal cells is 24 hours, while rumen retention of fluid is usually less than 10 hours. If protozoa left the rumen at the same rate as the fluid phase their survival rate would be very low.

Veira (1986) has shown that any effect that protozoa have on the nutrition of ruminants results from the effects they have on rumen function. The presence or absence of protozoa has been shown to affect

rumen pH, ammonia concentration, volume and dilution rate, and bacterial numbers and types (Veira 1986). The pH of the rumen was shown to be lower in defaunated animals than in faunated ones (Veira et al. 1983). This was probably due to protozoal uptake of soluble sugars and starches. This would remove sugars and starches from immediate fermentation by bacteria and therefore regulate ruminal lactate metabolism (Veira 1986). In this way rumen protozoa may prevent accumulation of excessive levels of lactate and thus help to prevent acidosis. The ammonia concentrations are consistently higher in the rumen in faunated animals than in defaunated ones. This is thought to be due to the greater recycling of microbial protein within the rumen of faunated animals with the result being fewer bacteria to utilize the ammonia, and increased dietary protein breakdown (Leng and Nolan 1984). This finding has led to speculation of inefficient utilization of nitrogen in faunated animals (Veira 1986). Nonammonia nitrogen (NAN) flow from the rumen is usually higher in defaunated animals than in faunated animals (Veira 1986). This is indicative of increased efficiency of microbial protein synthesis and a decrease in degradation of dietary protein in the defaunated animals. The active proteolytic enzymes found in ciliate protozoa and their ability to engulf feed particles are factors that contribute to increased dietary protein degradation in faunated animals.

Microbial Growth and Growth Factors

Microbial growth is an important part of the ruminant protein system. There is an optimum balance between microbial growth

requirements and substrate availability. The optimum is usually dictated by the utilization of degraded protein and carbohydrate from any of the feedstuffs or ingredients used in diets. If the nitrogen (N) level is excessive, then protein wastage will occur because energy is the limiting factor for efficient N utilization (Allison 1982). In contrast, if the energy level is excessive then carbohydrate digestion will be reduced because protein is the limiting factor (Allison 1982).

Bacterial growth can be rapid, doubling times can range from 14 minutes to 14 hours (Bull et al. 1985). The rate of bacterial growth is a partial function of the availability of substrate at any given time interval. Bacterial growth is usually described as a change in mass per unit of time. At steady state conditions in the rumen, bacteria grow or multiply at a rate only sufficient to replace those passing out of the rumen or lysing. Growth rate is an index of the rate at which cells are replaced (Bull et al. 1985). Microbial yield is commonly calculated as the multiple of substrate used. The preferred way to express microbial yield is by the amount of carbohydrate substrate fermented (Ørskov 1982).

Russell and Hespell (1981) divided the microbial mass into two major categories: primary and secondary fermenters. The primary fermenters degraded the cell wall, starch and sugars. The secondary fermenters utilized the products produced by the primary group. Readily available carbohydrate such as starches and sugars provide the greatest amount of energy for microbial growth both in vitro and in vivo (Stern et al. 1978). When starch is added to a high cellulose diet or replaces part of the cellulose, increased nitrogen utilization has been reported

(Stern and Hoover 1979).

Microbial nitrogen requirements vary quantitatively. The microbes that digest fiber require ammonia and may require branched chain acids for protein synthesis and growth (Russell and Sniffen 1984). Primary and secondary fermenters also seem to require ammonia. Ørskov (1982) questions the ability of compounds like ammonia, or compounds which upon degradation yield ammonia, to supply the sole source of N to achieve a maximal yield of microbial protein. Ørskov (1982) suggests that some preformed amino acids are required to supply the sole source of N. Amino acids are stimulatory to a few microorganisms such as Ruminococcus albus, R. flavefaciens and Megasphera elsdenii (Russell et al. 1983). Cotta and Russell (1982) have shown that amino acids and short peptides are essential to some species such as Streptococcus bovis. Since lysing of bacteria is a natural ongoing process, some bacterial amino acids will always be available in the rumen. Maeng and Baldwin (1975) clearly demonstrated that the yield of the microbial biomass was increased by 100% when 25% of the urea N in a purified diet was replaced by a mixture of amino acids. The division time in this experiment was also reduced from 6.7 hours to 3.4 hours. Teather et al. (1980) reported that diets containing urea-silage or soybean protein supported ruminal bacteria populations that were 70% greater than with equivalent urea (12.5% CP) as the sole supplement. It is yet to be determined whether branched chain fatty acids produced from the degradation of added protein and amino acids are responsible for these observations.

There is evidence that many rumen bacteria excrete amino acids during growth in media with ammonia as the main N source (Allison 1982).

The amino acids excreted by pure cultures in greatest amounts were alanine, glutamic acid, valine, aspartic acid, and glycine. These amino acids were found in highest concentration in the rumen fluid. The degradation of excreted amino acids may partially explain the presence of branched-chain fatty acids in the rumen of animals fed diets that do not contain branched-chain fatty acids (Allison 1982).

The sulphur containing amino acids make up a constant proportion of microbial amino acids. The microbial biomass can contain as much as 8 g sulphur/kg dry matter (Ørskov 1982). The requirement for sulphur may be expected to be related to the requirement for N. Microorganisms usually derive their sulphur from the degradation of protein. Therefore a deficiency of sulphur is likely to occur only if there is also a deficiency for nitrogen from protein sources (Ørskov 1982).

Sources of Nitrogen for Microorganisms

The most important source of nitrogen for rumen microorganisms is dietary protein and non-protein nitrogen (NPN). Rumen microorganisms are highly proteolytic so that most of the dietary protein that enters the rumen is degraded to peptides, amino acids and ultimately deaminated to ammonia. Proteolytic microorganisms use protein degradation as a source of energy so protein degradation is carried as far as possible (Ørskov 1982). The extent to which protein is broken down is influenced by a number of factors such as structure of the protein, solubility, processing and storage, and residence time in the rumen.

Access to the protein by proteolytic enzymes is influenced by the three-dimensional structure of the molecule. Proteins with extensive

cross-linking, such as disulfide bonds, are less accessible to proteolytic enzymes and are relatively resistant to degradation (Satter 1986). This fact is currently being used to protect protein from degradation (Satter 1986). Protein treated with formaldehyde contains sufficient methylene cross-linking to reduce the rate of proteolysis (Satter 1986). Cyclic features can also reduce the rate of proteolysis. Ovalbumin is a soluble protein, but it is a cyclic protein with no terminal amine or carboxyl groups. Ovalbumin is therefore highly resistant to degradation (Satter 1986).

Proteins that dissolve readily in the rumen are the most susceptible to microbial degradation, although this is not always true. Soluble proteins differ greatly in the rate at which they are hydrolyzed. This indicates that the difference in the rates of microbial hydrolysis of some proteins are caused by something other than solubility, such as structure (Satter 1986). Protein solubility therefore is a poor predictor for extent of ruminal degradation across a wide variety of feeds, but may be used to predict the protein degradation of similar feeds (Owens and Bergen 1983).

Processing and storage can effect degradability of protein. Satter (1986) shows that as heat input increases the amount of undegraded protein increases. However, the amount of unavailable protein in the small intestine will also increase, but initially the quantity of unavailable protein formed will be less than the amount of protein protected from degradation (Satter 1986). Therefore, the maximum amount of protein available for digestion in the small intestine will most likely occur when there is a modest amount of heat damage to the

protein. Feed processing techniques such as pelleting, extrusion and steam rolling may generate enough heat to alter protein degradation in the rumen.

Rumen retention time and feed intake can alter protein degradability to a certain degree. Usually only certain protein sources that have continuous degradation, such as soybean, sunflower and alfalfa meals, are affected by retention time and feed intake (Owens and Bergen 1983). Protein sources that are considered high bypass such as distillers grains, fish and meat meal have a lower rate of proteolysis after about 4 hours of incubation in the rumen (Owens and Bergen 1983). Increased feed intake can greatly increase protein bypass as shown by Tamminga (1979) and Zinn and Owens (1983a). Tamminga (1979) reported that the amount of undegraded protein, as a percent of total dietary protein, was 29 and 45% for dairy cows consuming 8.2 and 12.9 Kg of DM daily, respectively. Zinn and Owens (1983a) showed that a 10% increase in feed intake of a high concentrate diet increased the bypass of plant protein from the rumen by 6.5%. This increase in bypass may be due to both decreased residence time and to changed fermentation characteristics in the rumen. A change in fermentation characteristics may lower rumen pH which would decrease the amount of bacteria and therefore proteolytic activity. Rumen pH is normally between 5.5 and 7.0, so protein with an isoelectric point in this range would have altered solubility and possibly altered degradability (Satter 1986).

Increasing the dilution rate of rumen fluid can increase flow of protein from the rumen of sheep and steers (Cole et al. 1976; Harrison et al. 1975; Prigge et al. 1978). This is thought to be due to a net

increase in bacterial protein and an increase in the proportion of undegraded dietary protein (Satter 1986). Environmental temperature can influence residence time of feed in the rumen. Kennedy et al. (1976) showed that sheep in a cold environment had an increased rate of passage. This would increase the amount of microbial crude protein and of undegraded dietary protein reaching the small intestine.

Ruminal Ammonia and Nitrogen Recycling

Nitrogen recycling to the rumen, in the form of urea, is a characteristic unique to ruminants. This process serves to supplement low nitrogen diets and the urea can be used as a source of nitrogen by rumen microorganisms. Kennedy and Milligan (1980) showed that 23 to 92% of the plasma urea is recycled to the digestive tract, with the higher value associated with low nitrogen intake. Urea can be returned to the rumen via saliva and via the blood. The extent to which urea is returned via the saliva seems to be directly proportional to the blood urea concentration and to the amount of saliva excreted (Ørskov 1982). Saliva excretion is influenced by physical form of the diet, for it increases as the proportion of long fibres increases. The blood urea concentration is influenced by the extent to which absorbed amino acids are oxidized and on the absorption of ammonia from the rumen (Ørskov 1982). The entry of urea via the blood is more important than via saliva. It has been shown that up to 7.3 g of nitrogen enters the rumen of sheep daily as urea and only 15% of it is accounted for by salivary urea (Kennedy and Milligan 1980).

Ammonia is passively absorbed in the nonionized form. The pK of

ammonia is above 9 and therefore absorption is low at pH 7 and decreases as pH decreases (Visek 1968). Absorption is positively correlated with ammonia concentration in the rumen (Chalmers et al. 1954). The concentration of ammonia in the rumen can affect the transfer of urea across the rumen wall. The quantity of nitrogen recycled to the rumen appears to be negatively related to ruminal ammonia concentration and positively related to plasma urea concentration, and to organic matter fermentation (Owens and Bergen 1983). The transfer of urea across the rumen wall is thought to be an attenuated diffusion process (Chalmers et al. 1954). Bacterial urease in the rumen epithelium hydrolyzes urea diffusing into the mucosa from the blood stream (Cheng and Costerton 1980). Liberated ammonia rapidly diffuses into the rumen where it is trapped by conversion to the ammonium ion at the pH of the rumen (Cheng and Costerton 1980). High ruminal ammonia concentration reduces recycling either by inhibiting urease in the rumen wall or by decreasing the ammonia diffusion gradient (Owens and Bergen 1983).

Recycled nitrogen becomes useful to the ruminant animal when it is incorporated into microbial crude protein. This incorporation of recycled nitrogen can cause daily duodenal nitrogen flow to exceed nitrogen intake on a low nitrogen diet (Chamberlain and Thomas 1979). On a high nitrogen diet, however, a net loss rather than a net gain of nitrogen in the rumen is usually observed.

Endogenous Nitrogen

It has been suggested that endogenous nitrogen enters the rumen via sloughed epithelial cells (Nolan and Leng 1972). This type of

contribution to the total passage of protein to the duodenum appears small (Beever et al. 1974). However, Ørskov (1982) suggests that the quantity of nonammonia nitrogen from rumen epithelial cells is probably greater than the amount of nitrogen contained in enzyme secretions in the abomasum. It is also possible that under normal feeding conditions the abraded epithelial cells will be partially degraded by rumen microorganisms. The extent to which this fraction is really available is as yet unknown.

METHODS OF MEASURING RUMEN DEGRADABLE PROTEIN

Measuring rumen degradable protein is important, since the supply of amino acids to the small intestine of ruminants is determined by the amount of dietary protein that escapes rumen degradation, and the quantity of microbial protein synthesized in the rumen. There have been many techniques devised to evaluate protein degradability in the rumen. These include in vivo techniques with post ruminal collection of digesta; in vitro techniques such as ammonia release; and the in situ technique which utilizes artificial fibre bags.

In Vivo Method

The in vivo method involves the collection of digesta post ruminally. This involves surgical preparation of the animals with cannulae in the omasum, abomasum, or proximal duodenum. There are two types of cannulae commonly used, the re-entrant type usually placed in the small intestine, and the single t-type usually placed in the abomasum or proximal duodenum. The collection of duodenal flow can give an accurate assessment of the quantity of nitrogen which is passing into the small intestine.

If a duodenal re-entrant cannula is used, small quantities of digesta are collected, about 100 ml for sheep and up to 1 l for cattle, in a cylinder and placed on ice (Ørskov 1982). Of this, about 10% would actually be analyzed, the rest would be returned to the animal by way of the cannula, after being heated to body temperature. In some cases indigestible markers are included in the diet to distinguish between microbial protein and feed protein. Samples are then taken over one or

more days, the microbial protein is isolated and feed protein is calculated as the difference between total duodenal nitrogen and microbial nitrogen (Ørskov 1982).

The use of a single t-type cannula requires less surgery and only spot samples need be taken. Normal feed intake is usually maintained by the animal and the cannula is easier to maintain. Sampling takes place every hour or every two hours over a 24 or 48 hour period. The samples are frozen immediately until analyzed, which is usually the same as for the re-entrant cannula technique.

Stern et al. (1979a) used the single t-type cannula technique to estimate digesta flow to the duodenum. Chromium (Cr) EDTA and lanthanum (La) were sprayed onto portions of the grain mixture and fed four times daily at rates of 80 and 15 mg/Kg of total DM fed respectively. A 400 ml sample of duodenal digesta was collected over a 4 day period every 8 hours. If the duodenal digesta samples taken were representative of true digesta flow past the cannula, then the ratio of Cr:La in the duodenum should equal the Cr:La ratio in the feed. Overall, the mean ratios were 5.62, 5.35 and 5.36, and in the feed, duodenum, and feces respectively (Stern et al. 1979a). A 95% recovery was indicated from both the duodenum and the feces (Stern et al. 1979a). Other researchers who have used the single t-type cannula to determine N degradation and flow to the small intestine include Hvelplund et al. (1976), Merchen et al. (1980), Prange et al. (1980), Stern et al. (1980a), Stern and Satter (1982) and Tamminga et al. (1979).

The two mathematical methods used most often to estimate protein degradability from samples obtained, via digesta collection, are the

"regression technique" and the "by difference" technique. The regression 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 by difference technique measures dietary protein intake and the total protein flow to the duodenum. The microbial and endogenous sources of protein are estimated at the duodenum, and the undegraded dietary protein is calculated by difference. Since there is little data available regarding endogenous protein flow, undegraded protein is calculated as the difference between total protein flow at the duodenum and microbial protein (Merchen et al. 1980; Prange et al. 1980; Stern and Satter 1982).

The use of digesta collection to calculate undegraded feed N is a relatively inaccurate method. The reason for the large error is that the feed N is normally the smallest fraction and, since it is determined by difference, it means that the error of measurement is similar in magnitude to the error in determination of microbial protein (Ørskov 1982). The method requires a lot of effort in the collection and the analysis of samples. The animals used require an adjustment period of at least 2 weeks. The technique is too laborious for routine feed evaluation. The technique's most serious disadvantage is that it is only applicable to dietary conditions in which the rate of outflow was similar to that which occurred during the actual experiment and collection of digesta.

In Vitro Method

There are various in vitro techniques and these include; ammonia release using rumen inoculum; nitrogen solubility in buffers and other solvents; the rate of protein hydrolysis using various commercial proteases; and ammonia plus total amino acid release in rumen inoculum modified by an inhibitor of metabolism of protein degradation products.

The ammonia release technique has fallen to disuse for two reasons. Experiments were conducted using large quantities of protein source and were interpreted without regard for microbial uptake, as well as release of ammonia (Broderick 1982). Ammonia release from cottonseed meal was found to be faster than casein suggesting greater ruminal degradation. However, it is now known that casein is degraded to the greater extent (Broderick 1982). Experiments with corn and sorghum grains resulted in negative ammonia release because readily fermentable carbohydrate stimulated greater microbial uptake of ammonia. The presence of fermentable energy sources with most feed proteins make this technique unreliable.

The nitrogen solubility technique has been used by many researchers (Craig and Broderick 1981; Crooker et al. 1978; Hendrickx and Martin 1963; Mahadevan et al. 1980). Hendrickx and Martin (1963) found that the degradation of purified proteins during rumen fluid incubation was correlated ($r = .99$) with their solubility in Burrough's buffer. The solvents used most often to date for the nitrogen solubility technique have been: 1) 10% Burrough's buffer; 2) autoclaved rumen fluid ARF; 3) McDougall's buffer; 4) NaCl solution; 5) hot water; and 6) dilute NaOH (Broderick 1982). A series of experiments by Crooker et al. (1978)

showed that ARF was the least desirable solvent due to sampling and uniformity problems, and that NaCl was the best solvent to use since it was the simplest and most convenient to prepare. Broderick (1982) found that there was too much variation using the solubility technique. The soluble N fraction of one feedstuff was found to vary in different solvents. For example, the solubility of corn gluten meal was found to be very high in NaCl solution, but very low in Burrough's buffer (Broderick 1982). The solubility of different feedstuffs was found to be similar in the same solvent, such as SBM and oats (Broderick 1982). In a series of experiments using cottonseed meal and correlation techniques, Broderick and Craig (1980) concluded that NaOH ($r^2 = .93$) and McDougall's buffer ($r^2 = .83$) were the most accurate predictors of degradation and Burrough's buffer ($r^2 = .55$) was the poorest predictor. McDougall's buffer and NaOH were probably both sensitive to changes in the properties of the protein fractions which were normally classified as insoluble, but which quantitatively represent most of the degraded protein (Broderick and Craig 1980). Solubility alone is not the only limiting factor of protein degradation, protein structure also plays an important role in degradation. This, in combination with the variability of the nitrogen solubility technique make quantitative interpretation of nitrogen solubility data difficult.

The amino acid plus ammonia release technique is a new in vitro system for estimating ruminal protein degradation rate. Hydrazine sulfate, when added at 1.0 mM to an incubation medium consisting of strained rumen liquor (SRL) and McDougall's buffer, effectively inhibits removal of added amino acids and ammonia by rumen microorganisms

(Broderick 1978). Therefore, protein degradation may be estimated from the accumulation of these endproducts. Broderick (1978) applied this procedure to casein and observed mean in vitro and in vivo degradation rates for casein of .30% and .46%/hr, respectively. Casein escape was estimated to be 11.8% from in vitro data and 8.0% from in vivo data (Broderick 1978). In studies with cottonseed meal, Broderick and Craig (1980) determined protein degradation rates from a bioexponential interpretation of in vitro data on amino acid and ammonia release. The results were comparable to those previously reported from in vivo feeding studies. Estimating ruminal protein degradation from accumulation of endproduct may result in inaccuracies because this method does not take into account the rate of passage out of the rumen, which is also an important determinant of protein degradation.

Artificial Fibre Bag Technique

To date, the preferred (Ørskov 1982) method of obtaining quantitative estimates of degradability is the artificial fibre bag technique, also known as the nylon bag technique and the in situ technique. This technique allows for the estimation of rate of degradation which cannot be derived from digesta collection techniques. The method is not new, it has been documented since 1938 (Ørskov 1982). Artificial fibres, such as nylon or polyester, are now utilized since they are totally resistant to microbial degradation.

The technique has been subject to some uncertainty as to the period of incubation which would be most appropriate, this being dependent upon the time that protein is retained in the rumen (Ørskov and McDonald

1979). Pore size of the bags must also be adjusted for finely ground feeds, and the pore size must be a compromise to allow entry of microbes and escape of gas, but prevent losses of solid particles. Nocek (1985) investigated specific variables associated with the in situ digestion of SBM. Bag porosities of 6 and 20 μm gave the lowest, and 80 and 102 μm gave the highest rate constants of in situ DM and N disappearance (Nocek 1985). Bag porosities of 40 59 80 and 102 μm compared favorably with in vivo ruminal available protein studies (Nocek 1985). Ørskov and McDonald (1979) used dacron bags with pores of 50 μm , and Crawford et al. (1978) used bags with 35 to 70 μm pores. Therefore, bags with a pore size of 40-50 μm seem appropriate for use.

Sample size to surface area of the bag is also critical. Crawford et al. (1978) reported that apparent N disappearance from dacron bags increased with decreased ratio of sample mass to bag surface area, but N disappearance plateaued at 3.5 mg/cm^2 . Craig (1978) did not observe a plateau, N disappearance continued to increase with sample mass to surface area as low as .7 mg/cm^2 . Ørskov and McDonald (1979) and Mehrez et al. (1980) conducted in situ studies with ratios of sample mass to surface area of 8 mg/cm^2 . Nocek (1985) used a sample weight to surface area ratio of 12.6 mg/cm^2 that resulted in acceptable estimates of ruminal protein availability.

Another important problem is that microorganisms invade the bag and some may become attached to feed particles in the bags. This causes reduced apparent nitrogen disappearance. Not all of the microbes are readily rinsed away during the normal washing period after rumen incubation. Craig and Broderick (1980) tried to correct for this by

using blank (empty) in situ bags containing N free material. Microbial N contamination can be determined through analysis for diaminopimelic acid, the amino acid specific to the cell wall of most bacteria (Ørskov, 1982). Mehrez (1977) reported that bacterial protein amounted to less than 1% of the total protein in the bags.

DeBoer et al. (1987) reported that DM and N disappearances from small bags (3.5 x 5.5 cm) were lower than from large bags (7.0 x 11.0 cm). These differences were attributed to the variabilities in the hand washing technique. They devised a mechanical washing device which was designed to reduce the variability due to hand washing. After the utilization of this device, there were no significant differences for DM or N disappearance between bag sizes (DeBoer et al. 1987).

The feed sample to be incubated must represent the feed as it would appear in the rumen, i.e., as if it has been consumed by the animal. For dry protein supplements no preparation is required. For other dry materials, they should be passed through a hammermill with a screen size of 2.5-3.0 μm (Ørskov 1982). For green materials, succulant materials and silage, a mince is more appropriate with a 5.0 μm screen size (Ørskov, 1982). The diet given to the animal while incubation is taking place must be similar to the feedstuff for which the results are to be applied. The type of diet fed will affect the rate of protein degradation. An example of this is that a protein supplement of vegetable origin will be degraded more slowly in an animal given a high-concentrate diet, than in an animal given a high-forage diet (Ørskov 1982).

Different feedstuffs have different rates of degradation. The

simplest rate is when the substrate starts to degrade as soon as it is incubated in the rumen. It contains no water soluble fraction and in time will completely degrade. A formula developed by Ørskov (1982) illustrates this:

$$p = 100 (1 - e^{-ct})$$

where p is the amount degraded at time (t) and (c) the degradation rate for N disappearance. This is a very simplistic description and very few feeds degrade according to this formula. Most protein supplements that are incubated using the bag technique follow a pattern of rapid initial disappearance, reflecting N solubilization as well as protein degradation, followed by a slower rate of N disappearance during longer incubation times. This type of degradation can be described by the kinetic interpretation of Ørskov and McDonald (1979):

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

where p is the amount degraded at time (t) but (a) (b) and (c) are constants in the exponential equation. The proportion and degradation rate of this more slowly degraded fraction (b) was quantified from the regression on time of the log of the fraction of N remaining in the bags (Broderick 1982). The slope and intercept of this regression corresponded to degradation rate (c) and proportion (b) of the more slowly degraded fraction (Broderick 1982). The rapidly degraded fraction (a) was estimated by difference $a = 1 - b$ and assumed to be completely degraded in the rumen (Broderick 1982). That which is totally undegradable in the rumen can be shown by $100 - (a + b)$ (Ørskov 1982).

The (b) fraction of a feedstuff is of greatest concern, since the

(a) fraction is assumed to be degraded instantaneously, and the insoluble fraction will not be degraded at all. The degradation rate (c), that applies to the (b) fraction, must be applied to rumen outflow rate (K) (Ørskov 1982). Outflow rate is another important factor in measuring degradation. There are two possible fates for feedstuffs entering the rumen. They can continue to be degraded or they can flow out at any time and escape degradation. Outflow rate depends on the particle size of the basal feed (Ganev et al. 1979). If the basal feed consisted of long particles, then the outflow rate of small particles would be faster than if the basal feed itself also consisted of small particles. Therefore, the outflow rate of small particles is affected to a large extent by the structure of the feed and the level of feeding (Ørskov 1982). Outflow rates are difficult to determine, mainly due to the problem of distinguishing between dietary flow and microbial N flow from the rumen.

Calculating Rumen Outflow Rate

Uden et al. (1978) developed a method where chromium (Cr) was mordanted to the protein source under study. This process renders the protein insoluble and undegradable. The outflow of protein from the rumen can then be followed by determining the flow of chromium. This method has since been used by many researchers: Ganev et al. (1979); Mehrez et al. (1980); Ørskov and McDonald (1979); Ørskov et al. (1983); and Stern et al. (1980b). The rate of dilution of Cr in samples of rumen contents can therefore provide an estimate of the rate of passage of protein from the rumen. Ørskov and McDonald (1979) plotted Cr

concentrations on a logarithmic scale which showed a linear decrease with time. The rate of decrease (K) can be estimated by regression analysis and can be interpreted as the rate constant at which the treated protein passes from the rumen to the abomasum, if it is assumed that the weight of rumen contents remains approximately constant (Ørskov and McDonald 1979).

Another method of determining (K) is to take grab samples of the feces instead of rumen samples. Chromium treatment not only renders protein undegradable in the rumen, but indigestible in the lower tract. It can therefore be used with a method developed by Grovum and Williams (1973) where fractional outflow of Cr can be determined from the descending concentration of Cr in the feces. The correlation between fractional outflow rates of Cr as determined from the feces and the rumen was $r = .99$ (Ørskov 1982). The mean values for outflow rates were 0.21 and 0.23%/hr, estimated from the rumen and feces respectively (Ørskov 1982). Hartnell and Satter (1979) found no significant difference between grab sampling and other methods for determining the rate of passage for liquid, grain and hay.

Dhanao et al. (1985) question the use of a single exponential equation, and the assumption that the slower rate constant represents outflow from the rumen. Dhanao et al. (1985) reported that outflow from the caecum may also be slow. They proposed a new model that provides two rate constants, which in theory relate to the two compartments with the longest mean retention time. Their formula for describing fecal outflow rate was:

$$y = Ae^{-C_1 t} \exp [-Be^{-C_2 t}]$$

This model was found to be superior to all other published models, including that of Grovum and Williams (1973). However, it has yet to be demonstrated clearly which rate constant belongs to the rumen and which one belongs to the caecum (Dhanao et al. 1985).

There has been much discussion as to whether water soluble markers are as good as solid markers for measuring rumen outflow rate. Teeter and Owens (1983) examined five water soluble markers for measuring rumen outflow rate. They examined polyethylene glycol and ethylenediaminetetraacetic acid (EDTA) complexes of Cr, Co, Fe and Yb. They found all of the markers to be suitable for measuring rumen outflow rate (Teeter and Owens 1983).

To date many estimates have been made using the fecal concentration method. The values reported so far range from 1.0%/hr for maintenance feeding of ground diets to sheep (Mansbridge and Ørskov 1980), to 10.0%/hr for feeding at a high level of intake for dairy cows (Eliman and Ørskov 1985).

Once K has been determined, the percentage of protein actually degraded can be calculated from a formula developed by Ørskov (1982):

$$p = a + \frac{bc}{c + K}$$

where (a) (b) and (c) are the constants from the equation $p = a + b(1 - e^{-ct})$ describing degradation, and K is the outflow rate.

Protein supplements with a large (a) value and little or no (b) value, such as well preserved fishmeal, the outflow rate will have little effect on degradability (Ørskov 1982). Protein supplements that have a large (b) value and a high rate of degradability, such as sunflower meal, their actual degradability will change from 97.9% to

85.5% when K increases from 1.0% to 10.0%/hr (Ørskov 1982). Outflow rate, therefore, has the greatest effect on protein supplements that have a large (b) value and a low (c) value. Ørskov (1982) showed that the ranking of protein based on degradation can also change at different outflow rates. Therefore, some protein supplements are more suitable in feeding situations with low outflow rates, and others at high outflow rates.

Measurement of Microbial Nitrogen

Many methods have been developed to estimate bacterial nitrogen. Of these, diaminopimelic acid (DAPA) has been the most widely used (Amos and Evans 1976; El-Shazly and Hungate 1966; Evans et al. 1975; Hogan and Weston 1971; Hutton et al. 1971; Ørskov et al. 1971; Rahnema and Theurer 1986). The amino acids lysine and leucine have also been used to estimate microbial N (Muntifering et al. 1981; Potter et al. 1971; Rahnema and Theurer 1986). Nucleic acids and radioisotope tracers for microbial N determination have become more common over the years (Pilgrim et al. 1970; Prigge et al. 1978; Salter et al. 1979). Comparative in vivo experiments using various marker techniques have been used (Harmeyer et al. 1976; Ling and Buttery 1978; Nikolic and Jovanovic 1973; Smith et al. 1978; Walker et al. 1975). The use of 2-aminoethylphosphonic acid (AEP) as a marker for protozoal nitrogen has so far been unsuccessful (Abou-Akkada et al. 1968; Ling and Buttery 1978; Rahnema and Theurer 1986). The following are the most common methods in use today.

1. Protein-free purified diet

The use of a purified protein-free diet minimizes the complication of distinguishing between feed N and microbial N (Ørskov 1982). Animals are fitted with postruminal cannulae and all digesta flowing out of the rumen is collected to determine N flow. Ammonia N is then subtracted from this value. The uncertainty with using this method exists in the determination of the endogenous N contribution from abraded epithelial cells and enzymes.

2. Diaminopimelic acid (DAPA)

Diaminopimelic acid (DAPA) is the amino acid found in the cell wall of many, but not all, rumen bacteria. It has been used extensively to measure the microbial protein entering the duodenum. Samples are obtained from strained rumen fluid and compared with the DAPA content in the duodenum. The disadvantages of this method are that DAPA is contained in only some bacteria, usually from the fluid phase, and the amount of DAPA relative to bacteria N can vary between different species of rumen bacterial (Purser and Beuchler 1966). However, it has been demonstrated that on fixed dietary regimes the N:DAPA ratio of bacteria remains reasonably constant (Hutton et al. 1971). Another disadvantage, when using an amino acid analyzer, is that methionine and DAPA have similar elution patterns. This can be overcome, however, through conversion of methionine to methionine sulfone by performic acid (Ibrahim et al. 1970). This makes it possible to distinguish between DAPA and methionine. The assumptions inherent in the use of DAPA as a marker for bacterial N are that no feed DAPA is degraded in the rumen, the protozoal contribution to the abomasum is minimal and that

endogenous N in the abomasum contains no DAPA (Rahnema and Theurer 1986). In a study that compared DAPA, lysine, and leucine for estimating bacterial N, Rahnema and Theurer (1986) found that corrected DAPA consistently gave the best estimate of bacterial N.

3. Nucleic acids

This method is based on the assumption that there is a constant proportion of nucleic acid in microbial N. It includes protozoal N, but it assumes that the feed is free of RNA, or that RNA from the feed is degraded in the rumen. However, many feeds do contain RNA, especially protein supplements of animal origin (Ørskov 1982). Nucleic acids are therefore not very reliable markers of microbial N.

4. Amino acid profile in postruminal digesta

Microbial protein has a constant amino acid composition and is independent of the diet given. Therefore, variation in the amino acid composition of the digesta entering the duodenum should be mainly due to the variation in the amino acid composition and quantity of feed protein escaping rumen degradation (Varvikko 1986). A method was devised based on this theory, whereby the amino acid profile in abomasal or duodenal fluid could be used to measure microbial N. The disadvantage with this method is that the amino acid composition of endogenous secretions, and of many feedstuffs, are not different enough from microbial protein to make an accurate distinction. Varvikko (1986) reported that errors were particularly large with fibrous or starchy feeds of low protein content. This method gives the lowest measurement of microbial N in comparison with other methods that have been used.

5. Isotopes ^{35}S or ^{15}N and ^{32}P

The most reliable method, so far, for determining microbial N, is the use of a nuclide label. The most common labels are ^{35}S , ^{15}N and ^{32}P . These are incorporated into the microorganisms after infusion of the label into the rumen (Beever et al. 1974; Kennedy et al. 1984; Mathers and Miller 1980; Matheson and Milligan 1971). The disadvantages of this method are that the microorganisms have to be isolated from the rumen fluid. Many microbes become attached to digesta particles making it difficult to obtain a microbial fraction that is representative of the population. Kennedy et al. (1984) found that in vivo and nylon bag estimates of rumen degradation of forage diets were subject to unacceptable errors. Methods for accurate measurement of endogenous protein secretions and microbial flow from the rumen are required to make isotope use more practical.

INTESTINAL PROTEIN SUPPLY

The nitrogen that enters the duodenum is a combination of microbial crude protein, undegraded feed protein, and endogenous protein.

Bacterial crude protein and undegraded crude protein influence the supply of absorbable amino acids to the greatest extent. Digestion of protein in the abomasum and small intestine is essentially the same as for monogastrics except for a few differences (Van't Klooster and Boekholt 1972).

Microbial Protein Composition and Nutritive Value

A large part of the dietary N reaching the small intestine will be of microbial origin. Weller et al. (1958) reported that rumen microbial N accounted for 63-82% of the dietary N. In other studies, about 50% of the protein passing from the rumen to the small intestine of sheep and calves, was of microbial origin (Hogan and Weston 1970; Smith and McAllan 1971). Ørskov (1982) estimates that, under most dietary conditions, the microbial protein synthesized in the forestomach of ruminants accounts for 60-85% of the total amino acid N entering the small intestine.

The amino acid composition of microbial protein appears to remain constant regardless of dietary and animal conditions, and the differences in composition of microbial protein and of animal protein is quite small (Chamberlain and Thomas 1979; Merchen et al. 1986; Prange et al. 1984; Storm et al. 1983; Zinn and Owens 1982). Although amino acid N probably makes up about 79% of the total microbial N, Storm et al. (1983) found that in a large sample of isolated rumen microbial biomass

RNA was 11.3% and DNA 4.1%, giving a total value of nucleic acid N of 15.4% of the total microbial N. Nucleic acid N however is unlikely to be utilized to any great extent in the animal body. Microbial N may also contain other nonprotein compounds such as N-acetylglucosamine as cell wall components (Ørskov 1982).

Information on the digestibility of microbial N is difficult to obtain due to the problems of isolating microbial protein. Most of the work so far has been done with rats and determined as the digestibility in the whole digestive tract. In data summarized by Bergen (1978) digestion of pure cultures of rumen bacteria in vitro ranged from 0.44 to 0.93. Zinn and Owens (1982) reported true absorption of rumen bacterial and protozoal protein in rats to be 0.66 and 0.88 respectively. Studies with 35S yielded values of 0.74 (Bird 1972) and 0.85 (Salter and Smith 1977). A study using 15N gave a value of 0.79 (Salter and Smith 1977). Tas et al. (1981) used regression analysis and obtained an estimate for microbial amino acid digestibility of 0.87. Zinn and Owens (1982) used regression analysis and obtained a lower value of 0.73. Storm et al. (1983) reported that the true digestibility of microbial amino acids was 0.84, closer to the value reported by Tas et al. (1981). Storm et al. (1983) also estimated that the digestibility of RNA and DNA was 0.87 and 0.81, respectively. These values compare well with the digestibility estimates of Smith and McAllan (1971) of 0.89 and 0.80 for RNA and DNA respectively.

Many researchers have found the digestibility of microbial methionine to be higher than that of other amino acids. However, this was not confirmed by the work of Storm et al. (1983). Armstrong and

Hutton (1975) found the digestibility of cystine to be quite high, while Sharma et al. (1974) and Storm et al. (1983) did not. The digestibility of the different amino acids varied little and only cystine and histidine showed values that were significantly lower than the average (Sharma et al. 1974; Storm et al. 1983). In general, the true digestibility of amino acids in the small intestine appears to be about 85%, and there appears to be no difference among microbial amino acids in their digestibility.

Apparent Absorption of Feed N

Measuring the disappearance of N or amino acids between the duodenum and ileum provides an estimate of apparent absorption. In general, the apparent absorption of nonammonia N (NAN) appears to be .65 and .68 of the amino acids entering the duodenum (Bull et al. 1985). In an experiment using various protein sources, Santos et al. (1983) reported the apparent absorption of NAN to be 63.3% 70.9% 64.6% and 57.9% for soybean meal (SBM), corn gluten meal (CGM), wet brewer's grains (WBG) and dried distillers grains (DDG), respectively. The apparent digestibilities of total amino acids were 70.3% 76.5% 71.1% and 65.5% for SBM, CGM, WBG and DDG, respectively. Van't Klooster and Boekholt (1972) found the apparent absorption of NAN to be 65% and of amino acids to be 73%. In general, the apparent absorption of NAN seems to be less than the absorption of amino acids. Tamminga (1980) concluded from various experiments that apparent absorption of total N is usually .05 less than that of amino acids. Bull et al. (1985) suggests values for apparent absorption from the small intestine of NAN

and amino acids to be .65 and .70, respectively, of the amounts entering the duodenum.

The apparent absorption of essential amino acids (EAA) appears to be about .05 greater than nonessential amino acids (NEAA) (Tamminga 1980). Van't Klooster and Boekholt (1972) reported values of 75% for EAA and 72% for NEAA. However, Santos et al. (1983) reported values of 64% for EAA and 67% for NEAA. To date, apparent absorptions of EAA suggest that absorption of Lys and Arg is greater (Sharma et al. 1974; Armstrong and Hutton 1975; Santos et al. 1983; Bull et al. 1985), while absorption of Thr, Val and Phe is less (Sharma et al. 1974; Bull et al. 1985) than the absorption of total EAA.

True Absorption of Feed N

True absorption is the sum of apparent absorption and endogenous loss. Endogenous protein enters the small intestine in the form of enzymes, bile, mucus, serum albumin, lymph, epithelial cells, and other degradable products from the gastrointestinal lining. The N in duodenal contents from abomasal juice, pancreatic juice, bile and epithelial cells was estimated at $0.004 \times \text{DM consumed}$ (Tamminga et al. 1979). Hogan and Weston (1970) used regression analysis to calculate the endogenous loss from the small intestine that appeared in the feces, as $0.0016 \times \text{organic matter (OM) entering the duodenum}$. The endogenous loss appearing in the feces from the entire tract was $0.004 \times \text{OM consumed}$. Hogan and Weston (1970) and Hogan (1965) concluded that only about 1/3 of the N in the metabolic fecal portion is of endogenous origin, and the remaining 2/3 is of microbial origin.

To date, estimates of endogenous losses for nonlactating cattle are 0.77 g/d NAN (Zinn and Owens 1982; Sharma et al. 1974) and 98 g/d amino acids (Sharma et al. 1974). Estimates for lactating cattle are 0.13% of the N supply to the proximal duodenum (Merchen 1981; Merchen and Satter 1983).

Estimates of true absorption can also be obtained from regression analysis. The true absorption of NAN from the small intestine of sheep was reported to be 0.76, and of EAA it was 0.80 (Hogan and Weston 1970). Tas et al. (1981) reported values for NAN in sheep to be 0.80, and of amino acids to be 0.86. True absorption values obtained by isotopically labeling plant materials with ^{15}N gave values of 0.85 for leaf protein absorption (Salter and Smith 1977). Smith et al. (1974) used ^{14}C labeled chloroplast protein which gave a range of 0.73 to 0.82 for absorption. Zinn and Owens (1982) reported lower values for true absorption of NAN for nonlactating cattle of only 0.68. Bull et al. (1985) suggested the following values for true absorption of NAN and amino acids from the small intestine, 0.75 and 0.80 respectively, of amounts entering the duodenum.

Amino Acid Uptake

The mucosa of the small intestine contains uptake systems for free amino acids, peptides, nucleotides, and nucleosides. In studies with sheep the mid to lower ileum has been found to be the most active site for amino acid uptake (Bull et al. 1985). However, the highest rate of amino acid disappearance in situ from the digesta has been found in the mid jejunum (Bull et al. 1985). It has been demonstrated (Johns and

Bergen 1973) that amino acid uptake in sheep occurs against a concentration gradient, exhibits saturation kinetics, and depends upon metabolic energy. It has been shown by various researchers that EAA are preferentially absorbed over NEAA (Bull et al. 1985; Santos et al. 1983). Amino acid absorption has been ranked as follows, by Bull et al. (1985), using exteriorized intestinal loops:

Ile>Arg>Val>Leu>Met>Phe>Lys>Try
>Asp>Ser>Ala>Pro>His>Thr>Glu>Gly

Johns and Bergen (1973) used jejunal strips in vitro and found Met>Lys>Gly. Phillips et al. (1976) used everted sacs in vitro and found Met>Val>Thr. Prange et al. (1984) in a study with lactating cows found that the apparent absorption of Met Arg Gly Lys Val Thr and Phe was greater than the average for total amino acids. The apparent absorption of Ser Pro Ala and Ile was lower than the average for total amino acids (Prange et al. 1984). In general, the order of uptake of amino acids from the small intestine of sheep is similar to that of man (Bull et al. 1985).

PROTECTED PROTEIN

Numerous methods have been proposed for increasing protein bypass. The use of feeds that are relatively resistant to rumen degradation, heat treatment, and chemical treatment, all have potential for improving animal productivity through increased protein bypass. The ideal method for protein protection should decrease dietary protein degradation in the rumen, without adversely affecting other aspects of rumen metabolism, and should increase the total supply of nonammonia nitrogen to the small intestine.

Protein Sources that are Resistant to Rumen Degradation

Protein sources that are relatively resistant to rumen degradation are often desired for supplementation of diets for young growing ruminants or high producing dairy cows in early lactation. The feeds most often studied for these purposes are corn gluten meal and feed, distillers grains and brewers' grains.

Research carried out to date on corn gluten meal indicates that it is a relatively resistant protein source. In the wet-milling process corn is steeped in dilute acid and some solubilization of protein and hemicellulose may occur. Since solubility may influence the extent of rumen protein degradation, the protein in corn gluten meal and feed may be degraded more rapidly than protein from other byproducts (Stern et al. 1983). Research has shown that microbial degradation of protein in corn gluten meal ranged from 38% to 54% when fed to growing cattle (Waller 1978; Zinn et al. 1981). Stern et al. (1983) used regression technique to find that 57% of the protein in corn gluten meal left the

rumen undegraded. However, Firkins et al. (1984) found that the rumen escape protein for wet corn gluten feed was only 26% and for dry corn gluten feed only 14%. The difference in values for wet and dry corn gluten feed are attributed to the fact that the mean particle size is larger for wet corn gluten feed and this, therefore, decreased the susceptibility of the protein to microbial attack (Firkins et al. 1984).

Total amino acid degradation in the rumen of corn gluten meal was 43% as determined by linear regression (Stern et al. 1983). This compares well with the values found by Waller (1978) and Zinn et al. (1981) that ranged from 38 to 54% in growing cattle. Stern et al. (1983) found that the six most degradable amino acids were the essential amino acids, with the basic amino acids ranking near the top. The most degradable of the amino acids was lysine, of which only 38% escaped degradation (Stern et al. 1983). The basic amino acids have been shown to be relatively more degradable and threonine less degradable than the total amino acid degradability for several protein supplements (Stern and Satter 1982). Chalupa (1976), however, found that threonine was the second most degradable essential amino acid.

Stern et al. (1983) reports that corn gluten meal has potential as a resistant protein supplement for lactating dairy cattle, especially when complemented with a relatively resistant protein source high in lysine. Firkins et al. (1984) suggest that even though wet and dry corn gluten feed is degraded more rapidly in the rumen than other byproduct feeds, it could still be used to replace a very rapidly degraded protein source such as SBM.

The rumen escape of dry distillers grains (DDG) has been

extensively researched (Firkins et al. 1984; Satter et al. 1977; Waller et al. 1980). The rumen escape of wet distillers grains has not been researched as much (Firkins et al. 1984). The rumen escape of WDG was found by Firkins et al. (1984) to be 47% and of DDG to be 54%. Satter et al. (1977) found the value for DDG to be 60%, while Santos et al. (1983) determined that 53% of the protein in DDG escaped degradation compared with 30% for SBM. Firkins et al. (1984) found no difference in rumen degradation between DDG protein and WDG protein in the rumen of steers. The feeding of both may be beneficial to growing and lactating cattle.

In a study by Davis et al. (1983), pressed brewers' grains were substituted for ground corn and SBM at 0, 20, 30 and 40% of the ration DM and fed to lactating dairy cows. At the 40% level DM intake was suppressed, but 4% fat corrected milk was the same for all diets (Davis et al. 1983). At the 40% level of intake no effects were apparent on DM or N disappearance from dacron bags (Davis et al. 1983). Milk yield was not affected by dietary treatments and therefore efficiency of milk production favored the pressed brewers' grains diets over the control. Davis et al. (1983) theorize that the nutrients in wet brewers' grains are utilized more efficiently than those in the control diets. Porter and Conrad (1975) compared dried and wet brewers' grains in the rations of lactating dairy cows at 20% of the total DM. Milk production was reported to be equal for the two rations even though DM intake was significantly lower on the diet containing the wet brewers' grains. Conrad and Rogers (1977) also found DM intake depressed by rations containing wet products, but milk production was essentially the same

for all rations tested. Murdock et al. (1981) found no depression of DM intake when wet brewers' grains made up 30% of the total ration DM for dairy cows. Most of the research so far indicates that wet brewers' grains are equal to or possibly superior in nutritional value to dried brewers' grains.

Satter and Whitlow (1977) reported that the protein in brewers' dried grain was highly resistant to rumen degradation. In a study by Merchen et al. (1979) animals fed brewers' dried grain, or a combination of brewers' dried grain and urea, had consistently higher levels of total nonammonia nitrogen reaching the abomasum, than did animals fed all-urea supplements, and levels equal to those fed SBM and urea combinations. The bypass values of brewers' dried grain for two trials were reported at 61 and 48%, whereas values for SBM were reported to be only 24% (Merchen et al. 1979). The feeding of brewers' dried grain and brewers' dried grain-urea diets tended to produce more valine, leucine, phenylalanine and methionine entry into the intestinal tract than the feeding of urea or SBM-urea diets (Merchen et al. 1979). The flow of lysine into the lower tract was similar for all diets indicating that the lysine to protein ratio is lower for brewers' dried grain than for bacterial protein (Merchen et al. 1979). The results to date indicate that brewers' dried grain is resistant to rumen degradation and therefore has potential to supply more dietary amino acids to the lower tract for absorption than conventional protein supplements.

Heat Treatment

Heat is generated or applied in many procedures used to manufacture feed ingredients. Heat treatment often results in improved animal productivity. The effect is caused by the Maillard reaction which irreversibly binds sugar aldehyde groups and free amino groups, thereby decreasing the rumen degradability of the protein. However, as a result of this binding, the protection of protein by heat treatment is often accompanied by a reduction in availability in the small intestine.

Goering and Waldo (1974) summarized data that demonstrated decreased protein digestibility and animal performance attributable to heat damage in forages. Effective heating time, temperature, and moisture were all related to amount of damage in forages. Temperatures above 60° C for 24 hrs with moisture contents between 20 and 70% resulted in heat damage to forages, but differences in susceptibility of different forages were large (Goering et al. 1973). Soybean meal treated at 180° C for 25 min resulted in extensive rumen degradation (Crooker et al. 1986). Defatted soy flakes were heated at 250° C for 30 min; 250° C for 20 min; 215° C for 20 min; and 180° C for 25 min. The untreated and the heat treated SBM samples demonstrated the most frequent occurrence of changes in amino acid content due to rumen exposure (Crooker et al. 1986). Therefore, heat treatment is ineffective in preserving the amino acid profile of SBM exposed to rumen degradation. However, in vitro studies by Thomas et al. (1979) indicated that temperatures of 138° C up to 149° C for 4 hrs, were needed to minimize degradation without reducing availability in the small intestine. In feedlot trials, heat treatment of 138° C to 149° C,

resulted in 50% faster weight gains and 23% more efficient feed conversion over untreated SBM (Thomas et al. 1979). Sherrod and Tillman (1964) reported that cottonseed meal autoclaved for 60 min produced superior daily gains and feed efficiencies to nonautoclaved meals or meals treated for longer periods of time.

The results of studies to date, on heat treatment, have not been consistent. It is still difficult to arrive at a temperature and time period which will protect the protein from rumen degradation, and at the same time ensure the availability of protein in the small intestine.

Chemical Treatment

Some chemical agents form reversible cross linkages with amino and amide groups which decrease the solubility of proteins at the pH of the rumen. The advantage of the use of these chemical agents, is that the protein is subsequently made available to the host by destruction of these linkages in the acidic abomasum. The agents most investigated include aldehydes (Crawford and Hoover 1984; Crooker et al. 1986; Ferguson 1971; Fohman et al. 1981; Hatfield 1973); tanning materials (Ferguson 1975; Hatfield 1973; Zelter et al. 1970); acetic acid (Ames and Robeson 1976; Atwal et al. 1974; Vicini et al. 1983); and alcohol (van der Aar et al. 1982a; van der Aar et al. 1984).

Many studies so far have concentrated on the use of formaldehyde to protect protein. Many researchers have found that casein treated with formaldehyde generally results in increased N retention, wool growth, and muscle growth (Faichney 1971; Hemsley et al. 1973; MacRae et al. 1972; Reis and Tunks 1969; Wright 1971). The treatment of plant

proteins, however, has not yielded consistent responses, but growth rates and feed efficiencies have been improved (Driedger and Hatfield 1972; Faichney and Davies 1972; Nimrick et al. 1972; Nishimuta et al. 1972).

Many of the problems associated with formaldehyde treatment have been attributed to overprotection. Stanton et al. (1983) used beef cattle and commercially treated SBM at the following levels: .2 .3 and .6% formaldehyde. The in situ digestion rates appeared to be reduced with .2 and .6% formaldehyde treatment compared with untreated SBM (Stanton et al. 1983). Lactating cow performance and pepsin insoluble N suggest that a lower level of formaldehyde treatment may be more desirable for ruminants fed a low quality roughage. The lactating beef cows seemed sensitive to overprotection of protein, and the weight gain in calves is responsive to changes in protein level fed to the dam (Stanton et al. 1983). In a study by Crooker et al. (1983) SBM was treated with .3 g formaldehyde/100 g SBM and fed to lactating dairy cows. The digestibility of dietary crude protein by cows fed the formaldehyde treated SBM was lower than by cows fed untreated SBM (62.4 vs 65.4%) (Crooker et al. 1983). This indicates that treating SBM with .3 g formaldehyde/100 g SBM may decrease the availability of SBM protein for lactating dairy cows. Crawford and Hoover (1984) reported that formaldehyde treatment reduced the solubility of SBM from 22.7 to 2.9%, it also reduced milk protein (3.08 vs 2.85%) and solids-not-fat (8.51 vs 8.35%). Formaldehyde treatment did not affect intake or overall milk production. Crawford and Hoover (1984) attributed the lack of production response and the reduced milk protein to overprotection of

protein. Research by Crooker et al. (1986) using SBM treated with .3 .6 and .9% formaldehyde showed that formaldehyde preserved the amino acid profile of SBM very effectively. However, it was noted that a decrease in tyrosine and lysine content occurred as a result of formaldehyde treatment. This was attributed to the formation of crosslinked products that were resistant to the 6 N HCl hydrolysis (Crooker et al. 1986).

It has been suggested by Junkins (1981) that optimum treatment may be influenced by rate of passage of the treated feed. At a retention time for solids of 14 hours, treatment with .3% by weight of formaldehyde decreased crude protein digestion by 42% compared to a decrease of only 20% at 24 hrs retention (Junkins 1981). At a shorter retention maximal depression of crude protein digestion could be achieved with only .15% formaldehyde (Junkins 1981). At short retention times, especially for high producing dairy cattle consuming in excess of 3% of body weight, the amount of formaldehyde to adequately protect protein from rumen degradation may be less than for animals at lower intakes and longer rumen retentions (Junkins 1981). Thomas et al. (1979), in a study with beef cows, found increased weight gains and feed efficiencies by feeding SBM treated with .4 to .6% formaldehyde, a level considered to be overprotective. Spears et al. (1980) also found linear increases of gain and feed efficiency for beef cattle fed SBM treated with .9% formaldehyde. Fohman et al. (1981) fed SBM treated with 1.7% formaldehyde to dairy cows. This level was thought to be too low for adequate protection in dairy cows, yet production increased from 38.9 to 40.4 Kg milk/day.

Crawford and Hoover (1984) suggest, however, that dose related to

flow rate may affect the availability of protein postruminally. The rapid flow of digesta through the abomasum and small intestine would result in shorter exposure to acid hydrolysis. This then may reduce the maximum at which formaldehyde could be applied without adversely affecting the availability of amino acids in the small intestine (Crawford and Hoover 1984).

Formaldehyde treatment does inhibit rumen degradation and preserves the amino acid profile of protein supplements such as SBM. However, more research needs to be done to find the optimum level that can be applied without decreasing the availability of amino acids to the small intestine. To date, results have not been consistent; perhaps a single level of protein protection with formaldehyde is not suitable for all diets and production areas.

Other chemical agents have been studied for their ability to protect protein from rumen degradation. Schmidt et al. (1973) studied the effects of glyoxal and hexamethylenetetramine (HMT) treatment of SBM. The treatments were as follows; 1 2 3 4 or 5 ml of 40% glyoxal; or 3 6 12 18 or 36 ml of 41.6% HMT, diluted to a final volume of 18 ml (except the HMT-36 ml). The solutions were then sprayed on an amount of SBM equivalent to 100 g crude protein. All of the glyoxal treatments resulted in gains less than the controls, but these results were confounded by parallel depressions in intake (Schmidt et al. 1973). The response to increasing levels of HMT was not consistent over three totals. Animals consuming SBM treated with HMT-36 gained less than controls and retained less N (Schmidt et al. 1973). At the lower levels of HMT, such as HMT-12, ammonia release was effectively reduced without

corresponding decreases in gains or N retention (Schmidt et al. 1973). This study indicates that since HMT is safe to use and easy to handle, the potential is there for reducing rumen degradation. Glyoxal treatment, however, resulted consistently in depressed intakes and gain.

Thomas et al. (1979 I) conducted experiments to evaluate a wood molasses (WM) product in protecting protein. The WM was applied to SBM at levels of either 10 or 20%. In vitro studies showed that WM at either 10 or 20% was effective in decreasing rumen ammonia release and crude protein solubility (Thomas et al. 1979I). The WM was then used in a feeding trial where it increased body weight gains an average of 36% and improved feed conversion an average of 24% over cattle on control diets (Thomas et al. 1979II). Thomas et al. (1979II) estimated that the WM product protected about 50% of the SBM protein as compared with about 25% which escaped degradation from untreated SBM.

Some researchers have used acetic acid to protect protein and have achieved decreased ammonia production (Ames and Robeson 1976; Atwal et al. 1974; Vicini et al. 1983). Vicini et al. (1983) treated SBM with 8% acetic acid, and used the nylon bag technique to incubate samples for 1 2 4 8 12 16 and 24 hrs. Acetic acid treatment gave lower degradation rates than untreated SBM for DM and N (Vicini et al. 1983). The mode of action possibly involves the lower pH of about 5.0 which may decrease microbial degradation. The SBM treated with acetic acid, also released more amino-N than SBM treated with formaldehyde or untreated SBM, at 12 hrs of incubation (Vicini et al. 1983). This may indicate that acetic acid makes SBM more available than formaldehyde (Vicini et al. 1983).

The treatment of SBM with various alcohol-water mixtures has

resulted in lower protein solubilities in Burroughs' mineral mix, slower rates of in situ disappearance and lower in vitro ammonia release (van der Aar et al. 1982a). A study was conducted using five N sources: urea; hexane extracted dehulled SBM (control); SBM treated with 50% ethanol; SBM treated with 40% propanol; and SBM treated with ethanol in combination with heat and pressure (EHSBM) (van der Aar et al. 1984). The SBM treated with 50% ethanol and 40% propanol resulted in lower protein solubilities and slower rates of degradation during in situ digestion, than did the control SBM (van der Aar et al. 1984). The control SBM had the fastest rate of in situ degradation and the lowest percentage of escape, whereas the EHSBM was the most resistant to in situ degradation and resulted in the highest percentage of escape (van der Aar et al. 1984).

CANOLA MEAL

One of the most important oilseed crops in Canada is rapeseed (canola). Canola are new cultivars of rapeseed that are low in erucic acid and glucosinolates. The seed contains about 40-42% oil and yields a protein supplement containing 35-40% protein after the oil is extracted. The oil accounts for about 46% of the vegetable oils used by Canadian consumers and the meal is marketed widely both within Canada and abroad.

Rapeseed (*B. campestris*) was first introduced to Canada in 1936, and the *B. napus* type from Argentina was introduced a few years later (Bell 1984). In 1968, the first low erucic acid cultivar was produced in Canada, to be followed by several more until the first "double low" cultivar, Tower (*B. napus*), low in both erucic acid and glucosinolates was licensed in 1974. The low glucosinolate nature of Tower was obtained from a Polish cultivar, Bronowski (Bell 1984). Another new "double low" cultivar of *B. campestris* was soon produced afterward. The production of high glucosinolate cultivars had nearly ceased in Canada by 1981 (Bell 1984). The low glucosinolate cultivars include Tower, Regent, Candle and Altex. The name "canola" was adopted in 1979 to apply to all "double low" cultivars. Canola is characterized by having less than 3 mg of glucosinolates per gram, and less than 5% erucic acid (Bell 1984).

Composition of Canola Meal

Commercial canola meal (CM) contains 12.1% crude fibre, most of which is derived from the hulls (Bell 1984). The ether extract is 4.1%

and is composed of residual oil plus gums derived from oil refining and added back to the meal (Bell 1984). The N-free extract and gross energy value of the hulls and the meal are about equal. The amino acid composition of CM compares favorably with that of SBM. The CM contains more sulphur amino acids and slightly lower lysine values.

Canola meal has a lower energy level than SBM mainly due to the higher fiber content in the hull fraction. The hulls make up about 16% of the seed weight (Appelqvist and Ohlson 1972). This is equivalent to about 30% of the oil-free CM. To date reports on the crude protein content of the hulls are highly variable. This is attributed to the difficulty of separating the hulls from the seed embryos (Bell and Shires 1982). Finlayson (1974) took special care to remove the seed embryo and reported a crude protein value of 12% for the hulls. Other researchers have since reported values around 16%. It has been suggested that the protein of the hull fraction would be very poorly digested (Finlayson 1974).

It is difficult to assemble an accurate composition for canola hulls. There is a lot of uncertainty about the amounts of lignin and polyphenols present (Bell 1984). Cellulose seems to be the dominant carbohydrate and most of the remaining carbohydrates are pentosans (Bell 1984).

Canola meal has a higher B vitamin content than SBM, except for pantothenic acid. Most minerals are higher in CM than in SBM, especially calcium, phosphorus and selenium. However, the phytin content in CM is quite high and its effects on phosphorus, calcium, and zinc may require attention when formulating diets. It is possible that

two-thirds of the phosphorus in CM is bound in phytin (Bell 1984).

The Use of Canola Meal in Dairy Rations

Before the development of canola, rapeseed meal (RSM) was studied in dairy diets. Incorporation of RSM into the diet at the level of 13 to 27% of the concentrate was found to significantly reduce concentrate intake (Ingalls et al. 1968; Waldern 1973). It was also shown to reduce milk yield by Waldern (1973) but this was not shown by the work of Ingalls et al. (1968). The high glucosinolate content was attributed as the cause of the reduced animal performance.

Laarveld and Christensen (1976) reported that incorporation of a "double low" cultivar RSM in the ration at the level of 8 to 30% tended to increase milk yield and total solids. The feeding of a high glucosinolate RSM (Span) at the same levels had no effect on milk production (Laarveld and Christensen 1976). Ingalls and Sharma (1975) included RSM of the cultivar Bronowski into a lactating dairy cow diet, up to the 24% level without affecting grain consumption, milk yield or milk composition. Sharma et al. (1977) incorporated "double low" cultivars into the diet up to a level of 25%. Feed intake, milk yield, and fat content were not different when compared to SBM (Sharma et al. 1977). Papas et al. (1978) found that the incorporation of RSM at the 30% level actually increased the milk yield of cows. Incorporation of RSM at the 20 and 26% levels resulted in equal milk yields and feed intake with no effect on milk composition. Fisher and Walsh (1976) found that as the proportion of RSM was increased in the concentrate, there was a significant linear depression in milk yield. However, they

reported a high residual oil contamination containing erucic acid and hexane, which may have been related to the negative response.

Sanchez and Claypool (1983) compared CM with SBM and cottonseed meal (CSM) as single protein supplements in complete dairy rations. The levels used were 11.7% CM, 10.4% CSM, and 8.6% SBM. The results reported indicated that actual and 4% fat-corrected milk production did not differ among diets (Sanchez and Claypool 1983). However, the cows fed the CM produced 3.2 and 1.2 Kg more milk per day than the cows receiving SBM and CSM respectively (Sanchez and Claypool 1983). Depeters and Bath (1986) conducted four trials to evaluate the effects of incorporating CSM or CM in dairy diets. The yields of milk, milk components and feed intake were not affected by the different protein supplements (Depeters and Bath 1986).

These studies indicate that CM may be incorporated in dairy rations up to the level of 25% without adversely affecting the performance of lactating dairy cattle. These studies also indicate that CM can equal SBM in rations for dairy cows.

Rumen Degradability of Canola Meal

The increasing use of CM in ruminant rations makes it imperative that its degradability in the rumen be known, since this ultimately determines the amount of undegraded protein that will be available to the animal in the small intestine.

Ha and Kennelly (1983) reported an Effective Degradable Protein (EDP) value of 67.7% for CM and an EDP value of 53.5% for SBM, using the nylon bag technique. Ha and Kennelly (1984), again using the nylon bag

technique, reported that N disappearance from SBM was consistently less than that of CM across an incubation range of 4 8 12 and 24 hrs. The EDP for CM, at a K value of .05, was 65.8% and 53.6% for SBM (Ha and Kennelly 1984). The N in SBM disappeared at a slower rate than CM N during the first 12 hrs of incubation, but disappearance was similar after 24 hr incubation in the rumen (Ha and Kennelly 1984). Ha and Kennelly (1984) reported that the DM disappearance values of SBM and CM were not different, with the exception of 12 hrs incubation where CM DM disappearance was higher than for SBM. The Effective Degradable Dry Matter (EDDM) values were 82% and 95% for CM and SBM respectively. Bailey and Hironaka (1984) reported an EDP value of 58% for CM, and an EDDM value of 57% for CM at a K value of .06. Kirkpatrick and Kennelly (1985) reported that at higher protein levels (19 vs 16%), K at .05, EDP tended to decrease, 66.3 vs 64.5 for CM, and 65.0 vs 61.5 for SBM. The values reported by Kirkpatrick and Kennelly (1985) for EDDM followed the same pattern as EDP. Kennelly et al. (1986) reported protein degradability values for SBM to be 64.6% and for CM to be 70.1%. DeBoer et al. (1987) reported EDP values of SBM and CM to be 75.8 and 73.9% respectively, and EDDM values of SBM and CM to be 81.6 and 70.1% respectively. The K value used was .05. Most of the research to date shows that CM is more degradable in the rumen than SBM, which indicates that more undegraded protein will be available to an animal in the small intestine from SBM than from CM.

The degradability of CM in the rumen can be effectively reduced by chemical treatment. To date the most successful of these is formaldehyde treatment. Varvikko et al. (1983) treated rapeseed meal

(RSM) with 0.4 or 0.8 g formaldehyde/100 g CP. The disappearance of both DM and N was clearly reduced with increasing formaldehyde treatment (Varvikko et al. 1983). Setälä and Syrjälä-Qvist (1984) treated RSM with 0.4 or 0.8 g of formaldehyde/100 g CP, and they also reported a dramatic reduction in protein degradability. Ha and Kennelly (1984) also showed that degradability of CM can be effectively reduced by formaldehyde treatment. They reported that formaldehyde treatment reduced EDDM of CM by about 50%, and it reduced EDP of CM from 66 to 22%. Bailey and Hironaka (1984) added 5 g of formaldehyde/Kg of CM and substantially reduced the degradability of DM and N from CM. They reported that EDDM was reduced from 57% to 34%, and EDP was reduced from 58 to 6%.

In general, the amino acid composition of undegraded CM residue does not deviate from that of the original sample. Many researchers have observed, however, that methionine is the most easily degraded amino acid in the rumen (Setälä and Syrjälä-Qvist 1982; Setälä and Syrjälä-Qvist 1984; Varvikko et al. 1983). This finding may also be typical of CM protein, and indicates that a large proportion of methionine is located in the soluble fraction of CM. In addition to methionine, Setälä and Syrjälä-Qvist (1984) also found that histidine serine and glutamic acid were the most degradable amino acids in feed protein. The glutamic concentration was also found to decrease in protein supplements in experiments conducted by Ganev et al. (1979), Tamminga (1979) and Varvikko et al. (1983). However, Varvikko et al. (1983) did not find a decrease in glutamic acid concentration in SBM. Some researchers have found that alanine, valine, isoleucine and

glycine of CM protein were resistant to rumen degradation (Lewis and Emery 1962; Setälä and Syrjälä-Qvist 1984).

To date little research has been carried out on the digestibility of CM in the lower tract of ruminants. However, a new technique has been developed for the rapid determination of intestinal disappearance in ruminants. The technique is based on the Mobile Nylon Bag Technique designed for pigs by Sauer et al. (1983), and modified for ruminants by Kirkpatrick and Kennelly (1985). The technique involves isolating a small feed sample (1-2 g) in a small nylon bag (3.5 x 5.5 cm) and following it through the entire digestive tract. The animals used must be fitted with rumen and duodenal cannulae. The small nylon bags must be incubated in the rumen for a predetermined amount of time, removed and incubated in pepsin-HCl solution for 3 hrs at 39° C to simulate the effects of the abomasum, then inserted into the duodenum via the duodenal cannula and finally collected in the feces about 16-20 hrs later. This technique has so far yielded results that are similar to those obtained via conventional methods.

Kirkpatrick and Kennelly (1985) reported the digestibility of CM protein to be 70.6%, at a protein level of 16%, and 62.9% at a protein level of 19%. The protein digestibility of SBM was 71.9% at a protein level of 15%, and 79.4% at a protein level of 19% (Kirkpatrick and Kennelly 1985). Rae and Smithard (1985) found that the N digestibility of CM decreased with increasing retention time in the rumen. Intestinal disappearance of rumen undegraded N from CM was 79.1% at 8 hrs of rumen incubation, 74.5% at 12 hrs incubation, and 56.9% at 24 hrs incubation (Rae and Smithard 1985). Intestinal disappearance of rumen undegraded N

of SBM was 90.5% at 8 hrs rumen incubation and 88.5% at 12 hrs incubation (Rae and Smithard 1985). DeBoer et al. (1986) also reported a similar trend in intestinal disappearance of rumen undegraded N of CM, but did not find the same trend for SBM. The research to date shows that the rumen undegraded protein of CM is not as available in the lower tract of ruminants as the rumen undegraded protein of SBM.

MANUSCRIPT I:
INSACCO RUMEN DEGRADATION OF FIVE DIFFERENT
CANOLA MEAL SAMPLES, COMPARED TO SOYBEAN
MEAL, WITH STEERS RECEIVING A DIET
FORMULATED FOR HIGH PRODUCING COWS

ABSTRACT

The nylon bag technique was used to compare canola meal (CM) and soybean meal (SBM) degradability in the rumen with respect to dry matter, protein, energy, and essential amino acids (EAA). A sample of canola meal was obtained from five different processors and designated A, B, C, D and E. A SBM sample was obtained from a processor in Altona, Manitoba, and designated F. Two rumen fistulated steers were used. Small nylon bags 3.5 x 5.5 cm, containing .5 g of sample, were incubated in the rumen for various time intervals, removed and rinsed. Five trials were carried out with the incubation periods as follows: Trial 1 - 16 h; Trial 2 - 12 h; Trial 3 - 8 h; Trial 4 - 4 h; Trial 5 - 30 h. The N degradability value of SBM in the rumen fell within the range of values obtained for CM with 4 to 30 h fermentation periods. Nitrogen degradability of one sample of canola meal was greater than soybean meal after 0, 4 and 30 h of fermentation. With one exception the other four samples of canola meal resulted in similar or less ($P < 0.05$) rumen N degradation at the different fermentation time periods compared with soybean meal. Dry matter degradability of SBM is greater than CM at 30 h, but at the other time intervals it falls within the range of values obtained for CM. Energy disappearance from rumen incubation for SBM is lower than that of CM at 4 and 16 h, but at 8, 12 and 30 h, it is similar to the values obtained from some of the CM samples; however apparent energy digestibility did not appear to increase with longer fermentation periods. Disappearance of EAA from SBM falls in the range of values obtained for EAA disappearance from CM at all time intervals,

except for; histidine at 4 h; methionine at 12 h; methionine at 16 h and in some cases canola sample A. In general, these data suggest that at least four of the CM samples were similar or less degradable for DM, nitrogen and EAA, in the rumen than the SBM samples.

INTRODUCTION

The most important source of nitrogen for rumen microorganisms is dietary crude protein. Rumen microorganisms are highly proteolytic and a high percentage of the dietary protein that enters the rumen is degraded to peptides, amino acids and ultimately deaminated to ammonia. The extent to which a protein source is broken down is influenced by factors such as, protein structure, solubility, processing techniques and residence time in the rumen. The degradability of a protein source in the rumen also determines the amount available for utilization in the small intestine by the animal.

In light of the recently proposed protein systems for the feeding of ruminants, it has become increasingly important to accurately measure protein degradability. The nylon bag technique provides a method of obtaining quantitative estimates of degradability (DeBoer et al. 1987; Ha and Kennelly 1984; Kirkpatrick and Kennelly 1985; Ørskov and McDonald 1979; Nocek 1985; Stern and Satter 1982). This technique allows for the estimation of dry matter and protein degradability at various time intervals, and it allows for the estimation of rate of degradability.

One of the most important oilseed crops in Western Canada is canola. Canola is a cultivar of rapeseed that is low in erucic acid and glucosinolates. Canola meal (CM), used as a protein supplement, can contain up to 40% protein and is marketed widely. The increasing use of

CM in diets for dairy cows makes it imperative to obtain estimates of rumen degradability of CM, as this will determine how efficiently it can be used by a ruminant animal. Studies have been carried out on the use of CM in ruminant diets and its degradability (Bailey and Hironaka 1984; DeBoer et al. 1987; Ha and Kennelly 1983; Ha and Kennelly 1984; Kennelly et al. 1986; Kirkpatrick and Kennelly 1985; Varvikko et al. 1983). Little work has been carried out to compare different canola meal samples or amino acid degradability.

The objectives of this study were, using the nylon bag technique, to compare the rumen degradabilities of CM and SBM with respect to dry matter, protein, energy and essential amino acids.

MATERIALS AND METHODS

Animals and Diets

The rations formulated for high producing cows (Table 1, 2) were fed ad libitum to two rumen fistulated steers. The roughage source had to be changed from alfalfa to brome, after Trial 1, due to an unforeseen delay between Trial 1 and subsequent trials. Protein and fiber levels were similar (Table 2). Hay and concentrate were fed twice daily at a 35:65 ratio.

A sample of canola meal (CM) was obtained from five different processors (Table 3, 4) and designated A, B, C, D and E. A soybean meal (SBM) sample was obtained from a processor in southern Manitoba, and designated F (see Appendix F).

Bag Technique

Small nylon bags (3.5 x 5.5 cm) were made by heat-sealing pieces of nylon, pore size 50 micron (Felco Industries). The bags were weighed

Table 1. Ingredient composition of the rations fed to fistulated steers used in the rumen incubation study (% as fed)

Ingredient	Trial	
	#1 (%)	#2-5 (%)
Alfalfa hay	36.0	-
Brome hay	-	35.2
Canola meal	11.4	20.8
Barley (rolled)	51.7	43.1
Urea 281	0.25	0.25
Bio Phos	0.32	-
CaCO ₃	-	0.29
Salt-TM ^a	0.35	0.38

^aTrace Mineral

Table 2. Analysis of feed ingredients (%)

Ingredient	DM (as fed)	Crude Fibre (as fed)	Crude Protein (as fed)
Alfalfa hay	90.5	28.6	17.1
Brome hay	90.0	34.7	9.4
Canola meal	92.9	12.1	38.1
Barley	89.0	5.6	13.0
Diet (DM basis)	100.0	14.0	18.5

Table 3. Analysis of canola meal (CM) and soybean meal (SBM) samples received from different processors (% as fed)

Sample	DM	ADF (% DM)	ADIN (% DM)	ADIN (% N)	CP	Energy (kcal/g)
A (CM)	92.9	12.1	1.8	12	38.1	4.2
B (CM)	97.6	15.5	2.0	16	35.8	4.3
C (CM)	97.7	13.1	2.3	16	38.3	4.2
D (CM)	94.4	14.3	3.0	19	39.8	4.3
E (CM)	94.5	13.7	2.3	16	37.7	4.2
F (SBM)	91.5	3.4	2.2	10	45.7	4.2

Table 4. EAA and DAPA content of samples received from processors
(% DM)

EAA	Sample					
	A	B	C	D	E	F
Lys	1.92	1.73	1.84	1.76	1.86	2.69
His	1.30	1.20	1.32	1.40	1.28	1.27
Val	1.83	1.59	1.74	1.82	1.78	2.14
Thr	1.50	1.38	1.57	1.53	1.55	1.73
Ile	1.54	1.38	1.53	1.55	1.55	2.14
Leu	2.39	2.12	2.31	2.33	2.33	3.18
Phe	1.50	1.32	1.43	1.51	1.47	2.24
Met	.64	.52	.72	.72	.74	.31
DAPA	.042	.054	.068	.091	.059	.030

and then filled with .5 g of sample as received from the processor. Twenty bags of each sample were incubated per trial along with twenty empty bags (blanks). The blanks were used to correct for any feed particles and bacteria that adhered to the nylon. Five trials were carried out at different rumen incubation intervals: Trial 1 - 16 h; Trial 2 - 12 h; Trial 3 - 8 h; Trial 4 - 4 h; Trial 5 - 30 h. To estimate soluble DM and CP at time 0, two bags of each sample were rinsed in distilled water for 10 seconds. In total there were 140 bags incubated per trial. The small nylon bags were contained in women's panty hose during the rumen incubation period. Marbles were placed in the panty hose along with the bags to act as weights. Upon removal from the rumen, the bags were washed under cold tap water until the rinse water was colorless, and then dried at 60°C for 48 h.

Outflow Rate

The outflow rate from the rumen of the fistulated steers was calculated by injecting 250 ml of chromium ethylene diametetraacetic acid (Cr-EDTA) (Binnerts et al. 1968) into the rumen of each steer. Fecal grab samples (Grovum and Williams 1973) were then taken about every 4 h over a 48 h period. The descending concentrations of Cr in the feces were transformed to natural logarithms and a series of linear regressions were then performed on the post-peak values (Hartnell and Satter 1979). The slope with the best fit was then taken as the outflow rate from the rumen, see Appendix A. Once outflow rate was determined, the cumulative percentage of N and DM degradation was calculated according to the method of Ørskov and McDonald (1979).

Chemical and Statistical Analysis

Four bags per sample plus blanks from each trial were analyzed for N, DM, energy and Diaminopimelic acid (DAPA). Two bags per sample per trial were analyzed for regular amino acids, and 2 bags per sample per trial were analyzed for methionine and cystine. The entire bag and sample was subjected to Kjeldahl N analysis (Association of Official Analytical Chemists 1980). The entire bag and sample was analyzed for energy (cal/g) using a bomb calorimeter. Approximately 0.100 g of sample was removed from each bag and analyzed for amino acids (Association of Official Analytical Chemists 1980) using an amino acid analyzer (Association of Official Analytical Chemists 1980). The purpose of DAPA was to estimate bacterial contamination, since DAPA N is about .6% of total bacterial N (Hutton et al. 1971). The value obtained for DAPA N was divided by .6% to get a value for bacterial N and this value was then subtracted from the total N found in the bags, see Appendix B. The DM, energy and essential amino acids (EAA) values found for the blanks were subtracted from the total of each bag, see Appendix C. The percent disappearance of corrected N, DM, energy and EAA's, were calculated from the proportion remaining after incubation in the rumen, see Appendices D and E.

Results were analyzed statistically by analysis of variance to examine differences between samples within each incubation interval, with the Student Neuman Keul's test used to compare sample means with significant values (Snedecor and Cochran 1980). Each incubation interval was treated separately for statistical analysis.

RESULTS

The rumen escape of N (0 h) was lower ($P < 0.05$) for two samples (A, C) of CM compared with SBM (Fig. 1). At 4 h, SBM was similar ($P < 0.05$) to CM samples B, C. At 8 h, all samples had similar escape values, except A which had the lowest ($P < 0.05$) value. At 12 h, SBM had the highest ($P < 0.05$) value. The CM samples were similar at 12 h, except A which had the lowest ($P < 0.05$) escape value. At 16 h all samples were similar, however, this was due to high standard errors in the statistical analysis of this incubation interval. At 30 h all samples were similar with the exception of CM sample D, which had the highest ($P < 0.05$) value.

The rumen escape of DM for sample A was less ($P < 0.05$) at 0 and 4 h compared with the other CM samples (Fig. 2). At 12 h DM was similar ($P < 0.05$) between CM and SBM. At 16 h DM escape was similar ($P > 0.05$) except CM sample D was higher ($P < 0.05$) than SBM. At 30 h DM escape was high ($P < 0.05$) for all samples of CM compared with SBM while CM sample D had an even higher ($P < 0.05$) escape value than the remainder of the CM samples. At 8 h DM escape was similar among all samples, however, this was due to a loss of data resulting in a high standard error of 2.8. The mean standard error for the other incubation periods was 1.3.

At 4 h energy disappearance (Table 5) was greater ($P < 0.05$) for the CM samples compared with SBM with significant but small differences among the CM samples. The differences among samples appeared less at 8 h; however energy disappearance was greater for 3 of the CM samples (B, C, D) compared to SBM. At 12, 16 and 30 h energy disappearance was similar or greater ($P < 0.05$) for CM samples compared to SBM.

Rumen escape of lysine at 4 h was high ($P < 0.05$) for two CM samples

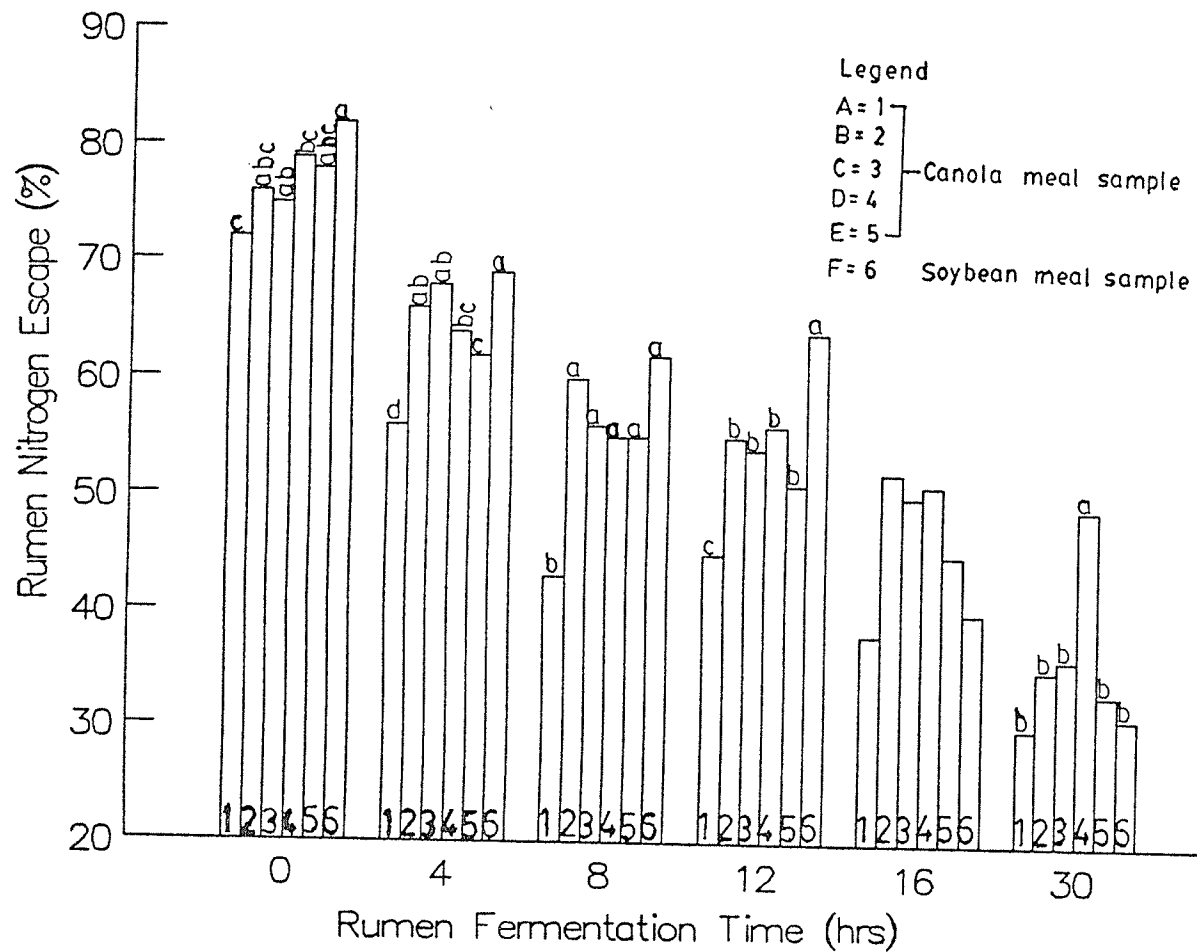


Fig.1
 Escape of canola meal and soybean meal N in the rumen of steers receiving a high energy and protein diet.
 a.b.c.d. - Means with different letters differ ($P < 0.05$)

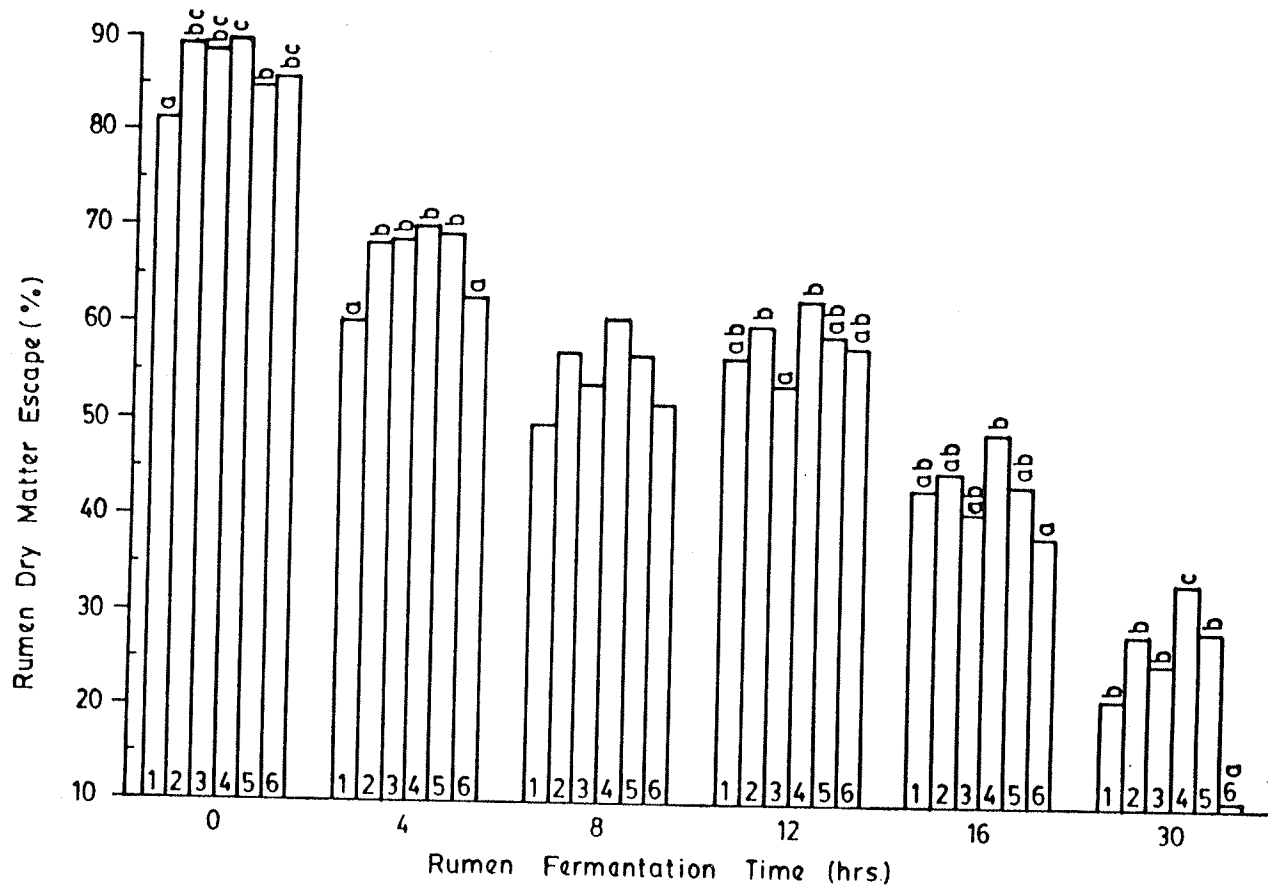


Fig 2. Escape of canola meal and soybean meal DM in the rumen of steers receiving a high energy and protein diet.

a,b,c.- Means with different letters differ ($P < 0.05$)

Table 5. Insacco disappearance of canola meal and soybean meal energy in the rumen of steers receiving a high energy and protein diet (%)

Sample	Rumen incubation time (h)				
	4 h	8 h	12 h	16 h	30 h
A	29.2b	20.6bc	23.0ab	8.12a	27.0bc
B	32.0a	23.8a	26.3a	11.6a	33.2a
C	30.2ab	22.1ab	20.1bc	6.2a	30.5abc
D	28.4b	23.2ab	21.3abc	8.3a	28.8abc
E	30.1ab	21.3abc	23.8ab	8.0a	31.6ab
F	23.3c	19.1c	17.6c	-0.2b	26.3c
SE	.6	.7	1.3	1.9	1.2

a,b,c - Means in the same column with different letters differ ($P < 0.05$).

(B, D) than SBM while all CM samples had lower ($P < 0.05$) histidine escape values than SBM (Fig. 3). Histidine escape value from CM was or appeared to be less than from SBM at 8, 12 and 30 h intervals but not at the 16 h interval (Fig.'s 3, 4, 5). In general EAA escape of canola meal A was less than the other samples except for methionine and this tended to be true for the 12 h interval. Escape of CM methionine at 4 h tended to be and was less ($P < 0.05$) at 12 and 16 h than that of SBM. This difference was not apparent at 8 and 30 h of fermentation. At 4, 8 and 12 h (Figure 3, 4) with the exception of one CM sample (A) the rumen escape of valine, threonine, isoleucine, leucine and phenylalanine from CM and SBM appeared similar while at 30 h the rumen escape values appeared lower for SBM compared with CM.

The effective degradable N for SBM appeared to fall within the range of values obtained for CM (Table 6 - statistical analysis was not carried out). The effective degradable DM in the rumen appeared to be less for CM than SBM (Table 7).

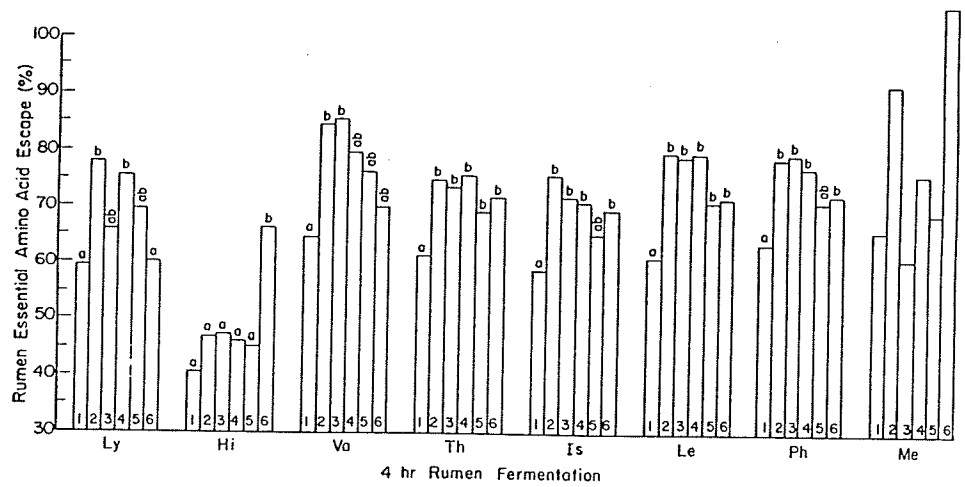
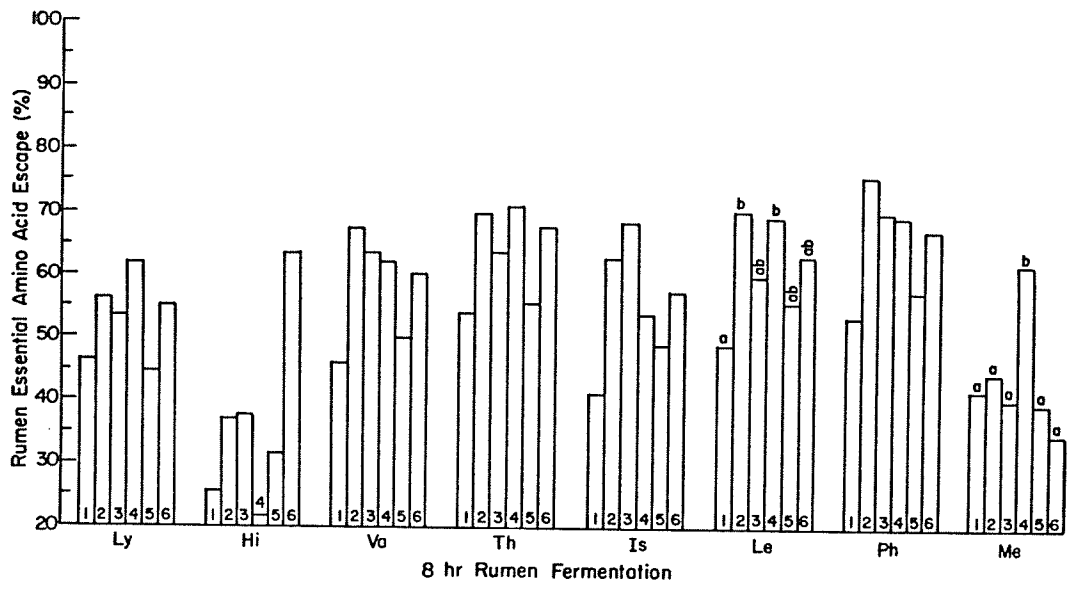


Fig. 9. Rumen escape of essential amino acids from canola meal and soybean meal.
 a,b,c- Means with different letters differ (P < 0.05)

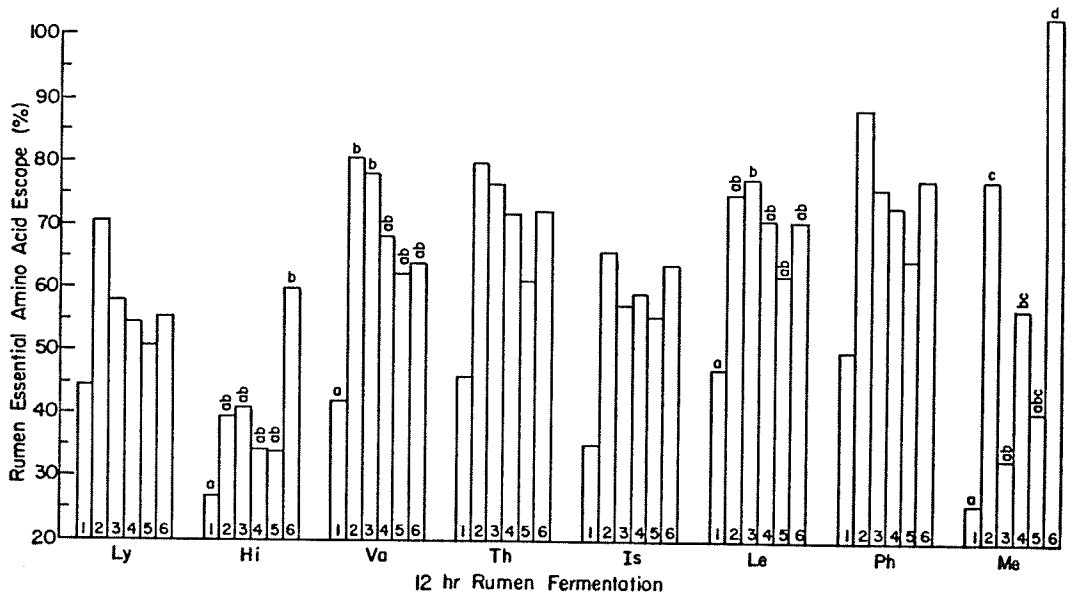
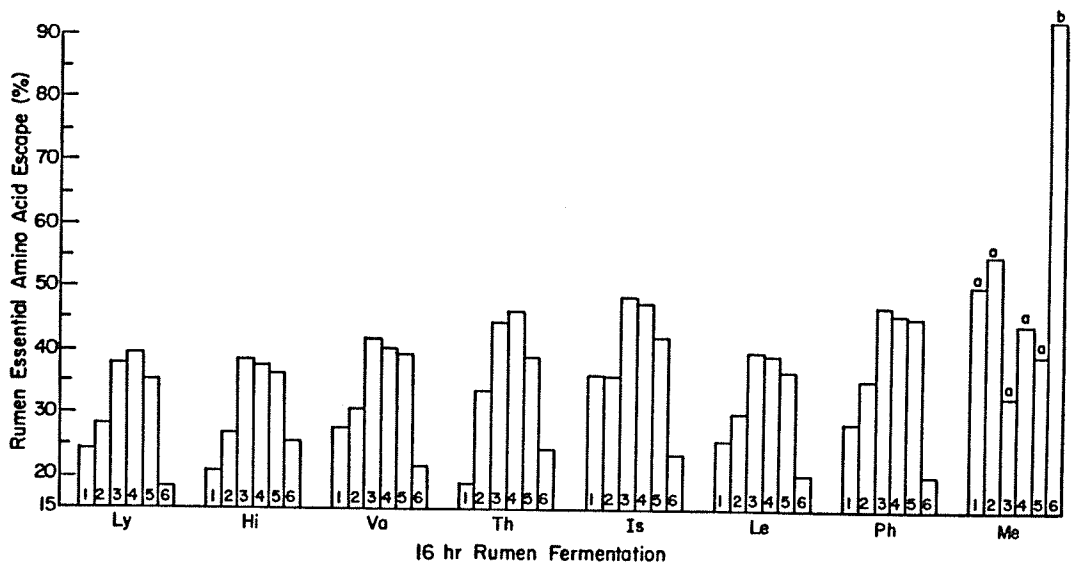


Fig. 4. Rumen escape of essential amino acids from canola meal and soybean meal. a,b,c-Means with different letters differ (P < 0.05)

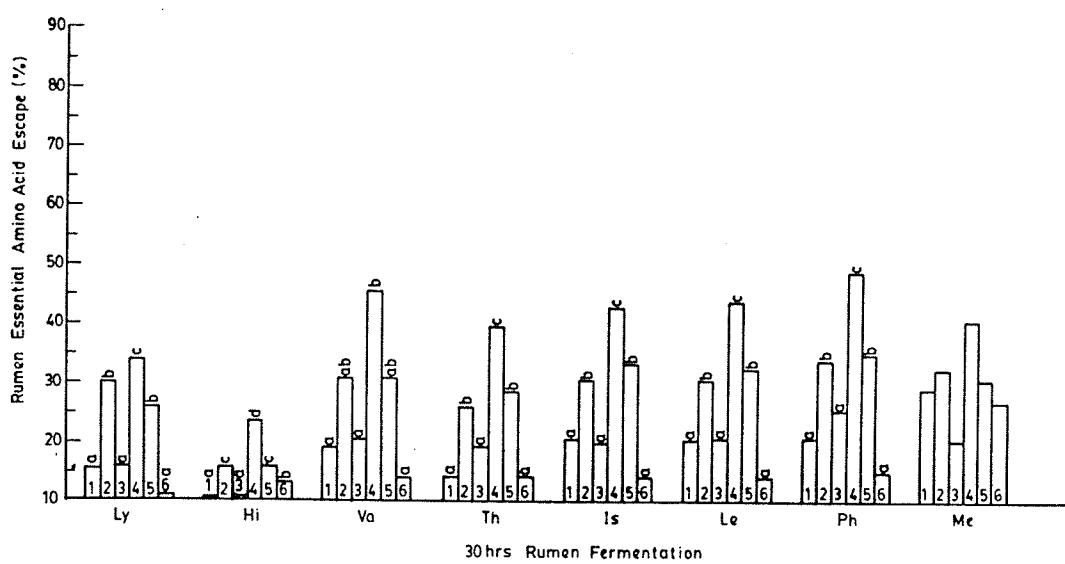


Fig. 5. Rumen escape of essential amino acids from canola meal and soybean meal.
 abc-Means with different letters differ ($P < 0.05$)

Table 6. Effective degradability of canola meal and soybean meal N (%)

Sample	Flow rate			
	K .02/h	K .04/h	K .05/h	K .08/h*
A	62.0	58.0	56.0	53.0
B	52.8	47.3	45.3	40.9
C	49.0	43.9	42.1	38.4
D	45.1	43.0	42.1	39.7
E	60.6	57.9	56.7	53.4
F	53.9	46.7	44.1	38.5

*Actual calculated value obtained from data.

Table 7. Effective degradability of canola meal and soybean meal DM (%)

Sample	Flow rate			
	K .02/h	K .04/h	K .05/h	K .08/h*
A	67.0	60.8	58.2	52.1
B	62.9	55.9	41.3	46.4
C	64.3	56.9	53.9	46.9
D	57.0	50.4	47.7	41.6
E	61.6	54.4	51.6	45.1
F	77.1	68.8	64.3	55.8

*Actual calculated value obtained from data.

DISCUSSION

The values for N and DM escape in the rumen of SBM and CM reported in this study, are similar to values reported by some other researchers. DeBoer et al. (1987) reported N escape for SBM at 0 and 4 h incubation to be 82 and 65% respectively. These values are similar to the values shown in Figure 1, 83 and 69% respectively. However, the values reported by DeBoer et al. (1987) for 8, 12 and 24 h incubation, 53, 39 and 24% respectively, are lower than those shown in Figure 1. The values in Figure 1 for 8, 12, and 30 h incubation, are 62, 64, and 31% respectively. Barrio et al. (1986) reported a N escape value from SBM at 4 h incubation to be 68%. This is similar to the value shown in Figure 1 of 69% for 4 h incubation. However, the values reported by Barrio et al. (1986) for 12 and 24 h incubation, 49 and 20% respectively are lower than the values shown in Figure 1. The values shown in Figure 1 are 64 and 31% for 12 and 30 h respectively. Comparing values at 24 h and 30 h is justified by the finding that measurements beyond 24 h add little to the estimate of degradability (Ørskov and McDonald 1979). The N escape values for SBM reported by Ha and Kennelly (1984) at 4, 8, 12 and 24 h were 70, 64, 58 and 28% respectively. These values are similar to the values shown in Figure 1 which were 69, 62, 64 and 31% for 4, 8, 12 and 30 h respectively. However, they reported a value of 92% for 0 h incubation, which is higher than the value of 83% shown in Figure 1. Ørskov and McDonald (1979) reported values, for SBM at 3, 9, 15 and 24 h incubation, of 62, 41, 21 and 11% respectively. These values are all lower than those shown in Figure 1, which were 69, 62, 40 and 31% for 4,

8, 16 and 30 h respectively. However, the values reported by Ørskov and McDonald (1979) are also lower than those reported by DeBoer et al. (1987), Barrio et al. (1986) and Ha and Kennelly (1984). The values reported by DeBoer et al. (1987) were also lower than those reported by Ha and Kennelly (1984) and Barrio et al. (1986).

Some of the differences between researchers may be explained by the use of DAPA as a microbial marker. The data reported in this study for rumen N escape may have been higher if DAPA in the feed sample (Table 4) had been subtracted from the rumen incubated samples. Recent research (Nocek 1988) has shown that the presence of DAPA is significant in some feeds, and therefore a correction must be made for it. The presence of DAPA in feeds may be attributed to the presence of bacteria in the feed, and/or protozoa, and also the presence of DAPA in the cell walls of the feed itself (Rahnema and Theurer 1986). At the time this study was conducted, it was generally assumed that DAPA in feed was negligible (Rahnema and Theurer 1986), and therefore correction for DAPA in the initial samples was not carried out. The other researchers discussed herein (Barrio et al. 1986; DeBoer et al. 1987; Ha and Kennelly 1984; Ørskov and McDonald 1979) made no mention in their papers of the method they used to correct for microbial contamination, or if they even attempted to correct for it.

DeBoer et al. (1987) reported DM escape values for SBM at 0, 4, 8, 12 and 24 h to be 64, 53, 42, 29 and 17% respectively. The values for 0, 4, 8 and 12 h are lower than those shown in Figure 2, which were 85, 63, 53 and 58% respectively. However the value for 24 h is similar to the value shown for 30 h in Figure 2 which was 11%. The values reported

by Ha and Kennelly (1984) for DM escape were 80, 62, 54, 48 and 26% at 0, 4, 8, 12 and 24 h respectively. The values at 4 and 8 h are similar to those shown in Figure 2, however the values at 0 and 12 h are lower than the values in Figure 2 and the value at 24 h is higher than the value in Figure 2 for 30 h. Barrio et al. (1986) reported values of 63, 42 and 24% at 4, 12 and 24 h respectively. The value of 4 h is similar to the value shown in Figure 2 but the value at 12 h is lower and the value at 24 h is higher than those reported in Figure 2. The values reported by Ha and Kennelly (1984) and Barrio et al. (1986) are similar, yet the values of DeBoer et al. (1987) are lower than those of the other researchers.

The DM escape values for CM reported by DeBoer et al. (1987) were 69, 59, 49, 31 and 17% at 0, 4, 8, 12 and 24 h respectively. The values shown in Figure 2 were 86, 67, 56, 59 and 29%, respectively. The values at 4 and 8 h were similar to those shown in Figure 2, however the values of 0, 12 and 24 h are lower than those shown in Figure 2. Ha and Kennelly (1984) reported values of 79, 63, 54, 42 and 29% at 0, 4, 8, 12 and 24 h respectively. The values at 4, 8 and 24 h were similar to those shown in Figure 2, however, the values at 0 and 12 h were lower than those in Figure 2. The values of DeBoer et al. (1987) are again lower than those of other researchers.

DeBoer et al. (1987) reports an effective degradable N value for SBM at $K = .05$ to be 76%. This value is higher than the value reported in Table 6 which was 44%. Effective degradable N values reported for SBM by Barrio et al. (1986) range from 60-66% at $K = .06$ and from 73-78% at $K = .03$. These values are higher than those obtained for $K = .05$ and

$K = .04$ in Table 6, which were 44.0 and 47.0% respectively. Ha and Kennelly (1984) reported effective degradable N values for SBM at $K = .05$ to be 54%, which is also higher than the value reported in Table 6. Stern et al. (1980b) reported values ranging from 63-68% for SBM at $K = .05$, also higher than the value reported in Table 6. Effective degradable N for SBM at $K = .05$ was reported by Ørskov et al. (1981) and Broderick et al. (1988) to be 63% again higher than the value reported in Table 6. The differences with respect to effective degradable N, may be due to the fact that the other researchers did not correct for microbial contamination.

The effective degradable N value reported by DeBoer et al. (1987) for CM at $K = .05$ is 74%, again higher than the values reported in Table 6 which ranged from 42-57%. The effective degradable N value reported for CM at $K = .05$ by Ha and Kennelly (1984) was 66%, which is also higher than the values reported in Table 6 for $K = .05$. The mean of the effective degradable N values for CM at $K = .05$ is 48%, this is similar to the value of 52% reported by Broderick et al. (1988).

The effective degradable DM value for SBM of $K = .05$ reported by DeBoer et al. (1987) was 82%, again higher than the value reported in Table 7 for $K = .05$ which was 64%. Ha and Kennelly (1984) reported an effective degradable DM value of 58% at $K = .05$ for SBM. The value at $K = .05$ is lower than the value reported in Table 7.

The effective degradable DM value for CM at $K = .05$ reported by DeBoer et al. (1987) at 70% is higher than the value reported in Table 7 which ranged from 41-58%. The effective degradable DM value for CM at $K = .05$ reported by Ha and Kennelly (1984) is 57%. This value is

similar to the values reported in Table 7. The general trend reported by most researchers to date is that as outflow rate (K) increases, effective degradable N and DM decrease (Ha and Kennelly 1984; Ørskov et al. 1981; Stern and Satter 1982).

The rapidly degradable protein fraction (a), taken at 0 h, was lowest for SBM, indicating that protein in SBM was less soluble than in CM. This was also reported by Ha and Kennelly (1984). The values in Figure 1 indicate that SBM and CM are not different with respect to N degradability in the rumen. This is in contrast to the report of Ha and Kennelly (1984) that states the N in SBM disappears at a slower rate than CM N during the first 12 h, but that disappearance is similar thereafter. Figure 2 shows that while SBM has a greater DM degradability at 30 h, SBM and CM are not different with respect to DM degradability. This is similar to what Ha and Kennelly (1984) reported, however they found that CM DM disappeared more at 12 h than SBM DM. The effective degradable DM values in Table 7 indicate that these samples of CM were similar or somewhat less degradable than SBM.

These data, and the data of many other researchers, further support the statement by Setälä and Syrjälä-Qvist (1984) that incubation period, experimental animals, and different diets, can all affect the results obtained with the nylon bag technique. Furthermore, the nylon bag technique only measures disappearance from the bag itself, and as suggested by Ha and Kennelly (1984) it is desirable to validate the technique by comparing values obtained for the same feeds in vivo.

Several researchers have observed that methionine was the most easily degraded (low escape value) amino acid in the rumen (Setälä and

Syrjälä-Qvist 1984; Varvikko et al. 1983). In this study methionine seems to degrade to the same extent as the other amino acids at 4 h, and in samples B and F (SBM) it seems resistant to degradation (Fig. 3). Methionine compared with the other EAA, except histidine, does appear to have a lower escape value at 8 h, for CM, however, methionine has the lowest escape value compared with the other EAA's in the SBM. At 12 h methionine has the lowest escape value in samples A and C, but it seems resistant to degradation in the SBM. At 16 h, methionine has the highest escape value in samples A, B and F (SBM). At 16 h methionine has the lowest escape value in sample C. At 30 h, methionine appears to escape to the same extent as the other amino acids in the CM samples, but it appears to have a higher escape value compared with the other EAA's in SBM.

In addition to methionine, Setälä and Syrjälä-Qvist (1984) found that histidine was a highly degradable (low escape) amino acid in feed protein. In this study, histidine appeared to have the lowest escape value in the CM samples at 4, 8 and 30 h (Fig.'s 3, 5). Histidine appeared to have the lowest escape value from CM samples B, D and E at 12 h and from CM samples A, B and D at 16 h (Fig. 4).

The observation has been made that valine and isoleucine of CM protein were resistant to ruminal degradation (Lewis and Emery 1962; Setälä and Syrjälä-Qvist 1984). At 4 h (Fig. 3) valine appears to have a high escape value in samples B and C, however it appears to degrade to the same extent as the other amino acids in the CM samples at 4 h. At 8, 12, 16 and 30 h, valine and isoleucine appear to be degraded to the same extent as the other amino acids in the CM samples (Fig.'s 3, 4,

5).

Lysine compared with other EAA's appears to have the lowest escape value in SBM at 4, 12, 16 and 30 h (Fig.'s 3, 4, 5). Methione compared with other EAA's appears to have the highest escape value in SBM at 4, 12, 16 and 30 h (Fig.'s 3, 4, 5).

It would seem that conclusions reached, with respect to EAA escape, using the nylon bag technique are in disagreement with those reached using more conventional methods. However, the escape of the two most limiting amino acids for ruminants, methionine and lysine, are similar between CM and SBM for 48 and 30 h incubation using this method.

MANUSCRIPT II:
THE DIGESTIBILITY OF CANOLA MEAL AND SOYBEAN MEAL
IN THE LOWER DIGESTIVE TRACT OF RUMINANTS

ABSTRACT

The "Mobile Nylon Bag Technique" was used to compare the digestibility of canola meal and soybean meal protein, dry matter, energy and essential amino acids in the lower digestive tract of steers. A sample of canola meal (CM) was obtained from five different processors and a sample of soybean meal (SBM) was obtained from a processor in Altona, Manitoba. Five trials were carried out at different rumen incubation intervals: 0 h; 4 h; 8 h; 12 h; and 16 h. The bags were then incubated in pepsin-HCl solution for 3 h at 39°C to simulate abomasal digestion. The bags were then allowed to pass through the lower digestive tract of duodenal cannulated Holstein steers, subsequently collected in the feces, and analyzed. Nitrogen, DM, and energy digestibility of SBM, is greater ($P < 0.05$) than that of CM in the lower digestive tract. This difference could be accounted for by a low digestibility of rapeseed hulls. Essential amino acids had digestibilities that were greater ($P < 0.05$) from SBM than from CM in the lower digestive tract, except: methionine at 0 h; methionine at 4 h; lysine, histidine, valine, threonine, isoleucine, leucine and methionine at 8 h; lysine, histidine, phenylalanine and methionine at 12 h; and all EAA'S at 16 h. There was a trend that the longer CM is retained in the rumen, the less digestible the N and DM becomes in the lower digestive tract, a trend that would be expected if low digestibility hulls make up a greater proportion of the digesta presented to the lower digestive tract. Soybean hulls are readily digested by ruminants. Energy digestibility in the lower tract appeared to increase as retention time increased for both CM and SBM. Rumen retention (fermentation time) had

no significant ($P < 0.05$) effect on digestibility of most EAA's from both CM and SBM. However, again a general trend appeared that although digestibility after 4, 8 and 12 h did not differ much, digestibility after 16 h tended to be reduced in the lower digestive tract.

INTRODUCTION

The supply of nutrients, such as amino acids, to the small intestine of a ruminant animal, is determined by the amount of dietary nutrients that escape rumen degradation and microbial synthesis of protein. The extent to which nutrients such as proteins and amino acids are broken down are influenced by factors such as residence time in the rumen, protein structure, solubility and processing techniques.

To date little work has been done on the quantitative absorption of nutrients, particularly amino acids, from the small intestine of ruminants. Clarke et al. (1966) discussed the absorption of individual amino acids from the small intestine and found that essential amino acids were preferentially absorbed over nonessential amino acids. This has since been confirmed by Coelho da Silva et al. (1972), Purser (1970), and van't Klooster and Boekholt (1972). In general, the order of amino acid uptake for sheep has been found to be similar to that of man (Bull et al. 1985). However, much of the information so far has been based on in vitro studies, and the in vivo work that has been done was performed on sheep. The biggest problem to date has been the partitioning of bacterial crude protein from undegraded feed protein, as well as to estimate endogenous contributions. These problems have made difficult the quantitative measurement of nutrients available for absorption from the small intestine of ruminants.

A more accurate method for determining the true digestibility of nutrients is required. A procedure recently developed by Sauer et al. (1983) for pigs, may be the most suitable method to date for determining digestibility in the lower gastro-intestinal (GI) tract of ruminants. Sauer et al. (1983) inserted small nylon bags containing feed samples, into the duodena of pigs, subsequently recovered the bags from the feces and determined digestibility. This technique has since been modified for ruminants (Kirkpatrick and Kennelly 1985), and named the "Modified Mobile Nylon Bag Technique". It has since been used successfully by DeBoer et al. (1986), Kirkpatrick and Kennelly (1985) and Rae and Smithard (1985).

The objective of this study was to determine the "true" digestibility of protein, dry matter (DM), energy, and essential amino acids (EAA) of canola meal and to compare these with similar measurements on soybean meal using the "Mobile Nylon Bag Technique". Also, to determine the effect of rumen retention time on the digestibility in the small intestine of nutrients remaining in the bag.

MATERIALS AND METHODS

Animals and Diets

Two rumen cannulated steers and three duodenally cannulated Holstein steers were used in this study.

The rations were formulated to meet the energy and protein requirements (19% CP - 14% CF) of high producing dairy cows, see Manuscript I. Hay and concentrate were fed ad libitum in a 40:60 ratio twice daily.

A sample of canola meal (CM) was obtained from five different

processors and designated A, B, C, D and E (Table 8) (see Appendix F). A soybean meal (SBM) sample was obtained from a processor in southern Manitoba, and designated F.

Small nylon bags (3.5 x 5.5 cm) were made by heat-sealing pieces of nylon with a pore size of 50 microns (Felco Industries). The bags were weighed and then filled with .5 g of the sample as it was received from the processing plant. Twenty bags of each sample were incubated per trial, along with twenty empty bags (blanks). The blanks were used to correct for any feed particles that adhered to or entered the nylon bags. Five trials were carried out at different rumen incubation intervals: Trial 1, 16 h; Trial 2, 12 h; Trial 3, 8 hr; Trial 4, 4 h; Trial 5, 0 h. These trials were carried out at the same time as those in Manuscript I. In total there were 140 bags per trial.

After removal from the rumen, these bags were incubated in a pepsin - HCl solution (1 g pepsin per 1 .01 N HCl) for 3 h at 39°C to simulate abomasal digestion. After pepsin - HCl incubation the bags were placed on ice at 4°C. The bags were then inserted into the proximal duodenum at the rate of 2 per h. The bags were subsequently separated from the feces, using a 30 x 60 x 30 cm wooden box with a .6 cm screen in the bottom. The fecal material was shovelled into the box and then washed through the screen with a garden hose, with the bags remaining on the screen. The bags were wiped dry with paper towels and then dried at 60°C for 48 h.

Chemical and Statistical Analysis

Four bags per sample per trial were analyzed for N, DM, energy and diaminopimelic acid (DAPA). Two bags per sample per trial were analyzed

Table 8. Analysis of canola meal and soybean meal samples (as fed) (%)

Sample	DM	ADF (% DM)	ADIN (% DM)	ADIN (% N)	CP	Energy (kcal/g)
A	92.9	12.1	1.8	12	38.1	4.2
B	97.6	15.5	2.0	16	35.8	4.3
C	97.7	13.1	2.3	16	38.3	4.2
D	94.4	14.3	3.0	19	39.8	4.3
E	94.5	13.7	2.3	16	37.7	4.2
F (SBM)	91.5	3.4	2.2	10	45.7	4.2

A,B,C,D,E - Canola meal samples.

F - Soybean meal sample.

for methionine and cystine and 2 bags per sample per trial were analyzed for the remaining EAA (see Manuscript I). The percent disappearance of corrected N, DM, energy and EAA's in the lower digestive tract, were calculated by subtracting the proportion remaining after recovery from the feces, from the proportion remaining after recovery from the rumen (see Manuscript I and Appendices D and E). Rumen digesta and fecal DM were corrected for DM found in the blanks, see Appendix C. Rumen digesta and fecal N were corrected for bacterial N as calculated from DAPA, see Appendix B.

Results were analyzed statistically by analysis of variance to examine differences between samples within incubation interval, with the Student Neuman Keuls test used to compare sample means with significant F values (Snedecor and Cochran 1980).

RESULTS

Digestibility of Nutrients from Rumen Residues

With no rumen incubation, N from SBM was more digestible ($P < 0.05$) than N from CM samples B and E (Fig. 6). After 4, 8 and 12 h rumen incubation, SBM N was more digestible ($P < 0.05$) than N from any of the CM samples. Nitrogen digestibility among the CM's were not different with the exception of CM C and B at 12 h. After 16 h rumen incubations SBM N digestibility was similar to CM samples C and D. There were differences among CM's after 16 h incubation, sample A was different from C and D.

After 0, 4, 12 and 16 h rumen incubation periods, DM from SBM was more digestible ($P < 0.05$) than DM from any of the CM samples with the exception of sample C at 4 h (Fig. 7). There were no significant

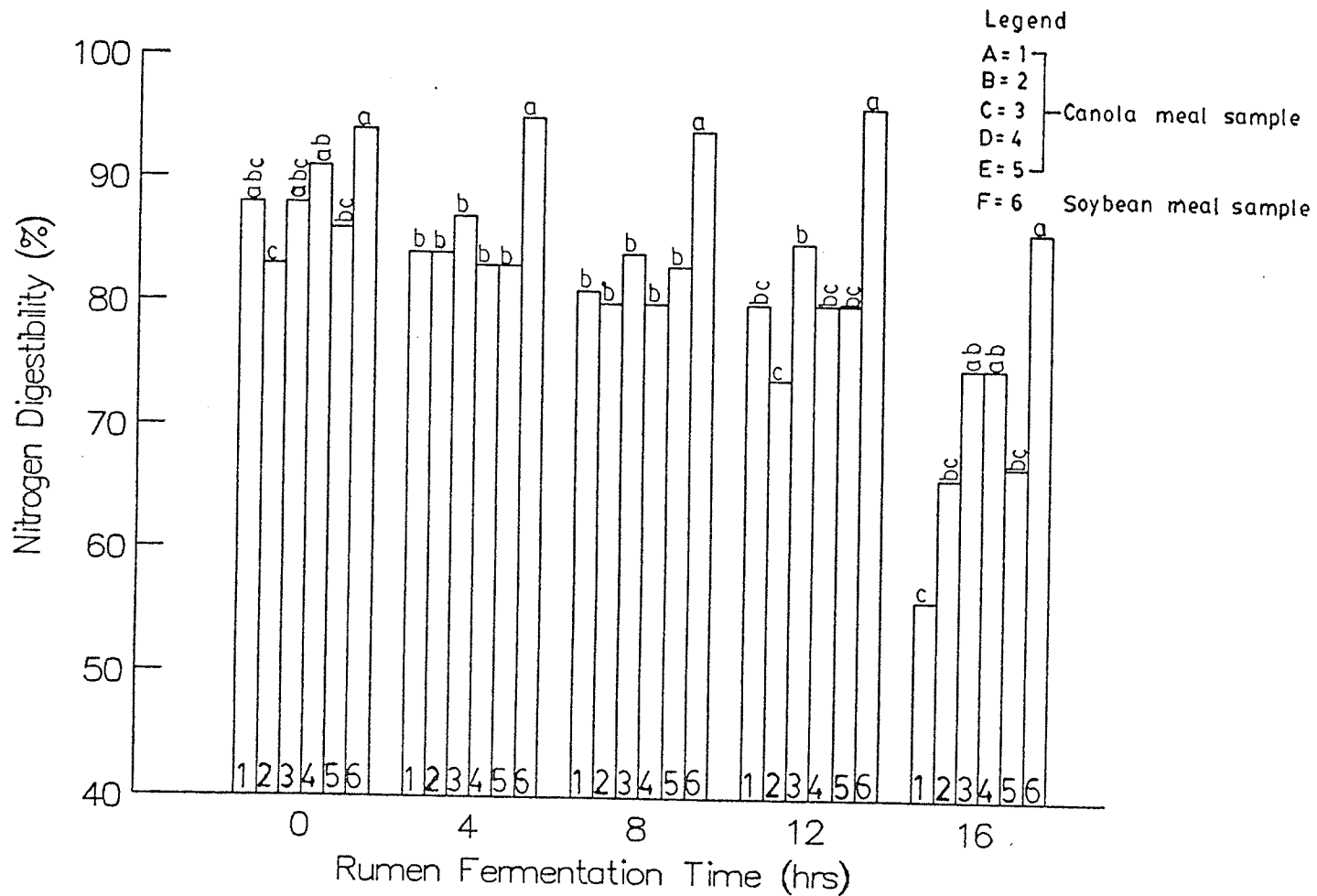


Fig. 6

The digestibility of N from rumen undegraded residues of canola meal and soybean meal in the lower digestive tract.

a, b, c - Means with different letters differ ($P < 0.05$)

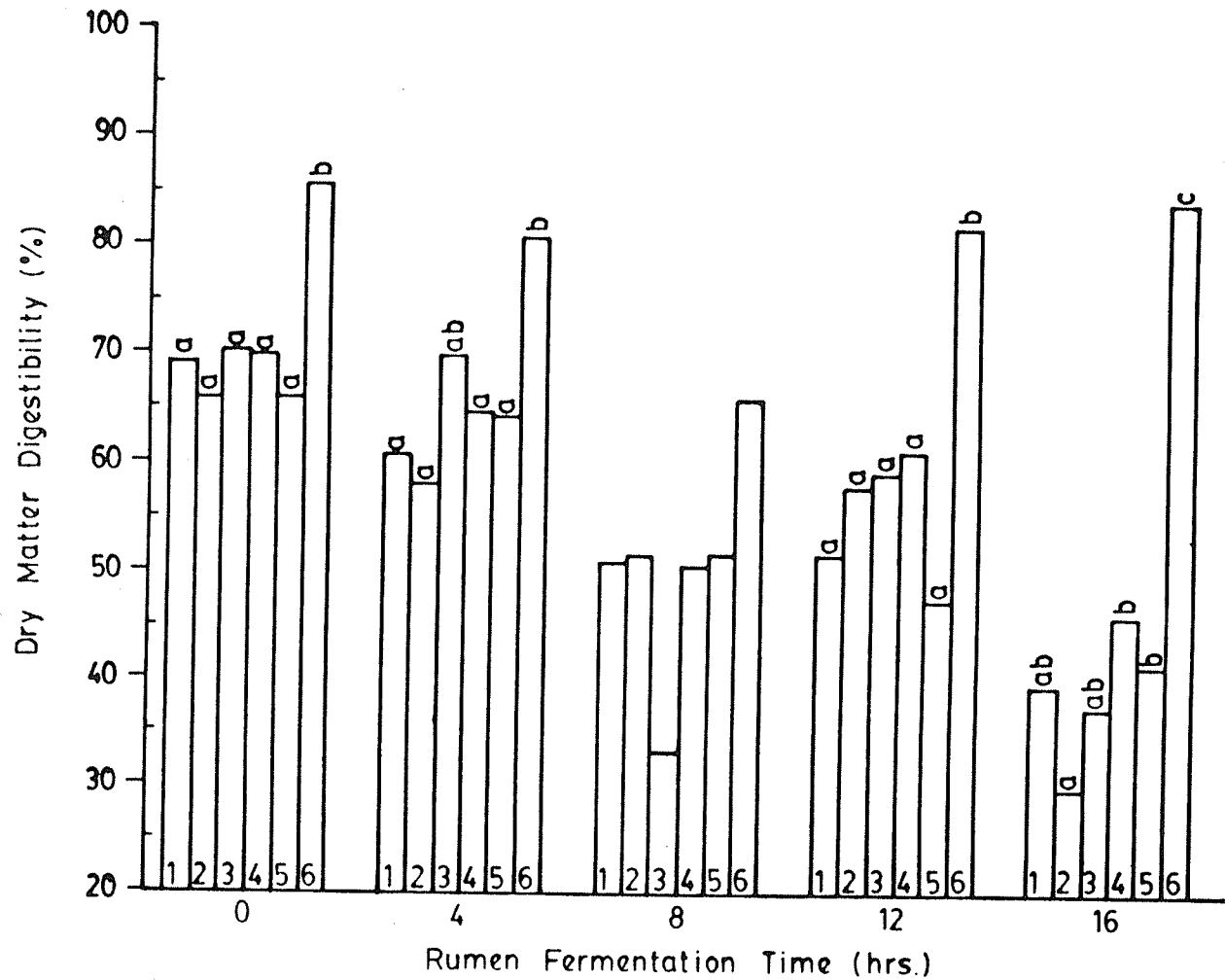


Fig.7 The digestibility of DM from rumen undegraded residues of canola meal and soybean meal in the lower digestive tract.

a,b,c- Means with different letters differ ($P < 0.05$)

($P > 0.05$) differences in DM digestibility among the CM samples with the exception that CM samples D and E were more digestible ($P < 0.05$) than CM sample B at 16 h. The DM digestibility data at 8 h was not significantly different, however, again this was due to a loss of bags. The statistical analysis had to be performed on 2 bags per sample, instead of 4. This resulted in a standard error of 11.50, compared with a standard error averaging 3.30 for the other incubation intervals.

Energy digestibility was lower ($P < 0.05$) for CM than SBM with 8, 12, and 16 h rumen incubation periods (Table 9). There appeared to be an increase of energy digestibility for 16 h rumen incubation samples over 4, 8 and 12 h samples. Relative to N and DM the digestibility values for energy are very low but similar for the 4, 8 and 12 h rumen fermentation samples with an apparent increase for the 16 h rumen samples.

With no rumen fermentation prior to pepsin-HCl digestion lysine, histidine, valine, threonine, isoleucine, leucine and phenylalanine (Fig. 8) were more digestible ($P < 0.05$) from SBM than any of the CM samples which were all similar (Fig. 8). Digestion of methionine however, was similar ($P > 0.05$) for CM and SBM after 0 and 4 h incubation (Fig. 8) with a trend of lower methionine digestibility for CM samples at 8 and 12 h incubation (Fig.'s 8, 9).

The digestibility of lysine, histidine, valine, threonine, isoleucine and phenylalanine after 4 h incubation were all more digestible ($P < 0.05$) from SBM than the CM samples which were similar with a few exceptions (Fig. 8). At 8 (Fig. 8) of incubation, EAA digestion appears to be lower for samples A and B compared with the other CM

Table 9. The insacco digestibility of energy from rumen undegraded residues of canola meal and soybean meal in the lower digestive tract (%)

Sample	Rumen incubation time (h)				
	0	4	8	12	16
A	33.3a	10.4ab	14.0b	11.3b	27.0b
B	36.8a	14.1ab	8.7b	6.2b	33.6b
C	32.7a	10.1ab	10.3b	12.5b	24.5b
D	33.0a	12.9ab	12.2b	13.2b	28.8b
E	33.1a	7.0b	9.8b	8.3b	26.4b
F	35.8a	23.0a	28.2a	23.3a	55.0a
SE	1.4	3.1	1.6	2.4	6.0

a,b - Means in the same column with different letters differ ($P < 0.05$).

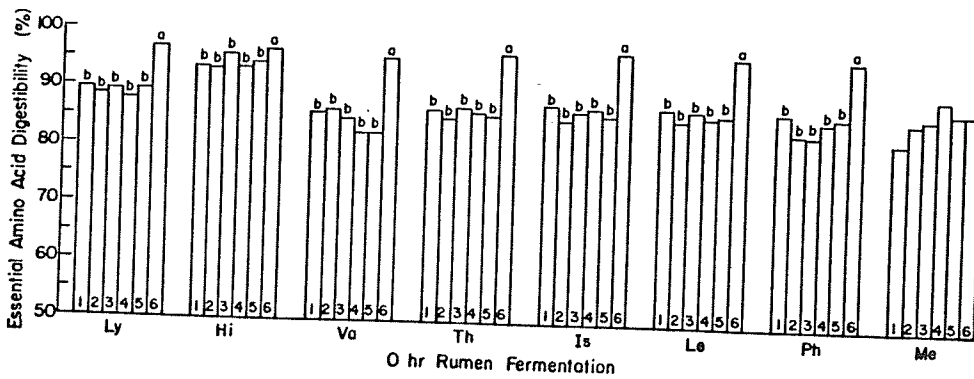
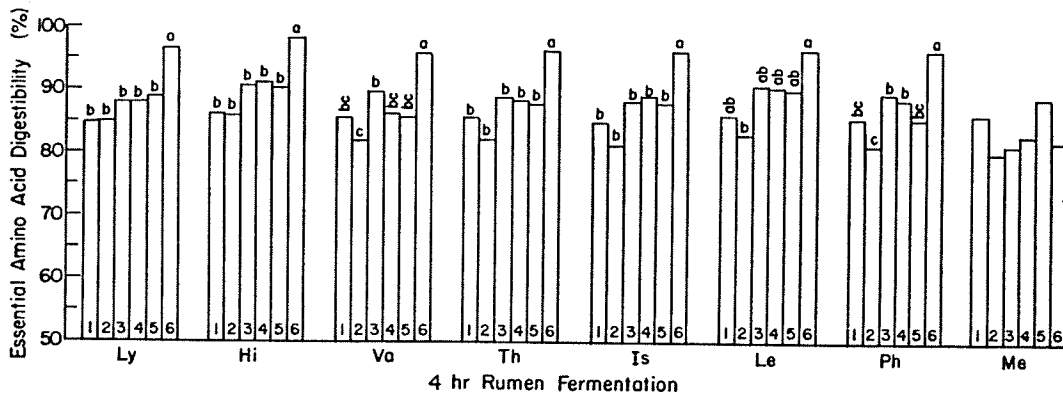
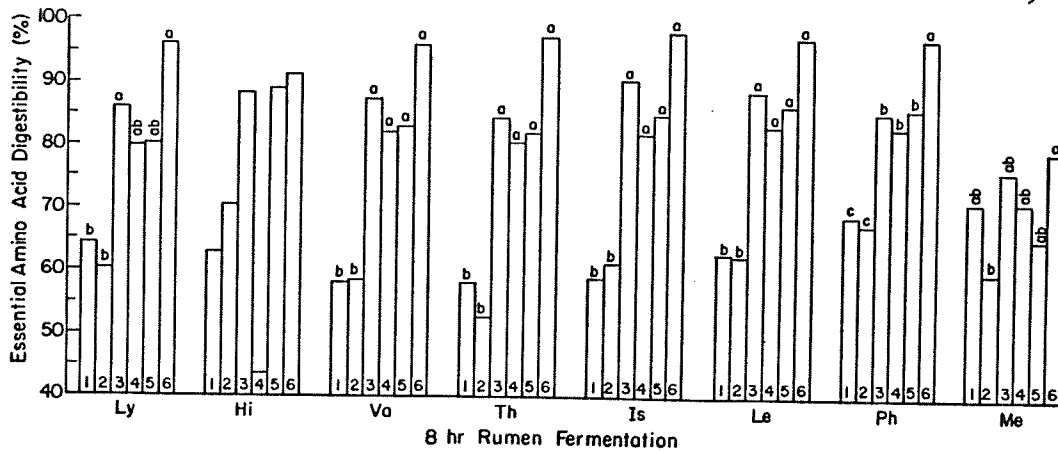


Fig. 8. The digestibility of essential amino acids from rumen undegraded residues of canola meal and soybean meal in the lower digestive tract.

a,b,c - Means with different letters differ ($P < 0.05$)

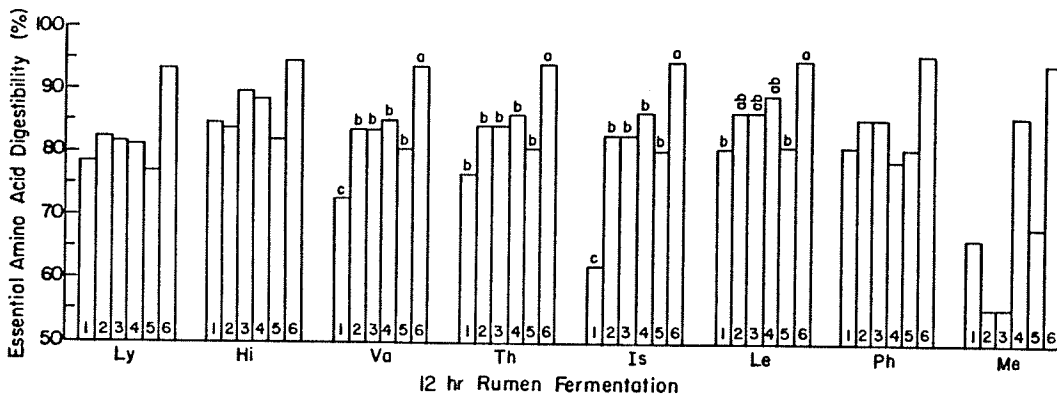
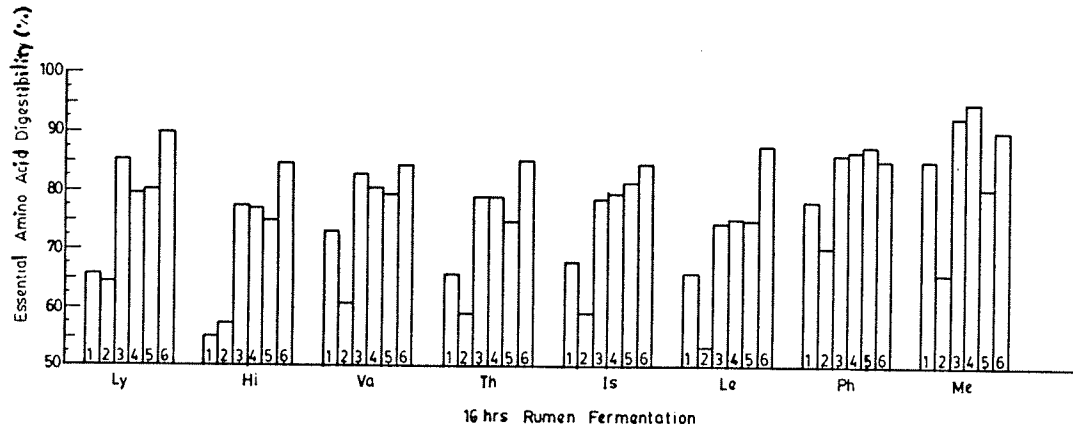


Fig. 9. The digestibility of essential amino acids from rumen undegraded residues of canola meal and soybean meal in the lower digestive tract.
 a,b,c - Means with different letters differ ($P < 0.05$)

samples.

The data suggest that at 8 and 12 h (Fig.'s 8, 9) EAA, except methionine, had digestibilities that were greater from SBM than from CM although the differences are not always significant ($P>0.05$). At 12 h valine, isoleucine, leucine and threonine all had digestibilities that were greater ($P<0.05$) from SBM than from CM.

Effect of Rumen Retention Time on Digestibility

The effect of rumen retention time on N digestibility (Table 10) was measured, however, the data must be viewed with caution because each rumen fermentation time represents a trial carried out on a different day. These data suggest a general trend for CM, though not always a significant one, that the longer CM is retained in the rumen, the less available the N becomes in the lower digestive tract (Table 10). This trend was not apparent for SBM N except at 16 h.

Rumen retention time did not appear to have an effect ($P<0.05$) on SBM DM digestibility (Table 11). For CM, a retention time of 16 vs 4 h resulted in a significantly lower ($P<0.05$) DM digestibility of sample A, B, C and D with a similar trend for sample E.

Energy digestibility (Table 12) in the lower GI tract appeared to be low compared with that of DM (Table 11). For CM samples A, C, D and E, and SBM, a rumen retention time of 16 h compared with 4, 8 or 12 h, resulted in a significantly higher ($P<0.05$) digestibility in the lower digestive tract.

Retention time of CM samples A, C, D, E had no significant ($P<0.05$) effect on the digestibility of EAA with the exception of sample C and D leucine, D and E methionine and E histidine (Tables 13, 15, 16, 17).

Table 10. The effect of rumen retention time on N digestibility in the lower digestive tract (%)

Retention Time (h)	Samples					
	A	B	C	D	E	F
4	84.0a	84.0a	87.0a	83.0	83.0a	95.0a
8	81.0a	80.0a	84.0a	80.0	83.0a	94.0ab
12	77.0a	74.0ab	85.0a	80.0	79.0a	96.0a
16	56.0b	66.0b	75.0b	75.0	67.0b	89.0b
SE	2.6	1.9	1.5	1.5	1.9	.94

a,b - Means in the same column with different letters differ ($P < 0.05$).

Table 11. The effect of rumen retention time on insacco DM digestibility in the lower digestive tract (%)

Retention Time (h)	Samples					
	A	B	C	D	E	F
4	60.7	57.0a	69.1a	64.6a	64.4	80.6
8	51.1ab	52.0a	33.3b	50.6a	52.3	66.3
12	51.8ab	56.6a	58.8ab	62.0a	48.0	82.0
16	39.0b	29.6b	37.2b	46.4b	41.6	84.5
SE	4.8	3.4	8.4	3.8	6.4	10.8

a,b - Means in the same column with different letters differ ($P < 0.05$).

Table 12. The effect of rumen retention time on insacco energy digestibility in the lower digestive tract (%)

Retention Time (h)	Samples					
	A	B	C	D	E	F
4	10.4b	14.1	10.1b	12.9	7.0b	23.0b
8	14.0b	8.7	10.3b	12.2b	9.8b	28.2b
12	11.3b	6.2a	12.5b	15.2b	8.3b	23.3b
16	27.0a	33.6	24.5a	28.8a	26.4a	55.0a
SE	2.1	7.3	1.9	2.1	2.8	2.8

a,b - Means in the same column with different letters differ ($P < 0.05$).

Table 13. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from Sample A (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	84.7	86.8	85.7	85.1	84.7	86.6	85.5	79.8
8	64.1	62.8	58.0	57.9	58.9	62.9	68.9	71.4
12	76.2	85.0	72.5	76.9	62.1	80.3	81.0	66.7
16	65.6	55.0	72.7	65.3	67.5	65.4	77.4	84.9
SE	7.6	11.5	7.3	8.6	8.3	7.8	4.6	7.9

a - Means in the same column with different letters differ ($P < 0.05$).

Table 14. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from Sample B (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	84.9a	86.7a	82.2a	82.2a	81.3a	82.7	81.8ab	76.4
8	60.2b	70.1b	58.3b	52.8b	61.8b	62.7	67.6b	59.9
12	81.4a	88.4a	83.6a	84.0a	82.2a	86.7	85.5a	55.7
16	64.0b	57.0c	60.3b	59.4b	58.7b	53.7	70.1b	66.1
SE	3.9	2.7	3.5	3.9	3.9	5.8	2.9	10.7

a,b,c - Means in the same column with different letters differ ($P < 0.05$).

Table 15. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from Sample C (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	87.9	90.6	89.0	88.2	87.8	90.3a	89.1	83.0
8	86.3	88.0	87.0	84.2	90.2	88.2a	85.6	76.3
12	81.4	88.4	83.6	84.0	82.2	86.7a	85.5	55.7
16	85.3	76.9	82.9	77.9	77.6	73.4b	85.7	91.7
SE	3.5	2.5	1.1	3.3	2.6	2.8	3.4	12.9

a,b - Means in the same column with different letters differ ($P < 0.05$).

Table 16. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from Sample D (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	87.9	91.4	86.2	87.7	88.2	90.2a	88.0	82.9ab
8	79.7	43.6	82.4	80.5	81.9	83.4b	82.7	71.7b
12	81.6	88.2	84.9	85.7	86.1	88.8a	78.2	85.7ab
16	78.8	76.5	80.3	77.9	78.8	74.8c	86.2	94.1a
SE	2.8	21.8	2.2	3.0	2.9	1.3	5.8	1.8

a,b,c - Means in the same column with different letters differ ($P < 0.05$).

Table 17. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from Sample E (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	88.7	90.0a	85.7	87.3	87.5	89.5	85.3	88.7a
8	80.0	88.7a	82.8	81.9	84.8	86.6	86.1	65.1b
12	78.1	82.2a	80.4	80.2	80.2	81.0	80.5	68.0b
16	79.8	74.8b	79.4	74.6	81.1	74.5	87.1	80.4ab
SE	3.6	1.9	3.2	3.1	2.4	4.0	1.8	4.0

a,b - Means in the same column with different letters differ ($P < 0.05$).

Rumen retention times of 8 and 16 h for CM sample B (Table 14) appeared ($P < 0.01$) to reduce EAAs digestibility compared with 4 and 12 h. A similar though nonsignificant ($P < 0.05$) result was noted for CM sample A (Table 13). The change for methionine did not appear to follow a similar pattern.

Digestion of EAA's from SBM appeared uniformly high and not different ($P > 0.05$) with rumen retention time although there appeared, except for leucine, to be a uniform non-significant decrease after 16 h rumen incubation (Table 18).

Table 18. The effect of rumen retention time on insacco EAA digestibility in the lower digestive tract from SBM (F) (%)

Retention Time (h)	Essential amino acids							
	Lys	His	Val	Thr	Ileu	Leu	Phe	Met
4	96.5	97.7	95.6	96.2	96.2	96.3a	96.7	83.1
8	96.5	91.2	95.4	96.5	97.3	97.0a	97.3	79.2
12	94.1	93.5	93.5	93.7	44.2	94.5b	95.4	94.1
16	89.7	84.3	83.8	85.0	83.6	87.0c	84.6	90.0
SE	1.9	4.1	2.9	3.7	3.2	0.2	4.2	8.3

a,b,c - Means in the same column with different letters differ ($P < 0.05$).

DISCUSSION

The values of N digestibility in the lower digestive tract reported in this study, are similar to the values reported by other researchers. Rae and Smithard (1985), using the modified mobile nylon bag techniques reported that the N digestibility of CM at 8 h rumen incubation was 79.1%, at 12 h it was 74.5% and at 24 h 56.9%. These values are similar to those reported in Figure 6 which were 81.3, 79.5 and 67.9% for 8, 12 and 16 h respectively. Although values for incubation at 24 h were not reported here, the same decreasing trend in digestibility can be seen by comparing 16 h values to 12 h values. Rae and Smithard (1985) reported that the N digestibility of SBM at 8 h was 90.5% and 88.5% at 12 h. The value for 8 h is similar to the one shown in Figure 6, 93.7%. However, the value for 12 h is lower than that reported in this study. Kirkpatrick and Kennelly (1985), also using the mobile nylon bag technique, reported N digestibilities of 70.6% with a dietary protein level of 16%, and 62.9% with a dietary protein level of 19%, for CM. The N digestibility of SBM was 71.9% at a dietary protein level of 15% and 79.4% at 19% protein (Kirkpatrick and Kennelly 1985). Other researchers have also reported that the longer CM is retained in the rumen, the less available it becomes in the lower digestive tract (DeBoer et al. 1986; Rae and Smithard 1985). This trend is not apparent for SBM (DeBoer et al. 1986). The difference in hull digestibility for CM and SBM may explain this difference between CM and SBM.

Energy digestibility (Table 9, 12) appears to be low using the mobile bag technique. Rumen fermentation times of 4, 8 or 12 h had

little effect on energy digestibility in the lower GI tract. The 16 h rumen fermentation resulted in increased ($P < 0.05$) energy digestibility compared with the 4, 8 and 12 h fermentation periods. It was shown previously in Manuscript I that energy disappearance from the samples in the rumen incubated for 16 h had an apparent lower energy digestibility compared with incubation periods of 4, 8, 12 and 30 h. This may not be a real difference. It may be experimental variation due to the day that the rumen fermentation was carried out, or due to the type of forage (alfalfa vs brome) the cannulated steers received.

Hull digestibility may explain the difference in digestibility between CM and SBM. Limited research (S. Thomke, personal communication) suggests that rapeseed hulls are 25-30% digestible. The hull fraction of CM could make up a significant proportion of the residue leaving the rumen and an even larger proportion of the fecal residue. In general, CM contains about 30% hulls (Bell 1984) which contain 14% C.P. of which 10% is available in the lower digestive tract (Bell 1984).

Lower GI tract DM and N digestibility appeared to decrease with longer rumen fermentation periods for CM but not for SBM. Digestibility of methionine appears to be equal from CM and SBM even though other EAA's tend to be less digestible in CM. These differences may be due to the relatively low digestibility of CM hulls compared with SBM hulls.

GENERAL DISCUSSION

Nylon Bag Technique

The supply of amino acids to the small intestine of ruminants is determined by the amount of dietary protein that escapes rumen degradation, and the quantity of microbial protein synthesized in the rumen. To date, many systems have been proposed to evaluate dietary protein supply to the lower digestive tract in ruminants. These include the in vivo method, the in vitro method and, most recently, the in situ method, or nylon bag technique.

The in vivo method involves collection of digesta post-ruminally and surgical preparation of the animals with various types of cannulae, in the omasum, abomasum, or proximal duodenum. The collection of duodenal flow can give an accurate estimate of the quantity of protein passing to the small intestine from the rumen.

In vitro techniques include: ammonia release using rumen inoculum; nitrogen solubility in buffers; and ammonia plus total amino acid release in rumen inoculum. The ammonia release technique has fallen into disuse largely because the results were interpreted without regard for microbial uptake. The problems with nitrogen solubility are that the proportion of soluble nitrogen for different feeds may be similar in different solvents, and the proportion of soluble nitrogen for the same feed may differ in different solvents. The amino acid plus ammonia release technique is a new system for estimating ruminal protein degradation rate. To date results obtained from this technique (Broderick and Craig 1980) have been comparable to those previously

reported from in vivo studies. This method may lead to more accurate estimates of degradation rate, however, it still requires modification.

The preferred method to date, of determining protein degradability, is the in situ, or the nylon bag technique. This simple technique allows for the rapid determination of rate of degradation and rate of passage, which cannot be derived from either the in vivo or in vitro techniques. The results reported in this study, using the nylon bag technique, indicate that CM and SBM are not different with respect to nitrogen degradability in the rumen. This observation is supported by Ha and Kennelly (1984) for samples incubated for 12 hrs or more, but not for less than 12 hrs. Ha and Kennelly (1984) observed that at less than 12 hrs, SBM nitrogen disappeared at a slower rate than CM nitrogen. However, it is important to remember that if DAPA is used as a marker for microbial contamination it must be analyzed for, and subtracted from, the initial feed samples. Canola meal and SBM were similar with respect to DM degradability and Ha and Kennelly (1984) have reported a similar result.

The results reported in this study, using the nylon bag technique, show that at all incubation periods EAA degradation is similar from both CM and SBM. However, the order of degradation of EAA is different from that reported by other researchers. Other researchers have reported that methionine is the most easily degraded amino acid in the rumen (Setälä and Syrjälä-Qvist 1982; Setälä and Syrjälä-Qvist 1984; Varvikko et al. 1983). In this study the degradability of methionine was similar to that of other amino acids, except at 8 hrs where it did appear to be more degradable. However, in SBM, methionine appeared to be the most

resistant amino acid at 4 12 16 and 30 hrs. Valine and isoleucine of CM protein have been reported to be the most resistant to ruminal degradation (Lewis and Emery 1962; Setälä and Syrjälä-Qvist 1984). However, in this study there were no apparent differences between valine and isoleucine degradability and that of the other EAA's. Histidine was found to be the most degradable amino acid from CM at 4, 8 and 30 hrs, and this observation is supported by that of Setälä and Syrjälä-Qvist (1984).

The data reported in this study, and that reported by other researchers, supports the statement that the nylon bag technique only measures disappearance from the bag itself at a particular point in time; experimental animals and the various diets used can all affect the results obtained using this technique (Setälä and Syrjälä-Qvist 1984). Furthermore, it has been suggested by Ha and Kennelly (1984) that the nylon bag technique can only be validated by comparing results obtained from it, to results obtained for the same feeds from in vivo techniques. The nylon bag technique is simple to use, and allows for rapid determination of rate of degradation of protein samples at a particular point in time, however, care must be taken in the interpretation of the results obtained.

Mobile Nylon Bag Technique

It is difficult to quantitate the absorption of nutrients, particularly amino acids, from the small intestine of ruminants. The biggest problem to date has been the partitioning of bacterial crude protein from undegraded feed protein. A procedure recently developed by

Sauer et al. (1983) for pigs may be the most suitable method to date for determining true digestibility. It has been modified for ruminants (Kirkpatrick and Kennelly 1985), and named the "Modified Mobile Nylon Bag Technique." It has been used by DeBoer et al. (1986), Kirkpatrick and Kennelly (1985) and Rae and Smithard (1985). The procedure involves isolating a small feed sample (1-2 g) in a small nylon bag (3.5 x 5.5 cm) and following it through the entire digestive tract. The animals must be fitted with rumen and duodenal cannulae. The nylon bags must be incubated in the rumen for a predetermined length of time, removed and incubated in pepsin-HCl solution for 3 hrs at 39° C to simulate abomasal effects, then inserted into the small intestine via the duodenal cannula, and finally collected in the feces about 16-20 hrs later. This technique is encouraging as it yields lower tract digestibility results that are similar to those obtained through conventional methods.

The results reported in this study show that both N and DM were more digestible in the lower tract from SBM than from CM. This is supported by Kirkpatrick and Kennelly (1985) and Rae and Smithard (1985). This study also shows that the longer CM is retained in the rumen, the less available CM in the digesta becomes in the lower digestive tract. The trend was not apparent for SBM. This observation was also reported by DeBoer et al. (1986) and Rae and Smithard (1985).

The results reported in this study show that at 0 and 4 hrs, all EAA, except Met were more digestible from SBM than from CM. At 8 hrs all EAA, except Met and Phe had digestibilities that were similar from both SBM and CM. At 12 hrs Lys and Met digestibilities were similar from both SBM and CM, and Val, Ile, Leu and Thr were more digestible

from SBM. At 16 hrs, all EAA had similar digestibilities from both SBM and CM. The effect of rumen retention time on EAA digestibility showed that the digestibility of most EAA's, between 4 and 12 hrs, did not differ much. However, a trend was apparent that the digestibility of most EAA's was reduced after 16 hrs, although this was not always significant. Most of the work to date on amino acid absorption in the lower digestive tract of ruminants has been done in vitro, and on sheep (Coello da Silva et al. 1972; Phillips et al. 1976; Santos et al. 1983). It, therefore, may not be appropriate to compare those results with the results obtained in this study.

The "Mobile Nylon Bag Technique" shows promise for the future. It allows determination of the digestibility in the lower digestive tract of nutrients that have escaped rumen degradation. This type of information is extremely important for ration formulation in light of the new protein systems for the feeding of ruminants. Energy digestibility data obtained raised some questions on the suitability of the technique for measuring energy availability. It is however the best method, so far, for partitioning bacterial CP from undegraded feed CP.

SUMMARY

Rumen Degradation of Canola Meal Compared to Soybean Meal for Ruminants

These data show that the N escape values of SBM in the rumen fall within the range of values obtained for the N escape of CM in the rumen. The DM escape value of SBM is lower than that of CM at 30 h, but for other incubation periods the values for SBM fall within the range of values obtained for CM. Energy disappearance from the rumen for SBM is lower than that of CM at 4 and 16 h. However, the energy disappearance values from the rumen for SBM at 8, 12 and 30 h were similar to some of the values obtained for CM. These data show that EAA escape for SBM falls within the range of values found for CM from all incubation periods, except His at 4 h which degraded less for SBM than from CM, and Met at 12 h, which degraded less for SBM than for CM.

Although the nylon bag technique to date is the best method of measuring the rumen degradability of feedstuffs, it only measures disappearance from the bag itself at a particular point in time. Therefore, great care should be taken in interpreting data obtained from the use of this technique. There is still a lot of variation involved in this type of experimentation and more work needs to be done to standardize the technique.

The Digestibility of Canola Meal and Soybean Meal in the Lower Digestive Tract of Ruminants

These data show that the N digestibility from SBM is greater than the N digestibility from CM in the lower digestive tract. The DM digestibility of SBM is greater than the DM digestibility of CM in the

lower tract, again except at 8 h rumen retention time where there is no apparent difference between SBM and CM in the lower tract. Energy is more available from SBM in the lower tract than from CM, except at 4 h rumen retention time, at which point availability is similar for both SBM and CM. These data show that all EAA digestibilities are greater from SBM than from CM at 0 and 4 h rumen retention time except Met which is similar for both SBM and CM. All EAA digestibilities are similar for SBM and CM at 8 12 and 16 h, except Met and Phe at 8 h which are more available for SBM, and Val, Ile, Leu and Thr at 12 h which are more available from SBM.

These data show a trend for CM, though not always a significant one, that the longer CM is retained in the rumen, the less available the N becomes in the lower digestive tract. This trend was not apparent for SBM. At a long retention period such as 16 h, the DM digestibility of CM is significantly reduced, again this trend is not apparent for SBM DM. A retention period of 16 h also results in a higher availability of energy in the lower tract for both SBM and CM. Retention periods between 4 and 12 h did not result in digestibility differences of EAA for SBM and CM, however, a retention period of 16 h resulted in reduced EAA digestibility for both SBM and CM. The trend for EAA digestibilities, however, was not always significant.

The "Mobile Nylon Bag Technique" is a very simple technique that allows for rapid determination of "true" digestibility in the lower digestive tract of ruminants. However, since this technique depends on nylon bag incubation in the rumen, which is a technique that still needs to be standardized, care must be taken in interpreting data obtained by

this technique. It is, however, the best method to date for partitioning bacterial CP from undegraded feed CP.

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APPENDIX

Appendix A

CrO₃ Concentrations %Initial Dose .75 g CrO₃/250 ml

Appropriate time interval 4 h (actually varied)

Trial #2 12 h

Time	Steer #1			Steer #2		
	^a S ₁	^b S ₂	Ave.	^a S ₁	^b S ₂	Ave.
t ₀	.0035	.0000	.0018	.0000	.0000	.0000
t ₁	.0012	.0000	.0006	.0000	.0000	.0000
t ₂	.0018	.0018	.0018	.0029	.0018	.0024
t ₃	.0029	.0064	.0047	.0029	.0006	.0018
t ₄	.0338	.0286	.0312	.0058	.0000	.0029
t ₅	.0366	.0326	.0346	.0016	.0158	.0087
t ₆	.0209	.0197	.0203	.0179	.0155	.0167
t ₇	.0151	.0157	.0308	.0139	.0146	.0143
t ₈	.0131	.0123	.0127	.0110	.0116	.0113
t ₉	.0058	.0070	.0064	.0099	.0117	.0108
t ₁₀	.0040	.0053	.0047	.0052	.0012	.0032

^aS₁ = Sample 1^bS₂ = Sample 2

Appendix A

Trial #3 8 h

Time	Steer #1			Steer #2		
	S ₁	S ₂	Ave.	S ₁	S ₂	Ave.
t ₀	.0018	.0012	.0015	.0006	.0000	.0003
t ₁	.0012	.0012	.0012	.0029	.0018	.0024
t ₂	.0000	.0012	.0006	.0018	.0053	.0036
t ₃	.0006	.0170	.0088	.0006	.0006	.0006
t ₄	.0215	.0245	.0230	.0041	.0053	.0047
t ₅	.0175	.0169	.0172	.0222	.0246	.0234
t ₆	.0128	.0158	.0143	.0187	.0181	.0184
t ₇	.0193	.0192	.0193	.0104	.0128	.0116
t ₈	.0140	.0169	.0155	.0199	.0181	.0190
t ₉	.0087	.0076	.0082	.0158	.0134	.0146

Trial #4 4 h

Time	Steer #1			Steer #2		
	S ₁	S ₂	Ave.	S ₁	S ₂	Ave.
t ₀	.0012	.0023	.0018	.0018	.0012	.0015
t ₁	.0006	.0023	.0015	.0012	.0023	.0018
t ₂	.0006	.0018	.0012	.0029	.0035	.0032
t ₃	.0257	.0239	.0248	.0245	.0186	.0216
t ₄	.0180	.0195	.0188	.0134	.0117	.0126
t ₅	.0093	.0145	.0119	.0093	.0140	.0117
t ₆	.0111	.0146	.0129	.0064	.0070	.0067
t ₇	.0052	.0041	.0047	.0029	.0059	.0044

Appendix A

Trial #5 30 h

Time	Steer #1			Steer #2		
	S ₁	S ₂	Ave.	S ₁	S ₂	Ave.
t ₀	.0018	.0012	.0015	.0000	.0023	.0012
t ₁	.0029	.0023	.0026	.0035	.0029	.0032
t ₂	.0023	.0023	.0023	.0006	.0000	.0003
t ₃	.0198	.0216	.0207	.0135	.0134	.0135
t ₄	.0344	.0369	.0357	.0157	.0181	.0169
t ₅	.0256	.0275	.0266	.0216	.0239	.0228
t ₆	.0251	.0211	.0231	.0175	.0158	.0167
t ₇	.0174	.0209	.0192	.0157	.0169	.0163
t ₈	.0052	.0076	.0064	.0058	.0076	.0067
t ₉	.0064	.0029	.0047	.0047	.0047	.0047

Appendix A

Outflow Rate Sample Calculation

Step 1. The concentrations of Cr in the feces are transformed to natural logs.

Rumen Incubation 4 h

<u>Steer #2</u>		
Sample Time t(h)	CR Conc %	ln
0	0	-
4	.0018	-6.32
8	.0032	-5.74
12	.0216	-3.84
16	.0126	-4.37
20	.0117	-4.45
24	.0067	-5.01
28	.0044	-5.43

Step 2. The absolute value of the regression coefficient obtained from ln of Cr concentration in the linear portion of the curve is K1. The highest or peak value (12 h) was used as the starting point for the regression.

Rumen Incubation 4 h

<u>Steer #2</u>			Mean of ln = -4.62
i = 20			
Deviations From the Mean	Square	Product	
i	ln	i ln	
-8	.78	64	-6.24
-4	.25	16	-1.00
0	.17	0	0
4	-.39	16	-1.56
8	-.81	64	-6.48
-----			-----
160			-15.28

$$\text{(Slope b) } K1 = \frac{15.28}{160} = .10$$

$$r^2 = \frac{(.10)(15.28)}{1.508} = \frac{1.528}{1.508} = 1.0$$

(in deviations from
the mean)

$$\text{Outflow rate (K1) = .10}$$

Appendix A

Outflow Rates Obtained Over the Entire Experiment

Rumen Incubation Period	Steer #1 K ₁	Steer #2 K ₁
12 h	.10	.04
8 h	.04	.07
4 h	.09	.10
0 h	.10	.10

Average K₁ over Entire Experiment = .08

Sample Calculation for Cumulative Degradation Rate

Protein Degradation of Sample A:

$$p = a + b (1 - e^{-ct}) \quad P = a + \frac{bc}{c+k}$$

Step 1. Calculate c, the rate per hour at which the "b" fraction is degrading.

$$a (0h) = 28.20\%$$

$$p (t 4h) = 44.4\% \text{ (a value from a sensitive part of the curve)}$$

$$a + b = 67.00\%$$

$$e^{-c4} = \frac{a + b - p}{b} = \frac{28.20 + 38.80 - 44.40}{38.80} = .5825$$

$$\text{Calculate } \ln \text{ of } .5825 = -.540$$

$$-c4 = -.540$$

$$c = .135$$

Step 2. Calculate P, cumulative degradation rate.

$$P = 28.20 + \frac{(42.15)(.343)}{.135 + .08}$$

$$= 28.20 + 24.37$$

$$= 52.60\%$$

Appendix B

Calculation of Bacterial N as a % of Total N, Content of the Bags Using DAPA N as a Marker.

Sample Calculation: Rumen 4 h

$$\text{Sample A. } \frac{0.0283}{0.6} = 0.047 \times 100 = 4.7\%$$

Rumen Incubation 4 h

Sample	Rumen		Lower Tract	
	DAPA N %	Bact. N%	DAPA N %	Bact. N%
A	0.0283	4.7	0.0182	3.0
B	0.0203	3.4	0.0212	3.5
C	0.0147	1.5	0.0207	3.5
D	0.0227	3.8	0.0242	4.0
E	0.0167	2.8	0.0180	3.0
F	0.0315	5.3	0.0230	3.8

Ruman Incubation 8 h

Sample	Rumen		Lower Tract	
	DAPA N %	Bact. N%	DAPA N %	Bact. N%
A	0.0357	6.0	0.0131	2.2
B	0.0162	2.7	0.0228	3.8
C	0.0164	2.7	0.0168	2.8
D	0.0554	9.2	0.0195	3.3
E	0.0311	5.2	0.0149	2.5
F	0.0332	5.5	0.0303	5.0

Ruman Incubation 12 h

Sample	Rumen		Lower Tract	
	DAPA N %	Bact. N%	DAPA N %	Bact. N%
A	0.0108	1.8	0.0141	2.4
B	0.0314	5.2	0.0159	2.7
C	0.0219	3.7	0.0794	13.2
D	0.0242	4.0	0.0146	2.4
E	0.0218	3.6	0.0171	2.9
F	0.0419	7.0	0.0273	4.6

Appendix B

Rumen Incubation 16 h

Sample	Rumen		Lower Tract	
	DAPA N %	Bact. N%	DAPA N %	Bact. N%
A	0.0104	1.7	0.0045	.75
B	0.0113	1.9	0.0060	1.00
C	0.0114	1.9	0.0125	2.1
D	0.0117	2.0	0.0044	.73
E	0.0107	1.8	0.0056	.93
F	0.0114	1.9	0.0252	4.2

Ruman Incubation 30 h

Sample	Rumen	
	DAPA N %	Bact. N%
A	0.0116	1.9
B	0.0134	2.2
C	0.0129	2.2
D	0.0170	2.8
E	0.0120	2.0
F	0.0125	2.1

Ruman Incubation 0 h

Sample	Lower Tract	
	DAPA N %	Bact. N%
A	0.0107	1.8
B	0.0143	2.4
C	0.0126	2.1
D	0.0111	1.9
E	0.0119	2.0
F	0.0129	2.2

Appendix C

Correction Factors

		N	DMg	Energy Cal/g

Nylon		.70%	-	3656.99
Blanks	0 h R	.0003g	.0036	1302.31
	4 h R	.0002	.0002	1241.62
	LT	.0003	.0028	1507.62
	8 h R	Neg	.0041	1491.81
	LT	Neg	.0004	1597.68
	12 h R	.0005	.0084	1584.95
	LT	.0002	.0100	1374.01
	16 h R	.0006	.0124	730.66
	LT	.0006	.0068	860.00
	30 h R	.0011	.0385	1703.78

R - Rumen

LT - Lower Digestive Tract

Appendix D

Solubility of Samples Rinsed in Distilled Water for 10 Seconds (Average of two samples) (g)

Sample	Initial Sample Wt	DM Residue	N Residue	Initial N
A	.5026	.4155	.0222	.0309
B	.5732	.4746	.0233	.0309
C	.5162	.4534	.0237	.0317
D	.5369	.4796	.0272	.0342
E	.5424	.4626	.0255	.0327
F	.5405	.4680	.0324	.0395

DM and N Residues, Corrected for Bacterial Contamination, From the Rumen 16 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.4677	.2031	.0308	.0123
B	.4926	.2227	.0291	.0158
C	.5065	.2105	.0332	.0171
D	.4821	.2304	.0339	.0179
E	.4810	.2090	.0325	.0151
F	.4628	.1694	.0389	.0162

Appendix D

DM and N Residues, Corrected for Bacterial Contamination From the Rumen
4 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.4768	.2884	.0315	.0175
B	.5092	.3423	.0301	.0203
C	.5132	.3463	.0321	.0219
D	.4873	.3458	.0332	.0215
E	.4835	.3296	.0309	.0193
F	.4857	.2468	.0385	.0262

DM and N Residues, Corrected for Bacterial Contamination, From the Rumen
8 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.4834	.2405	.0310	.0133
B	.4962	.2825	.0296	.0179
C	.5308	.2811	.0313	.0176
D	.4867	.2960	.0325	.0174
E	.4883	.2742	.0314	.0172
F	.4795	.2483	.0372	.0230

Appendix D

DM and N Residues, Corrected for Bacterial Contamination From the Rumen
12 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.5277	.2754	.0337	.0157
B	.5371	.3140	.0314	.0177
C	.5218	.2706	.0316	.0175
D	.5265	.3099	.0341	.0195
E	.5187	.2877	.0311	.0165
F	.5203	.2722	.0375	.0246

DM and N Residues, Corrected for Bacterial Contamination, From the Rumen
30 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.5142	.1177	.0315	.0104
B	.5345	.1433	.0300	.0115
C	.5310	.1016	.0315	.0132
D	.5091	.1622	.0331	.0173
E	.5294	.1398	.0309	.0112
F	.5239	.0488	.0383	.0131

Appendix D

DM and N Residues, Corrected for Bacterial Contamination From the Lower Tract, Rumen Incubation 0 h (g) (Average of 4 Samples)

Sample	Initial Sample Wt	DM Residue	Initial N	N Residue
A	.4851	.1581	.0317	.0042
B	.5037	.1742	.0297	.0053
C	.5130	.1530	.0319	.0035
D	.4850	.1462	.0332	.0034
E	.4893	.1688	.0317	.0046
F	.4722	.0683	.0380	.0026

DM and N Residues, Corrected for Bacterial Contamination, From the Lower Tract, Rumen Incubation 4 h (g) (Average of 4 Samples)

Sample	DM Residue	N Residue
A	.1134	.0035*
B	.1464	.0041
C	.1070	.0036
D	.1223	.0047
E	.1207	.0041
F	.0574	.0016

*Average of 3 samples

Appendix D

DM and N Residues, Corrected for Bacterial Contamination From the Lower Tract, Rumen Incubation 8 h (g)

Sample	DM Residue	N Residue
A	.1117	.0027
B	.1352	.0088*
C	.1075	.0032
D	.1439	.0041
E	.1285	.0032
F	.0349	.0014*

*Average of 3 samples

DM and N Residues, Corrected for Bacterial Contamination From the Lower Tract, Rumen Incubation 12 h (g)

Sample	DM Residue	N Residue
A	.1315	.0036
B	.1370	.0054
C	.1190	.0030
D	.1181	.0045
E	.1483	.0040
F	.0488	.0012

Appendix D

DM and N Residues, Corrected for Bacterial Contamination From the Lower Tract, Rumen Incubation 16 h (g)

Sample	DM Residue	N Residue
A	.1236	.0054
B	.1531	.0057
C	.1315	.0045
D	.1301	.0049
E	.1237	.0053
F	.0385	.0023

*Average of 3 samples

Corrected Energy Values Cal/g, Rumen Incubation 0 h

Sample	Initial Energy	Lower Tract Energy
A	4229.93	2822.55
B	4304.59	2721.70
C	4246.80	2859.20
D	4271.67	2862.92
E	4216.92	2823.02*
F	4163.21	2674.91*

*Average of 3 samples

Appendix D

Corrected Energy Values Cal/g, Rumen Incubation 4 h

Sample	Rumen Energy	Lower Tract Energy
A	2996.62	2942.43*
B	2927.05*	2872.09*
C	2963.23*	2872.86*
D	3057.54*	2859.88
E	2949.32	2898.88
F	3194.55*	2910.57*

*Average of 3 samples

Corrected Energy Values Cal/g, Rumen Incubation 8 h

Sample	Rumen Energy	Lower Tract Energy
A	3360.91	3404.37
B	3260.89	3239.00
C	3306.49	3251.03
D	3281.86	3318.69
E	3319.66	3291.25*
F	3366.49	3328.87

*Average of 3 samples

Appendix D

Corrected Energy Values Cal/g, Rumen Incubation 12 h

Sample	Rumen Energy	Lower Tract Energy
A	3256.12	3385.86
B	3173.08	2524.97
C	3393.88	3139.25
D	3362.13	3247.43
E	3212.99	3385.55
F	3431.93*	3329.76

*Average of 3 samples

Corrected Energy Values Cal/g, Rumen Incubation 16 h

Sample	Rumen Energy	Lower Tract Energy
A	3886.50	2829.66
B	3807.45	2993.70
C	3984.13	3009.64
D	3918.30	2779.85*
E	3977.90*	2855.48*
F	4171.55*	1883.29

*Average of 3 samples

Appendix D

Corrected Energy Values Cal/g, Rumen Incubation 30 h

Sample	Rumen Energy
A	3089.66
B	2876.14
C	2951.59
D	3042.20*
E	2885.15
F	3069.43

*Average of 3 samples

Appendix E

Essential Amino Acid Disappearance Values in the Lower Digestive Tract of Samples Incubated in the Rumen for Various Time Intervals.

0 h Rumen Incubation (Lower Tract Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0101	.0111	.0090	89.11
His	.0068	.0004	.0064	94.12
Val	.0096	.0014	.0082	85.42
Thr	.0078	.0011	.0067	85.90
Ile	.0081	.0010	.0071	87.65
Leu	.0125	.0016	.0109	87.20
Phe	.0078	.0010	.0068	87.18
Met	.0034	.0006	.0028	82.34

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0011	.0078	87.64
His	.0062	.0004	.0058	93.55
Val	.0083	.0012	.0071	85.54
Thr	.0071	.0011	.0060	84.51
Ile	.0071	.0011	.0060	84.51
Leu	.0109	.0017	.0092	84.40
Phe	.0068	.0011	.0057	83.82
Met	.0027	.0004	.0023	85.19

Appendix E

0 h Rumen Incubation (Lower Tract Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0096	.0010	.0086	89.58
His	.0069	.0003	.0066	95.65
Val	.0092	.0014	.0078	84.78
Thr	.0082	.0011	.0071	86.59
Ile	.0080	.0011	.0069	86.25
Leu	.0121	.0016	.0105	86.78
Phe	.0076	.0013	.0063	82.89
Met	.0037	.0005	.0032	86.49

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0011	.0078	87.64
His	.0071	.0004	.0067	94.37
Val	.0092	.0016	.0076	82.61
Thr	.0077	.0012	.0065	84.42
Ile	.0079	.0011	.0068	86.08
Leu	.0117	.0016	.0101	86.32
Phe	.0076	.0011	.0065	85.53
Met	.0037	.0004	.0033	89.19

Appendix E

0 h Rumen Incubation (Lower Tract Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0100	.0011	.0089	89.00
His	.0069	.0004	.0065	94.20
Val	.0096	.0016	.0080	83.33
Thr	.0083	.0013	.0070	84.34
Ile	.0083	.0012	.0071	85.54
Leu	.0125	.0017	.0108	86.40
Phe	.0079	.0011	.0068	86.08
Met	.0040	.0005	.0035	87.50

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0138	.0084	.0134	97.10
His	.0064	.0002	.0062	96.88
Val	.0109	.0005	.0104	95.41
Thr	.0088	.0003	.0085	96.59
Ile	.0109	.0004	.0105	96.33
Leu	.0163	.0006	.0157	96.32
Phe	.0115	.0004	.0111	96.52
Met	.0016	.0002	.0014	87.50

Appendix E

4 h Rumen Incubation

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0059	.0009	.0050	84.75
His	.0027	.0003	.0024	88.89
Val	.0060	.0009	.0051	85.00
Thr	.0047	.0007	.0040	85.11
Ile	.0046	.0007	.0039	84.78
Leu	.0075	.0010	.0065	86.67
Phe	.0048	.0007	.0041	85.42
Met	.0022	.0003	.0019	86.36

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0069	.0011	.0058	84.06
His	.0030	.0004	.0026	86.67
Val	.0070	.0013	.0057	81.43
Thr	.0053	.0010	.0043	81.13
Ile	.0054	.0010	.0044	81.48
Leu	.0087	.0015	.0072	82.76
Phe	.0054	.0010	.0044	81.48
Met	.0025	.0005	.0020	80.00

Appendix E

4 h Rumen Incubation

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0062	.0008	.0054	87.10
His	.0032	.0003	.0029	90.63
Val	.0077	.0009	.0068	88.31
Thr	.0059	.0007	.0052	88.14
Ile	.0057	.0007	.0050	87.72
Leu	.0093	.0009	.0084	90.32
Phe	.0059	.0006	.0053	89.83
Met	.0022	.0004	.0018	81.82

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0070	.0009	.0061	87.14
His	.0035	.0003	.0032	91.43
Val	.0077	.0010	.0067	87.01
Thr	.0061	.0008	.0053	86.89
Ile	.0059	.0007	.0052	88.14
Leu	.0097	.0009	.0088	90.72
Phe	.0062	.0007	.0055	88.71
Met	.0029	.0005	.0024	82.76

Appendix E

4 h Rumen Incubation

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0066	.0007	.0059	89.39
His	.0030	.0003	.0027	90.00
Val	.0070	.0009	.0061	87.14
Thr	.0055	.0007	.0048	87.26
Ile	.0052	.0007	.0045	86.54
Leu	.0086	.0009	.0077	89.53
Phe	.0054	.0008	.0046	85.19
Met	.0026	.0003	.0023	88.46

Canola Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0086	.0003	.0083	96.51
His	.0044	.0001	.0043	97.73
Val	.0079	.0004	.0075	94.94
Thr	.0066	.0002	.0064	96.97
Ile	.0078	.0002	.0076	97.44
Leu	.0123	.0005	.0118	95.93
Phe	.0091	.0003	.0088	96.70
Met	.0017	.0003	.0014	82.35

Appendix E

8 h Rumen Incubation

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0047	.0020	.0027	57.45
His	.0017	.0007	.0010	58.82
Val	.0044	.0023	.0021	47.73
Thr	.0041	.0021	.0020	48.78
Ile	.0033	.0017	.0011	48.48
Leu	.0060	.0028	.0032	53.33
Phe	.0041	.0015	.0026	63.41
Met	.0014	.0005	.0009	64.29

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0050	.0020	.0030	60.00
His	.0023	.0007	.0016	69.57
Val	.0055	.0023	.0032	58.18
Thr	.0049	.0021	.0028	57.14
Ile	.0044	.0017	.0027	61.36
Leu	.0075	.0028	.0047	62.67
Phe	.0048	.0015	.0033	68.75
Met	.0012	.0005	.0007	58.33

Appendix E

8 h Rumen Incubation

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0052	.0007	.0045	86.54
His	.0026	.0003	.0023	88.46
Val	.0058	.0008	.0050	86.21
Thr	.0052	.0008	.0044	84.62
Ile	.0055	.0005	.0050	86.21
Leu	.0072	.0008	.0064	88.89
Phe	.0053	.0007	.0046	86.79
Met	.0015	.0004	.0011	73.33

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0056	.0011	.0045	80.36
His	.0016	.0004	.0012	75.00
Val	.0057	.0011	.0046	80.70
Thr	.0055	.0010	.0045	81.82
Ile	.0042	.0008	.0034	80.95
Leu	.0081	.0014	.0067	82.72
Phe	.0052	.0009	.0043	82.69
Met	.0023	.0006	.0017	73.91

Appendix E

8 h Rumen Incubation

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0043	.0009	.0034	79.07
His	.0022	.0003	.0019	86.36
Val	.0046	.0008	.0038	82.61
Thr	.0044	.0008	.0036	81.82
Ile	.0039	.0006	.0033	84.62
Leu	.0067	.0009	.0058	86.57
Phe	.0044	.0006	.0038	86.36
Met	.0015	.0005	.0010	66.67

Canola Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0074	.0002	.0072	97.30
His	.0038	.0001	.0037	97.37
Val	.0064	.0002	.0062	96.88
Thr	.0058	.0002	.0056	96.55
Ile	.0058	.0002	.0056	96.55
Leu	.0100	.0003	.0097	97.00
Phe	.0075	.0002	.0073	97.33
Met	.0017	.0001	.0016	94.12

Appendix E

12 h Rumen Incubation

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0046	.0010	.0036	78.26
His	.0019	.0003	.0016	84.21
Val	.0042	.0011	.0031	73.81
Thr	.0037	.0009	.0028	75.68
Ile	.0029	.0007	.0022	75.86
Leu	.0061	.0012	.0049	80.33
Phe	.0040	.0008	.0032	80.00
Met	.0009	.0004	.0005	55.56

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0062	.0011	.0051	82.26
His	.0024	.0004	.0020	83.33
Val	.0065	.0011	.0054	83.08
Thr	.0055	.0020	.0035	63.64
Ile	.0045	.0009	.0036	80.00
Leu	.0079	.0014	.0065	82.28
Phe	.0054	.0009	.0045	83.33
Met	.0020	.0004	.0016	80.00

Appendix E

12 h Rumen Incubation

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0055	.0010	.0045	81.82
His	.0028	.0003	.0025	89.29
Val	.0070	.0011	.0059	84.29
Thr	.0061	.0009	.0052	85.25
Ile	.0045	.0008	.0037	82.22
Leu	.0092	.0012	.0080	86.96
Phe	.0054	.0008	.0046	85.19
Met	.0012	.0005	.0007	58.33

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0049	.0009	.0040	81.63
His	.0025	.0003	.0022	88.00
Val	.0063	.0010	.0053	84.13
Thr	.0056	.0009	.0047	83.93
Ile	.0047	.0007	.0040	85.11
Leu	.0085	.0010	.0075	88.24
Phe	.0054	.0006	.0048	88.89
Met	.0021	.0003	.0018	85.71

Appendix E

12 h Rumen Incubation

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0048	.0011	.0037	77.08
His	.0022	.0004	.0018	81.82
Val	.0056	.0011	.0045	80.36
Thr	.0048	.0010	.0038	79.17
Ile	.0043	.0008	.0035	81.40
Leu	.0073	.0014	.0059	80.82
Phe	.0046	.0009	.0037	80.43
Met	.0014	.0004	.0010	71.43

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0077	.0005	.0072	93.51
His	.0038	.0002	.0036	94.74
Val	.0069	.0005	.0064	92.75
Thr	.0064	.0004	.0060	93.75
Ile	.0069	.0004	.0065	94.20
Leu	.0114	.0006	.0108	94.74
Phe	.0085	.0004	.0081	95.29
Met	.0017	.0001	.0016	94.12

Appendix E

16 h Rumen Incubation

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0024	.0007	.0017	70.83
His	.0015	.0006	.0009	60.00
Val	.0026	.0006	.0020	76.92
Thr	.0014	.0003	.0011	78.57
Ile	.0029	.0007	.0022	75.86
Leu	.0032	.0010	.0022	68.75
Phe	.0022	.0005	.0017	77.27
Met	.0017	.0011	.0006	35.29

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0025	.0009	.0016	64.00
His	.0017	.0007	.0010	58.82
Val	.0026	.0010	.0016	61.54
Thr	.0024	.0010	.0014	58.33
Ile	.0026	.0011	.0015	57.69
Leu	.0033	.0015	.0018	54.55
Phe	.0024	.0007	.0017	70.83
Met	.0015	.0005	.0010	66.67

Appendix E

16 h Rumen Incubation

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0036	.0006	.0030	83.33
His	.0026	.0006	.0020	76.92
Val	.0038	.0008	.0030	78.95
Thr	.0036	.0008	.0028	77.78
Ile	.0038	.0008	.0030	78.95
Leu	.0047	.0013	.0034	72.34
Phe	.0035	.0005	.0030	85.71
Met	.0012	.0001	.0011	91.67

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0037	.0007	.0030	81.08
His	.0028	.0006	.0022	78.57
Val	.0039	.0008	.0031	79.49
Thr	.0038	.0008	.0030	78.95
Ile	.0039	.0008	.0031	79.49
Leu	.0048	.0012	.0036	75.00
Phe	.0037	.0005	.0032	86.49
Met	.0017	.0001	.0016	94.12

Appendix E

16 h Rumen Incubation

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0034	.0006	.0028	82.35
His	.0024	.0006	.0018	75.00
Val	.0036	.0007	.0029	80.56
Thr	.0031	.0007	.0024	77.42
Ile	.0034	.0008	.0026	76.47
Leu	.0045	.0011	.0034	75.56
Phe	.0035	.0005	.0030	85.71
Met	.0015	.0002	.0013	86.67

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0025	.0002	.0023	92.00
His	.0017	.0003	.0014	82.35
Val	.0025	.0004	.0021	84.00
Thr	.0022	.0003	.0019	86.36
Ile	.0026	.0004	.0022	84.62
Leu	.0035	.0007	.0028	80.00
Phe	.0025	.0004	.0021	84.00
Met	.0015	.0002	.0013	86.67

Appendix E

30 h Rumen Incubation (Rumen Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0102	.0016	.0086	84.31
His	.0069	.0007	.0062	89.86
Val	.0096	.0018	.0078	81.25
Thr	.0079	.0015	.0064	86.49
Ile	.0081	.0017	.0064	79.01
Leu	.0126	.0026	.0100	79.74
Phe	.0079	.0017	.0062	78.48
Met	.0034	.0009	.0025	73.53

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0090	.0027	.0063	70.00
His	.0063	.0010	.0053	84.13
Val	.0083	.0026	.0057	68.67
Thr	.0072	.0019	.0053	73.61
Ile	.0072	.0022	.0050	69.44
Leu	.0110	.0034	.0076	69.10
Phe	.0069	.0023	.0046	66.67
Met	.0028	.0009	.0019	67.86

Appendix E

30 h Rumen Incubation (Rumen Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0096	.0016	.0080	83.33
His	.0069	.0007	.0062	89.86
Val	.0092	.0019	.0073	79.35
Thr	.0082	.0015	.0067	81.71
Ile	.0080	.0016	.0064	80.00
Leu	.0121	.0026	.0095	78.51
Phe	.0076	.0017	.0059	74.68
Met	.0037	.0008	.0029	78.38

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0030	.0059	66.29
His	.0071	.0012	.0059	83.10
Val	.0092	.0040	.0052	56.52
Thr	.0078	.0031	.0047	60.26
Ile	.0079	.0034	.0045	56.96
Leu	.0118	.0051	.0067	56.78
Phe	.0077	.0037	.0040	51.95
Met	.0037	.0015	.0022	59.46

Appendix E

30 h Rumen Incubation (Rumen Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0098	.0026	.0072	73.47
His	.0068	.0011	.0057	83.82
Val	.0094	.0029	.0065	69.15
Thr	.0081	.0022	.0059	72.84
Ile	.0082	.0025	.0057	69.51
Leu	.0123	.0040	.0083	67.48
Phe	.0078	.0027	.0051	65.38
Met	.0039	.0012	.0027	69.23

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0136	.0015	.0121	88.97
His	.0063	.0008	.0055	87.30
Val	.0107	.0014	.0093	86.92
Thr	.0087	.0012	.0075	86.21
Ile	.0108	.0015	.0093	86.11
Leu	.0160	.0022	.0138	86.25
Phe	.0113	.0017	.0096	84.96
Met	.0015	.0004	.0011	73.33

Appendix E

4 h Rumen Incubation (Rumen Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0099	.0059	.0040	40.40
His	.0066	.0027	.0039	59.09
Val	.0093	.0060	.0033	35.48
Thr	.0076	.0047	.0029	38.16
Ile	.0079	.0046	.0033	41.77
Leu	.0122	.0075	.0047	38.42
Phe	.0076	.0048	.0028	36.84
Met	.0033	.0022	.0011	33.33

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0069	.0020	22.47
His	.0063	.0030	.0033	52.38
Val	.0083	.0070	.0013	15.66
Thr	.0071	.0053	.0018	25.35
Ile	.0071	.0054	.0017	23.94
Leu	.0109	.0087	.0022	20.18
Phe	.0069	.0054	.0015	21.74
Met	.0027	.0025	.0002	7.41

Appendix E

4 h Rumen Incubation (Rumen Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0094	.0062	.0032	34.04
His	.0067	.0032	.0035	52.24
Val	.0090	.0077	.0013	14.44
Thr	.0080	.0059	.0021	26.25
Ile	.0079	.0057	.0022	27.85
Leu	.0119	.0093	.0026	21.85
Phe	.0074	.0059	.0015	20.27
Met	.0036	.0022	.0014	38.89

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0093	.0070	.0023	24.73
His	.0075	.0035	.0040	53.33
Val	.0097	.0077	.0020	20.62
Thr	.0081	.0061	.0020	24.69
Ile	.0083	.0059	.0024	28.92
Leu	.0123	.0097	.0026	21.14
Phe	.0080	.0062	.0018	22.50
Met	.0038	.0029	.0009	23.68

Appendix E

4 h Rumen Incubation (Rumen Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0096	.0066	.0030	31.25
His	.0066	.0030	.0036	54.55
Val	.0092	.0070	.0022	23.91
Thr	.0080	.0055	.0025	31.25
Ile	.0080	.0052	.0028	35.00
Leu	.0120	.0086	.0034	28.33
Phe	.0076	.0054	.0022	28.95
Met	.0038	.0026	.0012	31.58

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0142	.0086	.0056	39.44
His	.0066	.0044	.0022	33.33
Val	.0113	.0079	.0034	30.09
Thr	.0091	.0066	.0025	27.47
Ile	.0113	.0078	.0035	30.97
Leu	.0169	.0123	.0046	27.22
Phe	.0118	.0091	.0027	22.88
Met	.0016	.0017	+.0001	+6.25

Appendix E

8 h Rumen Incubation (Rumen Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0100	.0047	.0053	53.00
His	.0067	.0017	.0050	74.63
Val	.0094	.0044	.0050	53.19
Thr	.0077	.0041	.0036	46.75
Ile	.0079	.0033	.0046	58.23
Leu	.0123	.0060	.0063	51.22
Phe	.0077	.0041	.0036	46.75
Met	.0033	.0014	.0019	57.58

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0088	.0050	.0038	43.18
His	.0062	.0023	.0039	62.90
Val	.0082	.0055	.0027	32.93
Thr	.0071	.0049	.0022	30.99
Ile	.0070	.0044	.0026	37.14
Leu				
Phe	.0068	.0048	.0020	29.41
Met	.0027	.0012	.0015	55.56

Appendix E

8 h Rumen Incubation (Rumen Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0097	.0052	.0045	46.39
His	.0069	.0026	.0043	62.32
Val	.0092	.0058	.0034	36.96
Thr	.0082	.0052	.0030	36.59
Ile	.0081	.0055	.0026	32.10
Leu	.0121	.0072	.0049	40.50
Phe	.0077	.0053	.0023	30.26
Met	.0037	.0015	.0022	59.46

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0056	.0033	37.08
His	.0071	.0016	.0055	77.46
Val	.0092	.0057	.0035	38.04
Thr	.0078	.0055	.0023	29.49
Ile	.0079	.0042	.0037	46.84
Leu	.0118	.0081	.0037	31.36
Phe	.0076	.0052	.0024	31.48
Met	.0037	.0023	.0014	37.84

Appendix E

8 h Rumen Incubation (Rumen Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0097	.0043	.0054	55.67
His	.0067	.0022	.0045	67.16
Val	.0093	.0046	.0047	50.54
Thr	.0080	.0044	.0036	45.00
Ile	.0081	.0039	.0042	51.85
Leu	.0121	.0067	.0054	44.63
Phe	.0077	.0044	.0033	42.86
Met	.0038	.0015	.0023	60.53

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0135	.0074	.0061	45.19
His	.0063	.0038	.0025	39.68
Val	.0107	.0064	.0043	40.19
Thr	.0086	.0058	.0028	32.56
Ile	.0107	.0058	.0049	45.79
Leu	.0159	.0100	.0059	37.11
Phe	.0112	.0075	.0037	33.04
Met	.0015	.0005	.0010	66.67

Appendix E

12 h Rumen Incubation (Rumen Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0104	.0046	.0058	55.77
His	.0070	.0019	.0051	72.86
Val	.0099	.0042	.0057	57.58
Thr	.0081	.0037	.0044	54.32
Ile	.0083	.0029	.0054	65.06
Leu	.0129	.0061	.0068	52.71
Phe	.0081	.0040	.0041	50.62
Met	.0035	.0009	.0026	74.29

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0087	.0062	.0025	28.74
His	.0061	.0024	.0037	60.66
Val	.0081	.0065	.0016	19.75
Thr	.0069	.0055	.0014	20.29
Ile	.0069	.0045	.0024	34.78
Leu	.0106	.0079	.0027	25.47
Phe	.0067	.0054	.0013	19.40
Met	.0027	.0020	.0007	25.93

Appendix E

12 h Rumen Incubation (Rumen Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0095	.0055	.0040	42.11
His	.0067	.0028	.0039	58.21
Val	.0090	.0070	.0020	22.22
Thr	.0080	.0061	.0019	23.75
Ile	.0079	.0045	.0034	43.04
Leu	.0119	.0092	.0027	22.69
Phe	.0074	.0054	.0020	27.03
Met	.0036	.0012	.0024	66.67

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0090	.0049	.0041	45.56
His	.0072	.0025	.0047	65.28
Val	.0093	.0063	.0030	32.26
Thr	.0078	.0056	.0022	28.21
Ile	.0080	.0047	.0033	41.25
Leu	.0119	.0085	.0034	28.57
Phe	.0077	.0054	.0023	29.87
Met	.0037	.0021	.0016	43.24

Appendix E

12 h Rumen Incubation (Rumen Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0094	.0048	.0046	48.94
His	.0064	.0022	.0042	65.63
Val	.0090	.0056	.0034	37.78
Thr	.0078	.0048	.0030	38.46
Ile	.0078	.0043	.0035	44.87
Leu	.0117	.0073	.0044	37.61
Phe	.0074	.0046	.0028	37.84
Met	.0037	.0015	.0022	59.46

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0137	.0077	.0060	43.80
His	.0064	.0038	.0026	40.63
Val	.0108	.0069	.0039	36.11
Thr	.0088	.0064	.0024	27.27
Ile	.0108	.0069	.0039	36.11
Leu	.0162	.0114	.0048	29.63
Phe	.0114	.0085	.0029	25.44
Met	.0016	.0017	+.0001	+6.25

Appendix E

16 h Rumen Incubation (Rumen Only)

Canola Meal Sample A

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0099	.0024	.0075	75.76
His	.0067	.0015	.0052	77.61
Val	.0094	.0026	.0068	72.34
Thr	.0077	.0014	.0063	81.82
Ile	.0079	.0029	.0050	63.29
Leu	.0123	.0032	.0091	73.98
Phe	.0077	.0022	.0055	71.43
Met	.0033	.0017	.0016	48.48

Canola Meal Sample B

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0089	.0025	.0064	71.91
His	.0062	.0017	.0045	72.58
Val	.0082	.0026	.0056	68.29
Thr	.0071	.0024	.0047	66.20
Ile	.0071	.0026	.0045	63.38
Leu	.0109	.0033	.0076	69.72
Phe	.0068	.0024	.0044	64.71
Met	.0027	.0015	.0012	44.44

Appendix E

16 h Rumen Incubation (Rumen Only)

Canola Meal Sample C

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0095	.0036	.0059	62.11
His	.0068	.0026	.0042	61.76
Val	.0090	.0038	.0052	57.78
Thr	.0081	.0036	.0045	55.56
Ile	.0079	.0038	.0041	51.90
Leu	.0119	.0047	.0072	60.50
Phe	.0074	.0035	.0039	52.70
Met	.0037	.0012	.0025	67.57

Canola Meal Sample D

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0093	.0037	.0056	60.22
His	.0074	.0028	.0046	62.16
Val	.0096	.0039	.0057	59.38
Thr	.0081	.0038	.0043	53.09
Ile	.0082	.0039	.0043	53.09
Leu	.0123	.0048	.0075	60.98
Phe	.0080	.0037	.0043	53.75
Met	.0038	.0017	.0027	55.26

Appendix E

16 h Rumen Incubation (Rumen Only)

Canola Meal Sample E

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0096	.0034	.0062	64.58
His	.0066	.0024	.0042	63.64
Val	.0092	.0036	.0056	60.87
Thr	.0080	.0031	.0049	61.25
Ile	.0080	.0034	.0046	57.50
Leu	.0120	.0045	.0075	62.50
Phe	.0076	.0035	.0041	53.94
Met	.0038	.0015	.0023	60.53

Soybean Meal Sample F

Amino Acid	Ave. Initial Wt. g	Ave. LGIT Wt. g	Dif. g	Dis. %
Lys	.0138	.0025	.0113	81.88
His	.0065	.0017	.0048	73.85
Val	.0110	.0025	.0085	77.27
Thr	.0089	.0022	.0067	75.28
Ile	.0110	.0026	.0084	76.36
Leu	.0163	.0035	.0128	78.53
Phe	.0115	.0025	.0090	78.26
Met	.0016	.0015	.0001	6.25

Appendix F

Origin of Protein Supplement Samples

A	CSP Foods Ltd. (Altona)
B	NARP Processors
C	Alberta Food Products
D	Canbra Foods
E	United Oilseed Products
F (SBM)	CSP Foods Ltd. (Altona)
