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

ISBN 0-315-51621-6

INSACCO RUMEN DEGRADATION, AND DIGESTIBILITY IN THE LOWER DIGESTIVE TRACT OF RUMINANTS, OF CANOLA MEAL AND SOYBEAN MEAL

BY

ELAINE M. KENDALL

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

MASTER OF SCIENCE

ACKNOWLEDGEMENTS

I sincerely wish to thank my advisor Dr. J. R. Ingalls for his patience and very valuable help. I wish to thank Mr. Peter Mills for the amino acid analysis. I wish to thank the Canola Council of Canada and Manitoba Trades and Technologies for their financial assistance. Finally, I would like to thank all of the Technical Staff and the Barn Staff of the Animal Science Department for their assistance in the completion of this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 accessable 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.