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By

SHAN CHUNG

A Thesis

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ABSTRACT

COMPLEMENTARY EFFECTS OF FEATHER MEAL WITH OTHER PROTEIN SOURCES IN CORN SILAGE DIETS FOR RUMINANT

By

SHAN CHUNG

An experiment was designed to determine the effects of different feed intake levels and sodium bicarbonate addition on the ruminal dry matter and crude protein degradation of six protein sources which include feather meal, corn gluten meal, blood meal, fish meal, soybean meal, and meat and bone meal. Six cannulated steers were assigned to three feed intake levels in a replicated 3 x 3 Latin square design. Dacron bags were removed from the rumen across time to determine ruminal dry matter and protein degradation; and amino acids composition of the undegraded residue. The results indicated that soybean meal had the highest and feather meal the lowest dry matter degradation value. Feather meal and corn gluten meal had lower ruminal degradability values. The amino acid composition of undegraded residue of all the protein sources was different than the original composition. Feather meal residue had greater sulfur - containing AA concentration than residue from other protein meals. A blend of various protein meals may supply a more balanced supply of AA than any single source.

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ABBREVIATION

AA . . . . . amino acid

ADIN . . . . . acid detergent insoluble nitrogen

ALA . . . . . alanine

ARG . . . . . arginine

ASP . . . . . aspartic acid

BLM . . . . . blood meal

BW . . . . . body weight

EAA . . . . . essential amino acid

CGM . . . . . corn gluten meal

CP . . . . . crude protein

CYS . . . . . cystine

DM . . . . . dry matter

FTH . . . . . feather meal

FSM . . . . . fish meal

GLU . . . . . glutamine

GLY . . . . . glycine

HIS . . . . . histidine

ILE . . . . . isoleucine

LEU . . . . . leucine

LYS . . . . . lysine

MET . . . . . methionine

MBM . . . . . meat and bone meal

MSE . . . . . mean square of error

NEAA . . . . . non-essential amino acid

PHE . . . . . phenylalanine

RDP . . . . . ruminal degradable protein

PRO . . . . . proline

SED . . . . . standard error of deviation

SEM . . . . . scanning electron microscope

SER . . . . . serine

SLG . . . . . corn silage

SBM . . . . . soybean meal

THR . . . . . threonine

TYR . . . . . tyrosine

UDP . . . . . ruminal undegradable protein

VAL . . . . . valine

CHAPTER I INTRODUCTION

Protein is an important dietary ingredient for the beef and dairy cattle industries. Protein supplementation is a major portion of the ration cost for ruminants. Consequently, utilizing various blends of protein sources that reduce the cost of protein supplementation could improve the profitability of dairy and beef production. Formulation of diets requires balancing nutrient needs with nutrient supply from various feed sources (Bergen, 1986). Corn silage is a popular feedstuff for dairy and beef cattle. Because of its low protein content and quality, special attention is given to protein supplementation.

Currently, protein requirements and supply are based on the crude protein system. To more precisely formulate diets that lower the cost of production, ruminant nutritionists need to develop different schemes to supply protein. One proposed method is based on metabolizable or net protein system (NRC, 1985.). The critical elements of these new systems involve matching amino acid supplies to requirements. The focus of this thesis is to determine the profile of amino acids in the ruminally undegraded residue from various protein sources. This profile represents the AA profile of undegraded dietary protein (UDP) reaching the small intestine.

Ideal protein formulation technology is characterized by both qualitative and quantitative features. The concept of qualitative features is based on the amino acid (AA) content, whereas the quantitative features describe the amount of each AA. Theoretically, lean tissue growth is a function of the first limiting amino acid (Hansen, 1992).

The amino acid present in rumen undegradable, dietary protein (UDP) fraction will ultimately determined its nutritive value. The amino acid content of microbial protein does not vary appreciably across diets (Bergen et al. 1968b). Therefore, feeding UDP with high levels of desired amino acids and feeding more total AA are the only strategies to increase supply of specific AA to the splanchnic tissues. Blake and Stern (1988) demonstrated that feeding combinations of slowly degradable protein sources can alter the blend of AA passing out of the rumen which more closely matches the AA content of lean tissue.

Previous research has established the rumen undegradable protein (UDP) value for different protein sources. For example, feather meal is a potential protein source with UDP value of 69.1% (Goedeken, et al., 1990b). Since the AA profile of feather meal (FTH) is quite different than lean tissue, the efficiency of animal performance may be improved by providing combinations with different protein sources. Complementary responses in growing calves between dietary blood meal (BLM) and FTH have been reported (Goedeken 1990b; Blasi et al., 1991).

The ruminal environment varies with type of diet fed. Degradation of protein from the protein sources was evaluated under different ruminal conditions (i.e. pH and

rate of passage) by feeding three different diets. Sodium bicarbonate was fed to prevent a rapid pH decline shortly after feeding. Dietary intake was varied (1 vs 2% BW) to establish different rates of digest passage from the rumen.

The main purpose of this research is to pursue the ideal protein formulation to increase the efficiency of utilization protein sources in corn silage diets fed to beef cattle. The research project is intended to identify the optimum blend of protein sources (feather meal, corn gluten meal, soybean meal, fish meal, blood meal and meat and bone meal) to maximize utilization of corn silage diets.

The working premise of this thesis is that the profile of AA presented to the small intestine of steers fed corn silage diets is inadequate to allow maximum growth of cattle. This study will characterize the degradability and AA profile of the undegraded residue from several supplemental, protein sources. This information will allow formulation of diets to test the hypothesis that the pattern of AA supply doesn't affect lean tissue gain from corn silage diets. The feeding studies will not be part of the current thesis.

The research objectives of this study was to determine the AA composition of the UDP fraction of various supplemental crude protein sources.

CHAPTER II REVIEW OF LITERATURE

Protein Metabolism in Ruminants

Ruminal Protein Degradation. Ingested feed passes into the rumen where it is subjected to microbial degradation. Microbial fermentation of carbonaceous compounds results in the production of volatile fatty acids (VFA) and microbial cells, which are the major energy and protein sources, respectively, available to the host animal (Cotta and Hespell, 1986).

As a consequence of ruminal fermentation, two major sources of protein are presented for absorption. One is microbial protein (MCP, Czerkawski, 1976) and other is undegraded dietary protein (UDP), which escapes microbial degradation in the rumen (Bergen, 1968b; Bergen et. al. 1978). Digestion of this mixture of microbial protein and UDP in the abomasum and small intestine yields the amino acids available to the host animal (Broderick et al. 1991).

In the rumen, ingested protein is degraded to peptide, amino acids, ammonia, VFA's and carbon dioxide. The process represents the sum of a number of microbial activities, including protein hydrolysis, amino acid deamination and fermentation of resultant carbon skeletons. The initial step requires the action of extracellular proteolytic enzymes produced by ruminal microorganisms (Cotta and Hespell, 1986). Brock et al.

(1982) conducted a detailed analysis of proteolytic activities in rumen contents. The authors reported that 75% of the proteolytic activity in whole rumen contents was associated with the particulate fraction, which illustrated the importance of rumen microorganisms attached to, or associated with, feed materials in the rumen. Kopecny and Wallace (1982) reported that approximately 80% of extracellular proteolytic activity was released from cells upon mild blending or shaking treatments. They suggested that the majority of protease produced by ruminal bacteria is periplasmic or associated with extracellular capsular or coat materials. Rumen bacteria have been found to possess enzymes with trypsin-, chymotrypsin-, carboxypeptidase- and aminopeptidase-like activities (Brock et al., 1982; Wallace, 1983).

Bacteria are the principal microorganisms involved in protein degradation. Wallace (1985) reported that the first step in protein degradation related to the adsorption of soluble protein to the bacterial surface, or attachment part of the bacteria to bound protein. Susceptibility of different proteins to hydrolysis has been correlated to their relative adsorption affinities (Cotta and Hespell, 1986). The authors concluded that the sites of bacterial adhesion and proteolysis on the surface of feed particles may be identical.

Variation in the extent of protein degradation by rumen microorganisms has been attributed to differences in animals, rumen retention time of dietary protein, diets, protein sources and concentrate levels in the diets (Bergen and Yokoyama, 1977). In some studies, protein degradation has been shown to be closely associated with cellulose

disappearance (Ørskov, 1982). However, definitive information on the important factors regulating ruminal proteolysis is still lacking.

Ruminal ecosystem influences dietary protein degradation as well. Loerch et al. (1983) reported that disappearance of soybean meal from dacron bags incubated in rumen decreased dramatically as the percent concentrate in the diet increased from 20 to 80%. It was suggested that protein degradation may depend on the ruminal environment. Wohlt et al. (1973) found that increasing pH from 5.5 to 7.5 elevated the soybean meal solubility from 27 to 57%. The protein sources were more highly degraded at a rumen pH between 6 and 6.5 than at 5.5 or 7. Berger (1986) showed that the relationship between pH and protein solubility is related to isoelectric point of the protein. Proteins were least soluble at the isoelectric point pH. When cattle were fed a high-concentrate diet and had a rumen pH below 6.2, the digestion of fiber was markedly depressed (Ørskov et al. 1990). The reduced fiber digestion may possibly be explained by lack of acid tolerance or inability to compete for substrates by the ruminal microorganisms (Owens and Goetsch, 1988; Ørskov et al. 1990). Therefore, rumen pH is critical for optimum protein and fiber digestion.

A method of maintaining a stable pH is to feed sodium bicarbonate ($NaHCO_3$), which is a buffer and has a pKa near 6.75. The use of $NaHCO_3$ has been shown to increase the amount of time rumen pH is in the optimum range for fiber digestion (Kronfeld,1979). At high levels of intake, ruminal pH tends to be reduced and feeding a buffer may be beneficial (Mertens, 1979). Kronfeld (1979) showed that the appropriate level of sodium bicarbonate would be 2% of DM intake in ruminant diets.

Cotta and Hespell (1986) reported that the physical and chemical characteristics of feedstuffs influence the degree of protein degradation by ruminal bacteria. For example, bovine serum albumin contains 16 disulfide bonds which help stabilize its structure and makes the protein resistant to microbial attachment. Mahadevan et al. (1980) confirmed that the disulfide bonds in protein render it resistant to proteolytic attack and disruption of disulfide bonding increases the rate of degradation.

The quantity of protein presented to the small intestine for absorption is the sum of the microbial protein and rumen, undegraded protein (Bergen, 1968b; Bergen et al. 1978). Bergen (1968b) also demonstrate that the amino acid content of microbial protein does not vary appreciably across diet. Therefore, a nutritional value of dietary protein is a major determinant by the amount of protein escaped from rumen degradation. For ruminants with high levels of production, microbial protein alone may be not adequate to meet the demands for amino acids. Under these conditions where microbial protein will not meet animal requirements, addition of dietary protein with a high UDP value may be necessary (Owens and Zinn, 1988).

Microbial Protein Synthesis. Microbial protein synthesis in the rumen is influenced by many factors such as nutrient supply, microbial population and ruminal conditions. Nutrient requirements for microbial protein synthesis vary with the microbial species present (Hobson, 1972).

The quantity of microbial protein synthesized is limited by the amount of energy (quantity of ATP or digestible organic matter) available to the microbes and the efficiency of energy utilization. The nitrogen substrates used for microbial growth

include NH_3, amino acids and peptide (Owens and Zinn, 1988). When the crude protein level was limited or large amounts of NPN were fed, Chalupa (1968) found that microbial protein synthesis was limited by the availability of amino acids and fatty acids or carbon skeletons which are needed for the synthesis of valine, leucine, isoleucine, phenylalanine and tryptophan. In addition, Owens and Zinn (1988) stated that a deficiency of branched - chain fatty acids, ammonia and other nutrients can cause energy (ATP) uncoupling and decreased efficiency of microbial protein synthesis.

Bergen (1986) stated that rumen fermentation is advantageous to the host animal, because non-protein nitrogen (NPN) can be utilized by the microbes to produce microbial protein which supplies amino acids upon passage to the small intestine. Since NPN is generally less expensive than plant protein, NPN utilization may lower the cost of production. Microbial protein production from NPN is generally assumed to be adequate for 1.1 kg live weight or 22 kg milk production per day. For production levels greater than this, additional dietary protein may be required (Owens and Zinn, 1988).

Estimation of Degradability Using Polyester Bags. The technique of using polyester bags (nylon or dacron) to study feedstuff degradation in the rumen was first introduced by Erwin and Elliston (1959). Mehrez and Ørskov (1977) developed a more precise system (utilizing polyester bags) to compare the rate and extent of protein degradation. Polyester bags incubated in rumen for various time intervals may be used to determine the rate and extent of DM, N and amino acid disappearance.

Several factors influence the disappearance of feedstuffs from polyester bags. Bag porosity is an important factor in the disappearance of nitrogen from incubated bags.

The pore size has a direct influence on the influx of enzymes and microbes into the bag and the passage of undegraded feed particles out of he bag (Michalet-Doreau and Ould-Bah 1992). A pore size of 3 μm or less strongly inhibits the penetration of microorganisms into the bag (Van Hellen and Ellis, 1977). Feed particles can wash out of the bags as well. Additionally, losses take place during post-incubation washing and drying. Wash out and rinsing losses increase as bag porosity increases or particle size decreases (Lindberg and Knutsson, 1981). Selection of pore size of the bag should minimize particle loss yet allow unrestricted access by microorganisms. Inevitably, some degree of compromise is required here, and generally, a pore size of 40 to 60 μm is satisfactory (Mehrez and Ørskov 1977; Lindberg and Varvikko, 1982).

Samples are ground prior to placement into the bag to obtain a homogenous sample (Mehrez and Ørskov, 1977). Optimum bag porosity can be influenced by the amount of sample processing. It is recommended that adequate description of feed processing and pore size relationships be reported in all polyester bag studies (American Dairy Science Association, 1970). Feedstuffs are most often ground through a 1.5 - 3 mm screen (Michalet-Doreau and Ould-Bah, 1992). Generally, a 3 - 5 g sample is placed into each bag (Mehrez and Ørskov 1977; Lindberg and Varvikko, 1982).

The washing of bags after rumen incubation has two main objectives: the first is to stop microbial activity, and the second is to remove rumen liquid and microorganisms from undegraded residues, without increasing the loss of feed particles through bag pores (Mehrez and Ørskov, 1977). However, microbial contamination becomes more problematic with longer incubation periods because of the low protein content of residual

materials in the bag (Nocek and Grant, 1987). Difficulties are even greater with starch materials such as grains because these low - protein substrates are colonized extensively (Varvikko, 1986). The post-incubation washing may not remove all bacterial materials and consequently, an underestimate of disappearance may occur.

The most appropriate times to withdraw bags from the rumen to describe the disappearance rate is variable. For many protein supplements, samples obtained at 2, 6, 12, 24 and 36 h give an adequate description. For hay, straw and other fibrous materials, longer incubation intervals are generally required, and for some succulent feeds, the intervals should be shorter (Ørskov, 1982).

In Situ Degradation Kinetics. Ørskov and McDonald (1979) distinguished three nitrogen feed fractions, quickly degradable, slowly degradable and undegradable. Their study found that nitrogen solubility in different solvents is highly correlated with in situ disappearance after short term (2 -4 h) ruminal incubations. The slowly degraded insoluble fraction has often been described by a first-order kinetic rate constant (Ørskov and McDonald, 1979). Two important assumptions in using first-order kinetics are that the pool of material is homogenous and that disappearance can be described by a single digestion rate constant (Nocek and English, 1986). However, heterogeneity of feed nitrogen degradation has been demonstrated with the in vitro protease technique (Michalet-Doreau and Ould-Bah, 1992).

Fraction of rapidly degraded protein usually is quantified as the proportion of total protein that is water or buffer soluble (Broderick, et. al., 1991). The availability of water soluble protein is variable, since some proteins may be resistant to be degradation

because of its secondary structure. Broderick (et al., 1991) recommended that an alternative approach would be to separate rapidly degradable protein into two fractions; non-protein nitrogen and precipitable protein.

The undegradable nitrogen fraction can be defined as the lowest percent residual beyond which no further degradation occurs (Nocek and English, 1986). It is necessary to utilize sufficient incubation times to detect the end-point of degradation. Acid detergent-insoluble nitrogen (ADIN) can be used as an estimate of the ultimately unavailable nitrogen fraction (Pichard and Van Soest, 1977), and the potentially digestible residual nitrogen can be obtained by subtracting ADIN from nylon bag residual nitrogen (Michalet-Doreau and Ould-Bah, 1992).

Nocek and English (1986) suggested that evaluation of *in situ* degradation rates must be considered to correct the soluble and undegradable pools of each chemical component. Potentially degradable fraction should be evaluated by multiple linear analysis. If only one rate appears to exist, L-T LSR (logarithmic transformation by least square regression) or nonlinear procedure can be used to provide comparable estimates of ruminal availability. If more than one rate constant is apparent, the curve peeling method is preferred, because it more closely approximates rumen availability estimates similar to in vivo determinations. One specific mathematical procedure probably will not adequately describe all possible degradation situations (Michalet-Doreau and Ould - Bah, 1992).

Essential Amino Acid Requirements of Ruminants. Essential amino acids are defined as amino acids that are not synthesized de novo in sufficient quantities to meet

requirements (Rawn, 1989). Essential amino acids can be supplied from dietary or microbial sources (Michalet-Doreau and Ould - Bah 1992). Essential amino acid requirements of non-ruminants have been studied extensively. Generally these studies involved feeding graded levels of individual, essential amino acids (the intake of all other dietary components was kept constant) and measuring weight gain, feed conversion efficiency, nitrogen balance and plasma amino acids. This approach, however, can not be used in ruminants since dietary proteins and amino acids are extensively degraded by ruminal microorganisms (Fenderson and Bergen, 1975). Consequently, quantitative data on essential amino acid needs for ruminants are unavailable, though several estimates have been made based on plasma AA concentration (Fenderson and Bergen, 1975), or net protein deposition coupled with an assumed ideal essential amino acid pattern (Owens and Zinn, 1988).

The amino acid content of microbial protein does not vary appreciably with diet (Bergen, et al. 1968b), so altering of AA composition of UDP is a method to manipulate the composition of amino acids reaching the abomasum.

As the major protein storage depot of the animal, skeletal muscle has a similar AA composition to the total body protein-bound amino acid pool. In contrast, skin shows marked difference from whole body pool because of the dominance of collagen proteins, which contain high levels of proline. Wool contains low levels of methionine and lysine, variable amounts of tyrosine, and large quantities of cysteine in the keratin protein structure. If the essential amino acid (+ cysteine) composition of these components is compared with that of the amino acids available from rumen microbial

protein (Storm and Ørskov, 1983) then, the first limiting amino acid for carcass growth is histidine, while that for skin and wool, the sulfur-containing amino acids are more limiting. However, Bergen et. al. (1968a) reported that some de novo synthesis of histidine occurs. Therefore, histidine is unlikely to be the first limiting AA.

Storm and Ørskov (1984) reported that the first limiting amino acid in microbial protein was methionine, followed by lysine, arginine, and histidine. A supplemental dietary protein would have maximal value if the amino acid pattern in the undegraded residue was complementary to microbial protein.

Essential amino acid requirements are influenced by the growth rate of the animal. Animals in metabolism stalls often have reduced feed intake and growth rates. Under these conditions, essential amino acid requirements will be underestimated (Fenderson and Bergen, 1975; Owens and Zinn, 1988). Thus, procedures to determine essential amino acid requirements under field conditions need to be developed (Bergen, 1986).

Methionine has been identified as the first limiting amino acid (AA) for growing cattle when microbial protein is the principal source of AA supply to the small intestine (Fenderson and Bergen,1975; Richardson and Hatfield, 1978). Titgemeyer and Merchen (1989a) reported that post-ruminal supplementation with nonsulfur-containing amino acids tended to increase the ability of growing steers to respond to methionine supplementation, when steers were fed a diet containing minimal true protein in the diet. This observation would suggest methionine was marginally deficient or was not the first limiting AA under the conditions of their study.

Amino acid requirements for milk production has been studied for over twenty years. Schwab and Satter (1976) found that lysine appears to be the first-limiting AA when corn-based rations are fed. Fraser et al. (1991) identified lysine, methionine and histidine as first, second and third limiting AA in the lactating dairy cow, where casein was the sole, supplemental, protein source. Schwab et al. (1992) further proved that lysine was the first-limiting AA in early lactation, lysine and methionine appeared to be first and second limiting AA at peak lactation.

Argyle and Baldwin (1989) demonstrated that the growth rate of rumen microorganism are more sensitive to the amount of AA supplied rather than the composition. Macrae and Lobley (1984) stated that amino acids provide an important supplemental source of metabolic intermediates for rumen microorganism which influence efficiency and utilization of other substrates, especially when poor-quality forage diets are fed. A deficiency of amino acids from RDP for the animal reduces production of meat, milk or wool. Supplementation with specific ruminal escape amino acids may prove economically feasible under certain feeding conditions, especially when amino acid demand is high or when protein intake is very low (Owens and Zinn, 1988).

Stern (1981) studied that feeding a combination of resistant protein sources that have complementary AA profiles may result in a more beneficial post-ruminal AA supply. Blake and Stern (1988) demonstrated that AA profiles of digest residue leaving the rumen can be modified by feeding protein sources resistant to microbial degradation and that combinations of complementary resistant proteins could improve intestinal AA supply and balance.

Characteristic and Processing of Feather Meal

Feather Meal Composition. Approximately 85% to 90% of the protein in feathers is keratin. Native keratin exists in a β - helical structure reinforced by cross-linking between the side chains. These chains tend to aggregate by hydrogen bonding to form cylindrical units which in turn associate into a cable-like structure. Theoretically, cystine stabilizes the cable by extensive disulfide bridging between the cylinders (Schor et al., 1961 a,b). Due to this high degree of polymerization, keratin protein is resistant to digestive enzymes in the intestinal tract (van der Poel and Boushy, 1990). Hydrolysis is necessary to disrupt chemical bonds in the keratin molecule in order to expose the feather protein to digestive enzymes. Feather meal is commonly hydrolyzed with steam and pressure to increase its nutrient availability (Steiner et al., 1983).

Processing. Several processing methods have been utilized to break down hydrogen bonds between molecular chains in keratin protein. Alkali treatment (Retrum, 1981) and enzymes (Papadopoulos, 1986) have been used with limited success. The most economical treatment has been heat denaturation (van der Poel et al., 1990).

Thermal processing is based on exposing the feathers to elevated temperatures under saturated vapors. The basic stages of processing feather meal are carried out in two steps: 1) hydrolysis/sterilization of raw feathers and 2) drying the hydrolyzed feathers. For sterilization, the raw material must be heated to a minimal temperature specified by law. The minimal conditions include heating to a temperature of 133°C for

at least 20 minutes at an internal pressure of 300 kPa (kilopascal, under atmospheric pressure). The processing conditions for sterilization result in denaturation of the proteins in feathers. Drying is finally used to achieve a moisture level of approximately 4 to 10% to facilitate storage, handling and desired final quality (van der Poel et al., 1990).

A major difference in amino acid composition between raw and processed feather meal is the form of sulfur-containing AA (Papadopoulos et al., 1985). Feather proteins in their helical structure exhibit high levels of cystine among its constituent proteins. When the disulfide cross-link is broken, cysteine is formed. During processing some cystine is converted into lanthionine, ornithine or other S containing compounds (Papadopoulos et al., 1985). Other AA in processed feather meal are similar to raw feathers .

Feather Meal Quality. The standard definition for feather meal as reported by the Association of American Feed Control Officials is as follows: "The product resulting from the treatment under pressure of clean, undecomposed feathers from slaughtered poultry, free of additives and accelerators". Not less than 70% of its crude protein content shall consist of digestible protein.

Conversion of feathers to feather meal involves physical and chemical changes. A principal chemical change is the loss of cystine, appearance of cysteine and lanthionine, increased susceptibility to enzymatic hydrolysis and increased *in vivo* degradability. Loss of cystine probably occurs through desulfurization reactions that may lead to unstable residues of dehydroalanine. The residues condense with cystine to form

lanthionine or with the ϵ- amino-group of lysine to form lysinoalanine (Bjanrnason and Carpenter, 1970).

The temperature used to denature the protein in feathers can influence the resulting digestibility. Overheating feathers results in nitrogen loss and formation of a gummy material. Overheating may destroy essential amino acids and cause racemization of amino acids to the D-form (de Wet, 1982). The re-establishment of cross-linking between amino acids has also been described (van der Poel et al., 1990). Feathers that have been underheated do not grind well, have low bulk density and are less digestible (Davis et al. ,1961). Blasi et al. (1991) found that a hydrolysis time less than 18 min did not improve the nutritional quality. It has been suggested (van der Poel, 1990) that further research is needed to study the effects of processing (time/pressure and moisture content) on amino acid quality of feather meal.

Hydrolyzed Feather Meal as a Protein Supplement in Ruminant Diets

Feather Meal Digestibility. Testing the digestibility is the current accepted method used to assess feather meal (FTH) nutritive value, which is the in vitro pepsin-HCl test (McCasland, et.al, 1966). McCasland and Richardson (1966) demonstrated that raw feathers were only 7.7% digestible and hydrolyzed feather meal was 81% digestible as measured by this test. Church et al. (1982) reported that FTH had 94% CP, of which 84.5% was digestible by pepsin. This experiment also showed that pepsin digestibility

is an accepted means of evaluating digestibility of proteins for monogastric species, but its value for ruminants remains to be established.

Feather Meal Degradability in the rumen. The nutrition value of dietary protein for ruminants can be estimated by the ruminal degradability. Goedeken et al. (1990b) estimated the escape protein value for FTH (69.1%) was less than that for blood meal (82.8%) or corn gluten meal (80.4%), but greater than soybean meal (26.6%) after 12 hours of in situ incubation. These results confirm previously reported studies by the same authors (Goedeken et al. 1990a). Meat and bone meal (MBM) has 40.4% CP and a rumen undegradable protein (UDP) value of 53.6% (Gibb et al., 1992b). Fish meal has 60.4 to 72% CP and a UDP value ranging from 30 to 70%. The variation in UDP results from variability of source of meal and type of processing (Hussein and Jordan, 1991). Based on these results, FTH can be classified as a high escape protein source and may be useful in diets for ruminant animals. Little information is available to describe the rate of availability of protein in FTH. The quality of ruminal degradable and undegradable protein can be measured from AA analysis and subsequent weight gain.

Protein Quality of Feather Meal Compared with other Protein Sources. The amino acids present in the protein leaving the rumen will ultimately determine its nutrition value (Bergen et al. 1968b). Amino acids profile of a dietary protein can be used as the standard to evaluate protein quality. Thomas and Beeson (1977) found that hydrolyzed feather meal contained a higher level of total sulfur-containing amino acids than soybean meal (SBM), but it was quite deficient in lysine and histidine. Church et al. (1982) reported similar results, where hydrolyzed feather meal was deficient in lysine

and histidine in comparison to SBM, but the concentrations methionine were only slightly lower than SBM.

Goedeken et al. (1990a) compared the amino acid profile of FTH with other protein sources and estimated amino acid flow to the small intestine using AA composition of residues after *in situ* incubation. Feather meal, like corn gluten meal (CGM), is rich in ruminal escape sulfur-containing amino acids, whereas, blood meal (BLM) is rich in lysine. Gibb et. al, (1992a) further showed that the profile of total sulfur-containing amino acids (methionine + cystine) was the highest with FTH (6.1%) followed by SBM (4.1%), BLM (3.3%) and MBM (2.9%). The lysine content expressed as a percent of protein for BLM, SBM, MBM and FTH protein was 8.3, 5.4, 4.9 and 2.0%, respectively. On balance FTH may be a good source of sulfur-containing amino acids in ruminant diets. Gibb et al. (1992b) demonstrated that histidine became the first-limiting amino acid in FTH diets, and lysine eventually became limiting as maximum rate of body weight gain was reached. These experimental results released that FTH may be a good source for providing the sulfur-containing amino acids to balance amino acids in the microbial protein.

Hussein et al. (1991) reviewed the studies on fish meal (FSM) as a protein supplement in ruminant diets and found that FSM was more effective in improving live-weight gain in younger than in finishing ruminants and in males than in females or castrated males. Daily gains and feed efficiencies were higher when FSM was added to poor- or medium- quality silage than high-quality silage diets. The study suggested that the quality of undegraded proteins in ruminant diets influences pattern and quantity of

amino acids presented to the small intestine for absorption. Overall, this study indicated that AA profile varied from different protein sources.

Few studies have evaluated the bioavailibility of UDP and its amino acid composition available for absorption in the small intestine from different protein sources. Tigemeyer et.al. (1989b) found that the digestibility of UDP from different protein sources followed similar trends and provided similar AA for absorption in the small intestine. Digestibility of UDP fraction from CGM was highest at 80%; BLM and FSM were intermediate at 74%, and SBM was the least digestible at 63%.

Titgemeyer et. al. (1989b) further compared the supply of absorbable individual essential amino acid from different protein sources. Corn gluten meal was a poor source of lysine and an excellent source of leucine; blood meal was poor in methionine, isoleucine and tyrosine and an excellent source of histidine; fish meal was a poor cysteine source. The same author also demonstrated that threonine, valine and isoleucine were more resistant to ruminal degradation; whereas, methionine, cysteine, histidine and arginine were more extensively degraded than the total AA supply.

In summary, the blending of different protein sources may increase the efficiency of nitrogen utilization. Undegradable protein would be maximal value if its amino acid pattern was complementary to microbial protein. Therefore, combinations of different protein sources may be necessary to meet the AA requirement of the ruminant animal. Feather meal could be a key source of sulfur-containing amino acids.

Effects of feather meal supplementation on Animal Performance. Animal growth studies from several species have been conducted to evaluated the nutritional value of

FTH. Combs et.al., (1958) demonstrated that the growth rate of pigs fed FTH plus lysine was similar to a soybean meal based diet. This study illustrated that FTH can be used as a protein source for growing-finishing swine. McCasland and Richardson (1966) evaluated that growth of rats fed hydrolysed feather meal with or without amino acids added to the diet. The rats fed diets without added AA gained no weight whereas rats fed diets supplemented with AA gained 120 g. In general, the feeding of FTH as the only protein supplement to nonruminants has not been very successful. This is understandable because the dietary amino acid balance is more important for nonruminants than for ruminants.

The effects of a specific, supplemental, protein source on the amount of weight gain per unit of feed consumed is an important determinant of its true value. Feeding efficiency is one of the most important ways to evaluate a dietary protein value. Wray et al. (1979) showed no differences in average daily gain, feed efficiency and carcass characteristics of steer calves fed combinations of SBM with FTH or hair meal (HM). Cattle fed combinations of FTH and HM required more feed per unit of gain. When heifers were fed supplements with 9.5 to 19% FTH, feed conversion efficiency was negatively influenced as compared to performance on similar diets fortified with SBM. Feather meal can replace up to 75% of the SBM protein without a decrease in DM, energy or nitrogen utilization (Wray et al. 1980).

Church et al. (1982) compared FTH and SBM in high concentrate diets and reported that daily gains were similar, further in this study, the addition of urea to FTH

improved feed conversion efficiency. Aderibigbe and Church (1983) found that FTH and HM proteins are more efficiently utilized with high concentrate diets than with high roughage diets.

Different concentrations of feather meal in the diets of lactating dairy cows resulted in similar DM intake and milk fat percentage (Harris et. al., 1992). In the same study, a curvilinear analysis of dietary FTH content, showed that the optimal level of FTH in diet should not exceed 6%. Milk protein percentage was affected adversely by feather meal concentration. In summary, FTH can be used as protein supplement, but further fortification may be necessary to balance the AA profile. Utilization of blends of other supplemental proteins with feather meal may be beneficial.

Complementary responses in growing calves to dietary BLM plus FTH has been reported in the literature (Goedeken 1990b; Blasi et al., 1991). These studies demonstrated FTH is more complementary to BLM than SBM. Likewise, Stock and Klopfenstein (1979) showed a combination of BLM and corn gluten meal (CGM) would result in a more balanced supply of EAA flowing to the small intestine, because the blood meal is high in lysine and CGM is high in sulfur-containing amino acids. Gibb et al. (1992a) studied the complementary effects between MBM + FTH, or MBM + BLM; and concluded that calves receiving FTH+MBM combination gained faster ($P<0.1$) and were more efficient than urea-supplemented calves. But there was no complementary response between MBM and BLM or FTH.

Evaluation of complementary effects of FTH, BLM and SBM for growing finishing calves was studied by Sindt (et al., 1993). Supplementing SBM/FTH/urea or

BLM/FTH/urea improved feed efficiency compared with supplementing FTH/urea alone. Evaluation of complementary effects between protein supplements needs further study.

The previous review suggests complementary effects may exist between plant and animal co-product protein sources. The challenge is to determine the least expensive formulation of supplemental protein to complement AA composition of microbial protein.

In summary, integrating data published in the last decade regarding FTH supplementation to ruminant diets illustrates the potential for this feedstuff. First, feather meal has a high UDP value. Second, feather meal protein can be a source of sulfur-containing amino acids to complement microbial protein. Third, the complementary effects between rendered protein sources and FTH protein may improve protein quality, because FTH has a poor A\A balance and lacks palatability in ruminant diets.

Corn Silage as a Feed Resource

Corn silage is a popular feedstuff where climatic conditions are favorable for its growth (Church, 1991). The objective of silage-making is to preserve the harvested crop by anaerobic fermentation and maintain nutritive quality. The end products of anaerobic fermentation lower the pH and create an environment that limits microbial respiration and metabolism. This process involves converting soluble carbohydrates to lactic acid, which lowers the pH to inhibit biological activity in the ensiled forage mass (Bolsen et al. 1991).

Silage preservation includes a primary fermentation which results in growth of lactic acid producing bacteria (LAB) and accumulation of lactic acid. The lactic acid producing bacteria metabolize soluble plant carbohydrates into lactic and acetic acid. Occasionally, the primary fermentation is incomplete and a secondary fermentation can occur. Clostridial microorganisms metabolize lactic acid, sugars, proteins and amino acids to butyric and higher fatty acids, amines, amides and ammonia. Hence, a secondary fermentation is undesirable. Quality of the product is normally judged according to ratio of the primary to secondary fermentation products. The higher the ratio, the better the quality (Woolford, 1991).

Silage Quality and Nutritional Value. Principal criteria typically employed to evaluate the adequacy of silage fermentation are silage color, smell and texture (Woolford, 1984). Previous research has established chemical criteria to determine silage quality as well. Criteria included but are not limited to pH, butyric acid, lactic acid and volatile nitrogen contents (Woolford, 1984). A good quality silage will have a pH below 4.2; butyric acid level less than 4.0 g/ kg fresh weight; lactic acid levels between 6.0 - 10.0 g/ kg fresh weight; volatile nitrogen contents less than 100 g / kg total N. Woolford (1984) recommended that a reliable indicator of the quality of conventionally fermented or acid-treated silage is pH. Pitt (1990) also suggested that silage pH can serve as an indicator of the quality of preservation, when combined with measurement of DM content. Silage with a high pH would suggest an incomplete fermentation occurred which results in less nutrient retention and lower bunk stability.

The nutritional value of well-preserved silage from plant materials is similar to the fresh forage from which it was made, but chemically it is different. In a review by Woolford (1984), McDonald et al. (1973) reported that digestible DM and OM were similar for fresh forage or its subsequent silage. The proportion of structural carbohydrates (NDF and ADF) to total dry matter increase during ensilement, but this is accompanied with a small increase in the digestibility of crude fiber. Woolford (1984) indicated that silage has a high proportion of its nitrogenous constituents in non-protein compounds. Likewise, the free sugar content is low, and lactic and other volatile fatty acids levels are elevated. However, both metabolizable and net energy values of silage are generally 5 to 6% higher than the corresponding values for the fresh plant.

Well preserved corn silage is a very palatable product with a moderate to high content of digestible energy, but is usually low in undegradable protein, particularly for the amount of energy it contains. On a dry basis, corn silage will usually have 8-9% crude protein, 65-70% TDN, 0.33% Ca and 0.2% P. Silage made from well-eared crops may have as much as 50% grain, particularly in silage made from mature plants, although average values are usually between 40-45% grain. High-yielding grain varieties of corn generally produce maximal yields of digestible nutrients. Even so, maximum growth rates or milk yields from corn silage diets cannot be obtained from cattle without energy and protein supplementation (Church, 1991).

Animal Performance. Early studies proved that corn silage may be an important ingredient for beef cattle diets. Calder, et al., (1976) showed that average daily gain was 1.05 kg for corn silage; 0.89 kg for grass-legume silage and 0.56 kg for grass silage.

Steers fed corn silage had higher USDA quality grades than cattle fed either of the other silage. Comerford et al. (1992) reported that steers fed corn silage diets had significantly greater energetic efficiency ($P < 0.05$) than those fed alfalfa haylage. Compared with other silage, corn silage is a good forage source for cattle.

Comerford et al. (1992) found that the type of protein supplements did not influence energy intake, feed efficiency or gain when steers were fed corn silage as the basic diet. Jesse et al. (1975 as cited by Shirley, 1986) fed Hereford steers diets that contained various ratios of corn grain to corn silage (30:70, 50:50, 70:30 and 80:20). Daily gain for these four dietary groups were 0.90, 1.06, 1.13, and 1.11 kg, respectively. Krause et al. (1980) fed steers droughty corn silage supplemented with either 0 or 2.7 kg corn grain and 0.57 kg of a protein-mineral supplement per head per day. The observation was that those fed the droughty silage gained as rapidly and efficiently as those fed the normal silage-containing diets. Steers fed corn grain gained faster and converted feed more efficiently than those fed only corn silage. Feeding combinations of corn silage and corn grain does result in small negative associative effects on DM digestibility (Joanning et al., 1981). This negative relationship was caused by incomplete starch digestibility. Even though corn silage is a good forage source, the relatively low crude protein content and poor protein quality necessitates addition of supplemental protein (Bergen et al., 1974).

Addition of NPN to Corn Silage. Bergen et al. (1974) found that the proportion of nitrogen in various fractions of chopped corn plant material changed during ensiling. Forty-two percent of total nitrogen in corn silage was water soluble, compared to 8.1%

in the fresh plant. The authors further stated that a portion of the soluble nitrogen appears to be undegraded in rumen.

Bergen et al (1978) reported that treatment of chopped corn plants at ensiling with ammonia increases the total and insoluble nitrogen contents of the resultant silage, and also demonstrated that ammoniation of corn silage extends the fermentation period and increases the lactic acid content. Soper and Owen (1977) reported that the addition of ammonia to chopped corn plant material at time of ensiling increased bunk stability after air exposure. In a previous study, Cook and Fox, (1977) demonstrated that treatment of corn silage with cold-flow anhydrous ammonia prior to ensiling was an effective and economical way to provide supplemental nitrogen to corn silage diets.

Buffering Capacity. Pre-ensilement addition of buffering agents that extend the length of fermentation, reduce the level of readily available carbohydrates and appear to improve the degree of preservation (Owens et al., 1969). The same author showed that limestone ($CaCO_3$) additions at ensiling increased levels of organic acids, principally lactic acid. Byers et. al. (1976) further showed that adding limestone before ensilement of corn silage increased ruminal cellulose and net energy utilization. Additional buffer added in corn silage may improve energy utilization. Byers (1980) found that monensin, limestone or the combination increased average net energy value [NEm +NEg]/2 by 8.8, 9.6 or 15.4%, respectively. Feed conversion efficiency was improved by 5.8, 9.1 and 14.3% for monensin, limestone or the combination, respectively.

Sodium bicarbonate has been added to corn silage diet as a method to improve DM intake. Silage pH or free acid content has been implicated as an inhibitor of silage

intake. Shaver et al. (1984) found that DM intake of a corn silage diet was increased by addition 2 to 4% sodium bicarbonate, but was reduced at 6%. In a companion study (Shaver et al., 1985), sodium bicarbonate was added to silage to increase pH from 3.79 to 7.11. Organic matter intake was increased 0.59 kg/d. The study demonstrated that silage pH can influence voluntary consumption of corn silage and that neutralizing the acidity with sodium bicarbonate can improve intake. Tucker et al. (1992) showed that dietary buffers increase both ruminal fluid pH and buffering capacity, which provided a more stable environment for microbial growth.

The issue of rumen and microbial adaptation to dietary change is the basic principal for manage beef cattle production. Allison (1975) demonstrated that free glucose accumulates in the rumen, when animal fed an amount of rapidly fermented carbohydrate beyond the normal rumen fermentative capacity. This can lead to rapid growth of Streptococcus bovis with production of lactic acid, reduced ruminal pH and subsequently to growth of lactobacilli and development of lactic acidosis. Allison (1975) proved that the adaptation periods of change high concentrate diets may be required as long as three weeks. These results suggested that the requirement of adaptation period for adding sodium bicarbonate in corn silage diet.

CHAPTER III Complementary effects of different protein sources in corn silage diets for ruminants

SUMMARY

An experiment was conducted to evaluate ruminal protein degradation and amino acid composition of undegraded residues from feather meal (FTH), corn gluten meal (CGM), blood meal (BLM), fish meal (FSM), soybean meal (SBM) and meat and bone meal (MBM). Six cannulated steers were assigned to one of three dietary treatments in a replicated 3 x 3 Latin square design. The three dietary treatments included corn silage fed at 2% of body weight (BW), corn silage fed at 2% of BW plus 2% of sodium bicarbonate and corn silage fed at 1.4% of BW. Five grams of substrate from the respective protein source was placed into dacron bags and placed into the rumen through the canulas. Dacron bags were removed from rumen at 2, 4, 8, 12, 24, 48 and 72 h to determine rumen dry matter, organic matter and protein degradation and amino acid profiles of the undegradable residue after 24 and 48 h. Rumen pH was similar among the three dietary regimes. Soybean meal had the highest and feather meal the lowest dry matter degradation value. The highest rumen degradable protein value was observed with SBM (99.9%). Feather meal had lower rumen degradable protein than BLM, FSM or MBM. The proportion of histidine was significantly reduced during incubation for BLM, FSM and MBM. Blood meal has the highest content in histidine. Total sulfur-

containing amino acids content after 24 h of incubation was greatest for FTH followed by FSM, CGM, BLM, MBM and SBM.

INTRODUCTION

Increasing costs of the conventional protein sources has generated interest in new and less expensive protein sources for beef cattle. Commonly used supplemental protein sources for growing cattle are soybean meal, corn gluten meal and fish meal. Opportunities exist to utilize other protein sources like feather meal, blood meal and meat and bone meal to reduce the cost of supplemental protein with corn silage diets.

Previous research has established the value of rumen undegradable protein (UDP) from different protein sources. Goedeken et al. (1990b) reported the UDP value of following protein sources: FTH (69.1%), BLM (82.8%), CGM (80.4%) and SBM (26.6%). MBM had 53.6% UDP value (Gibb and Klopfenstein, 1992). Fish meal had UDP values ranging from 30 to 70% (Hussein, et al. 1991).

Feathers are generated in huge quantities as a waste by-product or co-product in commercial poultry processing plants. The major component in feathers is keratin, a protein comprised of amino acids arranged in an extended β-helical chain (Schor and Krimm, 1961a,b). Cystine stabilizes the helices by extensive disulfide bonding. Currently, raw feathers are hydrolyzed prior to feeding to increase digestibility. Hydrolyzed feathers meal has about 80 to 90% crude protein (van der Poel, et al., 1990).

Church et al. (1982) demonstrated that FTH could be a rich sulfur-containing amino acids in the protein supplements. Gibb and Klopfenstein (1992) showed that sulfur-

containing AA were elevated in FTH. Wray et al. (1979) reported that no improvements in daily gains, feed conversion efficiencies or carcass characteristics of steer calves fed diets supplemented with various combination of SBM, FTH and hydrolyzed hair meal. Contrary to Wray et al. (1979), Goedeken et al (1990a, b) and Blasi et al. (1991) noted improved growth responses in calves fed combinations of BLM and FTH. The explanation for dissimilar results between the four studies may be associated with the quality of protein or AA composition reaching the small intestine. For example, blood meal and FTH may have AA profiles in the UDP fractions that when blended together, more closely represent the lean tissue composition of the animal. These results also indicated that the combination of different protein sources could be the potential way to improve the efficiency protein utilization.

The amino acids present in UDP fraction will ultimately determine its nutritive value. Bergen et al. (1968b) found that AA content of ruminal microbial protein does not vary appreciably in ruminants fed different diets. Blake and Stern (1988) also proved that combinations of protein sources resistant to microbial degradation improve intestinal AA supply and profile. Consequently, feeding with high UDP or increasing total AA supply are the only strategies to increase AA supply post-ruminally.

The concept of synchrony, providing available nitrogen in concert with energy in the rumen, has been proposed as important. This may be especially important when intakes exceed 3% of body weight, and high rates of passage may limit rumen degradability. Formulation of diets that optimizes the use of all nutrients to the utmost provides a unique challenge to the ruminant nutritionist. One approach that has been

proposed to provide a more synchronous fermentation is to divide each protein and energy source into three fractions: rapidly degradable, potentially degradable and undegradable portions. In addition, the rate of degradation is calculated for the potentially degradable protein and energy fractions and diets are formulated to match the rate of energy and protein degradation.

The research objectives of this study were to determine the amino acid composition of the UDP fraction of various supplemental crude protein sources.

MATERIALS AND METHODS

Six, ruminally, fistulated Holstein steers (AVG BW = 401 kg) were utilized in a replicated (n=2) 3x3 Latin square design to evaluate the effects of DM intake and sodium bicarbonate on ruminal, in situ degradability of several protein sources with low rumen degradability (UDP). Fistulas and canula insertions were performed by licensed veterinarians under approved procedures by the All-University Committee on Animal Use and Care.

Steers were fed diets consisting of an 88% corn silage and a 12% protein - mineral supplement. The supplement was fortified to meet requirements for vitamins (A,D,E) and minerals (NRC, 1984). The supplement (Table 1) contained a mixture of FTH, CGM, BLM, FSM, SBM and MBM. Each protein meal was added to the supplement to provide equal amounts of total nitrogen.

To provide a variety of ruminal conditions to evaluate AA composition of UDP for each protein source, three dietary regimes were utilized. Dietary regimes (on a DM

TABLE1. DIET COMPOSITION FOR THREE DIETARY TREATMENTS (% DM).

Diet composition [a]	2% BW	2% BW plus 2% $NaHCO_3$	1.4 % BW
Corn silage	93.5	91.65	94.33
Blood meal	1.03	1.01	0.90
Corn gluten meal	1.04	1.02	0.92
Fish meal	1.02	1.00	0.89
Meat & bone meal	1.00	0.98	0.88
Soybean meal	1.07	1.05	0.94
Feather meal	1.01	0.99	0.88
Trace mineral salt	0.20	0.20	0.18
Vitamin premix [b]	0.0059	0.0058	0.0052
Rumensin 60	0.026	0.025	0.035
Calcium carbonate	0.079	0.077	0.069
Dicalcuim phosphate	0.027	0.03	0.022
Sodium bicarbonate	0	2.0	0

[a] Diets were formulated to contain 12% CP and expressed on a percentage of DM.
[b] Premix contained 30,000 IU vitamin A, 3,000 IU vitamin D and 500 IU vitamin E per gram.

basis) included: 1) diet fed at 2% of BW; 2) diet fed at 2% of BW plus 2% sodium bicarbonate (DM basis) and; 3) diet fed at 1.4% of BW. Steers were housed in metabolism stalls (3x1.4 m) in a temperature-controlled room during the studies. Steers were allowed in exercise for 4 h once per week. Cattle were fed every 12 h and orts recorded daily. Each of the three periods consisted of a 15-d adaptation and a 9-day collection period. Rumen samples were collected at 0, 2, 4, 8, 12, 24, 48 and 72 h post-feeding on d-16. Protein and amino acid disappearance from free floating dacron bags (Ankom, Spenceport, NY) were evaluated from day 17 to 24.

Five grams of each feedstuff (FTH, CGM, FSM, SBM, BLM, MBM and corn silage) were placed into dacron bags (5x2cm). Dacron bags had a 50 μm pore size. Three bags per feedstuff were prepared for each sampling time. Twenty-one dacron bags without feedstuff were placed into the rumen and three removed at each time end point to adjust for microbial attachment and infiltration into the bags. Dacron bags were prewashed in warm water for 30 min prior to placement into the rumen and the amount solubilized assumed to represent the rapidly degradable fraction ("a"). Bags were removed at 2, 4, 8, 12, 24, 48 and 72 h to determine protein degradability (Ørskov and McDonald 1979). Because of the number of dacron bags to be incubated (126 bags/period) occupied a large portion of the rumen volume, one half the bags were incubated from day 17 to 21, the other half placed on day 21 and removed by day 24. The order in which protein sources were incubated was randomized across periods to prevent a systematic error. Dacron bags containing corn silage were incubated from day

17 to 23. Upon removal, bags were washed in flowing tap water until water leaving the bag was clear. After washing, all the bags were squeezed gently to remove water, dried overnight at 100 °C, cooled to room temperature, and weighed for calculation of dry matter disappearance. Rumen pH was measured with a pH meter equipped with a combination electrode at 2 h intervals for a 12 h cycle on day 16. Rumen fluid was collected from the dosal and ventral sac of the rumen and pH measured on each sample. Since location within the rumen did not influence pH ($P > 0.1$), the mean value from both rumen samples is presented.

Nitrogen concentration of dried samples was determined by Kjeldahl procedures (AOAC 1984). The amino acid profiles of each protein source were determined on the fresh sample and undegraded residue after 24 and 48 h of incubation. Samples were hydrolyzed in 6 N HCl for 24 h at 121 °C, derivitized with PITC (Phenylisothiocyanate) resulting in PTC (Phenylthiocarbamyl)-amino acid derivatives which were quantified by reverse phase (C_{18}) chromatography (Pico - Tag ™, 1986). The amino acid composition of undegraded residue provided an estimate of the composition of amino acids that would appear in the small intestine in the UDP or escape protein fraction.

The General Linear Models subroutine of SAS (SAS, 1987) was used to analyze protein degradation and amino acid composition. A model appropriate for a crossover double-split plot in a replicated, 3x3 Latin square design was used, that included square, animal within square, period, dietary regimes, protein sources and time.

Differences in DM, rumen degradable protein (RDP), UDP and AA from different protein sources were determined with repeated measurement procedures of Gill (1986). The effect of dietary regimes on DM, RDP, UDP and AA from six protein sources was tested by the animal within square x dietary regimes mean square error (MSE). The effects of protein sources were evaluated by the MSE from sub-split plot (animal within square x dietary regimes x protein source). The effects of incubation time on DM, RDP, UDP and AA was compared by sub-sub-plot MSE (animal within square x dietary regimes x source x time). When the F - test was significant, the *Bonferroni* - t test was used to separate the respective means for dietary regimes. The *Tukey* - t test was applied to compare DM, RDP, UDP and AA between protein sources at different times.

Protein characteristics of the feedstuffs were defined by two systems. The first system separates protein into rumen degradable and undegradable. Rumen degradable protein (RDP) is determined as the amount of protein that disappeared from the dacron bag after a specified period of time. Undegraded protein (UDP) is that portion of the total protein that remains in the bag after the specified time period (UDP =100 - RDP). The second system divides protein into three fractions, which are: rapidly degradable (*a*); potentially degradable (*b*); undegradable protein (*c*) and the degradation rate constant (*k*). The "*a*" fraction is defined as the portion that disappears from the bag during the 30 min washing or rinse phase. The (*b* + *c*) fraction were obtained from 100 - "*a*". Using non-linear regression technique ((SAS Institute, 1987), the rate constant and b

fraction could be solved from the following equation [100-a = $(b \times e^{-k \times time}) + (100-b)$] (SAS Institute, 1987). Fraction "*c*" was calculated subtracting fraction "*a*" and "*b*" from 100. The rate constant represented the protein ruminal degradation rate expressed in percent per hour. Protein fractions and degradation rates were analyzed using the analysis of variance model described previously.

RESULTS AND DISCUSSION

Rumen pH decreased from 6.8 to 6.2 within 2 h after feeding and then gradually returned to 6.8 before the next feeding. Rumen pH was similar ($P > 0.1$) between dietary regimes, and similar pH curves over time were observed (Figure 1).

The effects of dietary regimes on disappearance of DM and protein from the different protein sources across time are presented in Table 2. Since none of the three way interactions were significant, the two way interaction means for diet by time and protein source by time are presented. By 12 h, the low intake regime had lower ($P < 0.05$) DM degradation than the high intake regime (Table 3). This difference existed throughout the remainder of the incubation time. From 12 - 24 h, addition of 2% sodium bicarbonate slowed the rate of digestion as compared at similar intake levels.

The rumen degradable protein values were lower ($P < 0.05$) on the low intake treatment as compared to high intake groups. Addition of a dietary buffer did not influence DM disappearance or RDP content. The relatively small differences in rumen pH may explain lack of dietary effects on disappearance of DM or protein from the bags.

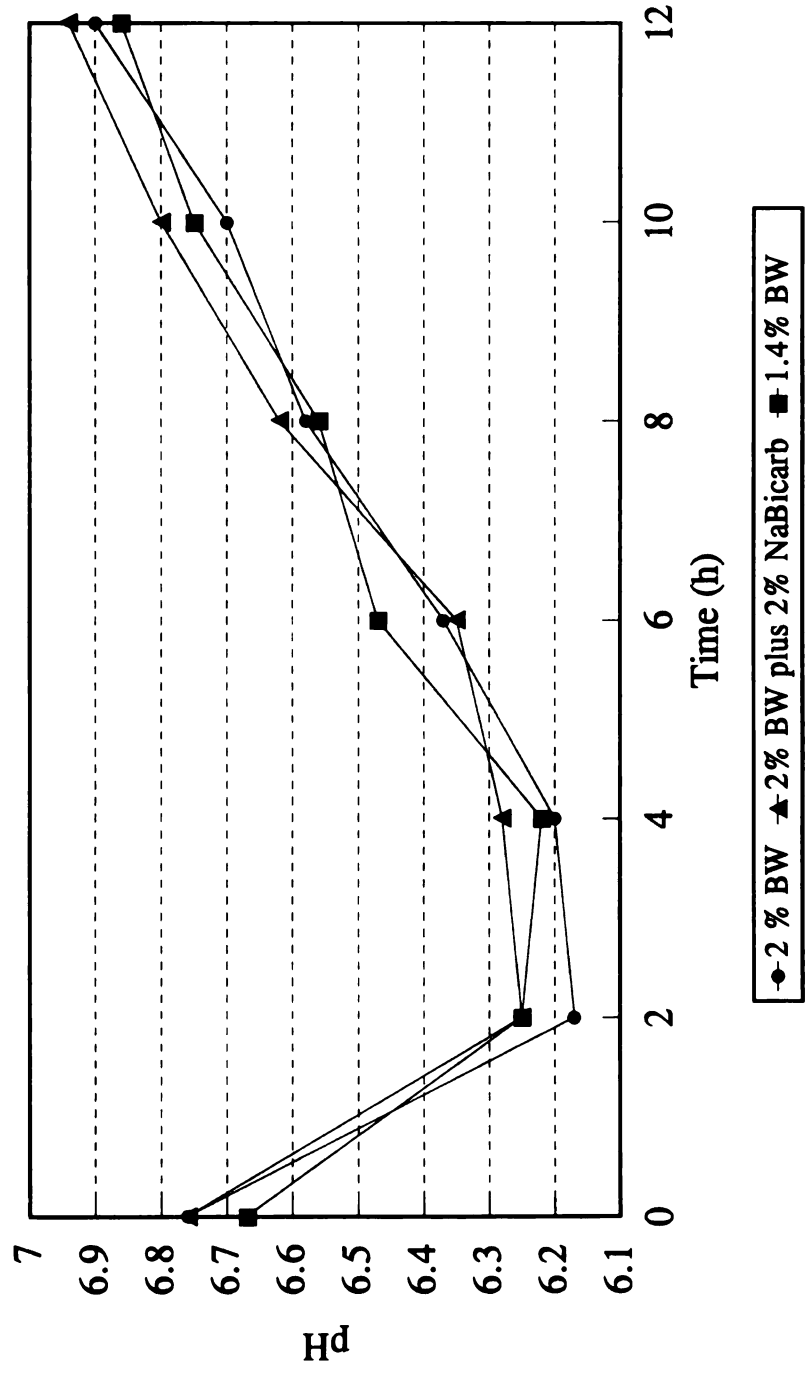


Figure 1. Effects of dietary regime on ruminal pH.
SED= 0.06.

TABLE 2. EFFECTS OF TIME AND DIET ON DM DEGRADATION AND RUMEN DEGRADABLE PROTEIN CONTENT FROM SIX PROTEIN SOURCES.

	FTH[a]			CGM			SBM			FSM			BLM			MBM		
TIME, h	2% BW	2%BW + B[b]	1.4% BW	2% BW	2%BW + B	1.4% BW	2% BW	2%BW +B	1.4% BW	2% BW	2%BW +B	1.4% BW	2% BW	2%BW +B	1.4 % BW	2% BW	2%BW +B	1.4% BW
DM, %[c]																		
0	9.16	9.16	9.16	14.76	14.76	14.76	37.59	37.59	37.59	22.47	22.47	22.47	17.41	17.41	17.41	25.64	25.64	25.64
2	14.58	14.30	15.93	19.41	19.35	21.00	41.10	40.06	39.91	28.87	28.74	30.68	27.32	29.19	29.00	36.11	35.40	39.43
4	16.23	15.86	17.40	22.16	21.25	23.40	45.61	43.92	42.61	31.80	31.07	32.35	27.95	29.20	28.31	37.25	38.05	40.77
8	20.66	22.19	21.68	26.20	25.51	27.03	60.30	57.25	55.81	32.13	33.82	33.58	32.44	33.91	32.98	41.09	41.92	42.41
12	26.22	27.13	27.07	31.15	31.14	31.72	73.97	75.95	67.00	35.76	37.32	35.91	37.45	39.00	37.05	46.26	46.63	48.09
24	29.01	31.11	30.20	37.09	38.11	36.35	91.66	95.25	88.65	37.86	39.09	39.32	39.51	40.74	39.85	50.04	50.02	48.94
48	38.66	39.30	34.57	55.61	55.07	48.57	97.75	98.38	97.97	42.63	45.37	44.61	45.19	46.00	45.72	57.00	55.03	52.30
72	42.81	48.05	38.10	77.45	78.76	70.42	98.54	98.61	98.46	49.92	51.62	50.06	50.01	49.68	47.23	57.20	58.49	54.73
RDP, % N[d]																		
0	17.11	17.11	17.11	18.44	18.44	18.44	34.28	34.28	34.28	32.28	32.28	32.28	23.31	23.31	23.31	39.56	39.56	39.56
2	17.94	18.85	20.22	18.20	17.38	19.92	41.77	39.73	38.56	33.10	31.91	33.01	31.47	33.31	32.26	46.50	45.91	46.71
4	19.98	19.96	21.67	19.38	17.74	21.00	46.29	43.69	41.64	34.86	34.31	33.50	33.00	34.70	31.35	48.29	48.71	50.01
8	23.11	25.12	25.32	19.06	18.49	20.77	59.06	55.20	53.94	35.70	37.88	38.72	36.16	36.88	35.58	52.03	52.21	51.16
12	27.66	27.80	29.47	22.26	21.44	22.70	73.22	74.83	64.85	40.29	40.81	39.54	41.19	42.13	39.84	54.42	57.04	57.05
24	30.59	32.46	32.66	25.93	24.68	25.03	93.84	97.51	90.69	43.71	43.88	45.21	43.70	44.53	42.57	60.55	62.43	59.42
48	39.91	41.04	36.49	47.19	45.64	40.46	99.15	99.42	99.30	50.18	51.40	51.32	49.37	49.78	48.48	69.26	67.78	62.96
72	44.25	49.29	40.83	73.89	75.19	65.38	99.87	99.86	99.83	58.87	59.91	57.31	54.86	55.14	51.79	70.26	72.21	66.86

[a] FTH = feather meal; CGM= corn gluten meal; SBM= soybean meal; FSM= fish meal; BLM= blood meal; MBM= meat and bone meal.
[b] 2% BW in diet plus 2% $NaHCO_3$. [c] DM SEM = 1.33. [d] RDP SEM = 1.14.

Previous research results would have predicted that 2% sodium bicarbonate would increase rumen pH and proteolysis (Kronfeld, 1979).

While intake level did influence disappearance of DM and protein from the dacron bags, the effects were relatively small. In addition, the differences were more pronounced after 48 h of residence time in the rumen. Since the normal residence time for degradation in the rumen is less than 24 h, the differences observed due to dietary regimes may have little practical significance. Dry matter and crude protein degradation of corn silage in dacron bags were not influenced by dietary treatments ($P>0.1$).

The six protein sources differed greatly ($P<0.05$) in disappearance of DM and protein from the bags over time. Soybean meal had 37.6% of the DM rinsed from the bag during the 30 min water soak before placement in the rumen (Table 4). Conversely, FTH had only 9.2% washed out ($P<0.05$). Fish meal (22.47%) and MBM (25.64%) had high wash out values as well. The amount of material that leaves the bag during this soaking process represents the soluble and small particle (less than 50 μ) fractions of the respective protein sources. By 24 h of incubation, nearly all of the DM from SBM had disappeared from the bag (92.3%) whereas, less than 50% of the other protein sources had disappeared from the bags. A previous literature summary (NRC, 1985) of in vivo RDP estimates for SBM range from 57 to 90%. The rumen degradable protein values observed between 8 and 24 h of incubation in the current study are within this range. Feather meal was only 43.6% degraded after the 72 h incubation. Of the potentially degradable DM, the actual amount placed into rumen, 37.9, 72.1, 97.7, 36.3, 38.5 and 42.3% were degraded after 72 h for FTH, CGM, SBM, FSM, BLM and MBM,

TABLE 3. EFFECT OF DIETARY REGIMES AND TIME ON DM DEGRADATION AND RUMEN DEGRADABLE PROTEIN (RDP).

	DM Degradation, %[a]			RDP, % of CP[a]		
TIME, h	2% BW	2% BW + B[b]	1.4% BW	2% BW	2% BW +B[b]	1.4% BW
0	21.17	21.17	21.17	27.50	27.50	27.50
2	27.90	27.84	29.32	31.49	31.18	31.78
4	30.17	29.89	30.81	33.63	33.18	33.19
8	35.47	35.77	35.58	37.52	37.63	37.58
12	41.80[cd]	42.86[c]	41.14[d]	43.17[cd]	44.00[d]	42.24[c]
24	47.53[cd]	49.05[c]	47.22[d]	50.57	51.33	50.81
48	56.14[c]	56.52[c]	53.96[d]	59.32[c]	59.27[c]	56.62[d]
72	62.65[c]	64.20[c]	59.83[d]	67.00[c]	68.60[c]	63.67[d]
SED[e]		0.76			0.65	

[a] Averaged across protein sources.
[b] 2% BW + B = 2% BW plus 2% sodium bicarbonate.
[cd] Means within a row with unlike superscripts differ ($P<0.05$).
[e] Standard error of the difference.

TABLE 4. DRY MATTER DISAPPEARANCE FROM PROTEIN SOURCES (%).

TIME, h	FTH	CGM	SBM	FSM	BLM	MBM
0	9.16[a]	14.76[b]	37.59[d]	22.47[c]	17.41[b]	25.64[c]
2	14.81[a]	19.78[b]	40.41[e]	29.28[c]	28.44[c]	36.67[d]
4	16.38[a]	22.13[b]	44.23[e]	31.66[c]	28.51[c]	38.43[d]
8	21.49[a]	26.15[b]	58.03[e]	33.12[c]	33.13[c]	41.73[d]
12	26.77[a]	31.29[b]	72.97[e]	36.38[c]	37.93[c]	46.86[d]
24	30.09[a]	37.29[b]	92.25[d]	38.68[b]	40.06[b]	49.76[c]
48	37.88[a]	53.65[c]	98.04[d]	44.15[b]	45.63[b]	55.08[c]
72	43.60[a]	76.18[d]	98.54[d]	50.59[b]	49.19[b]	57.07[c]

[abcde] Means within a row with unlike superscripts differ ($P< 0.05$).
[f] SED = 1.09.

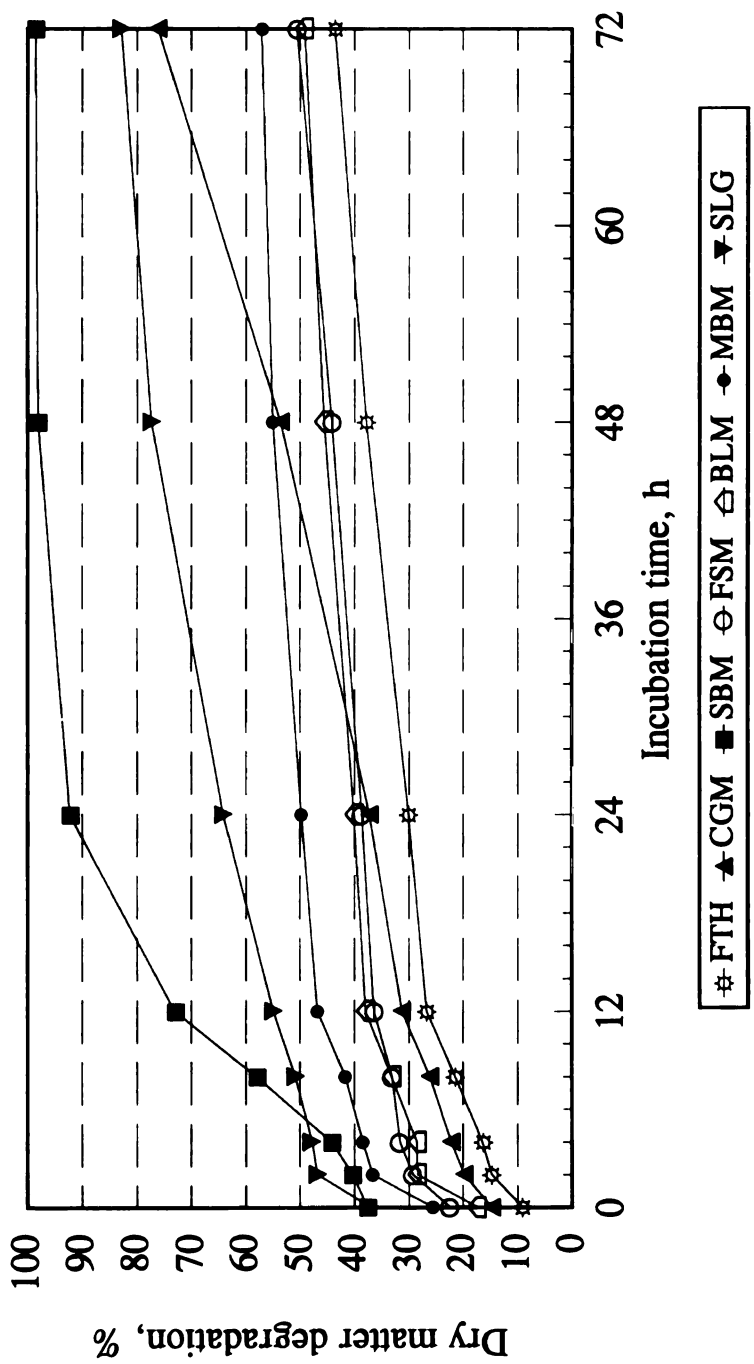


Figure 2. Ruminal dry matter degradation of six protein sources and corn silage.
SED = 0.76

respectively (Fig. 2). Clearly, the plant protein sources are potentially more degradable than the animal co-product, protein sources. Another point of interest, is the similarity between degradation estimates of the potentially degradable fractions (37.9, 36.3, 38.5 and 42.3%) for the animal byproduct protein meals. The differences in overall ruminal DM degradability for the four animal byproduct meals can be explained by the differences in washout during the 30 min rinsing phase.

Thirty-seven percent of the DM in corn silage disappeared from the bag during the rinse phase. After 72 h of incubation, 82.8% of the corn silage was degraded (Figure 2). The potentially degradable DM in corn silage was 84.2% (Table 5). After 120 h, 8.8% the total nitrogen was undegraded and represents the fraction which is totally unavailable. Almost all of the degradable nitrogen in corn silage had disappeared by 48 h of incubation.

The structural characteristics of animal proteins have been implicated in their resistance to degradation. Mahadevan et al. (1980) reported that the presence of disulfide bonds in a protein conferred resistance to proteolytic attack. Because keratin protein in feather meal has double β-helical structure with disulfide bonds, which stabilizes the tertiary structure of FTH protein, ruminal degradation by bacterial proteases is depressed and rumen undegradable protein is increased. G.A. Broderick (1990, personal communication) has suggested the low degradability of animal proteins results from an inability of microorganisms to attach to the particles. Even though attachment was not measured in this experiment, such a scenario would explain the low degradability

TABLE 5. EFFECT OF DIETARY REGIMES AND TIME ON DM DEGRADATION AND RUMEN DEGRADABLE PROTEIN (RDP) OF CORN SILAGE.

	DM Degradation, %			RDP, %		
TIME, h	2% BW	2% BW + B[a]	1.4% BW	2% BW	2% BW + B[a]	1.4% BW
0	37.1	37.1	37.1	81.6	81.5	81.5
2	46.7	45.6	48.5	84.1	83.3	84.8
4	46.8	47.9	49.3	83.9	83.3	84.4
8	50.4	51.1	51.2	83.8	84.4	84.4
12	54.9	54.7	55.4	86.0	85.8	86.2
24	64.4	65.4	61.8	88.1	87.4	86.8
48	77.5	79.5	73.8	90.6	90.4	89.5
72	82.4	84.5	80.9	92.4	92.5	91.9
96	84.5	85.6	82.2	91.9	92.5	91.8
120	84.2	85.7	82.7	90.3	91.2	89.2
SEM[b]		1.28			0.53	

[a] 2% BW + B = 2% BW plus 2% sodium bicarbonate.
[b] Standard error of the mean.
[c] Original crude protein content of corn silage was 6.98% and ADIN was 8.71% of total nitrogen.

estimated for animal protein sources. One interesting observation was the increased degradation of CGM at 48 and 72 h. Perhaps microbial attachment increased after 24h.

Ruminal protein degradability of the six protein sources is shown in Table 6. Soybean meal, FSM and MBM had greater than 30 percent of the crude protein disappear from the bag during the rinsing phase. After 24 h of incubation, SBM had the highest and CGM the lowest protein degradability ($P<0.05$). The proportion of potentially degradable protein washed out in the rinse phase was 53.8, 73.1, 36.3, 78.4, 53.3 and 64.9 percent for FTH, CGM, SBM, FSM, BLM and MBM, respectively. The highest rumen degradable protein value was observed with SBM (99.9%). Fish meal and BLM had similar protein degradation patterns (Figure 3). Feather meal had a lower ($P<0.05$) RDP than BLM, FSM or MBM. Similar results were reported by Goedeken et al. (1990a), who showed that the RDP of FTH was lower than BLM during a 12 h incubation period.

Nitrogen disappearance from corn silage during the 30 min rinse phase was 81.5% (Table 5). After 120 h, N degradability was 91.2%. Consequently, the degradable curve was essentially flat (Figure 3). The results from this study reported a greater water soluble fraction for corn silage (81.5 vs 42%) than Bergen et al. (1974). A partial explanation for this discrepancy may reside in the different procedures used to determine water solubility. Bergen et al. (1974) measured the nitrogen content of a filtered extract whereas our estimate measure retention of nitrogen inside the dacron bags, as a result, the present estimate also included nitrogen associated with particles that

TABLE 6. RUMEN DEGRADABLE PROTEIN VALUES FOR THE SIX PROTEIN SOURCES (% of crude protein).

TIME, h	FTH	CGM	SBM	FSM	BLM	MBM
0	17.11[a]	18.44[a]	34.28[c]	32.28[c]	23.31[b]	39.56[d]
2	18.85[a]	18.32[a]	40.20[c]	32.63[b]	32.36[b]	46.33[d]
4	20.40[a]	19.17[a]	44.15[c]	34.31[b]	33.22[b]	48.88[d]
8	24.41[b]	19.27[a]	56.33[e]	37.27[c]	36.29[c]	51.88[d]
12	28.16[b]	22.06[a]	71.73[e]	40.30[c]	41.20[c]	56.06[d]
24	31.81[b]	25.24[a]	94.43[e]	44.15[c]	43.73[c]	60.97[d]
48	39.48[b]	44.92[a]	99.29[e]	50.92[c]	49.30[c]	67.13[d]
72	45.28[a]	72.25[d]	99.86[e]	58.87[c]	54.19[b]	70.14[d]

[abcde] Means in a row with unlike superscripts differ ($P<0.05$).
[f] SED = 0.96.

TABLE 7. RUMEN UNDEGRADABLE PROTEIN (UDP) FROM THE SIX PROTEIN SOURCES (% of crude protein).

TIME, h	FTH	CGM	SBM	FSM	BLM	MBM
0	82.89[d]	81.56[d]	65.72[b]	67.72[b]	76.69[c]	60.44[a]
2	81.15[d]	81.68[d]	59.80[b]	67.37[c]	67.65[c]	53.67[a]
4	79.61[d]	80.83[d]	55.85[b]	65.69[c]	66.78[c]	51.12[a]
8	75.59[e]	80.73[d]	43.67[a]	62.73[c]	63.71[c]	48.12[b]
12	71.84[e]	77.94[d]	28.27[a]	59.70[c]	58.80[c]	43.94[b]
24	68.19[e]	74.76[d]	5.57[a]	55.85[c]	56.27[c]	39.03[b]
48	60.52[e]	55.08[d]	0.71[a]	49.08[c]	50.70[c]	32.87[b]
72	54.72[d]	27.75[b]	0.14[a]	41.13[c]	45.81[c]	29.86[b]

[abcde] Means within a row with unlike superscripts differ ($P< 0.05$).
[f] SED = 1.3.

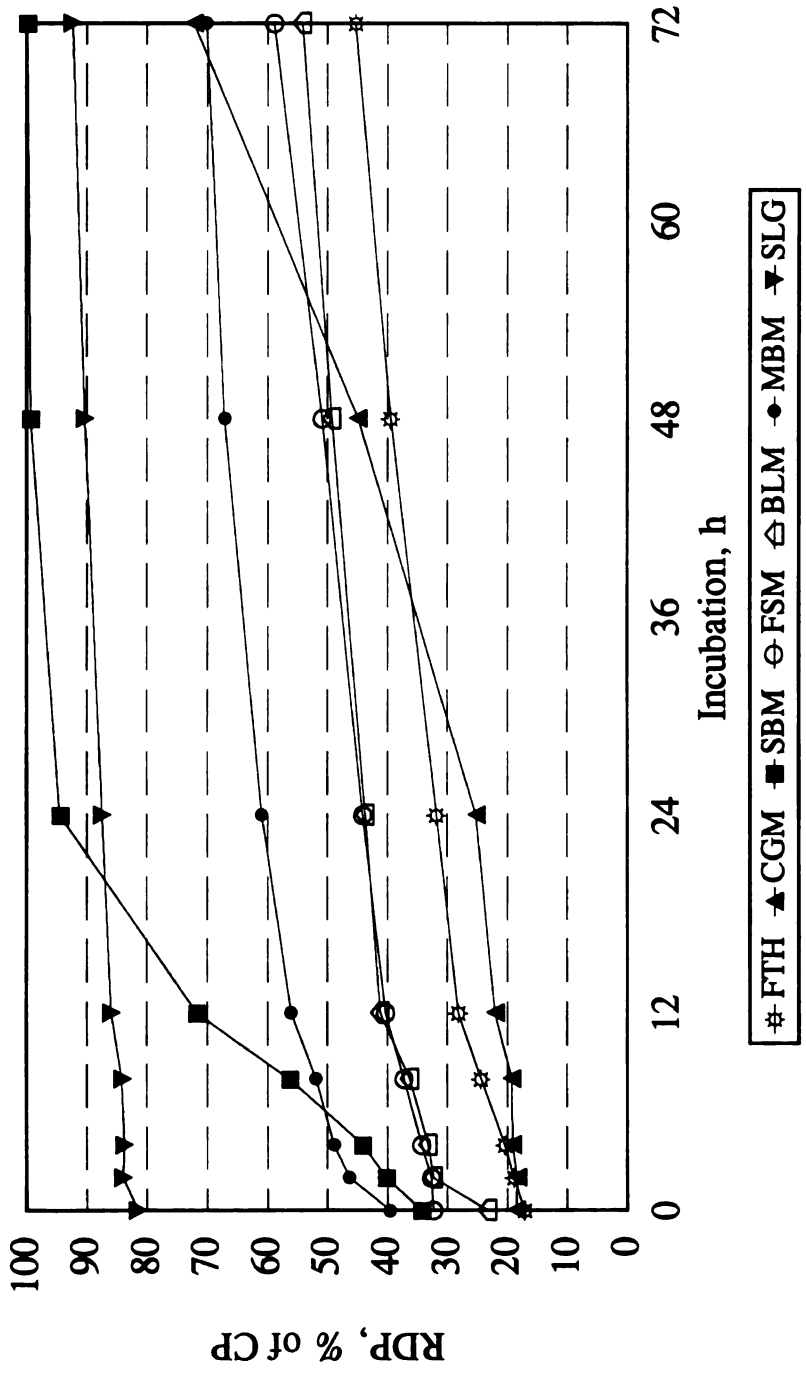


Figure 3. Rumen degradable protein of six protein sources and corn silage. SED =0.65.

escaped through pores of the bags. These particles are likely to be trapped on the filter used by Bergen. Given the rapid rate of availability of nitrogen from corn silage, it is possible that a synchrony exists between energy and nitrogen degradation. It may be possible to supplement corn silage diets with a more slowly degraded protein and increase microbial crude protein yield and fermentable organic matter.

The amount of rumen undegradable protein (UDP; 100-RDP) is not a constant as demonstrated in Table 7. For example, with FTH, UDP at 12 h was 71.8% compared to 54.7% at 72 h. Therefore, assigning a UDP value to feedstuffs is inappropriate unless the ruminal conditions are clearly defined (i.e. residence time). If one assumes 24 h represents the residence time, corn gluten meal had the highest UDP and SBM the lowest ($P<0.05$). The order for UDP content from highest to lowest was CGM (74.8%), FTH (68.2%), BLM (56.3%), FSM (55.9%), MBM (39.0%) and SBM (5.6%).

Dietary regimes had no effect on the protein degradation rate constants (*k*) or "*b*" and "*c*" fractions. Protein degradation rate constants of the "b" fraction were different ($P< 0.01$) among the five protein sources evaluated (Table 8). The degradation curve observed with CGM did not exhibit first order kinetics; consequently, the rate constant could not be determined. The degradation rate indicates how rapidly each "b" fraction is degraded. Meat and bone meal had the highest rate constant followed by BLM> SBM> FTH> FSM. The potential degradable protein ("*b*" fraction) in the rumen was highest for SBM ($P<0.01$) and the lowest for BLM (66.5 vs 27.1). However, the rate constant for degradation was greater for BLM than SBM (0.084 vs 0.070). Fish meal has a similar "*b*" fraction as MBM (33.6 vs 32.9%), but drastically different rate

TABLE 8. DRY MATTER, CRUDE PROTEIN, DEGRADATION RATE, ADIN and a, b, c FRACTIONS FROM PROTEIN SOURCES.

	FTH	SBM	FSM	BLM	MBM	CGM	Corn Silage	Prob
DM, %	94.7	88.8	93.6	92.7	95.6	91.2	33.6	-
CP, %	90.9	50.5	62.7	77.1	55.0	67.7	7.0	-
ADIN, % of N	14.9	4.1	3.4	7.3	2.9	10.6	8.7	-
k	$0.058^{ab} \pm 0.035$	$0.070^{b} \pm 0.015$	$0.030^{a} \pm 0.014$	$0.084^{b} \pm 0.064$	$0.129^{c} \pm 0.080$	[d]	[d]	0.01
a	15.7	33.3	29.5	27.6	35.8	18.4	81.6	0.01
b	$35.9^{a} \pm 18.6$	$66.5^{b} \pm 3.0$	$33.6^{a} \pm 9.5$	$27.1^{a} \pm 7.7$	$32.9^{a} \pm 4.2$	[d]	[d]	0.01
c	$48.4^{b} \pm 18.4$	$0.0^{a} \pm 0.0$	$38.4^{b} \pm 13.0$	$45.3^{b} \pm 7.1$	$31.8^{b} \pm 6.0$	[d]	[d]	0.01

[abc] Means within a row with unlike superscripts differ ($P < 0.1$).

[d] Degradation did not follow first order kinetics, therefore it was not possible to use non-linear regression to develop the rate constant.

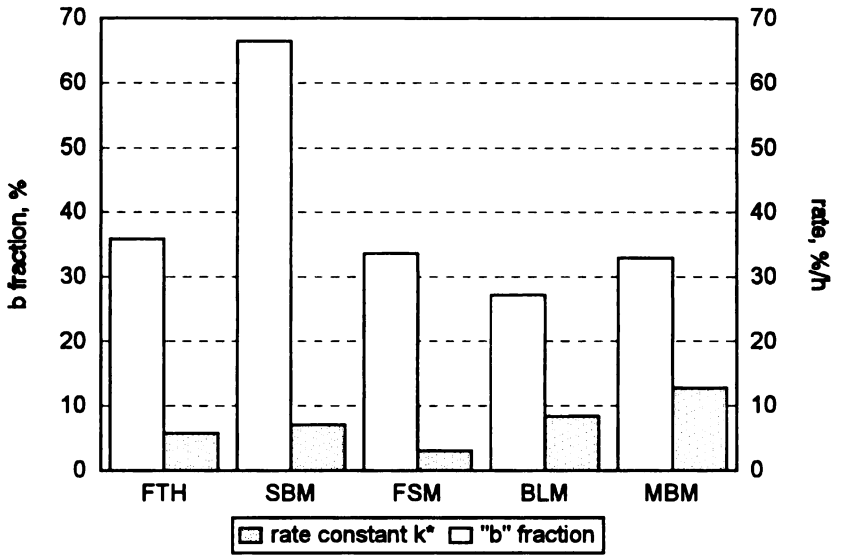


Figure 4. Comparison of the "b" fraction with rate constant k.

*rate constant k expressed as %/h

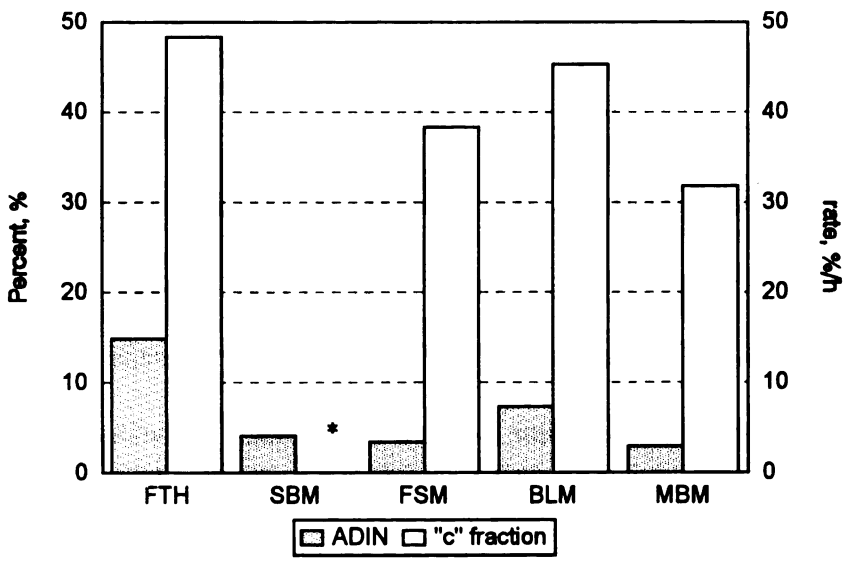


Figure 5. Comparison of ADIN (% of N) with fraction "c".

* "c" fraction of SBM = 0

constant($P<0.01$; 0.03 vs 0.128/h). There appears to be little relationship between the amount of potentially degradable protein and the rate of degradation (Figure 4). Feather meal had the ($P<0.01$) highest value of undegradable protein ("*c*" fraction). All of the SBM could be degraded rumanian as portrayed by the zero "*c*" fraction from the model (Figure 5).

The ADIN value has been recommended as a predictor of rumen unavailable nitrogen (Pichard and Van Soest, 1977). Acid detergent insoluble nitrogen of the six protein sources is presented in Table 8. Feather meal had the highest level (14.88%) and MBM the least (2.86%). The relationship between ADIN and fraction "c", the undegradable nitrogen, was poor. Based on this study, it appears ADIN was not a valuable predictor of unavailable nitrogen.

The AA profiles of the undegraded residues from the protein sources were similar between dietary regimes (Table 9). Amino acids profiles for each of the six protein sources at 0, 24 and 48 h of degradation is presented in Table 10. A comparison of the histidine contents of the six protein sources is shown in Figure 6. Blood meal had the highest content of histidine among the six protein sources. Even though histidine is considered an EAA, there is some de novo synthesis in the animal (Bergen et al. 1968a). Therefore, it is unlikely histidine would ever be a limiting AA. Threonine was reduced ($P<0.05$) in BLM but increased in the residue of FTH and MBM (Figure 7). The branched-AA (valine, leucine and isoleucine) contents were similar in the protein meals and undegraded residues after 48 h of incubation (Figure 8, 9 and 10). Feather meal and BLM had a higher proportion of valine. Methionine was higher ($P<0.05$) in the residue

TABLE 9. EFFECT OF DIETARY REGIMES ON AMINO ACID PROFILES OF UNDEGRADED RESIDUE FROM INCUBATED BAGS[a].

Dietary Regime	2% BW			2% BW plus 2% $NaHCO_3$			1.4% BW			SEM[b]
TIME, h	0	24	48	0	24	48	0	24	48	
ESSENTIAL AMINO ACIDS, g/100 g AA										
HIS	2.65	2.46	2.40	2.65	2.42	2.32	2.65	2.43	2.38	0.05
ARG	6.90	5.99	5.81	6.90	5.89	5.68	6.90	5.85	5.62	0.22
THR	3.51	3.71	3.62	3.51	3.68	3.60	3.51	3.55	3.46	0.07
VAL	6.35	6.87	6.88	6.35	6.90	6.84	6.35	7.08	6.95	0.07
MET	1.97	2.31	2.20	1.97	2.21	2.05	1.97	2.32	2.17	0.06
ILE	4.68	4.91	4.77	4.68	4.89	4.67	4.68	5.01	4.69	0.08
LEU	9.91	10.35	10.16	9.91	10.40	10.01	9.91	10.82	10.35	0.13
PHE	5.58	5.33	5.17	5.58	5.26	4.93	5.58	5.23	4.92	0.16
LYS	5.57	5.56	5.47	5.57	5.52	5.45	5.57	5.86	5.67	0.09
NON ESSENTIAL AMINO ACIDS, g/100 g AA										
CYS	0.75	0.79	0.78	0.75	0.79	0.79	0.75	0.72	0.87	0.05
ASP	7.79	7.79	6.86	7.79	7.65	6.98	7.79	7.36	7.11	0.15
GLU	14.50	13.16	12.53	14.50	12.87	12.34	14.50	12.60	12.35	0.24
SER	4.95	4.95	5.17	4.95	5.01	5.30	4.95	4.79	4.92	0.08
GLY	7.15	7.30	9.01	7.15	7.65	9.73	7.15	7.82	9.45	0.37
PRO	7.41	7.99	8.34	7.41	8.04	8.46	7.41	8.03	8.45	0.21
TYR	3.54	3.69	3.73	3.54	3.69	3.66	3.54	3.54	3.55	0.07
ALA	6.81	6.86	7.11	6.81	7.07	7.34	6.81	6.99	7.09	0.09

[a]Averaged across protein sources.
[b]SEM= standard error of the mean.

TABLE 10. AMINO ACID PROFILE OF THE SIX PROTEIN SOURCES AT TWO TIME ENDPOINTS.

	FTH			CGM			SBM			FSM			BLM			MBM			SEM[a]
TIME, h	0[b]	24	48	0	24	48	0	24	48	0	24	48	0	24	48	0	24	48	
ESSENTIAL AMINO ACIDS, g/ 100 g AA																			
HIS	0.74	0.79	0.75	1.76	1.97	1.84	2.79	2.64	2.60	2.74	2.36	2.36	5.05	4.60	4.45	2.84	2.28	2.20	0.08
ARG	7.69	7.41	7.37	3.34	3.23	3.10	8.28	6.17	4.91	7.83	5.89	5.94	5.82	4.93	4.90	8.45	7.86	8.05	0.38
THR	3.36	3.91	3.90	2.71	2.81	2.78	3.84	3.94	3.49	3.86	3.78	3.79	4.13	3.67	3.68	3.13	3.85	3.80	0.12
VAL	8.42	8.86	8.95	4.51	4.90	4.76	6.23	6.63	6.31	5.95	6.67	6.74	8.04	8.77	8.90	4.92	5.76	5.64	0.12
MET	1.58	2.49	2.36	2.04	2.20	2.16	1.22	1.35	0.88	3.01	3.47	3.43	1.89	1.95	1.90	2.08	2.21	2.11	0.10
ILE	5.32	5.30	5.27	4.50	4.68	4.62	5.78	5.81	4.56	5.15	5.41	5.51	3.38	3.35	3.41	3.97	5.01	4.91	0.13
LEU	8.16	8.52	8.52	16.24	15.89	15.81	8.87	9.30	7.79	8.13	9.05	9.06	10.99	11.75	11.78	7.07	8.39	7.96	0.22
PHE	5.84	5.75	5.58	6.26	6.53	6.42	5.73	5.27	3.97	4.62	3.90	3.95	6.80	5.36	5.32	4.20	4.86	4.85	0.27
LYS	1.96	1.74	1.75	1.60	1.61	1.48	7.03	6.65	6.75	8.97	8.99	8.83	7.82	7.86	7.54	6.02	6.86	6.72	0.15
Total Sulfur AA	3.40	4.46	4.48	2.64	2.88	2.84	1.61	1.89	1.41	3.40	3.87	3.82	2.80	2.63	2.59	2.48	2.59	2.53	0.12
NON-ESSENTIAL AMINO ACIDS, g/100 g AA																			
CYS	1.82	1.97	2.12	0.60	0.68	0.68	0.39	0.54	0.53	0.39	0.40	0.39	0.91	0.68	0.69	0.40	0.38	0.42	0.08
ASP	7.57	7.01	7.12	6.28	5.59	5.99	8.89	10.24	6.49	8.09	8.12	7.43	9.11	9.15	9.06	6.78	5.68	5.72	0.26
GLU	11.09	10.34	10.41	21.21	20.39	20.82	17.28	14.22	11.62	14.38	11.66	11.17	9.81	8.69	8.67	13.25	12.17	11.77	0.42
SER	9.80	9.82	9.80	4.16	4.19	4.25	4.44	4.68	5.97	3.39	3.11	3.14	4.70	4.50	4.56	3.22	3.30	3.22	0.14
GLY	8.41	8.22	8.19	2.76	2.68	2.63	4.71	6.74	16.08	7.74	9.31	9.97	5.67	7.76	8.04	13.60	10.65	11.42	0.64
PRO	10.79	10.09	10.08	8.69	8.76	8.60	5.67	5.90	7.35	5.35	7.58	7.75	5.36	6.66	6.74	8.58	9.12	9.94	0.37
TYR	2.68	2.99	2.99	5.03	5.25	5.37	3.91	4.28	4.36	3.48	3.28	3.28	3.28	2.71	2.69	2.86	3.42	3.25	0.12
ALA	2.68	4.80	4.84	8.32	8.64	8.68	4.94	5.67	6.33	6.92	7.03	7.27	7.25	7.61	7.67	8.64	8.09	8.37	0.15

[a] SEM = Pooled standard error of the mean.
[b] Content in original protein source.

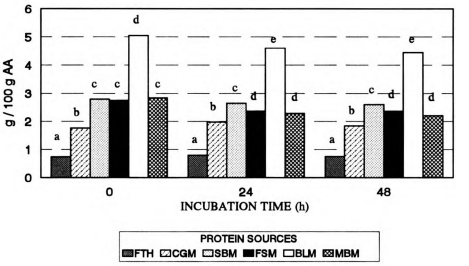


Figure 6. Histidine content of the six protein sources.
[abcde] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.063.

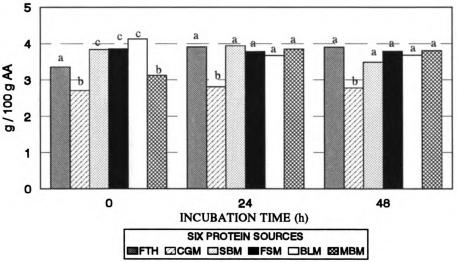


Figure 7. Threonine content of the six protein sources.
[abcd] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.1.

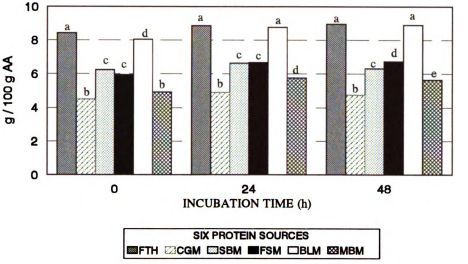


Figure 8. Valine content of the six protein sources.
[abcde]Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.097.

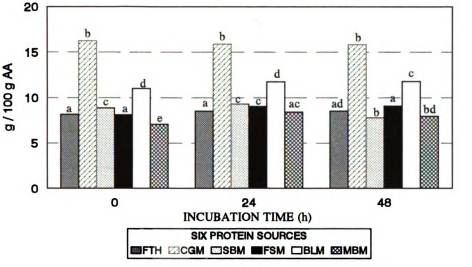


Figure 9. Leucine content of the six protein sources.
[abcd] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.172.

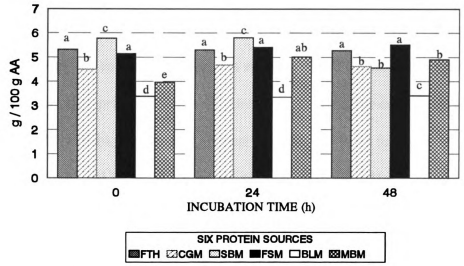


Figure 10. Isoleucine content of the six protein sources.
[abcde]Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.094.

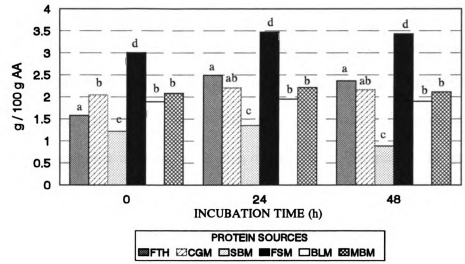


Figure 11. Methionine content of the six protein sources.
[abcd] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.075.

from FSM and FTH (Figure 11). The other sulfur-containing amino acid cystine showed the highest content in FTH and lesser amounts in the other protein sources (Figure 12). Total sulfur-containing AA content of the protein meal and UDP fraction was greater in FTH ($P<0.05$) than other protein sources (Figure 13). Total sulfur-containing amino acids content after 12 h of incubation was greatest for FTH (4.46%) followed by FSM (3.87%), CGM (2.88%), BLM (2.63%), MBM (2.59%) and SBM (1.89%).

Goedeken et al. (1990b) reported that FTH could provide sulfur-containing amino acids post-rumanian and alleviate subtle deficiencies of methionine and potentially stimulate growth. This study supports their conclusions that FTH is a potential source of sulfur-containing AA for ruminants. The intestinal availability of the protein was not evaluated in that study. In FSM, lysine levels were higher in the residue than the protein meal which suggested resistance to degradation by rumen microorganisms (Figure 14). Arginine levels were reduced ($P<0.05$) in the UDP fraction of SBM, FSM and BLM as compared to the other protein meals (Figure 15). Leucine levels were higher in CGM than the other protein sources and less degraded (Figure 9). In agreement with this study, Titgemeyer (et al. 1989) reported threonine, valine and isoleucine in SBM, CGM, FSM and BLM were more resistant to ruminal degradation than other AA. In this study, arginine was more susceptible to degradation than other AA. In contrast to this study, Titgemeyer (et al. 1989) reported methionine and cysteine contents of UDP were lower than in the protein meal.

The length of incubation had a significant effect on the AA value from each protein source. The proportion of methionine, threonine and valine increased ($P<0.05$)

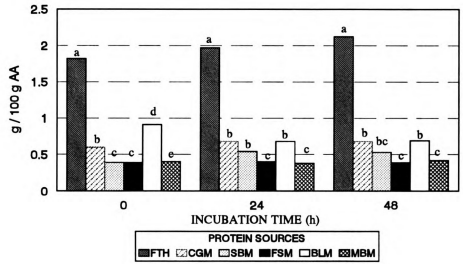


Figure 12. Cystine content of the six protein sources.
[abcd] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.056.

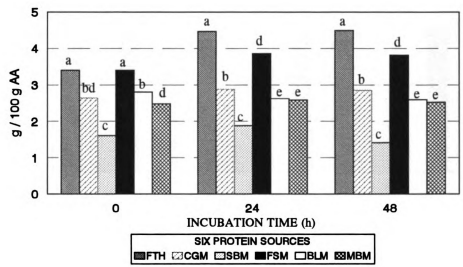


Figure 13. Sulfur-containing amino acids content of the six protein sources.
[abcde] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.124.

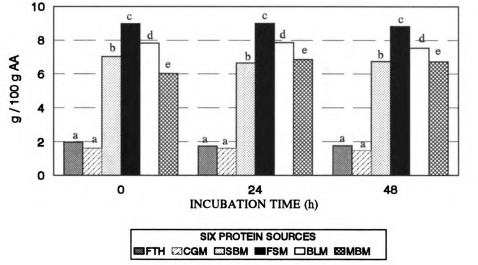


Figure 14. Lysine content of the six protein sources.
[abcde] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.12.

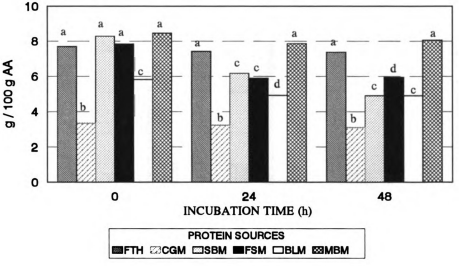


Figure 15. Arginine content of the six protein sources.
[abcd] Means within an incubation period with unlike superscripts differ ($P < 0.05$). SED = 0.19.

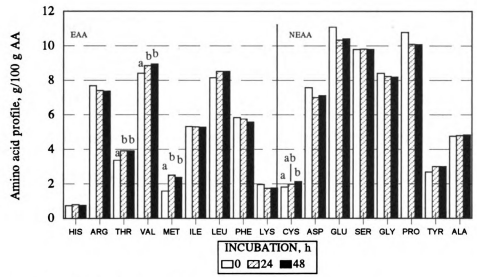


Figure 16. Changes in AA profiles over time for feather meal.

ab Within an amino acid, bars with unlike superscripts differ ($P < .05$)

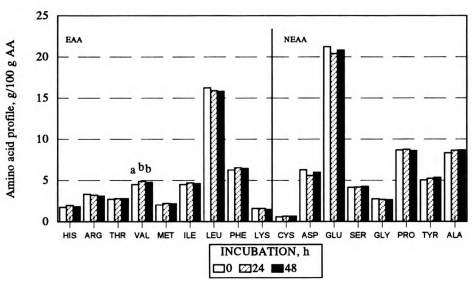


Figure 17. Changes in AA profiles over time for corn gluten meal.

ab Within an amino acid, bars with unlike superscripts differ ($P < .05$)

in the UDP residue of FTH as the length of incubation increased ($P<0.0001$), because the proportion of non-essential amino acid (NEAA) decreased (Figure 16). Percentage of EAA in CGM was similar (Figure 17) at 0, 24 and 48 h of incubation. Figure 16 illustrates all the amino acids changes across time in SBM. The content of arginine, methionine, isoleucine, leucine, phenylalanine and lysine were decreased after 48 h of incubation ($P<0.05$). Both fish meal and MBM showed (Figure 19 and 20) increased levels of EAA and decreased NEAA ($P<0.05$) in the residue. Arginine, threonine, phenylalanine and cystine percentages in BLM were decreased ($P<0.05$) whereas valine and leucine increased ($P<0.05$) after 24 h of incubation (Figure 21). The proportion of histidine was significantly reduced ($P<0.05$) during incubation for FSM, BLM and MBM (Figure 19, 20, 21).

The design of the current experiment was sufficiently replicated to allow the detection of small differences in protein and AA disappearance across dietary regimes and protein sources. Consequently small differences in EAA profiles of the undegradable residues were detected. The lack of accurate AA requirements for ruminants limits the implementation of these results into practical feeding situations. A 20% difference in AA percentage in the residue doesn't result in a 20% increase in AA flow to the small intestine. For instance, the 20% increase in methionine content in FTH residue after 24 h of incubation would increase total methionine supply post - ruminally from 5.63 to 5.98 g/d (+ 6 %), if one assumes a 5% passage rate.

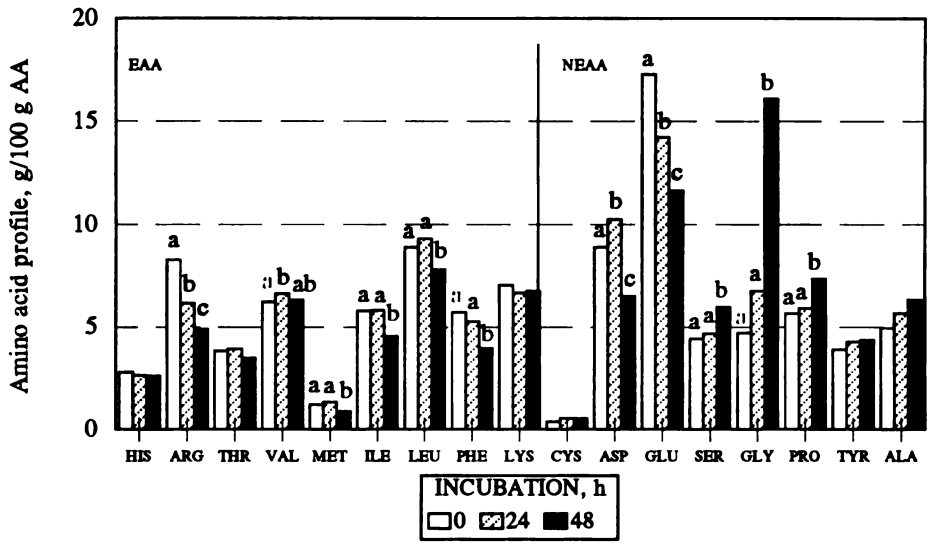


Figure 18. Changes in AA profiles over time for soybean meal.
abc
Within an amino acid, bars with unlike superscripts differ ($P < .05$).

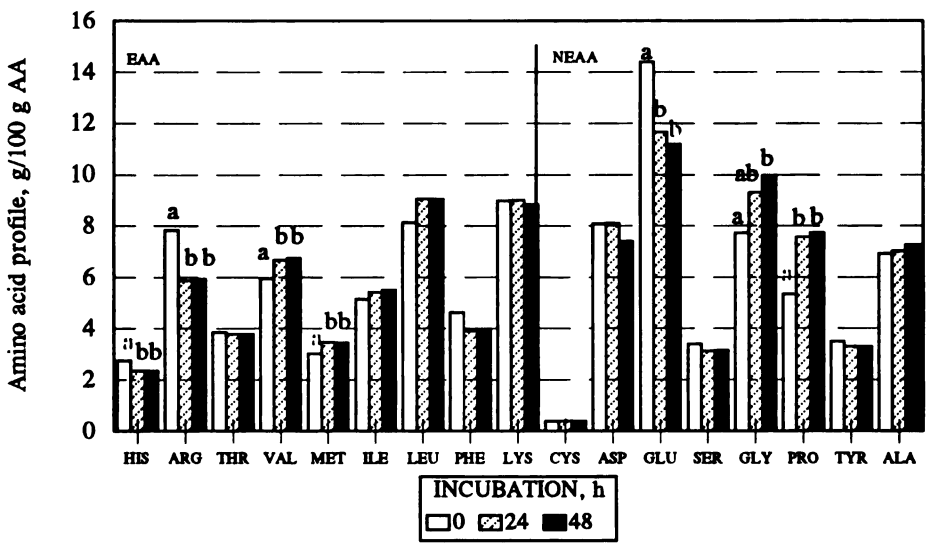


Figure 19. Changes in AA profiles over time for fish meal.
ab
Within an amino acid, bars with unlike superscripts differ ($P < .05$)

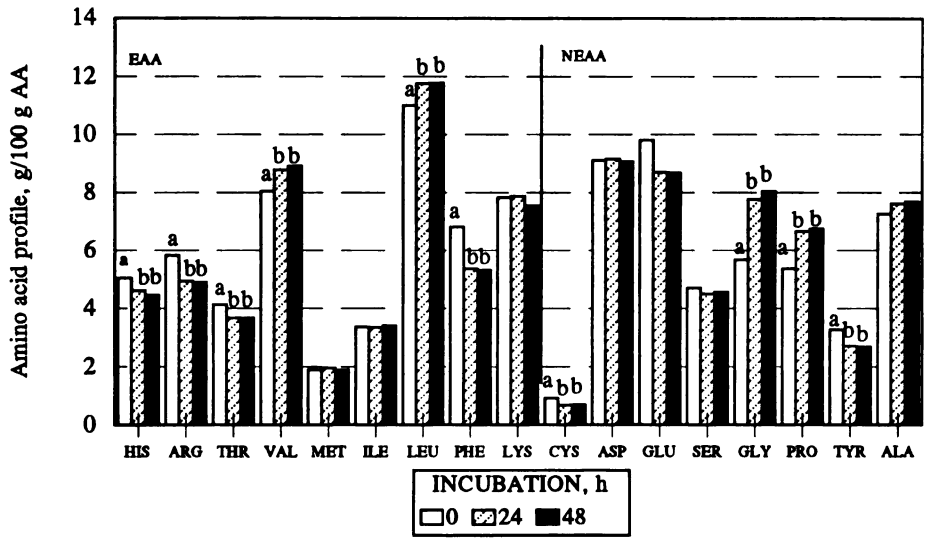


Figure 20. Changes in AA profiles over time for blood meal.

[ab] Within an amino acid, bars with unlike superscripts differ ($P < .05$)

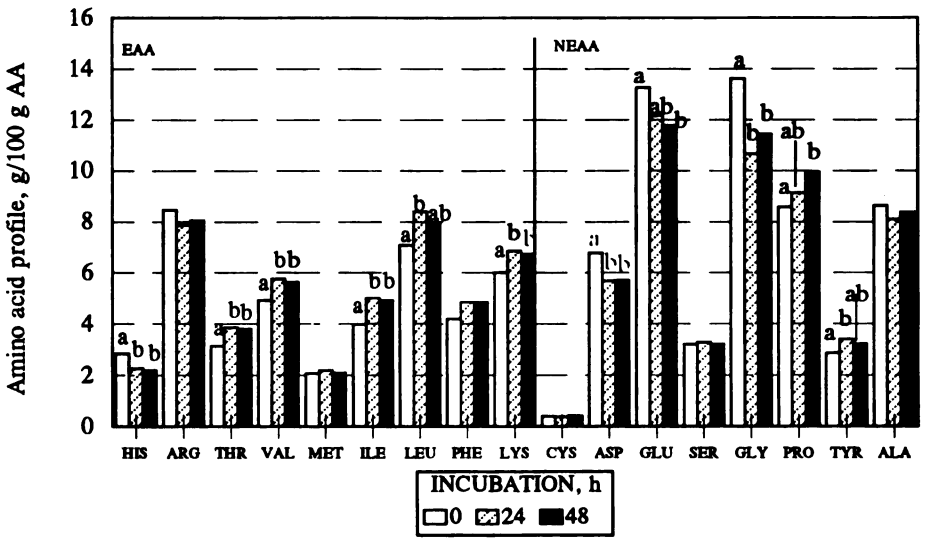


Figure 21. Changes in AA profiles over time for meat and bone meal.

[abc] Within an amino acid, bars with unlike superscripts differ ($P < .05$)

TABLE 11. EFFECT OF DIETARY REGIMES ON AMINO ACID PROFILES OF CORN SILAGE.

Dietary Regime	2% BW			2% BW plus 2% $NaHCO_3$			1.4% BW			SEM[a]
TIME, h	0	24	48	0	24	48	0	24	48	
ESSENTIAL AMINO ACIDS, g/100 g AA										
HIS	2.52	1.85	2.13	2.52	2.01	1.73	2.52	1.33	2.23	0.32
ARG	5.51	5.20	4.53	5.51	4.11	4.80	5.51	4.85	4.75	0.55
THR	3.80	4.77	4.85	3.80	4.45	4.66	3.80	4.49	4.50	0.16
VAL	6.00	6.45	7.02	6.00	6.51	6.96	6.00	6.09	6.66	0.28
MET	1.05	0.99	0.69	1.05	1.03	0.68	1.05	1.23	0.86	0.34
ILE	4.05	5.31	5.48	4.05	5.36	5.58	4.05	5.26	5.42	0.16
LEU	12.11	10.73	10.60	12.11	11.63	10.86	12.11	11.30	10.95	0.30
PHE	4.96	5.79	5.17	4.96	5.62	5.42	4.96	5.98	6.23	0.37
LYS	2.31	3.26	3.86	2.31	3.04	4.07	2.31	3.38	3.97	0.24
Total sulfur AA	1.82	1.76	1.61	1.82	1.45	0.79	1.82	2.01	1.32	0.36
NON ESSENTIAL AMINO ACIDS, g/100 g AA										
CYS	0.77	0.77	0.92	0.77	0.42	0.11	0.77	0.78	0.46	0.35
ASP	7.36	10.16	10.14	7.36	10.33	10.51	7.36	10.42	10.18	0.47
GLU	21.63	16.90	16.30	21.63	16.91	17.18	21.63	17.64	16.29	0.48
SER	3.92	3.67	3.66	3.92	3.94	3.38	3.92	3.89	3.67	0.28
GLY	3.78	4.85	5.10	3.78	5.09	4.93	3.78	5.18	5.26	0.26
PRO	9.16	7.94	7.61	9.16	8.25	7.63	9.16	7.62	7.08	0.57
TYR	3.34	4.26	4.50	3.34	3.85	4.11	3.34	3.48	4.15	0.41
ALA	7.73	7.11	7.44	7.73	7.44	7.40	7.73	7.07	7.37	0.13

[a]SEM= standard error of the mean.

TABLE 12. AMINO ACID PROFILE OF CORN SILAGE AT TWO ENDPOINTS.

TIME, h	0	24	48	SEM[a]
ESSENTIAL AMINO ACIDS, g/100 g AA				
HIS	2.52[b]	1.78[c]	2.00[b]	0.23
ARG	5.51	4.70	4.68	0.39
THR	3.80[b]	4.58[c]	4.69[c]	0.11
VAL	6.00[b]	6.38[bc]	6.91[c]	0.20
MET	1.05	1.06	0.73	0.24
ILE	4.05[b]	5.32[c]	5.50[c]	0.11
LEU	12.11[b]	11.21[c]	10.79[c]	0.21
PHE	4.96[b]	5.78[c]	5.53[c]	0.26
LYS	2.31[b]	3.21[c]	3.97[d]	0.17
Total sulfur AA	1.82	1.70	1.23	0.25
Total EAA	42.31	44.02	44.80	-
NON ESSENTIAL AMINO ACIDS, g/ 100 g AA				
CYS	0.77	0.64	0.50	0.25
ASP	7.36[b]	10.29[c]	10.29[c]	0.33
GLU	21.63[b]	17.09[c]	16.62[c]	0.34
SER	3.92	3.83	3.56	0.20
GLY	3.78[b]	5.02[c]	5.08[c]	0.18
PRO	9.16[b]	7.98[c]	7.48[d]	0.40
TYR	3.34	3.91	4.26	0.29
ALA	7.73[b]	7.22[c]	7.41[c]	0.09
Total NEAA	57.69	55.98	55.20	-

[a]SEM= standard error of the mean.

[bcd] Means within a row with unlike superscripts differ ($P<0.05$).

In previous studies (Bergen et al., 1968; Storm and Ørskov, 1984), the most limiting amino acids in microbial protein were methionine, followed by lysine, arginine and histidine. For growing calves, sulfur-containing amino acids and lysine were also considered to be first and second-limiting AA in microbial protein (Nimrick et al., 1970; Fenderson and Bergen, 1975; Richardson and Hatfield, 1978). Based on the results of this study, feather meal is rich in sulfur-containing amino acids, but low in lysine. Once the requirements are established for different levels of production, different protein meals can be blended to meet the AA requirements.

Dietary treatments had no effect on amino acids contents of the undegraded residue from corn silage (Table 11). Prior to incubation, corn silage has a high leucine content (12.11%), but low lysine (2.31%) and methionine (1.05%). Similar results were reported by Bergen et al. (1974). Contents of AA in corn silage residue over time of incubation are shown in (Table 12). Histidine and leucine content in the residue was decreased ($P < 0.001$), but branch-chain AA contents (valine and isoleucine) were increased. Lysine and threonine levels were increased also ($P < 0.001$). For the non-essential AA, the amount of aspartic acid, glycine and tyrosine increased after incubation whereas, proline and glutamic acid decreased.

In summary, rumen undegradable protein values were determined for the six protein sources. Accurate prediction of the amino acid flow to the small intestine should improve CP utilization in ruminant diets. Based on these results, feather meal can be classified as a highly undegraded protein source that provides a source of sulfur - containing amino acids. Fish meal is rich in lysine and blood meal has very high

histidine contents. This study demonstrated that the combination of different protein sources can be used to balance AA reaching the small intestine, and that potentially decrease the cost of performance for the beef cattle industry. Combination of different protein sources should be able to increase nitrogen utilization and as a supplemental protein in corn silage diet to increase feed efficiency in future.

IMPLICATION

The value of ruminal degraded protein (RDP) may be an effective strategy to define protein quality. Because RDP and UDP values changed with length of incubation time, neither RDP or UDP provide an adequate method to formulate diets unless rumen retention time can be sufficiently defined. Secondly, defining proteins by degradation rate constants has limitation because some protein sources did not follow the first order kinetics. Use of ADIN value as a predictor of bioavailability also has limitation, because of the poor relationship between ADIN and UDP. Even though the AA profile of the six protein sources changed during the incubation, the proportion of AA may not significantly impact EAA flowing to small intestine. Consequently, the AA profile of the diet before ingestion may be used to estimate the profile of AA flowing to small intestine from the undegraded feed residue.

Chapter IV Using Scanning Electron Microscopy to Observe Feather Meal Degradation by Rumen Bacteria

SUMMARY

Feather meal samples were selected from a steer fed a corn silage diet at 2% of body weight to observe the physical action of rumen bacteria colonization. The microstructure of original feather meal protein was ovoid in shape and comprised many closely associate strands as an unit under low magnification. Small pieces were dissociated from the structural integrity of the feather meal between 8 to 12 h of incubation. After 24 h of incubation, some strands were degraded and separated from the feather meal particles. After 48 h of incubation, most of the noticeable strands had disappeared. Under higher magnification, feather meal particles developed ragged edges and became pitted. Finally, after 72 h, extensive surface pitting, unraveling of the strands and disruption of microstructure of feather meal occurred. Consequently, the feather meal particles passing to the small intestine were physically altered by the rumen bacteria.

INTRODUCTION

Ruminant animals depend on the rumen microorganisms to degrade feedstuffs and synthesize microbial protein. Dietary protein is degraded by rumen microorganisms and converted to microbial protein, which usually have a lower biological value than the original dietary protein. Dietary protein sources vary in degradability of the crude protein fraction and AA content of that undegraded residues. Previous results demonstrated that FTH has a low ruminal degradability. The physical structure may be limiting microbial attachment.

Feather meal contains 80% to 90% CP (DM basis) and provides sulfur-containing amino acids to ruminant animals (Goedeken et al., 1990). Eighty-five to ninety percent of the protein in feather is keratin which is an extended β -helical chain which coils slowly to form a helix of relatively large pitch as the structural unit. Such helices tend to aggregate by hydrogen bonding to form cylindrical and form cable-like structures (Schor et al., 1961 a,b). This dense configuration and strong bonding limit the amount of ruminal degradation and observed in feather meal. The resistance of feather meal protein to ruminal degradation may be due to poor colonization by rumen bacteria (McAllister et al. 1990). Williams et al. (1990) demonstrated that feather-degrading bacteria altered the spacing of the rectangular array of intracellular crystals in feather. In additional to these information, it will be extremely beneficial for the manipulation of protein supplements by understanding the physical changes of FTH during the incubation.

Scanning electron microscopy (SEM) provide a powerful tool to explore the microstructure of material surface. The process of ruminal degradation is that rumen microorganisms interacted with feed particles. This process involves attachment or association between microorganisms and the feed particles (Brock et al. 1982; Wallace, 1985 a). Currently, there is very limited information about the physical structure of FTH in rumen by using SEM to observe FTH surface change.

The objectives of this study were to explore the physical differences in external features and microstructure of FTH particle that disappeared during ruminal incubation. This results can reveal the physical change of protein particles during rumen incubation and should provide the beneficial information to utilize FTH protein in rumen diets.

MATERIALS AND METHODS

The previous study provided a set of FTH ruminant degraded samples. The feather meal samples were incubated in the rumen of a fistulated steer fed a corn silage diet at a level equal to 2% of body weight. A mixture of proteins, feather meal, corn gluten meal, blood meal, fish meal, soybean meal and meat and bone meal was added to bring ration crude protein to 12%. Five gram of feather meal was placed into dacron bags (Ankom, Spenceport, NY) and incubated in for 8, 12, 24, 48 and 72 h. Undegraded residue was removed from the bag after washing and dried 100°C over night.

Undegraded residue from feather meal (1 μg) was evenly placed on a small metal cylinder (called a stub) and adhesive film was used to adhere the feather meal particles

to the stubs (Flegler 1992). Mounted samples were allowed to dry for 10 minutes. After mounting and drying, the fixed sample was coated with gold-palladium to a thickness of approximately 20-30 nm in a sputter coater. Coating time required about 2 minutes. This coating process generated the electric conductive sample. If a sample is not conducive, it will result in abnormal artifacts on the photographs. The coated feather meal samples were stored in a desiccator (Flegler 1990).

An scanning electron microscope (SEM) JEOL JSM-350 was used to observe the microstructure of the feather meal. A sample stub was inserted into the SEM fitted with 400 condenser lens, 15mm WD and operated at 10kV. The filament was saturated to obtain an image, which can be observed through the computer screen. The specimen is scanned at low magnification (under 800 x magnification) to select the proper contrast, brightness and position before the photograph is taken.

RESULTS AND DISCUSSION

The microstructure of FTH protein particles was ovoid in shape and comprised many closely associated strands as a unit (Figure 22-1, 0 h, 440 × magnification). By 8 h of incubation, the FTH particle start to lose and dissociate from the main unit (Figure 22-2, 8 h, 600 x magnification). After 12 h of incubation, some strands were detached from the main particles (Figure 22-3, 12 h, 400 x magnification). At 24 h of incubation, significant physical change can be observed in each particle unit, strands were separated from the particles (Figure 22-4, 24 h, 1300 x magnification). After 48 h of incubation,

Figure 22. Scanning electron photomicrographs of feather meal particles after various incubation periods in the rumens of cattle. The white bar on the picture is one micron.

1. particles of original feather meal (440 X);
2. particles after 8 h of incubation (600 X);
3. particles after 12 h of incubation (400 X);
4. particles after 24 h of incubation (1300 X);
5. particles after 48 h of incubation (600 X);
6. particles after 72 h of incubation (600 X).

1
2
3
4
5
6

Figure 23. Scanning electron photomicrographs of feather meal particles after various incubation periods in the rumens of cattle. The white bar on the picture is five micron.

7. particles of original feather meal (2000 X);

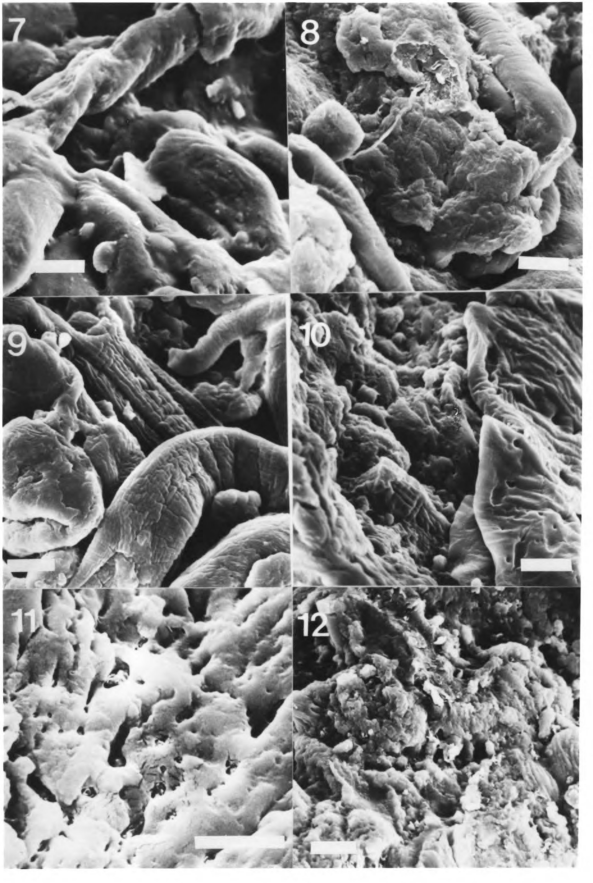
8. particles after 8 h of incubation (2000 X);

9. particles after 12 h of incubation (2000 X);

10. particles after 24 h of incubation (2000 X);

11. particles after 48 h of incubation (2000 X);

12. particles after 72 h of incubation (2000 X).


7
8
9
10
11
12

most of the noticeable strands had disappeared (Figure 22- 5, 22- 6; 48 and 72 h, 600 x magnification).

The physical differences on the surface of FTH particles were investigated at high magnification. Initially, the external surface of FTH was very smooth with few jagged edges (Figure 23-7, 0 h, 2000 x magnification). By 8 h of incubation, the rough edges appeared on the FTH surface (Figure 23-8, 8 h, 2000 x magnification). Significant amount of pitting was observed on the surface after the 24 h incubation of FTH (Figure 23-10, 12 h, 2000 x magnification). The surface of FTH was entirely disrupted after 48 or 72 h of incubation but the underlying helical structure was still intact (Figure 23-11 and 12, 2000 x magnification).

The washing and drying of the FTH residue may have dislodged the bacteria. There is limited evidence of bacterial attachment on the surface of FTH in these microphotographs. By 8 h of incubation, only 25% of DM in FTH was disappeared. Within the next 40 h, approximately 30% of the degradable material had disappeared. In the process of feather meal degradation, some microbial colonization or attachment was noticed. It is unclear from this study, how the bacteria interacted with feather meal particles to accomplished degradation. The majority of the protein degradation may have been performed by free floating extracellular enzymes closely associated with the surface attachment site. The microphotographs from 48 to 72 h samples showed extensive degradation of the ultrastructure of FTH.

Cotta and Hespell (1986) found that the physical and chemical characteristics of feedstuffs influence the degree of protein degradation by ruminal bacteria. Proteins that

have helical strands tend to have low degradability. Feather meal particles are composed of closely associated helical strands. Mahadevan et al. (1980) reported that the presence of disulfide bonds in protein made it resistance to proteolytic attack. Feather meal has a high content in keratin helded together by disulfide bonds which may render FTH resistant to degradation by ruminal microorganisms. During the early portion of incubation, the major physical appearance change of FTH was the unraveling of helical structure. After 12 h of incubation, FTH surface pitting was observed. Ruminal bacterial attachment was not observed. This indirectly confirmed that in situ method of washing procedure for FTH can eliminate the microbial protein contamination into undegraded protein analysis.

CONCLUSION

This study demonstrated that the major physical surface change of FTH in the process of 72 h ruminal incubation. Feather meal particles were changed from the unraveling tertiary structure units to full of surface pitting and lose strands units, as the length of incubation increased. Microbial attachment was not detected.

CHAPTER V RECOMMENDATIONS

This study demonstrated the possibility of utilizing mixtures of protein sources that have complementary AA profiles to increase efficiency of nitrogen utilization. For example, a mixture of soybean meal (high rumen degradation rate) with feather meal (lower rumen degradation rate) should decrease the amount of excess EAA (better balance of EAA). Combination of these two protein sources have potential to complement nitrogen availability in the rumen and improve efficiency of protein utilization. The amino acid profiles indicated that blending different protein sources which are rich in certain essential amino acids could balance the requirement of amino acids for growing ruminants.

Based on the outcome of this research, some general methodology recommendations for future study can be suggested. It is important to utilize sufficient experimental units to minimize the impact of experimental variation between replicate bags on the same treatment. It would be helpful, if a different sampling protocol were used. The sample collection times used in this study were 2, 4, 8, 12, 24, 48 and 72 h. Dry matter and CP degradation were essentially complete within 48 h except for CGM. In future studies, the last collection period could be 48 rather than 72 h. Secondly, more intensive sampling would improve the prediction of rate constants, especially during the

first 24h of incubation. The dacron bag technique was an effective method to measure degradation in rumen, however, the system still needs to be standardized. For example, the number of bags per animal, the position of bags within rumen, the order of removing bags from animals and the washing technique should be standardized (Nocek, 1988).

Evaluation of a protein source should consider several characteristics of the protein. First, the solubility of a protein source provides an estimate of rumen degradability but provides little information on rate of degradation or AA balance. Additionally, changes in rumen conditions, such as pH, can impact solubility. Second, it has been suggested that ADIN is closely related to rumen degradability, however, the relationship between ADIN and UDP was poor in this study. Developing a combination of protein characteristics such as ADIN, rate constant, RDP and UDP might be a feasible approach to characterize a protein source. Third, the degradation and uptake of amino acids from a protein source requires further study. Because of the different molecular structures among protein sources, the rate and extent of essential amino acid degradation in the rumen and uptake subsequently by the small intestine needs to be determined.

This study demonstrated the possibilities of using combination of protein sources to improve nitrogen utilization. The combination of FTH with SBM, SBM with BLM, SBM with MBM appear to minimize supply of excess EAA. Further studies should focus on the combination of different protein sources on animal feed efficiency, composition of carcass tissues and growth rate.

The results of this research also indicate that feather meal is rich in sulfur amino acids. The surface characteristics of each protein source is different. An important question that remains to be answered involves the attachment of microbes to these protein meals. Comparison of the microstructure of each protein source should enhance our understanding of ruminal degradation.

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