INFLUENCE OF DIET ON RUMINAL IN SITU DISAPPEARANCE OF DRY MATTER AND NITROGEN

By

JUAN RUBEN BARRIO

Bachelor of Science

Chihuahua University

Chihuahua, Mexico

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AND NITROGEN

Thesis Approved:

F. N. Owens
Thesis Adviser

D. G. Wagner

Robert Totter

Norman N. Durham
Dean of the Graduate College

1184025

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CHAPTER I

INTRODUCTION

Protein supplements for ruminant animals are of special concern since they are generally the most expensive ingredients of the diet. Though a small portion of the total diet, protein supplements can markedly affect production or performance of ruminant animals. Many producers commonly feed protein levels in excess of estimated requirements as a safety measure.

Two nitrogen requirements must be considered for ruminant animals--needs of ruminal microbes and of the host animal. Under many conditions, ruminant animals can survive and produce using only the nitrogen derived from microbial protein, but for highly productive dairy cows and young, rapidly growing animals, amino acid requirements of the host may exceed that supplied by microbial protein alone. In such cases, supplemental dietary protein which will partially bypass the rumen and supply amino acids in the small intestine will be needed for maximum performance.

In vivo (animal), in vitro (laboratory) and in situ (polyester bags suspended in the rumen) methods have been developed to estimate ruminal digestion of dry matter, fiber and protein. Each of these procedures has advantages and limitations. The in vivo system is desirable since it most closely approximates the production state of the animal and allows factors to change naturally, but such

experiments require considerable input of time, animals, labor and money. In vitro procedures are rapid and controllable but cannot reflect dynamic conditions in the rumen of an animal and are generally static fermentation systems. In situ techniques are a hybrid which offers the much of the simplicity of in vitro systems while allowing animal variables to operate.

With the in situ or in sacco procedure, dry matter disappearance from feedstuffs placed in artificial bags suspended in the rumen is measured. This technique has been employed for decades (Quin et al., 1938). This technique has more recently been used to assess degradation of protein in the rumen after Mehrez and Ørskov (1977) reported that protein bypass could be successfully predicted with in situ procedures.

Recently, methods to mathematically describe nitrogen disappearance from in situ methods have been reported (Ørskov and McDonald, 1979; Mathers and Miller, 1981; McDonald, 1981; Stern and Satter, 1982; Waldo, 1983; Zinn and Owens, 1983). The objectives of this thesis research were to 1) furnish in situ protein disappearance data for a wide variety of feedstuffs to fit one of these models, 2) determine to what degree in situ protein disappearance is affected by diet, time and animal differences, 3) examine the relationship between solubility of protein and in situ protein disappearance and 4) investigate possible interactions between two protein sources when incubated together in situ.

CHAPTER II

LITERATURE REVIEW

Protein reaching the small intestine of ruminants may be subdivided into fractions of microbial, dietary and endogenous origin. Reliable techniques to predict the quantity of feed protein leaving the rumen undegraded are needed to properly evaluate dietary protein sources. Current methods which have been developed to estimate protein bypass include in vitro, in vivo and in situ procedures. These are discussed below.

Methods for Bypass Prediction

In vitro procedures. The major advantage of in vitro procedures over in vivo methods is simplicity according to Satter (1982). In vitro methods have employed simple solubility procedures as well as incubation with fluid obtained from the rumen of cattle or sheep. The latter requires access to animals and may add variability, but results may be more closely related to the biological effects in the rumen.

Solubility of protein in the rumen has been suggested to be one of the most important factors affecting protein degradation in the rumen (Broderick, 1978; Waldo and Goering, 1979). Since the initial studies by Hendrickx and Martin (1963), soluble protein has been considered to be degraded at a very rapid rate. However, more

recent studies have questioned this concept (Mangan, 1972; Mahadevan et al. (1980) for certain soluble proteins. Though a rapid liquid dilution rate would enhance ruminal outflow of soluble protein, the low levels of soluble protein in the rumen under normal conditions (Borchers, 1965) would reduce the potential for bypass of soluble protein.

Waldo and Goering (1979) measured solubility of N from various common feedstuffs in four different solvents. These were 1) water, at 39 C for 6 h or boiling for 1 h; 2) 10% Burroughs buffer solution; 3) autoclaved ruminal fluid; and 4) .15 M NaCl. Solubilities among feedstuffs and solvents differed. Autoclaved ruminal fluid might be considered the most physiological solvent to simulate ruminal solubility of protein from feedstuffs. Krishnamoorthy et al. (1982) observed that fluctations in solvent pH can affect protein solubility. These workers proposed the use of a borate-phosphate buffer because of its pH stability during storage.

Many workers have incubated feedstuffs with ruminal fluid and measured ammonia accumulation over time. Ammonia release has been expected to reflect protein degradation, but since microbes use a variable amount of the liberated ammonia for protein synthesis, an undetermined amount of released ammonia will be reincorporated into protein during incubation. With feedstuffs which differ in the amount of microbial growth which they support, ammonia release may be a misleading index of protein degradation. It has proven difficult to separate or differentiate between microbial protein and feed protein.

To prevent microbial use of released amino acids and ammonia, Broderick (1978) added hydrazine sulfate to his incubation mixture and used release of ammonia plus total amino acids following incubation as an index of protein degradation. Interpretation of the release rates became complex since rates varied over time. Incubation times may need to be regulated to reflect differences in ruminal outflow rates among feedstuffs.

In vivo procedures. Stern and Satter (1982) stressed that in vivo results are the standard by which in vitro and in situ results are evaluated. In vivo trials must be conducted to validate or refute theories developed from either in vitro or in situ results. Comparisons among in vitro and in situ procedures are useless without in vivo measurements.

To measure protein degradation in the rumen in vivo requires the use of animals equipped with cannulas at some point in the digestive tract, usually in the omasum, abomasum or proximal duodenum. From these cannulas, samples of digesta can be obtained past the site of ruminal digestion. Flow rate of digesta at this point also must be estimated using either some indigestible dietary marker or a continuous sampling device. Finally, the nitrogen in the sample must assigned into fractions of microbial origin and feed origin. For this, some reliable microbial marker or feed protein marker is needed.

To calculate in vivo bypass or escape of a single component of a multi-component diet, further calculations are needed. Flow of N from the test source of protein must be differentiated from N contributed by other sources of N in the diet. For this, two

methods may be employed, the regression and the difference technique. With the regression method, several levels of the test protein are fed and the proportion of undegraded dietary protein is estimated from the relationship of duodenal protein flow to intake of protein. With the difference method, a basal (unsupplemented) diet must be fed. The difference in ruminal outflow of non-microbial protein between the basal diet and the basal diet with the test protein added is attributed to the test source of N.

The needs for surgical preparation of animals and the large amount of analytical work has hindered data acquisition. Also, the uncertainty of the accuracy with which N fractions can be subdivided, the adequacy of both microbial and passage markers, and the variation and imprecision of in vivo results has stimulated the search for alternative techniques.

In situ procedures. Midway in complexity between the in vitro (laboratory) and in vivo (animal) procedures stands the in situ or in sacco method. This method retains much of the simplicity of in vitro procedures while permitting both animal and microbial factors to influence results. Access to cattle or sheep with ruminal cannulas limits the wide application of this technique, but the influence of animal and microbial factors on ruminal proteolysis are often important factors which cannot be assessed in vitro.

Dry matter disappearance of feedstuffs suspended in the rumen has been studied for several decades. Quin et al. (1938) placed a variety of feedstuffs in silk bags and incubated these bags in the rumens of fistulated sheep. Since that time, the in situ technique has become an accepted practice for estimating ruminal disappearance

of dry matter and fiber (Hopson et al., 1963). In situ methods also have been used to quantitate protein disappearance (Crawford et al., 1978; Mehrez and Ørskov, 1977; Zinn and Owens, 1982; Weakley et al., 1983). Weakley et al. (1983) concluded that several intrinsic factors involved with this technique may affect results and must be controlled. These included particle size of the substrate, accumulation of gas inside the bag, animal effects, animal's diet, pore size, and the ratio of sample weight to surface area of the bag. These will be discussed later as they apply to protein.

Application of In Situ Nitrogen Disappearance Data to Nitrogen Flow in Ruminants

Nitrogen flow in ruminants. Protein supplements often are the most expensive ingredients in livestock diets, especially for high producing ruminants with high protein requirements. Thus, ruminant nutritionists must utilize protein supplements to maximize their efficiency. Nitrogen requirements of the host and the ruminal microbes must be considered. Owens and Bergen (1983) observed that the quantity of protein absorbed in the small intestine is the sum of microbial protein plus the dietary protein which escaped degradation during passage through the rumen. Waldo (1983) suggested that dietary protein should be subdivided into several fractions--a rapidly degraded portion called "A", a slowly digested fraction called "B", and an indigestible fraction called "C". For the slowly digested fraction, the fractional rate of digestion and the rate of passage from the rumen both must be considered to

calculate the extent of ruminal degradation and passage from the rumen. Fractional rates of digestion and passage are difficult to quantitate. Yet, this approach permits bypass or escape of dietary protein to be calculated from rate of digestion, an in situ or in vitro measurement, and rate of passage, an animal measurement. These factors--rate of digestion and rate of passage form the core of most models for calculating protein bypass. One other component, microbial protein synthesized in the rumen, must be added to equal the total flow of amino acids to the small intestine. Models to calculate total amino acid flow sum these two together, so that underestimates in one may be compensated by overestimates in the other.

Satter (1982) evaluated five different models for their capacity to predict total flow of amino acids from the abomasum to the small intestine which has been measured in a number of trials with sheep and cattle. Burroughs (1974) proposed bypass values for a number of feedstuffs. The model of Burroughs et al. (1975) underestimated amino acid flow to the small intestine, especially with high protein diets. The model of Journet and Verite (1977) estimated postruminal amino acid flow adequately; however, urea additions to high protein diets caused flow to be overestimated. This model accounts for differences in nitrogen metabolism between cattle and sheep. The model described by Kaufmann (1977) more greatly underestimated amino acid flow for cattle than for sheep. The ARC (1980) system is based largely on estimates by Roy et al. (1977). A large number of feeds are deemed 20% resistant to ruminal degradation in the model of Roy et al. (1977); therefore, an

underestimation of amino acid flow could result, especially with cattle. Total amino acid flow was most accurately predicted for cattle and sheep by the model of Satter and Roffler (1975). This model incorporates a variable rather than a fixed value for protein degradation in the rumen. Satter (1982) concluded that use of these models to increase animal performance is unquestionably an improvement over the crude protein system.

Microbial protein synthesis. Nitrogen requirements of ruminal microbes are met only by dietary and endogenous sources of nitrogen. Ruminal microbes degrade dietary protein to volatile fatty acids and ammonia (Van Soest, 1982). This process continues whether the supply of amino acids for the host animal is excessive or deficient. Excess ammonia is lost by absorption through the ruminal wall into the bloodstream or by passage to the omasum and abomasum. Owens and Bergen (1983) indicated that 40 to 80% of the total protein normally reaching the small intestine is of microbial origin but that dietary and animal factors will influence this fraction. Amounts of microbial nitrogen reaching the small intestine are affected by ruminal dilution rate, fermentation rate, and other factors which are poorly understood (Ørskov, 1982). Rate of protein degradation affects the availability of nitrogen for incorporation into microbial protein (Lindberg, 1981). Dietary protein escaping ruminal degradation can significantly alter efficiency of ruminal fermentation (Ørskov, 1982; Van Soest, 1982; Owens and Bergen, 1983). Diets containing substantial quantities of recalcitant proteinaceous feeds might limit microbial fermentation and (or) growth by supplying suboptimal ruminal levels of ammonia

nitrogen, amino acids or branched chained volatile fatty acids, etc., at some times within a feeding interval.

Application of in situ nitrogen disappearance rate. The goal of nutritionists when dealing with high producing ruminants is to satisfy the nitrogen requirements of ruminal microbes while attaining a high level of ruminal bypass of feed protein. To be of value to the animal, such bypassed protein must be highly digestible in the small intestine and be of higher quality than microbial protein (Owens, 1978). Hence, it is necessary to contrast chemically the protein fractions degraded in the rumen with those bypassing to the small intestine. Currently, the Agricultural Research Council (1980) uses in situ nitrogen disappearance data to predict ruminal degradation of protein. Klopfenstein et al. (1982) concluded that an accurate evaluation of protein degradability and its net effect on protein synthesis and animal growth or production must be made before various systems (Ørskov and McDonald, 1979; Broderick, 1982; Stern and Satter, 1982; Zinn and Owens, 1983) for meeting protein requirements can be used. Zinn and Owens (1983) suggested that a solubility measurement combined with in situ data, when adjusted by comparison with a standard protein source, such as soybean meal, correlated well with in vivo bypass values. Based on these findings, the objective of this study was to determine nitrogen solubilities and in situ disappearance values for a wide array of feedstuffs and to use these values to develop prediction equations for protein bypass.

Factors Affecting Ruminal Protein Degradation

The main variable affecting the nutritive value of dietary protein for ruminant animals is their degradation in the rumen according to Miller (1982). Degradation of dietary protein in turn is affected by several dietary and animal factors (Tamminga, 1979). These will be considered separately below.

Dietary factors. Several chemical and physical characteristics of the protein dictate its susceptibility to microbial attack. Soluble protein supposedly can be rapidly degraded by ruminal microbes, resulting in production of ammonia, volatile fatty acids, carbon dioxide and other metabolites (Church, 1976). Insoluble protein is less subject to microbial metabolism than soluble protein (Van Soest, 1982). Consequently, insoluble protein is more likely to escape ruminal fermentation (Pichard and Van Soest, 1977; Owens, 1978). Wohlt et al. (1973) observed that protein solubility is influenced by several factor inherent to a protein. Highly soluble feed proteins contain substantial quantities of albumins and globulins while lower solubilities reflect larger amounts of the prolamine and glutelin fractions. Based on this concept, several methods to estimate protein solubility in feedstuffs have been proposed. Waldo and Goering (1979) determined solubility in four different solvents. Due to the high amount of variability, they suggested that any single solvent has limited usefulness in prediction of ruminal protein degradation. That protein solubility alone can be used to predict bypass has been refuted by the poor correlation between solubilities and in vivo bypass (Satter et al., 1977). This is not surprising since the percentage of protein which is soluble usually is very much

lower than the percentage of protein which is degraded in the rumen.

Pichard and Van Soest (1977) proposed to divide feed protein into at least four categories differing in rate of degradation. These were:

1) Fraction A; water-soluble non protein nitrogen (NPN); includes nitrates, ammonia, amines and free amino acids.

2) Fraction B1; water-soluble, rapidly degraded protein.

3) Fraction B2; more slowly degraded protein.

4) Fraction C; unavailable protein.

Chemically defining these entities and their rates of degradation and passage has proven to be complex although further subdivisions of the B2 fraction have now been proposed.

Feed processing and storage. High temperatures during processing or storage of feeds or forages will decrease ruminal degradation of protein (Ørskov, 1982; Thomas et al., 1982). As heat is applied, albumins and globulins disappear, presumably by binding with cell wall constituents. Hence, protein solubility is reduced (Wohlt et al., 1973). Heat can be used to decrease in situ protein disappearance of soybean (Kung et al., 1983) and rapeseed meal (Lindberg et al., 1982). With in vitro techniques, Broderick and Craig (1980) suggested that heat treatment decreases protein degradation by reducing solubility and by blocking reaction sites for microbial proteolytic enzymes.

Formaldehyde treatment also has been used to protect proteins from degradation in the rumen (Ørskov, 1982). Kaufmann and Lupping (1982) indicated that the effectiveness of treatment depends on the

amount of formaldehyde used and the form of application. Protein content and structure, carbohydrate content and particle size also play roles in success of formaldehyde treatment. In vivo studies have shown that formaldehyde treatment can decrease ruminal degradation and increase the flow of dietary protein to the small intestine (Faichney and White, 1977). But overtreatment can reduce digestibility in the small intestine, as well. So the optimal level is an elusive target. Tamminga (1979) observed that intake of silage treated with formaldehyde at ensiling was greater than intake of untreated silage. Whether this is due to an increased supply of amino acids at the small intestine or reduced levels of amines and other intake depressing substances in the silage has not been determined.

Level of feed intake. High producing ruminant animals with high feed intakes tend to have larger percentages of dietary protein escaping ruminal fermentation (Satter, 1983). Zinn and Owens (1980) observed that bypass of protein increased by 6.5% for every 10% increase in feed intake above the maintenance level. Tamminga (1979) fed dairy cows at two feed intake levels (8.2 and 12.9 kg of dry matter per day) and found that protein escape from the rumen increased from 29 to 45% of the protein fed. This equals a 55% increase with a 57% increase in feed intake. Increasing feed intake increased flow of both feed and microbial nitrogen to the small intestine as well as efficiency of microbial growth in steers (Zinn and Owens, 1983). They also suggested that with lower feed intake, the need for protein to be degraded in the rumen is higher; hence, extent of ruminal dry matter fermentation is enhanced.

Rate of digesta passage. Under practical feeding conditions, extent of ruminal proteolysis can be considered a function of rate of proteolysis and retention time (Tamminga, 1977). Ruminal retention time is affected by feed structure and level of feeding. Ruminal outflow rate of protein supplements was 1% per hour for sheep fed ground diets at maintenance intakes and also was 10% per hour for dairy cows fed mixed diets at high intake levels (Ørskov, 1982). Satter (1983) observed that intakes in excess of 2% of body weight caused decreased ruminal retention times. Increased ruminal turnover appears to enhance bacterial protein production and to increase ruminal acetate concentration and methane production (Owens and Isaacson, 1977).

Determining the outflow rate of feed protein is difficult due to problems in distinguishing between dietary and microbial protein (Ørskov, 1982). Ganev et al. (1979) used sodium dichromate as a marker for protein supplements. They observed that particle size of the diet significantly influenced ruminal outflow rates. Outflow rate of smaller particles was faster when the diet consisted of large than of small particles. Grinding can alter specific gravity which may influence retention time in the rumen and exit rate from the rumen. Time for ruminal degradation may have a greater effect on bypass of more readily degraded protein sources than on more resistant ones (Owens and Bergen, 1983).

In Situ Disappearance and Protein Solubility.

Solubility has been used for many years as an index of ruminal protein degradation. This is based partly on classical research

work with purified protein sources such as casein and zein. Stern and Satter (1982) correlated in situ disappearance of nitrogen with nitrogen solubility. They found that solubility was highly correlated with in situ nitrogen disappearance during the first hours of incubation, but as ruminal incubation time increased, correlations decreased. This corroborates results of Crawford et al. (1978) and Zinn et al.(1981) who found that nitrogen disappearance from dacron bags during the first 2 to 4 h of ruminal incubation was closely related to solubility. Owens (1978) observed that in situ protein disappearance curves possessed two slopes. He suggested that the steeper slope seen during the first period of incubation represents loss of soluble protein from the dacron bag while the subsequent slower slope reflects solubilization and digestion leading to disappearance of the insoluble protein. Ørskov (1982) reported that for some feedstuffs (fish meal, silage) solubility was an adequate indicator of protein degradation. In contrast, Owens and Bergen (1983) concluded that solubilities should be combined with in situ disappearance measurement and adjusted by comparison with a standard protein source to be correlated with in vivo bypass.

The amount of protein soluble in an aqueous solvent can be changed by pH. This has led to the suggestion that the extent of proteolysis in the rumen is modified by diets which alter ruminal pH simply because pH influences protein solubility. Diets which alter pH can have additional effects on the microbial population in the rumen which may or may not be important in controlling the extent of proteolysis. Ruminal pH is normally between 5.5 and 7.0. Isaacs

and Owens (1972) found that pH altered the amount of protein soluble in ruminal fluid, but that feedstuffs differed in their isoelectric point. With most protein supplements, protein solubility decreased as pH decreased, but with protein from corn grain, solubility increased as pH decreased. Wohlt et al. (1973) reported that differences in solubilities between pH 6.5 and 7.5 were small, but Loerch et al. (1983) found that decreasing solvent pH from 7 to 5 decreased solubility of nitrogen from soybean meal and casein. As pH decreased from 7.5 to 5.5, mean solubility of dietary nitrogen decreased from 57 to 27%. In vitro results indicate that proteolytic activity of ruminal organisms are altered by pH (Erfle et al., 1981), but results with animals are conflicting (Satter, 1983).

Factors Influencing In Situ Measurements

Several factors need to be controlled for in situ experiments to yield meaningful data. These include control of certain animal, dietary and experimental factor, the most important of which appear to be ammonia concentration, time, pore size, substrate particle size, surface area of the bag and bag washing procedure. These factors will be discussed individually though they may interact in situ.

Animal factors. In situ dry matter disappearance differed among with steers fed a sorghum grain diet in a trial by Figroid et al. (1972). However, when steers were fed a barley diet, animal effects accounted for only 1 to 1.7% of the total variation. In contrast, Mehrez and Ørskov (1977) reported that animal differences

were the major source of variation for in situ disappearance of barley when sheep had ad libitum access to dried grass. Weakley et al.(1983) detected no significant animal effects for in situ protein disappearance in cows fed a high concentrate diet though differences approached significance after 12 h of incubation. They suggested that animal effects should be minor when substrates similar to soybean meal are used. Ruminal pH, viscosity and mixing would be more variable with younger animals, with higher concentrate diets and ad libitum access to feed. Consequently, with such animals and diets, it may be useful to use crossover or latin square designs to adjust for animal differences in the experimental model.

Diet factors. In situ nitrogen disappearance rate from plant proteins varies with level of concentrate in the diet. Figroid et al. (1972) fed steers alfalfa hay, sorghum grain or barley and measured in situ disappearance of barley or sorghum grain. Dry matter disappearance in situ was greatest when steers received an alfalfa hay diet. Playne et al. (1978) suggested that the basal diet should include some hay to maximize the rate of in situ disappearance. Ganev et al. (1979) reported that rates of in situ protein disappearance for soybean meal, ground nut meal and sunflower meal were lower when a higher level of concentrate was included in the diet for sheep. Disappearance of brome grass from nylon bags also was depressed as concentrate in the diet of sheep was increased (Franklin et al., 1982). Weakley (1983) suspended dacron bags containing meat or soybean meal in the rumens of steers fed high concentrate or high roughage diets. Disappearance of soybean meal was more rapid with the high roughage diet.

Two explanations for the effect of concentrate level on in situ digestion of protein have been offered. First, protein solubility may change with pH and this may alter rate of in situ degradation of protein. Secondly, microbial attack of protein may be limited by fiber barriers, and the higher rate of attack of fiber barriers would increase the exposure of protein and its disappearance rate. In support of this concept, dietary roughage level had little effect on in situ disappearance of meat meal, a protein source which contains very little cell wall material. Weakley (1983) also observed only small differences in disappearance of meat meal when animals received diets containing different concentrate levels. They suggested that vegetable protein sources are more greatly affected by dietary roughage level than animal protein sources due to fiber content.

Disappearance of protein sources with limited degradation in the rumen such as fish or meat meal, may not be greatly affected by many of the factors known to influence in situ results. Effects of dietary roughage level on ruminal pH, which may alter proteolytic enzyme activity, also deserves consideration. Associative effects of protein and starch, similar to those proposed for protein and fiber, are possible and might be affected by ruminal pH or concentrate level. Loerch et al. (1983) observed that numbers of cellulolytic bacteria decreased when animals were fed high grain diets. They suggested that lowering ruminal pH also decreases the numbers of ruminal proteolytic bacterial species. Other explanations for greater substrate disappearance with roughage diets are possible. Rubbing action between the bag surface and the

fibrous, tumbling mat formed in the rumen of animals fed high roughage diets could act to clear foreign material from the pores of dacron bags and increase both inoculation and removal of the substrate. Removal of influx protein should be of limited importance in this comparison since bags are usually scrubbed free of washable residues following incubation.

Ammonia concentration. Mehrez et al. (1977) evaluated the effects of ruminal ammonia concentrations on dry matter disappearance of barley contained in polyester bags. Sheep were continuously fed whole barley diets with different urea levels. Higher urea levels elevated ammonia concentrations and increased the rate of in situ dry matter disappearance. Ruminal ammonia concentrations of 200 to 270 mg/l were necessary to maximize the rate of disappearance. Wallace (1979) fed sheep whole barley with or without 3% urea added. Rate of dry matter disappearance of rolled barley from dacron bags was increased by 90% with the higher ammonia level. The authors suggested that elevated ruminal ammonia concentration increased microbial mass and hydrolytic activity.

In contrast to the above findings, other reports show little effect of ammonia concentration. Ortega et al. (1979) fed 10 and 13% protein diets and intraruminally infused urea creating an intake level equivalent to 16% protein diets. Dry matter disappearance of soybean meal, alfalfa hay, oat straw and corn did not differ with ruminal ammonia concentrations which ranged from 63 to 275 mg/l. Forster et al. (1983) also observed no effect of ruminal ammonia level on in situ protein disappearance rates in cows fed diets similar in all aspects except ruminal ammonia concentration.

Nikolic and Filipovic (1981) also detected no effect of low ammonia concentrations on in vitro protein degradation of corn protein. In conclusion, it appears that with certain diets, an ammonia deficiency may reduce rate of dry matter and protein disappearance in the rumen and from bags incubated in the rumen. Whether this effect is due to ammonia directly or to pH or other factors remains to be determined.

Time effects. Besides the effect of time of incubation on disappearance from dacron bags, time after feeding and day to day variation in ruminal digestion may alter in situ results. The effect of time after feeding on in situ disappearance was examined by Weakley and Owens (1982). They concluded that this source of variation may be removed by feeding test animals at frequent intervals. Day to day variation may reflect feeding, drinking or rumination patterns or variation in handling the dacron bags before placing them in the rumen or after removal from the rumen. Rate of dry matter disappearance or sorghum grain in situ was measured in steers fed ground alfalfa hay, 68% flaked sorghum and 75% flaked barley diets by Figroid et al. (1972). Dry matter disappearance differed between days, but this variation was small compared to the treatment differences due to diet. In contrast, Mehrez and Ørskov (1977) reported that day of incubation was the second greatest source of variation in situ. These workers recommended introducing all bags at once and withdrawing bags at various intervals. In contrast, Weakley et al. (1983) preferred to add bags at intervals and remove them for washing simultaneously. They found no period effect at any incubation interval and suggested that day or time is

of little concern. Owens (1982) proposed that in situ results could be adjusted for day and animal effects by incubating a standard feedstuff for comparison. This would be similar to adjusting in vitro values with results from a standard forage. Soybean meal was selected as a standard reference protein because of its consistency, availability and relatively constant rate of degradation.

Pore size. Several workers have stressed the importance of size of pores in the polyester bags used for in situ studies since influx and efflux of materials will bias results. Microbial matter, being high in protein, is of special concern when protein disappearance is measured in situ. Fine particles of feeds placed in the bag, also, can sift or be washed through coarse cloth and be lost. To avoid influx and efflux, some workers have employed bags with very small pores. Small pores prevent some of the rapid loss of material from the bags during the first hours of incubation in the rumen. However, other procedures can be used to adjust for efflux such as running a negative but washed control, ignoring the initial disappearance, or sieving feed prior to placing it in the bag.

Large pore sizes render the bag more prone to influx of ruminal microbes and ruminal particulates. Conversely, a very small pore size inhibits entry of liquids and microbes into the bag and exit of solubilized material from the bag (Playne et al., 1978; Linderberg and Varvikko, 1982). Van Hellen and Ellis (1977) examined several bag materials with various (10, 75, 100 and 100 by 135 microns) pore sizes. The largest pore size allowed dietary neutral detergent fiber to enter the bag. Material with a pore size of about 10

microns prevented loss of undigested substrate and gain of dietary particulates. Forster et al. (1983) also observed little influx with a 10 micron pore size. Playne et al. (1978) studied particulate matter losses through bags in steers fed low quality hay. Bags containing forage samples were enclosed in perforated jars or fixed to steel ring weights and placed in the rumen. Bags fixed to steel rings moved freely in the rumen. Bags enclosed in perforated jars had lower rates of dry matter disappearance as compared to the weighted bags (27.6 vs 39.4%, respectively). Playne et al. (1978) summarized that dry matter loss through bag cloth is a function of sample grind size, bag pore size, preparation prior to milling, and milling technique. Their observations suggested that such losses can lead to substantial errors in determination of rates of dry matter disappearance.

Khatabb and Tamminga (1982) ruminally incubated ground hay in dacron and nylon bags with various pore sizes containing ground hay. No differences in dry matter disappearance between dacron and nylon or between pore sizes of 37 to 57 microns were observed, but with pore sizes under 37 microns, dry matter disappearance was reduced. Among nylon bags of 10, 20 and 36 microns, dry matter disappearances for soybean meal, rapeseed meal, barley, hay, silage and oat straw were greatest with the 36 micron pore size.

In summary, some of these concerns about pore size reflect inherent problems with the in situ procedure while others are due to improper handling of the bags. If bags are not thoroughly washed following incubation, particulates and minerals which enter the bag

during incubation will remain in the bags and be considered to be undigested feed residues.

Substrate particle size. In situ substrate disappearance is affected by substrate particle size and length of time of exposure in the rumen. In the first minutes of incubation, fine materials can rinse through the bag pores. Often, this loss has been erroneously defined as digestion. In reality, all in situ and in vitro results should be described as "disappearance", not digestion.

Figroid et al. (1972) incubated sorghum grain of various particle sizes (.4 to .6 mm, .6 to .8 mm, and .8 to 2.0 mm) in situ. Dry matter disappearance increased with decreasing particle size. Van Keuren and Heinemann (1962) observed that as time of incubation increased, the relationship of particle size to disappearance rate dissipated. Playne et al. (1978) reported that such losses were higher with samples milled through a 1 mm than through a 2 mm screen. Khattab and Tamminga (1982) found that dry matter disappearance was greater for hay with a 1 mm than a .5 mm particle size, and Ehle et al. (1982) found that substrates with a 150 micron size were degraded faster than substrates of 300 micron size. Weakley et al. (1983) used bags of dacron (mean pore size 52 microns) and rip stop nylon (no discernible pore size). Commercial soybean meal and dry distillers grain were either ground through a 2 mm screen or pulverized in a ball mill which produced a particle size between 50 and 150 microns. Dry matter and nitrogen disappearance were greater for the pulverized feedstuffs, and greatest differences occurred during the first hours of incubation. However, considering the large difference in particle size,

differences were less than expected. Decreasing particle size also increase the ratio of substrate surface area to substrate weight exposing more surface for microbial attack (Weakley et al., 1983). If surface area limits digestion rate, a smaller particle size should be digested at a more rapid rate. Galyean et al. (1977) found that dry matter degradation of dry rolled corn roughly doubled as particle size was halved from 1.5 mm (9.74%) to 0.75 mm (18.38%). Reduction of substrate particle size within dacron bags may be attributed to action of ruminal microbes, solubilization and, possibly, physical effects of rumen contents (Ehle et al., 1982).

Ratio of sample size to bag surface area. Reducing the substrate sample size to bag surface area ratio increased disappearance of substrate in a study by Weakley et al. (1983). Figroid et al. (1972) incubated 6, 10, 14 and 18 g samples of sorghum and barley in bags 5.1, 7.6 or 10.2 cm wide for 3 or 6 hours in the rumen or in an agitating 39 C water bath. Dry matter disappearance increased as width of sample bags increased. The 10.2 cm bag gave most repeatable results and was most convenient to fill, wash and empty. Uden et al. (1974) tested sample size to surface area ratios from 6.5 to 50 mg/cm^2. Cell wall digestibility of Guinea grass decreased from 54 to 38% as the ratio increased but bag sizes were not listed. Van Hellen and Ellis (1977) recommended a maximum of 10 mg/cm^2. Effects of sample weights (3, 6, and 9 g) in bags with a constant weight to surface area ratio of 41.6 mg/cm^2 were measured by Playne et al. (1978). A bag size of 17 by 19 cm was recommended by Mehrez and Ørskov as adequate for 5 g dry matter samples. This combination produced 15.5 mg/cm^2. Weakley

(1982) observed that increasing the sample size in relation to bag surface area enhances the probability for excluding digesting agents from the substrate mass. Particle size may interact with both sample weight and the weight to surface area ratio. If the substrate becomes isolated from inoculation or from rinsing with ruminal fluid, a micro-niche may be established which could alter digestion rate. If large, finely ground samples become lodged in the bottom or one corner of the bag, fermentation may not be similar to that occurring in the free mass of the rumen.

Bag washing. At the end of the fermentation period, bags are removed from the rumen and washed, typically under tap water until the rinsing fluid becomes colorless (Mehrez and Ørskov, 1977). The variability associated with the washing procedure was examined by these workers. After incubating in the rumen of sheep for 24 h, bags were removed and either washed or rumen contents attached to the bag surface were simply removed. Washing only removed an additional 50 mg of material for a difference in dry matter disappearance of only 1.2%. Figroid et al. (1972) allowed nylon bags removed from the rumen to drain or washed them for 8 h in a shaking water bath at 39 C. Dry matter disappearance was four times greater for washed than drained bags. Crawford et al. (1978) also observed that washing of bags after removal from the rumen was essential to remove particulate matter as well as soluble material remaining in the bag. Abnormal physical manipulation during washing, however, can affect disappearance. Variation among individuals in the extent of loss during washing has been reported.

As influx and efflux of material from in situ bags would differ with 1) prevalence of particulate material in the rumen of a size which could enter the bag and 2) pore size of the bags, the inconsistency in results is not surprising. The need for thorough washing would probably be greatest with medium or large pore size bags and higher concentrate diets.

Predicting Ruminal Protein Degradation from In Situ Data

Several methods to predict nitrogen disappearance from in situ data have been proposed (Ørskov and McDonald, 1979; Mathers and Miller, 1981; Zinn et al., 1981; McDonald, 1981; Stern and Satter, 1982; Waldo, 1983). However, mathematical interpretation of in situ disappearance data has proven to be complex. Most models have combined in situ disappearance with ruminal passage rate to calculate bypass of dietary protein. A brief description of these systems is presented. For clarity and consistency, some letter designations have been altered and capital letter are used for pools and small letters for rates of passage or digestion.

According to the model of Ørskov and McDonald (1979), potential bypass or escape (E) of ultimately degradable protein (B) is measured by in situ nitrogen disappearance over time (t). The effective percentage of the supplement degraded at any time after consumption allowing for rate of passage from the fractional outflow or passage rate of protein (p) is calculated as follows:

$$E = A + [Bd/(d+p)][1-e^{-(d+p)t}]$$

where the constant "A" is the rapidly solubilized protein fraction which is also the intercept of the in situ disappearance curve and "d" is rate of in situ digestion. As time after feeding increases, E increases asymptotically so that total bypass at time equals infinity is:

$$E = A + B\ [d/(d+p)].$$

They suggested that a 24 h incubation time is adequate for most feedstuffs.

McDonald (1981) proposed a modification of the previous model when he noted that the equation for "E" is inadequate with short incubation times. These occur when there are low quantities of soluble protein and when a lag time precedes digestion. Therefore a lag time was introduced and the constraint that "A" plus "B" must equal 100% was relaxed.

The model of Mathers and Miller (1981) calculates protein degradation (D) using "d" to represent rate of in situ nitrogen disappearance and "p" to represent rate of passage from the rumen. Their model is:

$$D = A + (1-A)[d/(p+d)]$$

with other factors described as above. A constant, .046, was employed for "p" as estimated by Ganev et al. (1979), however, a single passage rate constant may be inadequate (Satter, 1983).

Stern and Satter (1982) described a model combining ruminal passage rate and in situ nitrogen disappearance using the equation of Mathers and Miller (1981). However, a single rate constant for describing the rate of passage was not used. Hence, predicted ruminal protein degradation was allowed to vary dependent upon

passage rate. Waldo (1983) fitted in vitro and in situ fermentations to a general model with three pools. Fraction "A" is nonprotein nitrogen or protein which is rapidly degraded, while fraction "B" is that protein which is degraded at a rate similar to the rate of passage (.02 to .071 per h). Lastly, "C" is bound or unavailable protein. Hence degradation in the rumen is described as;

$$D = A + B\ [r/(r+p)].$$

In a similar fashion, the total protein passed to the omasum is:

$$E = C + B\ [p/(r+p)].$$

Degradation and passage rates in the equation relate to the "B" fraction only. Fractional rates of degradation also could be applied to fraction A, but degradation of A was considered to be almost complete.

Zinn and Owens (1983) presented a modification of the Zinn et al. (1981) proposal based on bypass and in situ degradation rates of protein sources relative to a standard reference protein, soybean meal. Solubility in .15 N NaCl was considered to estimate fraction "A" described above. In situ disappearance the first 4 h of incubation was ignored as this should represent in situ loss of soluble protein which has already been considered and of finely ground substrate which should not be considered. Only subsequent rates of in situ loss are considered. The earlier of these rates, R1, represents the rate of protein loss in situ from 4 to 12 h while the later rate, R2, is the rate of in situ loss from 12 to 24 h. In situ degradation curves were generated for each protein source as

well as soybean meal. Time for ruminal digestion was then calculated by solving for incubation time of soybean meal at which calculated bypass equalled measured bypass. This time is used for other protein sources. For example, if time for digestion with a high concentrate diet is calculated to be 11.3 h, then the protein would be digested at rate R1 for 8 h and at rate R2 for 3.3 h (11.3 - 8h) and bypass (E) would be calculated as follows:

$$E = (1 - A)(1-8R1)(1-3.3R2).$$

Rates R1 and R2 will vary with dietary and ruminal conditions. Bypass of a number of protein sources was well predicted using this procedure. This is the only procedure that has been tested and verified using in vivo measurements.

CHAPTER III

MATERIALS AND METHODS

Dacron cloth (100% dacron Polyester, Poly-Air, N. Erlanger, Blumgart & Co., Inc. 1450 Broadway, New York, NY 10018) with a mean pore size between 50 and 75 microns was used for bag construction due to its availability, low cost and success in previous studies (Weakley et al., 1983). Single pieces of cloth 12 by 16 cm in size were folded and sewn along two of the open edges. The top was left open for insertion of the sample. Seams were double stitched with polyester thread and waterproof glue (Duco cement, Du Pont Co., Wilmington, DE 19898) was applied over the sewing thread to prevent loss of particles through the needle holes. Edges were singed with a hot knife to prevent fraying of threads. The constructed bags, 8 by 12 cm, had a surface area of about 192 cm^2. Approximately 1 g of substrate (not dried) was introduced into each bag yielding a sample weight to surface area ratio of 5.2 mg of air dry substrate per cm^2 bag surface. Bags were numbered with a water insoluble felt marker for identification. Prior to filling, bags were dried for 24 h at 100 C, cooled in a dessicator and weighed. After addition of samples, bags were tied with silk thread. In all experiments, bags of each substrate were tied at 1 cm intervals along a 60 cm nylon cord which was weighted with a 30 g steel nut.

In experiment 1, duplicate bags with each substrate were incubated in the rumen for 4, 12 and 24 h and in experiment 2, incubation times were 4, 12, 24, 48 and 72 h. Individual cords were used for each incubation period. Bags were introduced at different times so that all bags could be retrieved and washed at the same time. The number of bags incubated in the rumen of a steer at any time never exceeded 50 so as to avoid difficulties in removal and reduce interference with normal ruminal digestion.

After removal from the rumen, bags were washed under a gently flowing stream of lukewarm water until the rinse fluid was clear. About 120 seconds were required to wash each bag, though with some substrates incubated for longer times, 180 seconds or more were needed. Bags were drained, dried for 24 h at 100 C, cooled in a dessicator and weighed.

Animals were fed four times each day prior to and during sampling periods to stabilize ruminal conditions. Rumen fluid samples of about 400 ml were collected at each incubation time. The pH was measured immediately with a combination electrode after which samples were strained through 4 layers of cheesecloth and frozen. Later, samples were thawed at room temperature and ammonia-nitrogen content was determined by distillation (AOAC, 1975).

Experiment 1

Dry matter and protein disappearance were determined in situ with 21 substrates and a total of 18 feedstuffs. These included two samples each of cottonseed meal, soybean meal and meat meal and single samples of corn gluten meal, sunflower meal, linseed meal,

feather meal, fish meal, ground milo, ground wheat, ground corn, high moisture corn (25% moisture), cracked corn, rolled oats, prairie hay, alfalfa hay, corn silage and sorghum silage (table I). Protein supplements were incubated in the commercial form while other samples were ground through a 2 mm screen.

Two Hereford-Brahman steers (263 kg) fitted with permanent ruminal cannulas (10.2 cm i.d.) were housed in individual pens with free access to water were used in each crossover experiment with 18 day periods. Animals were fed at a level equivalent to 1.7% of body weight as dry matter per day. For the first 6 days of each period, steers were fed twice daily (0800 and 1700 h), and for the remainder of each period, steers were fed at 0500, 1100, 1700 and 2300 h. Diets were 60% roughage or 80% concentrate (table II) formulated to be somewhat similar to a dairy or to a feedlot diet. Diets were made isonitrogenous at 12% crude protein by addition of urea.

Four-1 day incubation periods with one day intervals were used to minimize effects of disturbance on ruminal digestion. Duplicate substrates were incubated on day 4 for 4 and 12 h to determine the degree of contamination of dacron bag residues with microbial nitrogen. Residues were removed and analyzed for purine content (Zinn and Owens, 1982). Bags containing hair curlers were incubated in the rumen for 24 h during the first three incubation periods to estimate the amount of influx of dry matter and nitrogen from the rumen. During each incubation period, duplicate bags containing soybean meal were incubated for 4, 12 and 24 h to serve as an internal standard to adjust for animal or period effects if necessary.

Experiment 2

Two Hereford heifers (219 kg) fitted with permanent ruminal cannulas (10.2 cm i.d.) were used in a crossover experiment with 15 day periods. Housing, feed intakes and feeding intervals were as described for experiment 1 except that diets consisted of 80% prairie hay or an 80% concentrate (table III) made isonitrogenous (13% crude protein) by addition of soybean meal.

Substrates, special preparations and incubation times are presented in table IV. Extractions with McDougall's buffer (McDougall, 1948) adjusted to pH 5.0 or 7.0 was for 6 h at 39 C in a shaking water bath. Another set of substrates were rinsed with the buffer at pH 6. After extraction, insoluble residues were retained by a dacron cloth sieve, dried at 60 C for 10 h and the dry samples filtered through a 2 mm screen prior to placing into bags.

Disappearance of dry matter (DMD) and nitrogen (ND) were calculated as follows:

$$DMD = [A - (B - C)]/A;$$

$$ND = [E - (6.25F)]/E;$$

where A = initial substrate dry weight, B = bag plus residue dry weight, C = dry bag weight, E = initial substrate protein content and F = bag plus residue nitrogen content.

Nitrogen was determined by macro-kjeldahl procedure on bag plus contents to avoid problems with removal and sampling of bag residues. Nitrogen content of the dacron bags was determined to be less than .5%.

Statistical comparisons in both trials used the Analysis of Variance procedure followed by comparison of means using the Duncan's Multiple Range components of the Statistical Analysis System (SAS, 1979). For the crossover experiment, periods were ignored and animal, feedstuff and time differences were used in the model.

CHAPTER IV

RESULTS AND DISCUSSION

Experiment 1

Since ruminal conditions may influence digestion rate, ruminal pH and ammonia concentration were measured and results are presented in table V. Ruminal pH averaged for the three sampling times was higher with the 60% roughage diet (R) than with the 80% concentrate diet (C) while ruminal ammonia was slightly lower. Differences in pH due to diet were less than expected (6.58 vs 6.34). Animals were fed at 1.7% (feed dry matter) of body weight daily divided into four meals per day during in situ measurements. This moderate level of feed intake may have prevented a large drop in ruminal pH and frequent feeding would have decreased post-prandial fluctuation in pH. Frequent feeding also may have stabilized the ruminal ammonia concentration. Frequently fed animals may possess microbial populations more adept to capture of ruminal ammonia and maintain a lower ruminal ammonia concentration.

Ruminal pH has been shown to influence fiber digestion and protein solubility (Wohlt et al., 1973; Isaacs and Owens, 1977). However, Wohlt et al. (1973) detected little difference in protein solubility between pH 6.5 and 7.5. In contrast, Loerch et al. (1983) observed that as pH increased from 5 to 7, solubilities of soybean meal (SBM) and casein protein increased by 3.6 and 23.3

fold, respectively. However, N solubilities of blood, meat and bone, pelleted dehydrated alfalfa and corn gluten meals were not affected by solvent pH. In addition, they reported a faster rate of in situ ND of SBM with a higher roughage diet. They suggested that greater solubility of protein at the higher ruminal pH probably was responsible for the greater degradation of SBM. However, rate of proteolysis also could be altered by a change in the microbial population in the rumen. Numbers of cellulolytic bacteria generally decrease when animals are fed high grain diets, and have a low ruminal pH.

Many workers have observed higher in situ rates of DMD and ND with R than with C diets (Figroid et al., 1972; Mehrez and Ørskov, 1977; Playne et al., 1978; Ganev et al., 1979; Franklin et al., 1980; Loerch et al., 1983; Weakley et al., 1983; Zinn and Owens, 1983). Some authors have suggested that microbial degradation of vegetable protein is limited by fiber barriers. When animals are fed roughage, rate of fiber digestion in the rumen increases. This, in turn, exposes more protein for degradation. In support of this hypothesis, roughage level had no effect on ND of fish meal or meat meal, both of which contain no cellulose (Ganev et al., 1979; Weakley, 1983). The high correlation between rates of protein and of dry matter disappearance from dacron bags also supports this theory. Owens and Zinn (1982) postulated that variable rates of fiber digestion may be responsible for the associative effects of protein with fiber and different rates of fiber digestion.

Ammonia-nitrogen concentrations in these studies were all above 5 mg/dl, the level suggested as necessary for maximum production of

microbial protein (Satter and Slyter, 1974). Whether this level is adequate to maximize in situ DMD is not certain. Mehrez et al. (1977) found 23.5 mg/dl maximized the extent of in situ fermentation, while Ortega et al. (1979) observed that in situ DMD of SBM, alfalfa hay, oat straw or corn grain was not changed when ruminal ammonia-nitrogen concentration was increased from 6.4 and 27.5 mg/dl. What factors are responsible for this difference in the amount of ammonia required to maximize digestion rate remain to be determined.

Two animals were used in this experiment in a crossover design. In situ DMD and ND after 4 h were affected by diet of the animals used for in situ digestion ($P<.05$; table VI) however ND at 24 h was not affected by diet. Averaged across feedstuffs tested, DMD was higher at 12 and 24 h for steers fed the R diet, ND at 12h also was greater for steers fed the R diet.

Nucleic acid composition of residues remaining in the dacron bags following in situ incubation also are presented in table VI. These would represent residual nucleic acids from the feedstuff, which should be minor, plus nucleic acids from microbial RNA and DNA. Thus, the amount of nucleic acid would be an index of the degree of microbial contamination of the feedstuff in the dacron bag.

As values were generally small as a percent of the residual N, this indicates that the degree of microbial contamination of the residues was minor and for the 18 feedstuffs averaged .75% of feed N at 4 and 12 h of incubation. Greater nucleic acid levels were found with some feed than others. Levels were highest for soybean meal

(1.87%), cracked corn (1.63%) and milo grain (1.57%), and lowest for corn silage, ground wheat, and meat meal (<.03%). The degree of washing of the bags following incubation would be expected to influence this percentage. A higher level of nucleic acid contamination was noted with the roughage than the concentrate diet. Other workers (Mathers and Aitchison, 1981) also reported that N contamination of residual fish meal from dacron bags was less than 2% while for alfalfa, the value was close to 20%. Without thorough washing, levels would be expected to be higher.

In situ results from the two animals differed slightly in DMD and ND but only at 24 hours. Weakley et al. (1983) found no effects of animal on in situ ND of soybean meal incubated in four cows receiving a high concentrate diet. However, Mehrez and Ørskov (1977) observed that animal effects were responsible for the greatest variation in in situ ND and DMD of barley with sheep ad libitum fed dried grass. Figroid et al. (1972) also observed animal effects with sorghum grain and barley diets. However, these effects accounted for only 1.7% of total variation with the sorghum grain ration.

Feedstuffs. Rate and extent of DMD and ND differed among time and substrates tested, and, for some feeds, by the diet fed to the steer (tables VII, VIII and IX). Feedstuff solubilities and digestion rates are discussed individually below.

Protein Supplements

Cottonseed meal (CSM). Two commercial cottonseed meal (CSM) samples were used in this experiment. DMD and ND of both sources

(table VII) were greater ($P<.05$ and $<.10$, respectively) 12 h with the R than with the C diet. Nitrogen disappearance at 12 h for the C diet (41.6) was similar to measures of CSM ND obtained with a typical dairy ration (44.4; Nocek et al., 1979), but DMD was much higher (24.4 vs 39.6) Predicted ruminal digested values of 51 and 67% for CSM with 80 and 40% concentrate diets, respectively (Zinn and Owens, 1983), are very similar to disappearance values for N at 24 h with the C diet in our study. However, ND at 24 h for R was lower than predicted with their 40% concentrate diet (57.0 vs 42.8).

Soybean meal (SBM). Nitrogen and dry matter disappearance of two commercial soybean meals (table VII) were greater ($P>.05$) with the R than with the C diet (55.16 vs 44.37). In situ substrate disappearance of plant proteins generally is higher with roughage than with concentrate diets (Weakley, 1983). Our estimates of in situ DMD and ND of SBM at 12 and 24 h of rumen incubation are similar to his values for dairy cows fed 75% concentrate diets. Ganev et al.(1979) reported greater degradation of plant proteins in sheep fed grass than sheep fed barley. Our 24 h ND for the C diet is similar to their results with barley, but measures with the R diet are slightly lower than they obtained with dried grass (89.0 vs 79.2). Zinn and Owens (1983) reported elevated in vivo ruminal protein bypass of SBM with steers fed 80 versus 40% concentrate diets (43.0 vs 24.0). Four h ND for R animals is similar to ND reported with an 80% roughage ration (34.1 vs 31.50) by Crawford et al. (1978).

Meat meal (MM). Protein from the two commercial MM sources used in this experiment disappeared slowly. Dry matter

disappearance and ND at 4 h tended ($P<.05$) to be higher for the R ration (60.50 vs 53.15). ND at 12 h are higher than reported by Zinn and Owens (1983) for steers fed similar diets, while DMD values were similar. Their in vivo protein bypass value for MM (76%) is higher than our 24 residual N measures (36.9%). DMD and ND at 12 and 24 h were similar to those reported by Weakley (1983b) with similar diets (C and R). Results suggest that rates of in situ ND for MM is less affected by dietary roughage content than for CSM and SBM.

Corn gluten meal (CGM). ND and DMD of CGM at 4, 12 and 24 h were similar for steers fed C and R diets except for DMD at 24 h which tended to be higher ($P<.05$) for steers fed the C diet. Nitrogen disappearance for CGM at 12 h for the C diet was similar to that reported (13.2 vs 19.0) by Nocek et al. (1979) with cows fed a high concentrate diet. Our dry matter disappearance at 12 h is slightly higher than their measurement (21.5 vs 12.48). However, all corn gluten products are not alike. Crawford et al. (1978) found considerably higher ND of corn gluten feed incubated for 4 h with an 80% roughage diet. This difference may have been expected due to higher protein solubility of corn gluten feed than CGM.

Sunflower meal (SFM). Both DMD at 12 h and ND at 4 h tended ($P<.05$) to be higher at 4 and 12 h of incubation for the R versus the C ration. However, the value for ND at 4 h for R steers is higher than results reported by Crawford et al. (1978) with a high roughage diet (58.0 vs 46.2). Ganev et al. (1979) found considerably higher ND for 24 h incubation of SFM in sheep receiving dried grass than barley diets (91.9 vs 79.9). With the C diet, our

DMD value was similar to their DMD with a barley ration, but DMD with their dried grass diets were higher than obtained with our R diet. Miller et al. (1982) fixed SFM values in Ørskov and McDonald's (1979) equations for prediction of ND and they estimated total nitrogen degradation of SFM protein to be slightly lower (65.5) than 24 h ND in our study (74.0).

Linseed meal (LSM). Dry matter disappearance of LSM at 12 and 24 h were generally higher ($P<.05$) for the R diet. However ND at 4 and 12 h was not affected by diet. At 4 h ND was greater ($P<.05$) with C than with the R diet.

Feather meal (FTM). Both DMD and ND were not affected by roughage level at any of the incubation times. In vitro studies by Daugherty and Church (1982) showed higher DMD of FTM than value observed in this work for 24 h DMD (37.8 vs 18.9).

Fish meal (FSM). ND at 4 h was greater ($P<.05$) for the C diet. In contrast, at 12 h, ND was higher ($P<.10$) with the R diet. Our DMD and ND values at 24 h for the R and the C diets were lower than those reported by Ganev et al. (1979) (58.1 vs 69.8) and similar to Mehrez et al. (1980) (28.7 vs 37.2) who incubated FSM processed in various ways in sheep fed a barley diet. Nocek et al. (1979) reported lower DMD and ND values (8.5 vs 15.1) than our values at 12 h for cows fed a concentrate-silage diet. Sheep fed whole barley diets displayed lower DMD and ND for FSM than detected with our C steers. Mehrez et al. (1978) suggested that ND could be predicted from DMD of FSM. Our results do not support this hypothesis, since ND exceeded DMD at all three incubation intervals in this study. In agreement with work of Ganev et al. (1979), DMD

and ND were similar for both diets suggesting that roughage level does not alter N disappearance of proteins of animal origin.

Energy Feedstuffs

In situ disappearance of dry matter and nitrogen for energy feedstuffs also were measured and are presented in table VIII. ND values for these feeds proved more variable than ND values for protein sources, probably due to the lower protein content and greater chance for analytical error. Since the majority of dietary protein is usually provided by such high energy feeds, bypass estimates for these feedstuffs are critical in formulation of diets.

Ground milo (GM). No differences in ND or DMD due to R or C diet were detected ($P<.05$). Figroid et al. (1972) observed a similar value for DMD of ground sorghum grain at 3 h of rumen incubation in a steer fed sorghum grain (23.6) as our 4 h DMD with C diet. Galyean et al. (1977) determined in situ DMD of ground sorghum grain with steers ad libitum fed a 63% dry rolled corn diet. Their 4 h DMD values were lower (15.5 vs 25.7) than our 4 h DMD value with the C diet.

Ground wheat (GW). Disappearance of both protein and dry matter were very rapid for GW. No effect of R or C feeding on ND and DMD of GW at 12 and 24 h was detected, but trends ($P<.05$) were present at 4 h. DMD tended to be greater in C steers. In situ DMD and ND values by Nocek et al. (1979) at 12 h for whole wheat with cows fed silage rations were (18.9 and 61.9) lower than our values for ground wheat at 12 h. Providing wheat in the whole form would have reduced exposure to microbial attack as compared with the

ground form. In situ ND results of Crawford et al. (1978) with a high roughage diet agree well with our R measurements (77.3 vs 68.6).

Ground corn (GC). No effect of C or R diet on DM and N disappearance at 4 and 12 h of incubation, though at 24 h of incubation, ND with the 40% C diet was drastically different ($P<.05$). This may be an artifact as no explanation is apparent. ND at 4 h for the R diet was considerably higher than reported by Crawford et al. (1978) with a high roughage diet (7.6). Dry matter and ND at 12 h with the C diet were similar to results of Nocek et al. (1979) of (36.48 and 44.52). The high ND values may reflect the high N solubility in salt solution of the sample of corn used in our work (40%) compared with more typical corn grain which has a nitrogen solubility of 10 to 15%. No explanation for such high N solubility is apparent. Possibly, corn harvested at a high moisture content and artificially dried would retain a high N solubility.

High moisture corn (HMC). In our study, N solubility of HMC was less than N solubility of GC. This is not typical as N solubility generally increases during the fermentation process (Prigge et al., 1976). No effect of R versus C diet on DMD at 4, 12 and 24 h were present, but ND at 24 h was greater ($P<.05$) with the C diet. Galyean et al. (1977) previously observed a similar 4 h DMD (25.0%) for HMC incubated in steers fed a high concentrate diet.Nitrogen disappearance at 24 h for both diets appear abnormal and may reflect interference or technique problems.

Cracked corn (CC). DMD for cracked corn was less than for ground corn. DMD at 12 h for the R diet was higher ($P<.05$) than for

the C diet. Diet composition did not affect DMD at 4 h. ND was not affected by diet. However, ND at 24 h was very low for the steers fed the R diet for no apparent reason. Possibly, slime found in the rumen of steers fed the C diet prevented thorough washing of the bags. DMD at 12 h was slightly lower than that reported by Galyean et al. (1977) for dry rolled corn. (22.0%). Their rolled corn may have had a smaller particle size than our cracked corn.

Rolled oats (RO). Diet did not affect (P>.05) DMD and ND at 4, 12 and 24 h of rumen exposure. ND of oats was very high, markedly exceeding DMD. At 12 h of ruminal incubation, ND was similar to that reported by Nocek et al. (1979) with dairy cows of 68.5%. However they reported higher DMD than we found (53.3 vs 40.6). ND at 4 h for rolled oats reported by Crawford et al. (1978) with animals fed a roughage diet (75.9) was similar to ND in our study.

Forage Feeds

Rate and extent of nitrogen and dry matter disappearance for forages also were estimated in this study and are presented in table IX. High N solubilities and low levels of N complicate the interpretation of results with these feedstuffs.

Prairie hay (PH). Dietary effects on DMD of PH were not detected at 4 and 24 h. DMD values were similar for steers fed for both diets. Greater ND at 4 h than 12 and 24 suggests that analytical problems occurred either during in situ digestion or during nitrogen determination of the bag-residue. The very low nitrogen content of the prairie hay magnifies changes in N content

of the bags. Influx of N associated with microbes which attach to the feed fibers could be responsible for some of this change.

Alfalfa hay (AH). No effect ($P<.05$) of diet on ND was detected, however, DMD at 4 and 12 h tended to be higher ($P<.05$) for the roughage diet. Values of ND at 4 h with a roughage diet, reported by Crawford et al. (1978) were slightly lower than our values (30.5 vs 45.8). Mathers and Aitchison (1981) obtained similar in situ ND values for alfalfa at 24 h with sheep consuming a high roughage diet. Nitrogen disappearance values for alfalfa hay reported by Miller et al. (1982) were similar to our values at 24 h for both diets (70.2 vs 75.4). The N solubility of alfalfa hay was higher than expected. Alfalfa products dehydrated with heat often have lower N solubility.

Corn silage (CS). Dietary effects were not detected for DMD. ND was very high for all incubation intervals with both diets. This could be explained for the low nitrogen content of CS and its very high N solubility. Again, analytical problems during the nitrogen determination of the residues and possible microbial attachment to feed fibers may be involved. Crawford et al. (1978) and Nocek et al. (1979) reported markedly lower ND values at 4 h of rumen exposure for CS with roughage and concentrate diets fed to cattle than observed in this experiment (29.9 and 64.3 vs 102.3).

Sorghum silage (SS). Dry matter disappearance was not affected by diet at 4, 12 and 24 h. ND was high at 4 and 12 h matching the high N solubility of the silage tested. At 24 h, analytical problems as well as possible microbial attachment to feed fibers complicated estimates of ND.

Experiment 2

Ruminal pH was suggested to be the primary cause of altered protein digestion in the rumen, presumably due to a changed solubility of protein (Loerch et al., 1983). These workers used in vitro and in situ techniques to predict ND of soybean meal (SB), blood meal (BM), casein (CN), meat and bone meal (MBM), dehydrated alfalfa (DA) and corn gluten meal (CGM). Substrates were incubated in vitro in four different solvents at pH 5, 6, and 7. The same materials were incubated in situ for 3, 6, 12 and 24 h in two Holstein cows receiving basal diets based on 3% NaOH treated corn or high moisture corn (HMC) at 20, 40, 60 or 80% of the total ration. SBM and DA protein solubilities were reduced in vitro at pH 5 and in situ ND was lower with the 80% HMC diet which produced a lower pH. They concluded that solubility of these feed proteins was a more important regulator of in situ ND than shifts in the microbial population.

Effects of pH on protein solubility are generally agreed upon. Solubility should have its greatest influence on in situ substrate disappearance in the first few hours of incubation. Possible effects of ruminal pH on rate of substrate disappearance after rapidly solubilized materials are gone are not well defined. Additional factors involved could include effects of protein solubilization on subsequent protein disappearance, and effects of pH on microbial degradation capacity.

The objectives of this experiment were to detect and quantitate effects of removal of soluble materials from soybean meal and meat

meal on in situ DMD and ND in steers fed a concentrate or a roughage diet.

Ruminal pH was higher ($p<.05$) with the R diet (table X), while ruminal NH_3-N concentration was markedly lower with the R diet. Ruminal pH has been observed previously to cause shifts in bacterial growth and species and has been recognized to affect protein solubility (Loerch et al., 1983). In agreement with results from the first experiment and other research (Ganev et al., 1979; Loerch et al., 1983; Mehrez and Ørskov., 1977; Weakley, 1983; Zinn and Owens., 1983), DMD and ND for SBM was higher ($P<.05$) with heifers fed the R diet than those fed the C diet. Possible explanations for this effect have been discussed earlier.

In situ DMD and ND averaged over feedstuffs (table XI) were greater with the heifers feed the R diet than steers fed the C diet at 4, 12, 24 and 48h ($P<.05$). Diet effects differences were no longer apparent at 72h.

DMD and ND of SBM and MM with their respective preincubation treatments are presented in table XII. With vegetable protein sources, diet effects were apparent. But DMD and ND of meat meal at 12 and 24 h were not affected by diet, in agreement with work by Weakley (1983). Rate of ND at all times was lower for rinsed than unrinsed SBM before 24 h, and after 12 h, rate of ND was almost always higher with the R than the C diet. This could have resulted from the rubbing action between bag surface and the fibrous mat in the rumen of animals fed the R diet. Such abrasion would aid inoculation and help to clear foreign material from the bag pores to aid in substrate removal.

Soybean meal and meat meal were rinsed with water or extracted at pH 5 or pH 7 to study effects of removal of the soluble proteins on in situ digestion. Rinsing SBM did not alter DMD or ND with steers fed the C diet, except that 4 h DMD was higher for the untreated SBM than rinsed SBM. Removal of soluble components can explain this difference. But rate of DMD from 4-48 h was higher for rinsed than unaltered SBM with the R diet. ND was not different between the two SBM types with the C diet, but unaltered SBM tended to have greater ND at 12, 24 and 48 h with both diets. For unaltered SBM, 4 h ND was greater for the R diet. This difference could be due to solubility differences in rumen fluid from animals fed roughage versus concentrate diets. Differences in 4 h ND within diet and between SBM type are interesting. Rinsing caused no change in ND at 4 h with the R diet, but rinsing increased ND at 4 h with the C diet. Perhaps soluble protein removal from the bags in animals fed concentrate stimulates subsequent degradation. However, since soluble protein should not be present in large quantities at 4 h, it is surprising that rate of ND was greater ($P<.05$) for unaltered than for rinsed SBM. This might suggest that removal of soluble protein may decrease subsequent ND.

Extraction of SBM at pH 5 and 7 did not significantly alter DMD or ND rates or extent. Higher rates of DMD and ND were observed for the R diet than for the C diet ($P<.05$) at all incubation times. Since this was observed despite extraction, results suggest that differences in ND rates between C and R diets cannot be ascribed simply to removal of soluble protein. Rates of ND from 4 to 12 h for the R diet were generally higher with the SBM that was extracted

at pH 5. Likewise values tended to be greater for the C diet with SBM extracted at pH 7. Differences at later times were smaller, possibly reflecting different quantities and (or) types of proteins being lost during preincubation treatment.

Meat meal was rinsed at pH 6 and extracted at pH 5 and 7. Effects of soluble protein on in situ digestion after 4 h were again detected. DMD and ND were not greatly altered by extraction of MM at pH 5 and 7. However in situ rates of DMD and ND from 4 to 12 h were altered for the C diet, being lower with extraction at pH 5. Rates of ND from 12 to 24 h with the C diet were generally greater for extracted than the rinsed MM. This may indicate that removal of soluble protein will enhance MM degradation with concentrate diets. Similar effects on SBM were noted at earlier hours of incubation.

Meat meal may possess a longer lag time of degradation than SBM. rates of ND with the R diet were similar, possibly due to greater solubilization capacity. Dry matter and ND were higher for the R diet than for the C diet ($P<.05$), in agreement with previous observations.

To determine whether presence of soluble protein altered DMD or ND in situ, rinsed SBM and MM were incubated together. Digestion rates were midway between rinsed MM and SBM suggesting that degradation of the two protein sources was occurring independently. Previously some workers (Waldo, 1983) had suggested that in situ results were not additive since digestion rates of mixed feeds differed from rate of each feed digested alone. Our results indicate that the non-additivity of in situ digestion rates previously reported may be due to interference with washing

procedures or factors other than having various types of protein present together.

Several methods for bypass estimation have been proposed and were discussed in the literature review. Our N disappearance values were applied in two different equations (table XIII) as reported by Zinn et al. (1981) and Miller and Mathers (1981). Digestion times in the Standard Reference system were 8 and 16 h, as might be expected with high and low intakes of feed. For the Miller equation, ruminal dilution rates of 6 and 3% per hour were again estimated for high and low levels of feed intake. Comparing in situ bypass estimates from the two different equations reveals general agreement for many of the feedstuffs tested. However, unrealistically high bypass values were obtained with roughages.

Workers have suggested to estimate solubility using various solvents to estimate the quantity of immediately degraded protein. Waldo and Goering (1979) compared several solvents and concluded that variability limited their usefulness in such estimation. The listing in table XIV presents literature values and our determined values for nitrogen solubilities. Despite a few discrepancies, general agreement among these various studies is evident. Variation within a feedstuff also may be large, as is illustrated by meat meal from our two sources. For comparison with solubility, loss of N from dacron bags was extrapolated to initial time to estimate immediate loss in situ. Initial losses almost always exceeded N solubility as would be expected. But with some feedstuffs, such as oats grain, wheat grain, high moisture and cracked corn and meat meals, initial loss drastically exceeded solubility. This is

presumably due to loss of fine particles of feed through the pores of the dacron bag. This loss illustrates one of the problems with the in situ procedure and suggests that some other index of the amount of N available for immediate digestion would be more appropriate than early N loss from the dacron bag.

In vivo N bypass values are presented together with amounts of residual N in dacron bags at 24 h from this study in table XV. This residue should represent a minimum of protein bypass and for many feedstuffs matches the in vivo estimate reasonably well. The major exceptions are meat meal which loses material through the bag very readily, and corn gluten meal with its gluten nature inhibiting passage through the dacron bag. Bypass estimates for energy and roughage feeds were quite variable but are very important for calculating the total protein bypass of a diet. More effort with these feedstuffs is required to obtain reasonable estimates of in vivo bypass.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two experiments were conducted to furnish in situ nitrogen disappearance data and detect effects of dietary roughage level and protein solubility on degradation of dry matter and nitrogen in the rumen of cattle. In experiment 1, 18 different feedstuffs were placed in dacron bags and suspended in the rumen of two ruminally cannulated steers fed diets containing either 20% or 60% roughage for 4, 12 and 24 hours. The influence of diet on loss of dry matter and nitrogen from the bags for each feedstuff was measured. In experiment 2, soybean meal and meat meal were incubated in situ without treatment or following extraction at pH 5 or pH 7 to determine if removal of soluble protein influenced disappearance rate. In addition, soybean and meat meals were incubated together to determine if these two protein sources would interact in situ.

Experiment 1 results illustrated that rates and extents of in situ disappearance in the rumen varied among feedstuffs. Disappearance of nitrogen from soybean meal, cottonseed meal and linseed meal was more extensive at 12 and 24 hours of incubation with steers fed the higher roughage diet than with steers fed the lower roughage diet, but diet did not influence disappearance with other feedstuffs. In situ loss at 4 hours of incubation was much greater than soluble nitrogen content of certain feedstuffs

including feather meal, meat meal, high moisture corn, oats, and wheat. These findings suggest that with certain feeds, fine particles were filtering through pores in the bag. With low protein feedstuffs, low nitrogen content caused technical problems in estimating nitrogen disappearance. In experiment 2, disappearance rate was not altered by extraction of proteins at pH 5 or pH 7 prior to in situ incubation. No interaction of soybean and meat meals on rate or extent of in situ disappearance of dry matter or nitrogen was evident.

APPENDIXES

TABLE I. DRY MATTER, CRUDE PROTEIN AND SOLUBLE PROTEIN OF FEEDSTUFFS.

FEEDSTUFF	IFN	Dry Matter %	Crude Protein % of DM	Soluble Protein[a] % of protein
PROTEIN SUPPLEMENTS				
Corn gluten meal	5-02-900	91.1	63.0	12.1
Cottonseed meal (a)	5-01-608	93.7	44.2	17.9
Cottonseed meal (b)	5-01-608	92.4	44.3	24.7
Feather meal	5-03-795	91.2	85.5	13.3
Fish meal	5-02-009	93.1	73.5	24.3
Linseed meal	5-02-045	89.7	40.2	38.7
Meat meal (a)	6-00-400	96.1	52.7	20.9
Meat meal (b)	6-00-400	94.8	52.2	42.0
Soybean meal (a)	5-04-604	89.0	50.2	27.2
Soybean meal (b)	5-04-604	88.2	54.1	24.2
Sunflower meal	5-04-738	92.8	31.0	37.5
ENERGY FEEDS				
Corn grain, cracked	4-02-931	89.3	9.2	11.7
Corn grain, rolled	4-02-931	86.4	9.2	40.0
Corn grain, high moisture	4-02-931	81.2	8.7	31.8
Oats, rolled	4-03-309	91.2	12.8	16.9
Sorghum grain, milo, ground	4-04-444	87.2	9.3	26.5
Wheat, ground	4-05-211	85.6	15.0	37.9
ROUGHAGES				
Alfalfa hay, chopped	1-00-071	91.4	20.4	41.2
Corn silage	3-08-152	36.5	9.0	89.0
Prairie hay, chopped	1-03-187	93.1	4.3	42.1
Sorghum silage	3-04-468	46.4	7.0	60.3

[a] In .15 N NaCl solution.

TABLE II. COMPOSITION OF DIETS USED IN EXPERIMENT 1.

CONCENTRATE PERCENTAGE	80(C)	40(R)
Ingredient	Percent of dry matter	
Prairie hay, chopped (IFN 1-07-957)	20.0	60.0
Corn grain, rolled (IFN 4-02-931)	75.4	26.2
Soybean meal (IFN 5-04-604)	.0	9.9
Molasses (IFN 4-04-696)	1.5	1.5
Urea (IFN 5-05-070)	1.20	.54
Salt, trace mineralized	.30	.30
Limestone (IFN 6-01-069)	.60	.0
Dicalcium phosphate (IFN 6-01-080)	.30	1.00
Sodium sulfate (IFN 6-04-292)	.30	.20
Potassium chloride (IFN 6-03-756)	.40	.40

TABLE III. COMPOSITION OF DIETS USED IN EXPERIMENT 2.

CONCENTRATE PERCENTAGE	80(C)	20(R)
Ingredient	Percent of dry matter	
Corn grain, rolled (IFN 4-02-931)	62.5	.0
Prairie hay, chopped (IFN 1-07-957)	.0	80.0
Cottonseed hulls (IFN 1-01-599)	14.0	.0
Soybean meal (IFN 5-04-604)	10.0	18.0
Alfalfa hay, chopped (IFN 1-00-063)	6.0	.0
Molasses (IFN 4-04-696)	6.0	1.0
Salt, trace mineralized	.50	.40
Limestone (IFN 6-01-080)	.50	.30
Dicalcium phosphate (IFN 6-01-080)	.50	.30

TABLE IV. SUBSTRATES, PREPARATION AND INCUBATIONS TIMES FOR EXPERIMENT 2.

Substrate	Preparation	Incubation times, h
Soybean meal	None	4, 12, 24, 48
Meat meal	Extracted, pH 5	4, 12, 24
Soybean meal	Extracted, pH 5	4, 12, 24
Meat meal	Extracted, pH 7	4, 12, 24
Soybean meal	Extracted, pH 7	4, 12, 24
Meat meal	Rinsed, pH 6	4, 12, 24, 48, 72
Soybean meal	Rinsed, pH 6	4, 12, 24, 48
Meat meal plus soybean meal	Rinsed, pH 6	4, 12, 24, 48, 72

TABLE V. RUMINAL MEASUREMENTS OF STEERS FED THE HIGH CONCENTRATE OR THE HIGH ROUGHAGE DIET IN EXPERIMENT 1.

MEASUREMENT	CONCENTRATE PERCENTAGE		SE
	80(C)	40(R)	
pH	6.34[a]	6.58[b]	.04
Ammonia-N, mg/dl	6.91	5.51	.98

[a,b] Means in a row with different superscripts differ ($P<.05$).

TABLE VI. DIET EFFECTS ON DRY MATTER AND NITROGEN DISAPPEARANCE AVERAGED ACROSS FEEDSTUFFS IN EXPERIMENT 1.

MEASUREMENT	INCUBATION TIME	CONCENTRATE PERCENTAGE 80(C)	20(R)	SE
Dry matter disappearance	4 h	29.1	29.6	2.10
	12 h	36.7	39.4	2.33
	24 h	47.0	46.7	3.22
Protein disappearance	4 h	47.9	44.7	3.94
	12 h	50.0	50.2	4.08
	24 h	54.6	32.1	8.64
Nucleic acid N % of residual N	4 h	.64[a]	.73[b]	.12
	12 h	.57[a]	1.07[b]	.14

[a,b] Means in a row with different superscripts differ ($P<.05$).

TABLE VII. EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF PROTEIN SUBSTRATES.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance: Dry matter 80(C)	Dry matter 40(R)	Dry matter SE	Nitrogen 80(C)	Nitrogen 40(R)	Nitrogen SE
Cottonseed meal (a)	4	30.2[c]	29.7[d]	.31	31.5[e]	32.0[f]	1.25
	12	38.9[c]	41.1[d]	.70	38.9[e]	42.1[f]	1.17
	24	46.2	49.7	1.04	43.8	47.2	1.50
	4-12[a]	3.57	4.82	.33	3.16	4.01	1.15
	4-12[b]	3.14	4.07	.24	2.76	3.47	.91
	12-24[a]	1.57	1.73	.26	1.05	.99	.80
	12-24[b]	1.43	1.57	.22	.98	.88	.71
	4-24[a]	2.64	3.37	.33	2.03	2.35	.47
	4-24[b]	2.12	2.57	.20	1.70	1.91	.32
Cottonseed meal (b)	4	27.2	27.4	.43	33.3	36.4	2.61
	12	40.2[e]	40.6[f]	2.04	49.9	43.8	4.14
	24	50.7[e]	57.8[f]	1.97	58.3	62.9	3.46
	4-12[a]	6.00	6.08	2.13	6.27	2.38	1.35
	4-12[b]	4.90	4.77	1.47	5.08	2.08	1.13
	12-24[a]	2.17	3.69	.79	1.36	4.04	1.46
	12-24[b]	1.92	3.02	.55	1.25	3.18	1.01
	4-24[a]	4.33	5.56	.62	3.72	3.66	.32
	4-24[b]	3.12	3.73	.30	2.78	2.74	.18
Soybean meal (a)	4	33.3[c]	36.5[d]	.74	27.7[c]	32.1[d]	1.11
	12	45.3[c]	57.9[d]	3.44	40.4[c]	51.4[d]	5.85
	24	61.1[c]	80.3[d]	1.62	54.9[c]	82.3[d]	2.76
	4-12[a]	4.57	7.45	1.97	5.60	8.25	4.34
	4-12[b]	3.89	5.72	1.25	4.62	5.77	2.70
	12-24[a]	2.88	3.30	.68	3.08	5.50	1.92
	12-24[b]	2.47	2.76	.49	2.61	4.06	1.18
	4-24[a]	4.20	6.03	.60	4.91	7.99	.97
	4-24[b]	3.04	3.94	.29	3.42	4.75	.38
Soybean meal (b)	4	32.4[c]	36.5[d]	.77	25.9[c]	30.9[d]	.95
	12	45.6[c]	57.9[d]	1.85	39.1[c]	51.5[d]	2.87
	24	65.9	72.8	3.29	61.5[c]	76.1[d]	4.33
	4-12[a]	5.07	7.36	1.13	6.11	8.47	1.93
	4-12[b]	4.23	5.77	.75	4.90	6.42	1.24
	12-24[a]	3.64	2.18	.79	4.53	3.99	.70
	12-24[b]	2.98	1.93	.55	3.60	3.25	.45
	4-24[a]	5.15	5.03	1.20	6.59	7.42	1.73
	4-24[b]	3.49	3.47	.60	4.12	4.52	.74

[a] [b] Calculated assuming a linear rate of disappearance. Calculated assuming a logarithmic rate of disappearance.

[c,d] Means in a row with different superscripts differ ($P<.05$).

[e,f] Means in a row with different superscripts differ ($P<.10$).

TABLE VII (continued). EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF PROTEIN SUBSTRATES.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance					
		Dry matter			Nitrogen		
		80(C)	40(R)	SE	80(C)	40(R)	SE
Meat meal (a)	4	32.9	32.1	3.53	44.1	47.3	1.52
	12	37.7	35.2	1.33	49.3[e]	49.3[f]	.77
	24	39.1	32.4	2.59	54.4[e]	47.7[f]	2.19
	4-12[a]	1.86	1.26	.63	1.53	.53	.38
	4-12[b]	1.72	1.18	.57	1.44	.52	.34
	12-24[a]	.31	.65	.67	.93	.26	.50
	12-24[b]	.29	-.72	.71	.86	.26	.46
	4-24[a]	.96	.05	.42	1.25	-.05	.48
	4-24[b]	.87	.04	.37	1.09	.05	.38
Meat meal (b)	4	36.9[e]	54.7[f]	5.12	62.2[c]	73.7[d]	2.96
	12	47.4[c]	46.5[d]	1.40	71.0	71.3	.97
	24	52.2[c]	44.7[d]	1.08	71.8	72.7	1.82
	4-12[a]	3.57	-1.15	1.79	1.77	-.23	.75
	4-12[b]	3.14	-1.53	2.00	1.65	-.27	.77
	12-24[a]	.84	-.29	.49	.09	.21	.36
	12-24[b]	.80	-.32	.51	.09	.19	.35
	4-24[a]	2.07	-.53	.96	.77	.06	.52
	4-24[b]	1.74	.81	1.10	.72	.00	.52
Corn Gluten meal	4	17.6	15.3	1.08	18.4	14.8	1.46
	12	23.1[c]	22.2[d]	.65	18.9	21.5	1.70
	24	27.6[c]	25.0[d]	.62	19.8	17.0	2.73
	4-12[a]	3.98	5.92	1.32	.33	5.70	1.06
	4-12[b]	3.46	4.78	.90	.28	4.68	.92
	12-24[a]	1.61	1.02	.45	.54	-1.83	1.10
	12-24[b]	1.46	.95	.39	.42	-2.10	1.12
	4-24[a]	2.87	3.23	.47	.39	.72	.68
	4-24[b]	2.26	2.48	.29	.36	.61	.60
Sunflower meal	4	30.9	35.4[d]	1.23	47.4[c]	58.0[d]	1.10
	12	42.6[c]	48.0[d]	1.24	64.3	67.5	4.70
	24	51.8	48.7	1.99	76.4	71.6	3.59
	4-12[a]	4.80	4.45	.38	4.18	2.11	.62
	4-12[b]	4.06	3.80	.28	3.61	1.93	.53
	12-24[a]	1.76	.13	.60	1.71	.51	.26
	12-24[b]	1.58	.11	.53	1.55	.49	.23
	4-24[a]	3.38	1.89	.55	3.05	1.21	.45
	4-24[b]	2.57	1.58	.37	2.38	1.06	.35

[a,b] Calculated assuming a linear rate of disappearance.
[c,d] Calculated assuming a logarithmic rate of disappearance.
[e,f] Means in a row with different superscripts differ ($P<.05$).
Means in a row with different superscripts differ ($P<.10$).

TABLE VII (continued). EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF PROTEIN SUBSTRATES.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance: Dry matter 80(C)	40(R)	SE	Nitrogen 80(C)	40(R)	SE
Linseed meal	4	34.5	35.4	3.87	52.0	38.7	6.34
	12	45.9[c]	52.5[d]	1.04	55.5[e]	65.7[f]	4.44
	24	55.0[c]	63.2[d]	.94	68.2[e]	76.9[f]	2.83
	4-12[a]	4.14	6.35	1.34	1.48	8.69	1.67
	4-12[b]	3.58	5.07	.89	1.39	6.52	1.02
	12-24[a]	1.66	1.78	.24	1.48	1.51	.67
	12-24[b]	1.51	1.61	.20	1.35	1.36	.56
	4-24[a]	2.98	4.13	.52	1.57	5.08	1.30
	4-24[b]	2.33	2.99	.29	1.37	3.43	.65
Feather meal	4	19.2	18.4	.60	32.9	30.0	1.55
	12	21.7	21.3	1.21	32.6	35.6	1.08
	24	23.0	23.4	.82	33.3	34.6	1.21
	4-12[a]	1.57	2.00	1.03	-.12	2.30	1.18
	4-12[b]	1.47	1.80	.90	-.12	2.03	1.02
	12-24[a]	.52	.80	.48	.18	-.19	.32
	12-24[b]	.49	.75	.45	.18	-.21	.33
	4-24[a]	.96	1.39	.67	.06	.74	.24
	4-24[b]	.88	1.17	.53	.06	.69	.20
Fish meal	4	20.5	20.0	1.01	37.0[c]	33.7[d]	.83
	12	25.5	23.3	2.83	39.0[e]	41.0[f]	.57
	24	25.5	23.4	2.69	47.0	47.9	1.34
	4-12[a]	3.21	1.99	2.26	.71	2.87	.91
	4-12[b]	2.84	1.56	1.97	.69	2.53	.74
	12-24[a]	-.06	.67	1.96	1.79	1.40	.74
	12-24[b]	-.07	.24	1.87	1.57	1.29	.61
	4-24[a]	1.29	.85	.40	1.41	2.20	.70
	4-24[b]	1.10	.76	.34	1.22	1.79	.51

[a] Calculated assuming a linear rate of disappearance.

[b] Calculated assuming a logarithmic rate of disappearance.

[c,d] Means in a row with different superscripts differ ($P<.05$).

[e,f] Means in a row with different superscripts differ ($P<.10$).

TABLE VIII. EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF ENERGY FEEDSTUFFS.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance					
		Dry matter			Nitrogen		
		80(C)	40(R)	SE	80(C)	40(R)	SE
Ground milo	4	24.7	24.1	1.39	25.4	33.7	6.35
	12	34.0	36.6	1.36	35.9	33.6	5.87
	24	53.9	52.0	1.15	47.0	32.1	7.20
	4-12[a]	4.83	6.49	1.13	7.49	-.51	1.69
	4-12[b]	4.02	5.21	1.04	5.87	-.77	1.75
	12-24[a]	4.85	3.50	.21	3.06	-1.66	3.55
	12-24[b]	3.82	2.92	.13	2.17	-3.19	3.94
	4-24[a]	5.94	5.78	.73	6.03	-.76	3.28
	4-24[b]	3.90	3.84	.34	3.65	-2.22	3.03
Ground Wheat	4	76.6[c]	60.0[d]	3.40	95.5[c]	68.6[d]	3.23
	12	80.8	75.4	2.49	96.8	89.7	4.64
	24	84.5	81.6	2.09	103.2	92.7	6.73
	4-12[a]	.69	3.52	1.28	.08	3.81	.93
	4-12[b]	.67	3.02	1.01	.02	3.32	.90
	12-24[a]	.38	.72	.42	.69	2.45	.61
	12-24[b]	.37	.67	.39	.63	2.39	.57
	4-24[a]	.52	2.39	.86	.40	1.72	.24
	4-24[b]	.49	1.61	.62	.39	1.47	.18
Ground corn	4	30.7	30.9	.90	40.1	42.2	3.70
	12	38.5	41.7	2.69	39.9	41.6	8.98
	24	64.2	66.2	3.35	50.0[c]	26.1[d]	4.13
	4-12[a]	3.23	4.37	.10	1.56	-.95	2.43
	4-12[b]	2.87	3.75	.08	1.42	-1.48	2.63
	12-24[a]	5.57	4.89	.86	1.06	-3.88	3.11
	12-24[b]	4.25	3.84	.53	.95	-15.9	11.50
	4-24[a]	5.50	5.71	.61	1.28	-1.88	2.16
	4-24[b]	3.70	3.80	.29	1.14	-10.1	7.90

[a] Calculated assuming a linear rate of disappearance.

[b] Calculated assuming a logarithmic rate of disappearance.

[c,d] Means in a row with different superscripts differ ($P<.05$).

[e,f] Means in a row with different superscripts differ ($P<.10$).

TABLE VIII (continued). EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF ENERGY FEEDSTUFFS.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance					
		Dry matter			Nitrogen		
		80(C)	40(R)	SE	80(C)	40(R)	SE
High moisture corn							
	4	30.0	28.6	1.46	39.7	45.3	7.02
	12	40.1	43.4	4.99	29.4[e]	54.2[f]	7.77
	24	63.2	68.7	5.29	39.7[e]	10.2[f]	10.2
	4-12[a]	4.31	7.71	1.25	-2.08	-3.24	3.71
	4-12[b]	3.68	5.97	.83	-3.12	-3.76	.22
	12-24[a]	4.73	3.95	.83	1.52	-8.20	1.23
	12-24[b]	3.71	3.23	.53	1.33	-34.2	1.04
	4-24[a]	5.60	5.97	1.32	-.29	-2.18	2.11
	4-24[b]	3.70	4.32	.60	-.45	-10.7	8.03
Cracked corn	4	3.7[c]	3.6[d]	.61	28.1	18.3	7.22
	12	7.5[c]	11.4[d]	1.07	23.4[c]	19.2[d]	4.79
	24	18.6	24.0	2.12	28.5[c]	1.7[d]	7.31
	4-12[a]	12.65	28.42	4.78	-.22	2.96	8.63
	4-12[b]	8.73	14.65	1.48	-.59	-2.91	9.22
	12-24[a]	13.81	9.24	4.04	3.76	-11.0	4.67
	12-24[b]	7.87	6.19	1.58	2.57	-10.4	3.01
	4-24[a]	21.5	30.00	7.38	2.71	-4.10	3.35
	4-24[b]	8.21	9.57	1.20	1.31	-2.32	3.02
Rolled oats	4	30.0	29.6	2.23	71.8	70.6	13.10
	12	38.7	38.0	2.91	75.6	72.1	10.90
	24	38.1	40.2	3.44	77.5	65.4	10.30
	4-12[a]	3.57	4.17	2.98	1.26	.40	1.32
	4-12[b]	3.12	3.21	2.28	1.11	.38	1.21
	12-24[a]	.05	.45	1.11	.39	-.86	.64
	12-24[b]	-.09	.44	1.11	.35	-.93	.65
	4-24[a]	1.39	2.09	1.50	.82	-.39	.84
	4-24[b]	1.19	1.54	1.10	.65	-.41	.73

[a] [b] Calculated assuming a linear rate of disappearance.
Calculated assuming a logarithmic rate of disappearance.
[c,d] Means in a row with different superscripts differ ($P<.05$).
[e,f] Means in a row with different superscripts differ ($P<.10$).

TABLE IX. EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF FORAGE FEEDSTUFFS.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance: Dry matter 80(C)	40(R)	SE	Nitrogen 80(C)	40(R)	SE
Prairie hay	4	10.4	10.1	.63	66.1[c]	36.3[d]	10.30
	12	12.1	12.7	1.29	38.7[c]	-12.0[d]	10.10
	24	19.8	17.2	6.77	24.1	-120.5	51.40
	4-12[a]	2.06	3.13	1.16	-5.70	-21.6	10.60
	4-12[b]	1.91	2.73	.93	-9.83	-10.5	7.68
	12-24[a]	5.67	4.66	8.25	-2.71	-48.9	31.90
	12-24[b]	4.06	-1.79	7.68	-3.33		
	4-24[a]	4.79	3.88	5.47	-3.28	-18.6	6.04
	4-24[b]	3.20	.02	4.25	-5.94		
Alfalfa hay	4	29.1[c]	31.1[d]	.68	49.4	42.3	1.39
	12	37.4[c]	48.6[d]	1.47	50.1	61.5	4.74
	24	56.2	56.4	4.16	77.5	73.3	6.33
	4-12[a]	3.55	7.10	1.02	.29	5.58	1.41
	4-12[b]	3.11	5.60	.72	.17	4.59	1.29
	12-24[a]	4.32	1.31	1.65	4.76	1.91	2.59
	12-24[b]	3.36	1.17	1.16	3.55	1.52	1.86
	4-24[a]	4.66	4.12	1.25	2.79	3.79	1.12
	4-24[b]	3.25	2.95	.67	2.20	2.75	.66
Corn silage	4	36.5	37.4	.77	108.7	96.7	8.77
	12	43.1	43.0	1.63	80.4	90.8	10.10
	24	53.6	31.1	19.82	105.6	-27.8	73.45
	4-12[a]	2.27	1.86	.55	-3.53	-1.66	1.80
	4-12[b]	2.07	1.74	.47	-4.26	-2.06	2.20
	12-24[a]	2.05	-2.27	4.32	3.07	-6.30	5.86
	12-24[b]	1.80	-28.3	22.2	2.56	1.70	1.09
	4-24[a]	2.33	.85	13.3	.16	4.83	2.86
	4-24[b]	1.91	-16.3	.67	.16	.86	1.86
Sorghum silage	4	22.9	24.3	1.51	68.9	57.4	10.60
	12	28.6	27.3	1.97	65.8	65.1	8.34
	24	35.8	11.1	11.9	3.6	-151.	51.04
	4-12[a]	3.16	1.58	1.41	-.41	-8.09	6.47
	4-12[b]	2.71	1.49	1.13	-.42	4.87	4.23
	12-24[a]	2.07	-4.77	7.26	-7.07	-23.8	7.60
	12-24[b]	1.84	4.21	.19	6.76		
	4-24[a]	2.83	-2.60	4.97	-4.21	-15.6	2.73
	4-24[b]	2.19	3.12	.85	-4.05		

[a] [b] Calculated assuming a linear rate of disappearance.
[c,d] Calculated assuming a logarithmic rate of disappearance.
[c,d] Means in a row with different superscripts differ ($P<.05$).

TABLE X. RUMINAL MEASUREMENTS OF STEERS FED THE HIGH CONCENTRATE OR THE HIGH ROUGHAGE DIET IN EXPERIMENT 2.

MEASUREMENT	CONCENTRATE PERCENTAGE		SE
	80(C)	20(R)	
pH	6.41[a]	6.90[b]	.09
Ammonia-N, mg/dl	11.97[a]	6.70[b]	1.04

[a,b] Means in a row with different superscripts differ ($P<.05$).

TABLE XI. DIET EFFECTS ON DRY MATTER AND NITROGEN DISAPPEARANCE AVERAGED ACROSS FEEDSTUFFS IN EXPERIMENT 2.

MEASUREMENT	INCUBATION TIME	CONCENTRATE PERCENTAGE 80(C)	20(R)	SE
Dry matter disappearance	4 h	16.8^{b}	21.7^{a}	.81
	12 h	31.5^{b}	36.3^{a}	1.15
	24 h	48.7^{b}	55.5^{a}	.85
	48 h	63.6^{b}	74.7^{a}	1.59
	72 h	50.7	60.1	2.92
Protein disappearance	4 h	17.6^{b}	25.6^{a}	1.69
	12 h	29.0^{b}	35.7^{a}	1.73
	24 h	50.9^{b}	60.4^{a}	1.41
	48 h	72.3^{b}	85.5^{a}	2.29
	72 h	63.9^{c}	78.6^{d}	3.79

^{a,b} Means in a row with different superscripts differ ($P<.05$).
^{c,d} Means in a row with different superscripts differ ($P<.10$).

TABLE XII. EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF PROTEIN SUBSTRATES.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance					
		Dry matter			Nitrogen		
		80(C)	40(R)	SE	80(C)	40(R)	SE
Soybean meal	4	36.7	40.0	2.39	14.2	29.4	2.04
	12	58.1	57.5	1.69	43.3	49.4	3.73
	24	82.0	77.1	5.04	74.2	73.0	6.63
	48	91.9	92.2	3.87	92.8	95.5	5.09
	4-12[a]	2.51	2.36	.26	2.72	3.42	.77
	4-24	1.91	2.17	.24	2.26	2.88	.43
	4-48	1.12	1.21	.02	1.50	1.66	.13
	12-24	1.57	2.06	.24	1.99	2.56	.31
Soybean meal (rinsed)							
	4	30.1	28.9	2.29	33.1	29.7	3.97
	12	51.3	45.6	1.83	39.2	40.5	4.70
	24	77.9	69.6	5.58	69.0	69.5	4.76
	48	93.2	84.7	5.33	92.2	87.3	5.74
	4-12	2.46	2.27	.30	1.42	.71	.77
	4-24	2.07	2.34	.23	1.91	1.97	.17
	4-48	1.23	1.39	.09	1.30	1.44	.04
	12-24	1.84	2.38	.34	2.19	2.71	.18
Soybean meal (pH 5 extracted)							
	4	24.1	19.0	6.62	11.3[b]	18.4[c]	1.60
	12	43.3	44.1	7.30	20.6	33.7	11.90
	24	75.4	67.3	7.34	70.3	61.8	9.30
	4-12	2.71	2.82	.58	.54	2.53	.96
	4-24	2.45	2.50	.11	2.27	2.96	.45
	12-24	2.30	2.32	.51	3.27	3.21	1.16
Soybean meal (pH 7 extracted)							
	4	13.7	15.5	3.31	18.6	17.9	10.00
	12	41.9	41.6	6.37	30.9	36.7	5.91
	24	71.9	68.1	4.94	66.3	67.9	9.21
	4-12	3.88	2.91	.84	2.75	1.13	1.71
	4-24	2.62	2.84	.16	2.41	2.53	.04
	12-24	2.80	1.90	.63	2.21	3.35	.99

[a] Disappearance rates calculated assuming linearity over time.
[b,c] Means in a row with different superscripts differ ($P<.10$).

TABLE XII (continued). EFFECT OF DIETARY CONCENTRATE ON DM AND N DISAPPEARANCE RATES OF PROTEIN SUBSTRATES.

Diet, % Concentrate Feedstuff	Time, h	Extent (%) or Rate (%/h) of Disappearance					
		Dry matter			Nitrogen		
		80(C)	40(R)	SE	80(C)	40(R)	SE
Meat meal (rinsed)	4	12.5	12.5	2.24	20.1	13.1	9.96
	12	20.9	16.0	1.53	26.8	28.5	2.50
	24	26.5	22.0	2.02	32.3	40.6	5.13
	48	31.7	32.0	6.11	54.9	51.3	14.38
	72	43.9	36.0	8.15	67.7	56.5	9.03
	4-12[a]	.60	.91	.46	2.49	.28	.56
	4-24	.50[b]	.66[c]	.11	1.24	.71	.46
	4-48	.30[e]	.56[d]	.01	.68	.91	.14
	4-72	.27[e]	.50[d]	.05	.58	.72	.07
	12-24	.45	.52	.11	.51	.96	.56
Meat meal (pH 5 extracted)							
	4	14.2	10.7	2.30	24.2	27.8	11.31
	12	17.6	13.0	4.71	17.3	17.5	5.22
	24	25.8	20.9	3.03	32.5	31.3	4.55
	4-12	.13	.60	.21	-.93	-1.23	.80
	4-24	.52	.59	.05	.35	.38	.44
	12-24	.74	.59	.18	1.10	1.32	.22
Meat meal (pH 7 extracted)							
	4	8.9	4.0	1.57	24.0	27.6	3.66
	12	14.2	10.3	2.34	33.8	35.2	6.38
	24	24.6	18.0	.77	41.0	43.6	1.58
	4-12	.60	.86	.08	.78	14.0	.20
	4-24	.84	.64	.05	1.05	.57	.10
	12-24	.99	.52	.12	1.21	.22	.44
Meat meal plus soybean meal (rinsed)							
	4	20.0	17.6	2.64	16.3	19.5	6.27
	12	31.9	34.8	3.40	26.9	37.1	4.03
	24	55.5	51.0	2.95	59.6	57.4	4.53
	48	60.0	67.3	7.49	79.9	77.0	4.75
	72	72.2	69.5	1.49	78.0	82.7	7.00
	4-12	1.74	1.91	.68	1.67	1.87	1.01
	4-24	1.71	1.73	.21	1.96	2.13	.30
	4-48	.86	1.12	.09	1.34	1.41	.15
	4-72	.70	.74	.01	.89	.96	.02
	12-24	1.68	1.62	.31	2.14	2.28	.53

[a] Disappearance rates calculated assuming linearity over time.
[b,c] Means in a row with different superscripts differ ($P<.05$).
[d,e] Means in a row with different superscripts differ ($P<.10$).

TABLE XIII. COMPARISON OF RUMINAL BYPASS CALCULATED FROM DIFFERENT EQUATIONS.

		STANDARD REFERENCE Time in rumen, h		MILLER EQUATIONS Ruminal dilution, %/hr	
FEEDSTUFF	DIET	8	16	.06	.03
COTTONSEED MEAL A	CONCENTRATE	70.64	61.98	70.17	61.27
	ROUGHAGE	64.60	60.29	68.04	58.09
COTTONSEED MEAL B	CONCENTRATE	47.17	40.60	54.57	42.78
	ROUGHAGE	47.72	29.45	51.58	39.22
SOYBEAN MEAL A	CONCENTRATE	47.82	36.90	52.25	40.75
	ROUGHAGE	15.83	-16.80	33.97	22.15
SOYBEAN MEAL B	CONCENTRATE	42.18	23.60	48.73	35.93
	ROUGHAGE	25.83	2.76	40.02	27.20
MEAT MEAL A	CONCENTRATE	66.33	61.29	67.70	59.18
	ROUGHAGE	77.61	79.19	78.84	78.58
MEAT MEAL B	CONCENTRATE	41.83	41.04	47.22	39.82
	ROUGHAGE	60.96	58.86	56.68	55.41
CORN GLUTEN MEAL	CONCENTRATE	86.67	86.10	86.74	85.62
	ROUGHAGE	83.70	86.70	86.74	85.62
SUNFLOWER MEAL	CONCENTRATE	27.72	17.15	37.65	26.94
	ROUGHAGE	42.30	38.12	47.53	38.34
LINSEED MEAL	CONCENTRATE	43.97	31.27	45.30	35.92
	ROUGHAGE	18.93	12.16	34.21	23.73
FEATHER MEAL	CONCENTRATE	86.48	85.87	86.27	85.84
	ROUGHAGE	80.29	81.10	82.57	78.82
FISH MEAL	CONCENTRATE	66.39	59.53	66.02	58.53
	ROUGHAGE	61.32	55.78	63.17	54.20
MILO GROUND	CONCENTRATE	54.45	46.54	57.27	46.91
	ROUGHAGE	74.71	75.80	75.00	76.56
WHEAT, GROUND	CONCENTRATE	No solutions		36.32	25.66
	ROUGHAGE	-5.49	-3.85	28.79	18.74
CORN, GROUND	CONCENTRATE	52.80	45.49	51.80	45.57
	ROUGHAGE	70.08	79.54	49.45	42.06
CORN, HIGH MOISTURE	CONCENTRATE	70.65	62.35	67.08	66.00
	ROUGHAGE	81.27	106.44	49.84	39.27
CORN, CRACKED	CONCENTRATE	89.58	85.27	87.28	86.29
	ROUGHAGE	98.73	110.15	90.41	92.62
OATS GRAIN	CONCENTRATE	67.02	62.97	70.03	60.51
	ROUGHAGE	90.05	101.35	72.37	64.09
PRAIRIE HAY	CONCENTRATE	110.23	128.26	200.81	-136.77
	ROUGHAGE	81.40	92.50	104.64	542.81
ALFALFA HAY	CONCENTRATE	27.19	-3.60	34.39	24.30
	ROUGHAGE	26.47	17.93	36.04	25.98
CORN SILAGE	CONCENTRATE	No solutions		11.00	11.00
	ROUGHAGE	40.86	59.77	-12.24	-3.93
SORGHUM SILAGE	CONCENTRATE	67.92	92.37	210.80	-63.69
	ROUGHAGE	53.27	74.76	150.76	-83.87

TABLE XIV. IN SITU DISAPPEARANCE EXTRAPOLATED TO INITIAL TIME AND PROTEIN SOLUBILITY ESTIMATES FROM THIS TRIAL AND THE LITERATURE.

FEEDSTUFF	IN SITU INITIAL LOSS[a]	NITROGEN SOLUBILITY IN VARIOUS BUFFER SOLUTIONS OURS	LITERATURE	CITATION[b]
PROTEIN SUPPLEMENTS				
Corn gluten meal	18.1[c],11.4[d]	12.1	3.4;7.2;4.5;4.2; 3.0;38.5;4.2;7.2 6.4;10.7	5,8,4,4, 4,4,3,7, 7,7
Cottonseed meal	26.9,27.8	17.9	7.2;11.3;15.7;9.4	8,2,6,6,
	25.0,32.8	24.7	32.4;10.3;15.3	7,7,7
Feather meal	33.1,27.3	13.3	4.1	6
Fish meal	35.8,30.1	24.3	10.8	6
Linseed meal	48.8,25.2	38.7	50.8	8
Meat meal	41.6,46.3	20.9	16.4;18.4;18.3;	8,4,4,
	57.8,74.9	42.0	20.3;28.0;12.9	4,4,6
Soybean meal	21.3,22.4	27.2	22.4;19.7;13.0;	5,1,1,
	20.1,20.7	24.2	6.3;13.0;36.7; 20.7;38.1;28.1; 83.4;20.4;41.8; 24.4;22.8	1,8,2, 4,4,4, 4,3,7, 7,7
Sunflower meal	39.4,53.2	37.5	34.1;30.7;24.0; 30.0	1,1,1, 8
ENERGY FEEDS				
Corn grain, cracked	30.4,17.8	11.7	16.1	6
Corn grain, ground	36.8,42.5	40.0	5.1;5.1;10.8; 1.9;32.6;11.1; 20.7;14.4;16.7	5,1,1, 1,2,3, 7,7,7
Corn grain, high moisture	46.3,50.0	31.8	16.2	6
Oats, rolled	70.0,69.8	16.9	31.3;26.8;9.2; 13.3;25.8;17.9	5,1,1, 1,8,6
Sorghum grain, milo, ground	20.2,33.8	26.5	12.4	6
Wheat, ground	94.8[d],58.0[c]	37.9	21.7;25.6;20.8; 29.7;19.7	1,1,1, 8,6
ROUGHAGES				
Alfalfa hay, chopped	49.0,32.7	41.2	29.4;32.1;34.5	1,1,1
Corn silage	122.8,99.6	89.0	31.5;34.2; 6.5;52.2	1,1, 1,3
Sorghum silage	70.4,53.6	60.3		
Prairie hay, chopped	172.3,150.5	42.1		

[a] Nitrogen loss extrapolated to time zero of in situ incubation with the roughage and the concentrate diet.

[b] Numerical citations listed on following page.

[cd] Means in a row with different superscripts differ ($P<.05$).

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TABLE XV. ESTIMATES OF THE BYPASS OR ESCAPE OF PROTEIN FROM COMMON FEEDSTUFFS FROM THIS TRIAL AND IN VIVO TRIALS.

FEEDSTUFF	IN SITU N RESIDUE AT 24 HOURS (%) Diet type		IN VIVO N BYPASS (%)	REFERENCE[a]
	Conc.	Forage		
PROTEIN SUPPLEMENTS				
Corn gluten meal	80.2	83.0	55,46-61,62	16,18,19
Cottonseed meal	56.2	52.8	24-61	18
	41.7	37.1		
Feather meal	66.7	65.4	71	2
Fish meal	53.0	52.1	71,69-100,78	4,13,19
Linseed meal	31.8	23.1	44	18
Meat meal	45.6	52.3	70,76	18,19
	28.2	27.3		
Soybean meal	45.1	17.1	27,29,61,32	17,16,4,7,
	38.5	23.9	15-18,20,24,	1,18,6
			20,24,48,24	6,11,19,19
Sunflower meal	23.6	28.4	19-28	13
ENERGY FEEDS				
Corn, cracked	71.5	98.3		
Corn, ground	50.0	73.8	73,58	19,19
Corn, high moisture				
	60.3	89.8		
Oats grain	22.4	34.6		
Sorghum grain	53.0	67.9	49,20,38,64	10,10,10,10
			58,52,69,65	3,3,8,8
Wheat grain	-3.2	7.3		
ROUGHAGES				
Alfalfa hay	22.5	26.7	30,41,21,	12,14,15,
			28,20-24	9,5
Corn silage	-5.6	33.5	27	19
Sorghum silage	79.5			

[a] From literature citation on following page.

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VITA 2

Juan Ruben Barrio

Candidate for the Degree of

Master of Science

Thesis: INFLUENCE OF DIET ON RUMINAL IN SITU DISAPPEARANCE OF DRY MATTER AND NITROGEN.

Major Field: Animal Science

Biographical:

Personal Data: Born in Chihuahua, Mexico, December 31, 1957, the son of Ruben and Martha Barrio.

Education: Graduated from I.E.S., high school, Chihuahua, Mexico, in May, 1976; received the Bachelor of Science Degree from Escuela Superior de Zootecnia, Chihuahua, Mexico, with a major in Animal Science in June, 1980; completed the requirements for the Master of Science Degree at Oklahoma State University in December, 1983.

Professional Experience: Raised on a ranch in Chihuahua, Mexico; employed by the S.A.R.H., in Chihuahua, Mexico 1978 - 1981; Laboratory and animal technician in the Animal Science Department, Oklahoma State University, 1983.