INFLUENCE OF ROUGHAGE LEVEL, RUMINAL PH AND AMMONIA CONCENTRATION ON RUMINAL PROTEIN DEGRADATION AND MICROBIAL PROTEIN SYNTHESIS IN CATTLE

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PREFACE

This thesis represents a small portion of my work at Oklahoma State University. The preceding year of work, unrelated to my thesis effort, plus the time involved in cooperative work and consultation aided my development considerably. The work reported here is not an end to itself, but rather created more questions than it answered. In retrospect, changes in a few experimental methods may have improved precision. Use of younger cannulated animals would have permitted greater feed intakes to allow greater applicability of derived relationships. More frequent sampling of duodenal flow, while probably not greatly altering mean measured responses, may have reduced variability enough to make more of the relationships significant, statistically. However, I am pleased with the experimental planning, execution and laboratory analysis, as I performed them myself, with the consultation of my advisor. The feeding and care of animals was superbly supervised by one of the finest animal care technicians I have encountered, Ken Poling.

My appreciation is extended to the members of my committee, Drs. F. N. Owens, chairman and advisor, D. R. Gill, R. G. Teeter, D. L. Weeks, E. C. Nelson and W. A. Phillips, for my use of their time and advice. Thanks is also extended to: Dr. R. A. Zinn for advice and surgical preparation of cannulated animals, Dr. W. L. Stockland of

International Multifoods for superb amino acid analyses, and to the Ralston Purina Company for their award of three Fellowships in support of my graduate training and my employment after graduate school. I am further grateful to have had the opportunity to interact with other graduate students, faculty, laboratory personnel, support staff and friends, all of whom have influenced my professional and social development and revealed many of the secrets of life during the course of my studies.

Of these individuals, some have had significant impact. Dr. Fred Owens and Dr. Larry Satter, my mentors at Oklahoma State University and the University of Wisconsin, respectively, have contributed significantly to my professional development. They encouraged imaginative thought and reasoning, and stressed the need for an integrated familiarity of the literature combined with well planned experimental pursuits of questions to pertinent problems. However, my initial development as a scientist was nurtured by my father's example of creative thought, dedication to precision and enthusiastic pursuits and applications of research.

My greatest appreciation, however, goes to my parents and family for their patient guidance, best wishes and for instilling in me the desire to succeed and the will to behave altruistically and with integrity. Jacob Bronowski (1973) adeptly remarked that "the ascent of man is....made by people who have two qualities: an immense integrity, and at least a little genius." Although my abilities do not place me in the company of geniuses, I shall continue to consider integrity, first, in conducting my life and my work.

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INTRODUCTION

In ruminants, the supply of protein reaching the small intestine for absorption originates primarily from microbial protein synthesized in the rumen and feed protein that has escaped microbial degradation. In addition, endogenous sources make a small contribution. Depending on animal and dietary factors, microbial protein can comprise 40 to 80% of the total nitrogen (N) entering the small intestine (Owens and Bergen, 1983). The relative importance of microbial or undegraded feed protein sources in meeting the animal's protein requirement depends on the production state of the animal. The wintering beef cow consuming low quality roughages which have protein of a low digestibility, depends heavily on the ability of ruminal microbes to convert the N contained therein into microbial protein which has a high digestibility and quality. Depending on the forage supply, microbial protein may be adequate to support the protein requirements of the typical sub-maintenance, non-lactating beef cow. An adequate supply of microbial protein and other required digestive end products depends upon efficient operation of the microbial population in the rumen.

On the other extreme of the production spectrum is the high producing dairy cow. Lactation requires both protein and energy in large amounts. Microbial protein synthesis can provide only

approximately 60 to 70% of the protein needed even though sufficient dietary energy may be available (Kaufmann and Lupping, 1982). Not only is the requirement for protein high, but a higher quality protein than microbial protein can provide may be needed. For the lactating cow, microbial protein production becomes less important and emphasis is diverted to increasing both the dietary supply and ruminal escape of higher quality protein. Feed protein escaping ruminal degradation is commonly called "bypass protein". Decreasing the microbial degradation of a high quality protein source in the rumen will also avoid the loss in efficiency of converting dietary protein to microbial protein. This efficiency has been estimated to be as low as 50 percent (Chalupa, 1975). Increasing the bypass of a protein source is not simply a matter of inhibiting microbial degradative processes, since such action could limit the digestibility of roughages. Consequently, considerable effort has been expended in chemically or physically modifying protein in feedstuffs to increase their resistance to microbial degradation (Chalupa, 1975; Ferguson, 1975).

Relatively less effort has been expended in understanding the various factors which may influence ruminal environment and alter both protein degradation in the rumen and efficiency of microbial protein synthesis. Several workers have suggested estimates of the extent of ruminal degradation of feedstuffs (Chalupa, 1975; Satter et al., 1977). Recent work (Ganev et al., 1979; Zinn and Owens, 1983a) suggests that protein degradation is not constant, but is subject to dietary influences. The influence of roughage level on the extent of degradation of feed protein is one of the research objectives of this dissertation.

A similar case can be stated for efficiency of microbial protein synthesis. Recent reviews (Czerkawski, 1978; Stern and Hoover, 1979) demonstrate a wide range in values for efficiency of microbial protein synthesis. Although some of this variation can be attributed to the analytical techniques employed in estimation, animal and dietary influences probably are involved also (Van Soest, 1982; Johnson and Bergen, 1982). Johnson and Bergen (1982) suggested that, in addition, estimates of ruminal organic matter (OM) digestion used in evaluating feedstuff potential to drive microbial protein synthesis in the rumen should consider feed type, feed processing, level of intake and dietary roughage level.

The objective of this dissertation was to evaluate the effect of dietary roughage level, ruminal ammonia supply and ruminal pH on feed protein digestion and efficiency of microbial protein synthesis in the rumen. The majority of the past research in the area of microbial efficiency has been conducted in vitro (Isaacson et al., 1975; Satter and Slyter, 1974; Schaefer et al., 1980) since these systems lend themselves to more refined control. But extrapolation from chemostats to the rumen can be erroneous. This thesis work was to designed to evaluate ruminal parameters in vivo and to allow for the interaction of animal factors. The major problem to this approach is the difficulty in changing only one ruminal fermentation characteristic at a time (e.g. pH, ammonia concentration, dilution rate, microbial species) (Harrison and McAllan, 1980). Nevertheless, application of research results to animals can only come from in vivo experimentation.

Relationships derived from these types of in vivo experiments are essential to improve the predictive capabilities of the various metabolizable protein models (Burroughs et al., 1975; Satter and Roffler, 1975; etc.). If such models are to predict metabolizable amino acid supply to the small intestine, more detailed information is needed concerning the composition of feed protein reaching the small intestine. This will also be addressed in this thesis study using the dacron bag technique to examine the change in amino acid composition of undigested protein.

CHAPTER II

REVIEW OF LITERATURE

Introduction

The study and quantitation of feed protein digestion and microbial protein synthesis within the rumen has been an active area of research for the past 15 to 20 years. Experimentation has been conducted both in vivo and in vitro. Both systems have improved over time. The batch culture has evolved to continuous, and now the intermittent, culture in vitro systems. Use has also shifted from re-entrant to simpler T-cannulas in the small intestine of animals. Although methodology has improved, both in vitro and in vivo systems have limitations in the interpretation and application of results. In vitro systems, with more precise experimental control than in vivo systems, are chided for being too artificial and static. In vivo systems, though more natural, are difficult to control experimentally. These concerns must be considered when surveying the literature. Many excellent recent reviews and books discuss microbial fermentation and feed digestion within the rumen (Hogan and Weston, 1970; Houpt, 1970; Chalupa, 1975; Smith, 1975; Mercer and Annison, 1976; Chalmers et al., 1976; Bergen and Yokayama, 1977; Chalupa, 1977; Satter et al., 1977; Czerkawski, 1978; Hespell, 1979; Hespell and Bryant, 1979; Smith, 1979; Stern and Hoover, 1979; Tamminga, 1979;

Theurer, 1979; Cheng and Costerton, 1980; Harrison and McAllan, 1980; Kennedy and Milligan, 1980; Russell and Hespell, 1981; Orskov, 1982; Van Soest, 1982; Owens et al., 1983; Owens and Bergen, 1983). In addition, an excellent review and discussion of the nutritional, methodological and modeling aspects of protein metabolism in cattle are contained in a recent symposium (Owens, 1982). Since these reviews exist, these topics will not be thoroughly covered here. Rather, this discussion will address specific areas of current concern and interest.

The initial portion of the review will discuss some of the limitations and concerns of the methods used in measuring microbial activity and feed protein degradation in the rumen of animal systems. The ultimate goal is to describe rumen metabolism in the animal. A discussion of in vitro systems will not be included, although they are useful for screening ideas, concepts and interactions. Instead, the reader is referred to the review by Broderick (1982). The review will conclude with discussion of current ideas about factors affecting efficiency of microbial protein synthesis and feed protein digestion within the rumen.

Measurement of Microbial Activity in Animal Systems

Microbial Markers

Since protein leaving the rumen is of feed, microbial and endogenous origins, separation is requires not only to assess feed protein degradation in the rumen, but also efficiency of microbial

protein synthesis. The microbial mass is usually quantitated by measuring the flow of some compound leaving the rumen that is solely associated with microbial cells. Estimates of microbial protein may vary by 10 to 36% depending on the microbial marker employed (Theurer, 1979).

Schelling et al. (1982) described the requirements for the successful use of nucleic acids or their constituents as microbial markers. Their comments, however, would pertain to any microbial marker. For a microbial marker to yield reliable estimates of microbial protein it should: (1) comprise a constant proportion of microbial nitrogen (N), (2) be present in only insignificant quantities in nonmicrobial material and (3) be analyzed by a procedure that is sensitive, repeatable, rapid and inexpensive.

A number of procedures have been developed for separating and quantitating compounds or markers unique to microbes. The more common of these include: nucleic acids (McAllan and Smith, 1969), diaminopimelic acid (DAP; Czerkawski, 1974), aminoethylphosphonic acid (AEP; Czerkawski, 1974), ^{35}S (Kennedy et al., 1980), ^{32}P (Smith et al., 1978) and adenosine triphosphate (ATP; Forsberg and Lam, 1977). These references may not necessarily refer to the original procedure, but in some cases describe a more recent adaptation or improvement of the original procedure. Total purines (Zinn and Owens, 1982) and D-alanine (Garrett et al., 1982) are two recent promising additions to the list.

A number of recent reviews (Smith, 1975; Czerkawski, 1978; Stern and Hoover, 1979; Bergen et al., 1982, Schelling et al., 1982; Theurer, 1982) and reports (Ling and Buttery, 1978; Siddons et al.,

1979) compare estimates based on various markers and discuss their limitations. This discussion will not reiterate marker comparisons or traditional criticisms in depth, but rather impart some of the more recent findings and concerns. Unfortunately, technique comparisons without a primary standard are never conclusive and may be misleading.

Diaminopimelic acid traditionally has been criticized for not representing the entire ruminal microbial population since it was thought to be contained only in the cell wall of bacteria. While some workers have found that most feeds are devoid of DAP (Dufva et al., 1982b), a desirable trait for a microbial marker, others (Theurer, 1982) have found DAP in a number of feedstuffs as well as bacteria and protozoa, probably due to protozoal engulfment of bacteria. Theurer (1982) reported that the DAP-N:total N in feeds was 12 to 78% of the DAP concentration in bacteria, suggesting that corrections for dietary DAP may be appropriate.

Aminoethylphosphonic acid has been considered, in the past, to measure protozoal protein, since it was thought to be found only in the lipid fraction of protozoa. However, Theurer (1982) also indicated that AEP is not detected in feed, mixed rumen protozoa or abomasal samples, precluding its usefulness as a protozoal marker. Dufva et al. (1982a) have reported that various feedstuffs and bacteria were devoid of AEP. However, AEP was present in protozoa. AEP concentration of microbes was found to vary with diet, possibly due to differences in bacterial lipid content. Still, others (Ling and Buttery, 1978) have found AEP not only in isolated protozoa but also in dietary and bacterial material. Any protozoal marker is going

to suffer from the incomplete isolation of protozoa from feed and bacteria, which are often adherent.

Nucleic acid polymers (RNA and DNA) or their derivatives (purines) have been popular microbial markers since they are representative of the entire microbial population. However, the procedure used to determine RNA (McAllan and Smith, 1969) is laborious. A major criticism leveled at these procedures is the amount of nucleic acids provided in feed which may survive ruminal degradation and contaminate a digesta sample, causing an overestimate of microbial N flow (Siddons et al., 1979). However, samples of pure RNA and DNA added to the rumen were observed to be rapidly degraded (McAllan and Smith, 1968). Nucleic acids from a sample of ground hay incubated in vitro were degraded almost as rapidly as pure nucleic acids (McAllan and Smith, 1973).

Microbial incorporation of ^{35}S, ^{15}N and ^{32}P have been thought to yield more precise estimates of microbial protein than the endogenous bacterial markers since only live microbial cells should theoretically incorporate them into their protein or nucleic acid structure in a uniform manner. Also, the pools from which these isotopes are drawn may change with diet and time post-feeding, especially for pulse-dosed labels. These isotopes have usually been compared with each other (Harmeyer et al., 1976) or with the traditional endogenous markers (Smith et al., 1978), while few critical evaluations in vivo on purified diets have been made.

Since quantitation of microbial N flow at any sampling site depends on knowledge of the microbial content of the marker used, considerable debate exists concerning the effect that diet and time

after feeding may have on microbial composition. Averaged over sheep and cattle fed various diets, the proportion of bacterial dry matter (DM) that is N has been reported to be 7.2% (Smith, 1975) and 7.77% (Orskov, 1982). Of the bacterial N, 10% has been reported to come from RNA and 5.2% from DNA with 82.5% being amino acid N (Orskov, 1982). Smith (1969) has reported that 75 to 85% of bacterial N is in the form of proteins, peptides or free amino acids. He further suggested that for every four parts of dietary N converted to bacterial protein or amino acids, about one part is converted to nucleic acids. This was based on earlier work (Smith et al., 1968) with calves fed various diets, where isolated microbes contained a fairly constant 19% of their total N in the form of nucleic acids regardless of the N content of the diet. Use of an average bacterial composition of an endogenous marker or N, however, may not be applicable in all situations as others have observed that microbial composition appears to change with diet and time after feeding.

Arambel et al. (1982), measured the nucleic acid content of 17 pure cultures of rumen bacteria as well as bacteria isolated from ruminally cannulated cattle fed either 85:15 or 15:85 alfalfa hay:concentrate diets. The gram negative bacteria contained slightly more nitrogen and a greater DNA-N:total N, while little difference existed in RNA-N:total N. Bacterial N was similar on both diets (averaging 7.3%). Ratios of DNA-N:total N and RNA-N:total N tended to be lower on the higher concentrate diets. They suggested these changes may be due to an increase in gram positive bacteria on the higher concentrate diets. They concluded, however, that bacterial RNA-N:total N was affected less by diet and sampling day than

DNA-N:total N. Zinn and Owens (1982) found that bacterial total nucleic acid N:total N from isolated ruminal bacteria was higher for typical high concentrate diets, contrary to the results of the previously mentioned workers. Smith and McAllan (1974), on the other hand, found that mixed bacteria taken 4 to 6 hr after feeding a high concentrate or high roughage diet were similar in N composition, RNA-N:total N and DNA-N:total N. Others (Ling and Buttery, 1978) have found that considerable variation existed for RNA-N:total N and DNA-N:total N for bacteria and protozoa, but these differences were not related to the effects of sheep, diets or sampling period. Czerkawski (1976) has noted that the concentration of some bacterial constituents varied with diet and time after feeding, but many of those differences disappeared when the results were expressed on a bacterial polysaccharide free basis. Czerkawski (1978) has observed that the polysaccharide content of bacteria can range from 8 to 35%. Similar observations have been made with DAP, where bacteria from cattle fed a high roughage diet contained more DAP-N:total N than those fed a high concentrate diet (Dufva et al., 1982).

Although some workers (Maeng and Baldwin, 1976) have found that the RNA-N:microbial N is fairly constant throughout the day, Smith and McAllan (1974) observed that mixed bacteria taken just before feeding showed significantly lower RNA-N:total N and slightly higher DNA-N:total N than bacteria 4 to 6 hr after feeding. However, others (Gillett et al., 1983) have observed that the RNA content (by the orcinol procedure) of the free floating ruminal bacteria increased with time after feeding, while the RNA composition of the feed adherent population was more constant. This appears to conflict with

the observation that cells grown at a high growth rate have a high RNA:protein while cells growing at a low growth rate have low ratios (Bergen et al., 1982), since bacteria would likely be in a higher growth rate phase soon after feeding. Gillett et al. (1983) suggested that the use of RNA as a microbial marker should be avoided when feeding high concentrate diets since more of the bacteria would be free floating and more variable with respect to time after feeding. Isaacson et al. (1975) observed that the N content of cells tended to increase as dilution rate increased in vitro. How this might affect bacterial RNA content is unknown. Time of sampling appears to have little influence on the DAP-N:bacterial N over the short term (Dufva et al., 1982) or long term (Hutton et al., 1971).

When differences are seen in bacterial composition, it is unknown how much of this is due to problems in bacterial isolation and analytical technique and how much is actually due to differences in ruminal environment. As a safety precaution, it may be advisable to isolate bacteria under each set of experimental conditions. This would serve not only to correct for ruminal environmental influences but also possible problems in analytical recovery of microbial markers. Such effort may still be inadequate, since parameters determined with bacteria isolated from strained rumen fluid may not be representative of the feed adherent bacteria (Gillett et al., 1983) or protozoa. Even if an adequate measure of bacterial composition is achieved, digesta flow indicators may have a more profound effect on microbial yield estimates since the flow of feed organic and microbial matter from the rumen is based on indigestible flow marker ratios (Theurer, 1982). With the magnitude of errors involved in animal to

animal differences, postprandial fluctuations in flow, nonrepresentative sampling and compositing of digesta, processing losses and analytical errors, the microbial marker concentration probably has limited impact on in vivo estimation of ruminal function.

In Situ Methods

In situ techniques had been employed extensively in the study of the ruminal degradation of forage fiber. Mehrez and Orskov (1977) suggested using artificial bags to study feed protein degradation in the rumen. Many had believed that microbial contamination of residues would preclude the technique's usefulness in the study of protein degradation. Mathers and Aitchison (1981) addressed this concern by suspending bags containing 5 g of dried lucerne or fish meal in the rumen for up to 24 hr. They found that microbial contamination after 24 hr exposure in situ was less than 2% of the residual N with fish meal but was nearly 20% with alfalfa. Microbial contamination was observed to increase with time. Correcting for contamination reduced the general mean degradation but had little effect on the pattern of degradation over time. Weakley et al. (1983) described some of the factors influencing the disappearance of feed components from bags. One of the greatest improvements needed in the technique is a repeatable method of washing bags after removal from the rumen. Placing bags into the rumen over time, in order to achieve the desired digestion intervals, and removing bags from the rumen at the same time, as opposed to the reverse situation, permits more similar conditions for washing of the bags. This procedure, however, requires frequent feeding of animals to maintain a constant ruminal

environment. When dietary effects on in situ protein disappearance are of interest, bags from all dietary treatments should be washed simultaneously. Failure to do so may have contributed to the results of Lindberg (1981) where the effect of diet on feedstuff N disappearance was studied in two different experiments. Not only were the patterns of degradation inconsistent, but roughage level effects on the disappearance of a number of the feedstuffs were the opposite of the results normally observed, as will be discussed later in this review.

Orskov (1982) devotes a considerable portion of his recent book to the interpretation of in situ protein degradation curves for predicting protein digestion in the rumen. One of the earliest suggestions was to proportionally divide in situ disappearance measurements according to the rate at which protein sources leave the rumen (Orskov and McDonald, 1979; McDonald, 1981). A more recent procedure (Zinn and Owens, 1983) suggests comparing degradation curves to a standard reference soybean meal (SBM) with a known extent of in vivo degradation to predict ruminal in vivo protein degradation of unknown feedstuffs under various dietary conditions. Both of these systems demonstrate the need for a more dynamic approach to the interpretation of bag results to predict ruminal degradation of feedstuffs under a variety of dietary conditions.

In Vivo Methods

To study the efficiency of microbial protein synthesis and feed protein degradation in the rumen and subsequent digestion in the small intestine, cannulas are needed in the rumen, terminal ileum and either

the omasum, abomasum or duodenum, proximal to entrance of the bile duct. Earlier intestinal fistulation efforts employed the use of re-entrant cannulas (MacRae, 1975) to facilitate total collections and return of intestinal contents. Due to the alterations in intestinal digesta flow and motility as a result of re-entrant cannulas, feed intake was often reduced, and the type of diet had to be restricted to prevent cannula blockage.

With the introduction of indigestibility markers, total collection of intestinal flow was no longer required. Intestinal re-entrant cannulas were replaced by simple T-type cannulas. These cannulas appeared to have only minimal effects on feed intake and intestinal motility (Wenham and Wyburn, 1980). Representative sampling of intestinal contents became and still remains a major concern, especially with omasal and abomasal fistulas. This concern led Faichney (1975, 1980) to suggest the use of the double marker or two marker techniques to ensure that samples obtained from simple T-type cannulas were representative of the solids:liquid distribution in the intestine. If a duodenal digesta sample is representative of the digesta flow past the cannula, the ratio of the solid marker (e.g. lanthanum, La) to the liquid marker (e.g. Chromium-EDTA, Cr-EDTA) in the sample should equal the ratio in the feed. Stern and Satter (1982) have reported in one of their experiments that the marker ratio in the duodenum was 95% of the marker ratio in the feed, suggesting that cannula sampling was reasonably representative. Nevertheless, Faichney (1980) stresses that single markers should not be used to estimate the flow of digesta and its constituents when samples are taken from a simple cannula. However, techniques to mechanically

separate and proportionately re-combine liquid and solid digesta have not been adequately tested. This may be a more serious concern with longer-term vs recently cannulated animals. Wenham and Wyburn (1980), using radiological techniques, found that the internal flange of their cannulas caused a permanent dilation of the intestine which contractions were unable to occlude to maintain onward pressure. If dilation became severe, a mixing pool may result, complicating representative sampling as well as particle and liquid passage rate measurements.

Surgical preparation and admonitions are discussed by McGilliard (1982). The methods used to measure protein degradation in the rumen are described by Stern and Satter (1982). The latter review summarizes methodology quite well; however, some additional comments on the use of indigestibility markers are necessary.

Since the major criteria for a successful indigestibility marker are the requirements for steady state flow and representative sampling, the marker need not necessarily be exclusively associated with any particular phase of the digesta flow, which would qualify most markers for the purpose. In fact, intimate association of a particulate or liquid marker with its respective phase may exaggerate erroneous intestinal flow measurements if unrepresentative sampling occurs. Of course, unrepresentative sampling will affect nutrient flow measurements regardless of the choice of marker.

The suggestion that chromium sesquioxide (chromic oxide) does not appear to associate exclusively with either the solid or liquid phase, does not render it less useful as an indigestibility marker unless it exhibits radical diurnal variation in its flow from the rumen. Prigge

et al. (1981) compared once or twice daily dosing of chromic oxide or ytterbium chloride with total collection of feces in beef cows. Fecal grab samples were taken every 4 hr for 48 hr. Twice daily dosing decreased diurnal variation in marker excretion pattern, and both markers estimated fecal output not significantly different from total collection. Diurnal patterns may be more evident at duodenal or abomasal sampling sites. Laughren and Young (1979), when feeding rams twice daily, noted that the flow of chromic oxide to the duodenum was not closely associated with the flow of N to the duodenum as indicated by the variation in the N to chromic oxide ratio in duodenal digesta with respect to time of feeding. An optimum frequency for marker administration and for sampling may exist which would minimize diurnal marker flow from the rumen.

Factors Affecting Efficiency of Microbial Protein Synthesis and Feed Protein Digestion Within the Rumen

Roughage Level

As more starch from concentrate feeds are added to the diet, cellulose digestion in the rumen decreases (MacRae and Armstrong, 1969). This demonstrates one of the basic metabolic principles on which cultured bacteria operate, that two substrates may be used at the same time, but the presence of one may suppress the utilization of the other (Hobson, 1972). Mertens (1979) proposed some theories to explain depression of ruminal cellulose digestion by added dietary starch: (1) production of inhibitors by starch digesting

microorganisms, (2) decrease in ruminal pH due to the increased acid production from starch fermentation (combined with decreased salivation), (3) competition for essential nutrients with proliferation of starch digesters, (4) shift in the microbial population to starch digesters and (5) repression of cellulase when more readily available carbohydrate is supplied. It is important to consider these ideas when observing and interpreting effects of roughage level on protein digestion and efficiency of microbial protein synthesis in the rumen.

Studies conducted using artificial bags suspended in the rumen demonstrated that the roughage level in the diet had an effect on protein degradation in situ. Ganev et al. (1979) observed that the rate of degradation of vegetable protein sources placed in dacron bags was greater in the rumen of grass fed than barley fed sheep. When fish meal was suspended in bags in the rumen, no effect of the sheep diet on rate of in situ degradation was seen. This led the authors to propose that fiber barriers may limit vegetable protein degradation. Dietary roughage level would, therefore, have little influence on the degradation of fish meal since animal sources of protein contain little fiber. This seems reasonable since DM and N disappearance from feedstuffs suspended in bags in the rumen have been shown to be highly correlated (Nocek et al., 1979; Lindberg, 1981). Similar effects by roughage level on disappearance of SBM nitrogen from bags suspended in the rumen have been seen in steers (Mohamed and Smith, 1977) and in dairy cows when changing the diet from 25 to 40% roughage (Weakley et al., 1983). Lindberg (1981) also observed that DM and N disappearance from hay, fish meal, sugar beet pulp and soybean meal suspended in

nylon bags in the rumen of two cows were significantly affected by dietary roughage level. However, their dietary responses appeared somewhat erratic, possibly since dietary comparisons were not conducted concurrently.

Franklin et al. (1981) shed further light on this relationship when they discovered that increasing the proportion of concentrate in the diet decreased disappearance of bromegrass DM from nylon bags suspended in the rumen of sheep only if a concomitant decrease in ruminal pH occurred. They observed that bromegrass DM disappearance increased as ruminal pH increased from about 5.8 to about 6.6, disregarding possible changes in the microbial population since the level of forage in the diet also increased. An ingenious study by Loerch et al. (1983) employed the use of diets containing various ratios of corn to ensiled corn stalks. The corn was fed as either NaOH treated corn, that maintained a constantly high ruminal pH at all concentrate levels, or high moisture corn, which resulted in a depression in ruminal pH at increasing inclusion. They felt that this would allow study of roughage level effects on disappearance of protein sources from dacron bags both in the presence or absence of pH changes. As in the previously described study, roughage level effects on SBM nitrogen disappearance were much less pronounced with the feeding of NaOH treated corn, which resulted in less obvious ruminal pH changes. They concluded that shifts in microbial populations are not an important contributing factor in roughage level effects on protein degradation. However, a shift in the microbial population in response to added increments of dietary forage may not have occurred in the absence of a concomitant pH change in the rumen. Such an

occurrence would confound their interpretations. It is unfortunate that a taxonomic evaluation of the ruminal flora was not conducted.

In an approach, similar to that of Loerch et al. (1983), Lindberg (1981) found that changing the roughage in the basal diet from ammonia-treated straw to untreated hay did not change the general degradative pattern of the test protein sources. However, ruminal pH responses to feeding of the two diets were not as distinct as in the previously discussed work. An enlightening observation was made by Henning et al. (1980) upon feeding wethers diets containing various ratios of straw and corn grain. Diets were formulated to support adequate ruminal levels of branched-chain volatile fatty acids and ammonia. As the proportion of maize in the diet increased, the number of cellulolytic bacteria decreased along with a concomitant decline in the mass of cellulose digestion in the rumen. The interesting point is that this depression was not related to ruminal pH since pH was unaffected by the lower level inclusions of corn into the diet, even though a depression in cellulose digestion occurred. Therefore, if fiber barriers are limiting protein digestion, ruminal pH would not be the sole factor influencing protein degradation in the rumen. More will be discussed concerning ruminal pH effects in a later section of this review.

Whether the influence of pH on protein degradation is direct or indirect through microbial cellulolysis may be gleaned from study of the effect of pH on microbial cellulose digestion. Mertens (1979) proposed three mechanisms by which decreasing pH depresses fiber digestion: (1) pH sensitivity of cellulase, (2) alteration of microbial metabolic pathways or (3) selected reduction of fiber

digesting microbial populations. His summary of three studies of the direct effect of pH on fiber digestion suggested that the digestion was most extensive at pH between 6.7 and 7.1. Using dual effluent continuous cultures charged with a mixed diet and infused with various levels of HCl or NaOH, Kincaid et al. (1981) observed that digestion of acid and neutral detergent fiber (ADF and NDF, respectively) and total VFA production increased as pH increased to 6.5, then declined. The addition of barley to an in vitro mixed culture depressed cellulolysis and the titer of bacteria which degrade filter paper, only when the pH was allowed to fall (Stewart, 1977). Slyter (1981) also observed that VFA production, fiber digestion and cellulolytic numbers decreased at pH less than 6 in vitro. These results suggest that decreasing pH decreases cellulolytic numbers and cellulolysis. The effect of added dietary concentrates on depressing fiber digestion may not occur in the absence of a concomitant pH depression.

Although most of the studies of roughage level effects on protein degradation have been conducted in situ, limited work in vivo has been conducted. Zinn and Owens (1983) fed Angus steers, with dual re-entrant intestinal cannulas, an 80% concentrate diet in two experiments and a 40% concentrate diet in a third experiment. Although comparisons were made across experiments, soybean meal and cottonseed meal protein bypass was observed to be greater on the higher concentrate diet, in agreement with findings in situ. Escape of soybean meal protein from ruminal degradation was observed to be 48% greater with steers fed a high concentrate diet vs a 50% roughage diet in the same experiment (Weakley et al., 1983). An additional physical influence by roughage in the rumen may compliment the

previously proposed ruminal factors and contribute to the effect of roughage on protein degradation in animal experiments. Sriskandarajah et al. (1981) noted a positive relationship between the retention time of fiber and over-treated formaldehyde casein in the rumen of sheep. The formation of a fibrous mat in the rumen with roughage feeding may serve to retain particles in the rumen for further degradation.

Dietary roughage levels appear to affect not only protein degradation in the rumen but efficiency of microbial protein synthesis as well. The results from a number of studies have demonstrated that higher dietary roughage levels support more efficient use of organic matter by ruminal microbes in supporting protein synthesis (Theurer, 1979; Johnson and Bergen, 1982). Mathers and Miller (1981) observed an increase in microbial efficiency when sheep were transferred, stepwise, from an all barley to an all lucerne diet, which they claimed was not associated with rumen fluid dilution rate. Chamberlain and Thomas (1979) found similar results in sheep which they speculated may have related to a low ruminal pH or a low ruminal dilution rate with the feeding of high concentrate diets. It is interesting to note that Cole et al. (1976) observed an increase in microbial efficiency with as little as a 7% inclusion of cottonseed hulls to a whole shelled corn diet. Larger increases in microbial efficiency were observed at a 21% addition of cottonseed hulls.

A number of reviewers in the area of dietary roughage level effects on microbial efficiency have reported some interesting relationships. McMeniman et al. (1976), summarizing results from 19 diets, reported that the average number of grams of microbial N synthesized per 100 g OM actually digested in the rumen was 3.3 for

forage diets and 2.2 for diets high in cereals. In a similar compilation of a number of trials with cattle and sheep, Van Soest (1982) reported similar averages of 2.11 for concentrate diets, 2.51 for mixed diets and 3.03 for forage diets. Bergen et al. (1982) observed that microbial efficiency values obtained with forage diets and sheep were higher than with concentrate diets and with cattle. Harrison and McAllan (1980) noticed that most in vivo microbial efficiency estimates were obtained with sheep fed at maintenance, so that the larger than usual increase in ruminal dilution rate with forage addition to the diet may exaggerate efficiency differences.

Van Soest (1982) has suggested that the lower microbial efficiencies associated with concentrate feeding may reflect slower rates of passage relative to digestion rate in the rumen. The summary by Rode (1981) would lend support to this theory, as he noted a positive correlation between microbial efficiency and solids turnover rate from the rumen.

In vitro findings would question the importance of energy supply in this relationship. Isaacson et al. (1975) noted that supplying four levels of glucose concentrations to continuous fermenters had little effect on the yield of microbial cells/mole glucose fermented at a constant growth rate. Stern et al. (1978) found that increasing the nonstructural carbohydrate levels in isocaloric diets supplied to continuous cultures increased microbial efficiency, in contrast to what is observed in vivo. This would suggest a considerable involvement of the physical influences of roughage in the rumen on microbial efficiency.

The new ARC requirements (1980) have assumed a value of 30 g microbial N/kg OM apparently digested in the rumen for all diets whether given to cattle or sheep. They admit, however, that this value is not a biological constant. A precautionary mention should also be made that increased efficiency of microbial protein synthesis associated with higher roughage feeding will not necessarily increase the supply of microbial protein to the small intestine. Total available digestible OM supply in the rumen, required to support microbial protein synthesis, may be limiting with lower quality roughages, reducing the total quantity of microbial protein synthesized in the rumen.

Ruminal Nitrogen Supply

Recycling. Before considering the influence of ruminal nitrogen concentration and source on microbial protein synthesis, mention of nitrogen recycling both to and within the rumen aids in understanding nitrogen availability between meals with various diets. Isotope dilution techniques have been used extensively to study the movements of urea-N and ammonia-N through the body (Nolan and Leng, 1972; Nolan et al., 1976; Norton et al., 1982). The earlier trials were conducted with sheep fed small amounts of forage diets. More recent work (Oldham et al., 1980) has been conducted with lactating dairy cows fed at a moderately high intake level. All of these studies have proposed models of N flow through the body which make interesting reading.

To conclude that findings concerning the mode and method of nitrogen flux in and through the rumen are controversial and conflicting, is an understatement. Some excellent reviews on this subject are available (Houpt, 1970; Chalmers et al., 1976; Kennedy and Milligan, 1980).

In general, it appears that blood urea, originating from either ammonia absorption from the rumen or tissue protein metabolism, is recycled to the rumen via saliva and by direct diffusion through the ruminal wall. The relative importance of these two routes has been debated and appears to depend on a number of factors. In addition to the external recycling of nitrogen to the rumen, as much as one-third of the N entering the ruminal ammonia pool appears to be recycled within the rumen via conversions from ammonia to amino acids to microbial protein to amino acids and back to ammonia (Nolan and Leng, 1972; Mercer and Annison, 1976).

The earlier work of Nolan and Leng with sheep fed lucerne hay (1972) noted that the quantity of urea appearing as ruminal ammonia could be almost entirely accounted for by total salivary flow. They concluded that the movement of urea into the rumen by passage from blood through the rumen wall was insignificant in comparison. This contrasts with estimates of only approximately 15 to 50% of the urea transfer to the rumen via saliva in sheep fed hay diets (Kennedy and Milligan, 1980). The relative significance of this route, however, should depend on the rate of salivary secretion and plasma urea concentration, both of which will depend on diet.

Kennedy and Milligan's (1980) summary of tracer studies in sheep demonstrated that urea transfer to the rumen: (1) was negatively

related with ruminal ammonia concentration, (2) positively related with plasma urea concentration and (3) positively related with OM apparently digested in the rumen. Possible explanations for this will be discussed later. The relationship between plasma urea and ruminal ammonia is curvilinear, suggesting that saturation of a carrier transport system in the rumen wall is occurring at high plasma urea concentrations. A model proposed by Houpt (1970) helped to explain urea transport from plasma, through the rumen wall, into the rumen. Earlier work had shown that net urea transport in either direction across the rumen epithelium was approximately proportional to urea concentration differences, suggesting the lack of a transport mechanism. Based on the observation that urea flux across rumen pouches was depressed following rinsing, Houpt (1970) proposed a transport hypothesis based on the presence of rumen wall bound urease. Blood urea would enter the rumen epithelium and either diffuse into the rumen to be rapidly hydrolyzed to ammonia by rumen wall associated bacterial urease, or be hydrolyzed by epithelial urease, after which ammonia would diffuse into the rumen. In either case, prevailing ruminal pH would trap ammonia in the ammonium form. The apparent lack of accumulation of either urea or ammonia would allow for continued simple diffusion of urea through the rumen wall, controlled by increasing urease inhibition with increasing ruminal ammonia concentrations (Cheng and Costerton, 1980; Kennedy and Milligan, 1980). The discovery of a significant population of ruminal epithelium associated bacteria, actively secreting urease (Cheng and Costerton, 1980), lent considerable support to the Houpt model.

The amount of ammonia absorbed from the rumen increases when either ammonia concentration or pH increases in the rumen (Chalmers et al., 1976). The pK for ammonia is 9.3. This means that at a pH of 7.3, 1% of urea is in the un-ionized form. A lower ruminal pH favors the presence of ionized ammonia, which decreased absorption, since ammonia absorption is by non-ionic diffusion through the rumen wall (Smith, 1975). For this reason, a common treatment for ammonia toxicity is ruminal acidification to decrease ammonia absorption. As ruminal pH decreases, more ammonium will pass out with the digest to be absorbed post ruminally (Smith, 1975; Chalmers et al., 1976). Post-ruminal absorption may explain why blood urea peaks several hours after ruminal ammonia peaks (Smith, 1975).

When dietary nitrogen is low, ruminants can conserve N by decreasing urinary output of urea, making considerable use of recycled N for microbial protein synthesis in the rumen. Recycling to the rumen and use by microbes in the rumen will cause total duodenal N flow to exceed intake of N on low protein diets. Hume et al. (1970a) observed this to occur in sheep fed urea supplemented, protein-free diets with ruminal ammonia concentrations as high as 13 mg/dl. Kropp et al. (1977b) observed negative apparent ruminal N digestibility with urea or SBM supplemented cottonseed hull based diets. Prigge et al. (1978) observed that daily abomasal N flow exceeded N intake with steers fed lower protein, processed corn diets. Stern and Satter (1982) reported total non-ammonia N leaving the abomasum daily was in excess of N intake in a number of Wisconsin experiments with dairy cows fed diets of rather high N content, coming primarily from true protein.

Concentration. Animal responses to dietary non-protein nitrogen (NPN) are presumably dependent on limited availability of N in the rumen. Responses occur when ammonia supply is below the microbial requirement. Supplemental NPN is not well utilized with dairy rations which contain more than 12 to 13% crude protein (Satter and Roffler, 1975). The in vitro work of Satter and Slyter (1974) demonstrated that when microbial N flow peaked, ammonia began to accumulate in the fermenters, precluding the usefulness of additional NPN. Considerable controversy exists concerning the optimum concentration of ruminal ammonia above which microbial protein production no longer increases. The value of 5 mg ammonia-N/100 dl rumen fluid (Satter and Slyter, 1974) determined in vitro has gained considerable popularity.

Literature estimates, however, range from 1.4 mg/dl with pure in vitro cultures (Schaefer et al., 1980) to as high as 23.5 mg/dl. The latter was required to maximize rate of fermentation of barley in polyester bags suspended in the rumen of sheep (Mehrez et al., 1977).

Incremental additions of urea or protein to low protein diets usually (1) increase efficiency of or total protein production by microbes or (2) increase dry matter digestion. Efficiency or total protein production by microbes plateaued at ruminal ammonia concentrations of 12 mg/dl with bull calves fed barley based diets (Leibholz, 1980), 8.5 mg/dl with cows fed corn based diets (Kang-Meznarich and Broderick, 1980), 7 mg/dl with wethers fed a semipurified diet (Okarie et al., 1977) and 2.2 mg/dl with steers fed 70% concentrate diets (Slyter et al., 1979). When sheep were fed

protein-free diets plus four levels of urea (Hume et al., 1970a), a trend for continued increases in efficiency of microbial protein synthesis occurred over the entire range of ruminal ammonia concentrations from 6.3 to 30.7 mg/dl rumen fluid. In this same study, concentrations of ruminal ammonia that supported maximum ruminal OM digestion were between 6.3 and 8.8 mg/dl. Oldham and Smith (1982) predicted that a 1% increase in the protein content of a dairy cow diet containing up to 18% crude protein would increase DM digestibility by 1 percent. Caution should be exercised in comparing microbial responses to ruminal ammonia concentrations across studies since such results can be confounded with differences in diet, response criteria and analytical methods.

In contrast to the previous studies, a number of workers have observed no microbial or digestibility responses to changed ruminal ammonia concentrations. Ruminal ammonia concentrations ranging from 6.3 to 27.5 mg/dl were observed to have little influence on the disappearance of corn, SBM, oat straw or alfalfa hay from dacron bags suspended in the rumen of cows infused with various levels of urea (Ortega et al., 1979). This suggests that all levels may have been adequate to support maximum DM disappearance in the rumen. Ruminal ammonia concentrations ranging from 1.9 to 12.7 mg/dl had no effect on rate of microbial synthesis or flow of bacterial N to the abomasum of calves fed corn based diets supplemented with three levels of supplemental SBM (Veira et al., 1980). Redman et al. (1980) fed steers an oaten chaff diet plus various protein sources. They observed little difference in the efficiency of microbial protein synthesis over the narrow range in ammonia concentrations of 3.4 to

8.8 mg/dl. Other work (Laughren and Young, 1979) with rams fed corn cob and corn based diets supplemented with four levels of SBM demonstrated no significant differences in amount of microbial protein synthesis among diets ranging from 8 to 17% crude protein.

Various ideas have been proposed to explain the great variation observed among estimates of minimum concentrations of ruminal ammonia required to support maximum microbial efficiency. Smith (1979) pointed out that variations in cell populations and permeability make it improbable that one concentration of ruminal ammonia would support maximum growth under all conditions. Orskov (1982) speculated that different substrates may require different concentrations of ammonia to achieve an optimum microbial yield. The optimum concentration for bacteria may be similar, but different ammonia concentrations in rumen fluid may be required to ensure adequate distribution throughout the rumen, especially when a roughage pad is present. In addition, response criteria among studies has differed. The rather high ammonia requirement (23.5 mg/dl) observed by Mehrez et al. (1977) was for optimum rate of digestion while other lower estimates were from measurements of microbial yield. Requirements for both functions may not necessarily be equal. This is further demonstrated by work with steers fed ground corn diets, where ruminal ammonia levels above 3 mg/dl appeared adequate for efficient protein synthesis by ruminal microbes but higher levels appeared to increase digestion of OM in the rumen (Weakley and Owens, 1983).

Pure cultures of ruminal bacteria grown in vitro have demonstrated maximum bacterial growth rates at very low concentrations of ruminal ammonia (Schaefer et al., 1980). Bacteria can efficiently

capture ammonia via glutamine synthetase when concentrations are low (Erfle et al., 1977). However, bacterial cell yields may not be maximized at low ammonia concentrations since bacteria must expend one mole of ATP for every mole ammonia fixed via glutamine synthetase. This information led Hespell (1979) to suggest that ruminal ammonia concentration cannot be affecting bacterial growth directly since concentrations as low as .25mM do not reduce bacterial growth rate in vitro. Rather, he felt that the beneficial effects of higher ammonia concentrations observed in vivo on cell yields and DM digestibility are probably an indirect effect of ammonia on other factors, such as ruminal pH. Smith (1979) suggested that bacteria may accumulate a labile N reserve which may buffer against fluctuations in supply or may use only the unionized form of ammonia. In vitro incubation studies with ^{15}N labeled maize (Nikolic and Filipovic, 1981) may lend support to this hypothesis. At low vs high in vitro concentrations of ammonia, the relative amount of N in the free ammonia pool derived from maize was decreased while the fractional turnover rate of the ammonia pool was increased, suggesting that a deficient supply of ammonia is compensated by an increased catabolism of N compounds derived from rumen microorganisms. Wallace (1979) speculated that hydrolytic bacteria may require higher ammonia concentrations either for effective ammonia assimilation by an unknown mechanism involving alanine or for full expression of enzyme activity.

Smith (1979) questioned whether ruminal ammonia supply becomes limiting with daily feeding. Since most ammonia is trapped in the rumen in the ammonium form at normal ruminal pH, a considerable amount of ammonia absorption probably would occur only after digesta has

flowed out of the rumen. With once or twice daily feeding of NPN and normal N intakes, quite high concentrations of ammonia may remain in the rumen and not necessarily be limiting or wasted.

Source. Considerable attention has been given to the possible relationship between solubility properties of proteins and their degradation in the rumen. Initial consideration should be directed to the methods of measuring protein solubility. Solvents commonly used are Burrough's buffer, autoclaved rumen fluid, McDougall's buffer, sodium chloride solutions, hot water, dilute sodium hydroxide and borate-phosphate buffer (Broderick, 1982; Krishnomoorthy et al., 1982). These solvents differ in pH, ionic strength and other factors important in solubilizing protein (Waldo and Goering, 1979), potentially yielding different estimates of protein solubility. This was well demonstrated by Waldo and Goering (1979) who observed a significant interaction between protein solubility and analytical method for 15 feeds by three methods. Furthermore, Satter et al. (1977) stressed that particle size and density, heat or chemical exposure or integrity of plant cell walls also can affect solubility measurements. Such differences can result in a rather wide variation in apparent solubility measurements within a feed class.

Generally, insoluble protein fractions are attacked more slowly than more soluble protein fractions and NPN. However, earlier workers had suggested a strong relationship between protein solubility and the total ruminal degradation of feedstuffs. Many feed companies accepted these earlier suggestions and formulated feedstuffs on the basis of

solubility. Unfortunately, protein solubility and ultimate ruminal degradability are not necessarily synonymous. Soybean meal, with a relatively low solubility, is often degraded in the rumen quite extensively (Kropp et al., 1977). Ovalbumin, a very soluble protein, is slowly degraded in the rumen (Mangan, 1972), possibly due to its cyclic chemical structure. Serum albumin and ribonuclease were found to be resistant to hydrolysis, although both proteins were very soluble (Mahadevan et al., 1980). Soluble proteins from soybean meal, rapeseed meal and casein were found to be hydrolyzed at different rates. Mahadevan et al. (1980) implicated disulfide bond cross-linking as one property of proteins conferring resistance to degradation. Some workers have attempted to resolve this problem by assuming that, in vivo, 100% of the soluble proteins and 40 to 50% of the insoluble proteins in feedstuffs are degraded in the rumen (Tamminga, 1979). In light of the previous information, such assumptions would not appear to be valid for all feedstuffs.

In conclusion, correlation studies between protein solubility and degradation demonstrate that solubility explains only one-third to one-half of the total variation in protein degradation in the rumen. Since many factors can influence feed protein degradation in the rumen (microbial types, particle size and density, particle wettability, feed intake, feeding frequency, ruminal turnover rate and diet), it is unlikely that any simple laboratory analysis, that does not consider many or most of these factors will satisfactorily predict protein degradation in the rumen.

Just as the source of N fed can influence the amount of protein degraded in the rumen, the source of N from which bacteria derive

their N requirements may influence their efficiency of protein synthesis. This belief was initially fostered by observations on the relative importance of ammonia and amino acids in meeting microbial N needs. A number of ^{15}N infusion studies have demonstrated that only 50 to 80% of the bacterial N is derived from ammonia (Mercer and Annison, 1976). The balance presumably is derived from amino acids. Peptides and amino acids are needed in vitro as precursors for branched-chain fatty acids for a number of bacterial species, particularly the cellulolytics (Bryant, 1973; Russell and Hespell, 1981). The branched-chain fatty acids are required by these species for synthesis of branched-chain amino acids, incorporation into higher fatty acids and components of cellular lipids (Allison, 1965; Bryant, 1973). Various forms of N shown to be required for optimum growth of some of the predominant culturable ruminal bacteria have been described by Bryant and Robinson (1962).

Recent work (Cotta and Russell, 1982) was conducted with five species of ruminal bacteria grown in vitro in continuous culture. Growth was maintained at a constant rate while the levels of amino acids and peptides were varied. Additions of amino acids increased the efficiency of energy use for synthesis of microbial protein. However, greater additions of amino acids were not associated with their efficient conversion to bacterial protein. Maeng and Baldwin (1976) observed a similar response upon supplementing small amounts of amino acids to replace urea in an in vitro media derived from a cow consuming a purified diet containing urea as the only source of nitrogen. A considerable improvement resulted in both yield and efficiency of microbial protein synthesis.

Some of the earlier in vivo research addressing this topic were the studies of Hume (1970b; 1970c). The diets fed in both these studies were virtually protein free, semipurified, based primarily on oat hulls, starch and sucrose. In one of the experiments (Hume, 1970b) urea was added to two diets to maintain adequate ruminal ammonia concentrations, while one of them was supplemented with an additional mixture of higher volatile fatty acids (VFA). While daily microbial protein production was increased with VFA supplementation, no influence was observed on efficiency of microbial protein synthesis. In a companion study (Hume, 1970c) the semipurified basal diet was supplemented with either 100% of the added N as urea or 50% of the added N as casein, gelatin or zein, the balance derived from urea. The all urea supplemented diet received a mixture of branched-chain VFA so that all diets would supply approximately the same amount of branched-chain VFA in the rumen. Both total daily yield of microbial protein and efficiency of microbial protein synthesis was decreased with the urea/VFA and gelatin supplemented diets. They suggested that this may have resulted from an inadequate supply or synthesis of one or more limiting amino acids for microbial growth.

Ben-Ghedalia et al. (1978) fed wethers a purified diet where either 100% of N was from urea, or 10% of the urea N was replaced by casein, maize gluten or fish meal. Efficiency of microbial N production was greatest with the maize gluten diet. No response was observed to casein supplementation, possibly due to the low level of replacement and rapid ruminal degradation. In other work (Jacobs and Leibholz, 1977), efficiency of energy use for microbial protein

synthesis was greater with SBM or uric acid than urea supplemented semipurified diets fed to calves.

Not all in vivo measurements of efficiency of microbial protein synthesis have demonstrated a response to supplemental N source. Most of these studies have been conducted with animals fed high roughage diets. In studies with steers fed high roughage diets, no improvement in efficiency of microbial protein synthesis was observed by supplementing urea supplemented basal diets with casein (Redman et al., 1980; Sriskandarajah et al., 1982) or SBM (Kropp et al., 1977a). In fact, in all of the above studies, urea supplemented high roughage diets produced slightly greater microbial efficiency. In studies with sheep fed high roughage diets, efficiency of microbial protein synthesis was not significantly different between urea or casein supplemented diets (Kempton et al., 1979; Leibholz and Kellaway, 1979). In studies with higher concentrate diets fed to sheep (Mercer et al., 1980) and steers (Smith et al., 1978), again, no improvement in efficiency of microbial protein synthesis was observed from substituting a variety of protein sources for urea.

In all experiments where improvements in efficiency of microbial protein synthesis were observed in response to protein replacement of urea N, purified or semipurified diets were fed. No such benefits have been observed with natural diets, suggesting that amino acid or branched-chain VFA supplementation of conventional diets may be of little benefit as long as N supply in the rumen is adequate to meet microbial N requirements. Teather et al. (1980) observed that urea was as effective as SBM in supporting numbers of cellulolytic bacteria with cows fed a basal diet of corn silage and concentrate. They

speculated that either VFA were not limiting microbial growth or added NPN in the diet had a sparing effect on the dietary precursors of VFA that are essential growth factors. Salter et al. (1979) infused ^{15}N urea into the rumen of steers fed straw diets supplemented with either groundnut meal or urea to determine the origin of bacterial nitrogen. They found no evidence that branched-chain amino acids were incorporated directly from the diet into bacterial protein to a greater extent than other amino acids. This would suggest that the required carbon skeletons were available in adequate amounts with both diets. Rather, these workers suggested that methionine and phenylalanine may be limiting bacterial growth on high NPN diets.

Therefore, it appears that benefits in bacterial growth to amino acid replacement of NPN in vitro or with purified diets in vivo, are not apparent with natural diets in vivo. Since many species of the bacteria population excrete amino acids that benefit symbionts that require amino acids (Allison, 1982), there is little reason to suspect a bacterial amino acid deficiency in mixed cultures. However, if any nutrient essential to the growth of bacteria is limiting, energy uncoupling supposedly can result, during which fermentation continues to produce ATP without a concomitant use of ATP for anabolic processes, resulting in an inefficient use of energy by bacteria (Hespell, 1979). In cases where amino acid addition to ammonia containing media stimulates cell yield, Hespell (1979) feels this results from decreased energetic uncoupling rather than from decreased ATP use for amino acid biosynthesis, since the cost for amino acid biosynthesis is small.

Ruminal pH

The influence of pH on ruminal bacteria has been studied with both isolated and mixed cultures in vitro. The effect of pH on cellulolytic organisms and cellulolysis was discussed previously, with respect to its role in the effect of roughage level on protein degradation. Russell et al. (1979) studied the effect of pH on on the growth rate of five pure cultures of rumen bacteria. The ranking of the species with respect to growth rate was pH dependent, suggesting that pH may determine competition among bacterial species in the rumen. Earlier work by Esdale and Satter (1972) demonstrated the effect of pH alteration on in vivo and in vitro VFA production. Volatile fatty acid production in vivo was not affected between pH 6.2 and 6.8, but acetate production was inhibited below a pH of 6.2. Similar results were observed in vitro where conditions other than pH were controlled. Changes in the proportion of VFA in the rumen are often observed when buffering agents are added to diets (Allison, 1976; Davis, 1979). It remains to be determined how much these changes reflect (1) shifts in microbial populations or (2) alterations in metabolic pathways used by microorganisms.

A similar controversy exists concerning the influence of pH on protein degradation in the rumen. Most work done in this area has been conducted in situ and in vitro. Okeke et al. (1983b) suspended bags containing SBM in the rumen of steers fed high or medium concentrate diets supplemented with three levels of buffer to change ruminal pH. A strong positive correlation existed between ruminal pH 4 hr after feeding and N disappearance from SBM placed in nylon bags

and suspended in the rumen for 24 hr. In a companion study these same workers (Okeke et al., 1983a) measured the in situ disappearance rate of SBM in cows fed medium roughage diets supplemented with two levels of sodium bicarbonate. Both the rate at which the slowly degraded SBM N fraction disappeared from nylon bags and passage rate of chromium mordanted SBM from the rumen were positively related to ruminal pH. They suggested that these two opposing factors, increased rate of degradation and decreased ruminal residence time, counteracted one another so that calculated SBM bypass remained relatively unchanged by buffer addition.

The question still remains concerning whether the interaction of pH with protein degradation is via: (1) affecting the solubility of feed protein, (2) reproportioning microbial species, (3) altering proteolytic activity of existing species. In his review, Tamminga (1979) stated that the optimum pH for proteolysis and deamination is between 6 and 7, being negligible below pH 4.5. In continuous culture, where pH was modified by infusions of artificial saliva and sodium carbonate, protease activity measured in bacteria maintained at pH 5 was only 22% of that of bacteria at pH 7 (Erfle et al., 1982). Proteolytic organisms were apparently washed out of the continuous culture when pH was below 6. The resulting decreased supply of amino acids in the culture may have contributed to the decreased measured cellulolytic activity below pH 6. The in situ work of Loerch et al. (1983), mentioned previously in this review, addressed this question more directly. Two cows were fed NaOH treated corn or high moisture corn (HMC) at 20, 40, 60 or 80% of the DM of an ensiled corn stalk diet. The NaOH treated corn maintained a higher and constant ruminal

pH over all levels of inclusion while ruminal pH decreased with increasing HMC inclusion in the diet. Various protein sources were placed in dacron bags and exposed in the rumen. Protein solubility at various pH was also measured in vitro. The mean 12 hr in situ N disappearance, averaged for all proteins and corn levels, was 54.5% with NaOH treated corn feeding and 47.7% with HMC feeding. Rates of N disappearance from SBM and dehydrated alfalfa meal were affected more by corn treatment and level than N disappearance from blood meal (BM), meat and bone meal (MBM) or corn gluten meal (CGM). Since SBM solubility in vitro and in situ degradation were both reduced at low pH, while BM and CGM in vitro solubility and in situ degradation were low and relatively unchanged at all pH, these workers concluded that protein solubility had more impact on ruminal degradation than alterations in bacterial populations or proteolytic activity. A taxonomic evaluation of predominant species of bacteria at extremes in pH would have aided in interpretation of these results.

Few studies are available evaluating the influence of pH on ruminal digestion or efficiency of microbial protein synthesis in vivo. A summary of two studies (Weakley and Owens, 1983a) involving steers fed ground corn diets and equipped with ruminal and intestinal cannulas demonstrated that efficiency of microbial protein synthesis was quite variable and not related to ruminal pH ranging from 5.8 to 6.7. However, a depression in ruminal OM digestion was observed at the extremes in ruminal pH, with a tendency for maximum ruminal OM digestion at a ruminal pH of approximately 6.3. Streptococcus bovis, one of the more prominant ruminal species with high starch diets, was observed to demonstrate its greatest growth rate in pure

culture at a pH of approximately 6.4 (Kistner et al., 1979), agreeing well with the effect of pH on the ruminal digestion of ground corn observed in the previous study. More in vivo work in this area is needed.

Level of Intake and Ruminal Outflow Rate.

The extent of protein degradation in the rumen is a function of microbial degradation of feed protein and retention time in the rumen. Zinn and Owens (1980) estimated that for every 10% increase in feed intake above maintenance, a 6.5% increase in feed protein bypass results. A portion of this can be explained by the direct influence of intake on protein passage rate from the rumen. When cows were fed a mixed diet at an intake level of three time maintenance, the flow rate (%/hr) of chromium-mordanted soybean meal, cottonseed meal and rapeseed meal from the rumen was an average of 40% greater than when the diet was fed at maintenance (Lindberg, 1982).

The influence of intake on ruminal degradation of feed protein and efficiency of microbial protein synthesis has been observed in vivo, but the exact mechanisms involved are difficult to identify due to the inadequate ability to control ruminal factors in vivo. Increasing the intake of a high concentrate diet, stepwise, from 1.2 to 2.1% of body weight in steers resulted in an increase of feed N bypass from 44 to 71% of fed N (Zinn and Owens, 1983). Efficiency of microbial protein synthesis increased as intake increased to 1.8% of body weight but decreased slightly at 2.1% of body weight, presumably due to a deficiency of degraded and recycled N for meeting microbial N requirements. Work with wethers maintained in warm or cold

environments (Kennedy et al., 1982), demonstrated that cold exposure increased escape of dietary N by 4 and 9% of the dietary N intake for lucerne and brome diets, respectively, presumably through a thyroid mediated increase in digestive tract motility. Environment had little effect on the bypass of fed N with a barley based diet.

It is uncertain whether the effect of increased intake (and the increased concomitant outflow of digesta from the rumen) on increasing efficiency of microbial protein synthesis is due to increases in ruminal outflow of liquid, of solids or of both. The effect of liquid dilution rate on efficiency of microbial protein synthesis has been demonstrated in vitro and in vivo. The early in vitro work of Isaacson et al. (1975) revealed that as dilution rate increased, efficiency of microbial growth increased as a result of dilution of maintenance expenditures. In a chemostat, however, all bacteria should flow with the liquid. Increases in ruminal turnover, for whatever reason, have been observed to increase efficiency of microbial protein synthesis in steers fed cottonseed hull based diets (Kropp et al., 1977), in steers fed processed corn diets (Prigge et al., 1978), in sheep fed pelleted bromegrass and exposed to cold (which increased ruminal turnover of both solids and liquid) (Kennedy and Milligan, 1978) and in sheep fed a mixed diet and infused with artificial saliva (Harrison et al., 1975).

Considerable discussion has occurred attempting to explain the effect of increased dilution rate on increased efficiency of microbial protein synthesis. It has been demonstrated that a lower proportion of ATP produced is used for maintenance functions as dilution rate and growth rate increase (Isaacson et al., 1975). Further, as dilution

rate increases, the mean age of the microbial population presumably is decreased due to a greater washout rate and these younger cells have a growth potential lacking in mature cells (Van Soest, 1982). At greater dilution rates there also may be a decline in cell density and concentration of inhibitory products as well as a reduction in bacterial autolysis and engulfment by protozoa, all contributing to increased efficiency of growth (Kennedy et al., 1976; Bergen et al., 1982).

In assessing the affect of intake on microbial efficiency, it is possible that turnover of ruminal solids may be as significant an influence as turnover of ruminal liquids. This consideration becomes even more important since 50 to 75% of the ruminal bacterial population is associated with feed particles (Cheng and Costerton, 1980). The effect of passage rate of solids has been studied in dual flow continuous cultures. In one experiment (Crawford et al., 1980) dual flow continuous cultures were operated with solids retention times (SRT) of 14, 22 and 30 hr and dilution rates (D) of .07, .11 and .15 volumes/hr. Efficiency of microbial protein synthesis tended to increase with decreasing SRT, while at all three levels of SRT, increasing D had no positive effect on microbial efficiency. The authors suggested that the dilution rates studied may have been above the range in which dilution rate has been shown to have a positive effect on microbial efficiency. In similar work (Hoover et al., 1982), dual flow continuous cultures were operated at a constant D (.06 volumes/hr) while SRT of 23, 28 or 40 hr were imposed. Changing SRT had no effect on efficiency of microbial protein synthesis, although there was a trend of decreasing microbial efficiency as SRT

was increased. More in vivo work on the effect of solids retention time on microbial efficiency is needed.

Amino Acid Composition of Protein Escaping Ruminal Digestion.

Models to predict the flow of amino acids to the small intestine will be considerably less complicated if the amino acid composition of ruminally undegraded feed protein entering the intestine is similar to the amino acid composition of the protein fed. Examination of 19 feedstuffs revealed that marked differences were observed between the amino acid profile of the total and insoluble protein fraction (MacGregor et al., 1978). Such was not the case with soybean meal, however. Since the soluble protein fraction is generally assumed to be completely degraded in the rumen, profiles of fed proteins and their ruminally undegraded residues may differ. However, solubilization conditions in vitro are most probably unlike those observed in the rumen.

In situ exposure of feedstuffs in the rumen is probably the most direct approach to this question. Such evaluations depend heavily on the assumption that microbial amino acid contamination of residues is insignificant. Microbial contamination is probably of little concern with concentrates but may be of greater concern with roughages studied in situ (Mathers and Aithchison, 1981). There was little evidence of selective amino acid degradation from 16 hr in situ degradation of soybean meal in cows fed mixed diets (Weakley et al., 1983b) or from 9 hr in situ degradation of soybean meal, groundnut meal or sunflower meal in sheep fed barley or dried grass (Ganev et al., 1979). In

another experiment (Crooker et al., 1981) soybean meal, cottonseed meal, fishmeal, linseed meal, distillers dried grains, wheat bran or alfalfa meal were suspended in the rumen of cows fed a mixed diet. Changes in amino acid profiles after digestion in situ were variable among feedstuffs. Threonine and leucine, however, tended to resist ruminal degradation more than other amino acids in all feedstuffs tested.

Although not as lucid as in situ disappearance findings, examination of the amino acid composition of digesta leaving the rumen yields some indication of the disappearance of amino acids in the rumen. For such results to be extrapolated to ruminal influences on degradation of feed protein, one must assume that the bulk amino acid composition of bacteria or protozoa is not affected by diet. This assumption may be fairly valid (Bergen et al., 1968). In many cases, investigations of the amino acid profile of protein flowing to the small intestine was relatively constant despite feeding of a variety of protein sources (Hagemeister et al., 1976; Oldham et al., 1977; Ben-Ghedalia et al., 1978; MacRae and Reeds, 1980).

However, some work in vivo has suggested that amino acid profiles of feedstuffs (or possibly bacteria) change following ruminal digestion. Digestion studies conducted with cattle and sheep have suggested that the degradation of lysine, histidine and arginine in the rumen was more extensive than degradation of other amino acids (Stern and Satter, 1982). In two other experiments with cows fed brome hay or mixed diets supplemented with four different protein sources (Arambel and Coon, 1981) or sheep fed a mixed diet supplemented with four levels of soybean meal (Laughren and Young,

1979), the essential to nonessential amino acid ratio was increased at the duodenum. Such results could suggest a possible natural chemical protection of some amino acids in feedstuffs or specific rather than mixed protease secretion by bacteria. The factor confounding any conclusions from such studies is that the contribution and turnover of amino acids from the microbial protein fraction is unknown.

CHAPTER III

INFLUENCE OF ROUGHAGE LEVEL ON SOYBEAN MEAL PROTEIN DEGRADATION AND MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

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Summary

Three experiments were conducted to determine the influence of dietary roughage level on the degradation of soybean meal (SBM) in the rumen of steers and release of amino acids from SBM placed in dacron bags and suspended in the rumen. In Exp 1, bags containing SBM or meat meal (MM) were placed in dacron bags and suspended in the rumen of steers fed either a high concentrate (C), high roughage (R) or mixed (M) diet. Disappearance of SBM nitrogen (N) and dry matter (DM) from the bags was greatest with R, least with C and intermediate with M, suggesting that fiber barriers may be limiting protein degradation of vegetable protein sources. Differences due to diet were very small with MM, an animal protein source. In Exp 2, four dairy steers (225 kg) fitted with ruminal and duodenal cannulas were fed a concentrate

or roughage-based diet with or without added SBM to determine the influence of roughage level on escape of SBM protein from ruminal degradation. Escape of SBM protein from ruminal degradation was 13.8% with a high roughage diet and 20.4% with a high concentrate diet. Efficiency of microbial protein synthesis was 19% greater with the high roughage diets ($P<.10$). Ruminal and total tract digestion of starch was not radically affected by including as much 13 to 54% of the DM as roughage. Acid detergent fiber (ADF) digestion in the rumen was greater with SBM than with urea supplementation on either a high concentrate or high roughage diet ($P<.05$), possibly due to addition of specific microbial growth factors with SBM. In Exp 3, dacron bags containing SBM or .15 N NaCl extracted SBM (ESBM) were suspended in the rumen for 4, 14 or 24 h with steers fed a high roughage or high concentrate diet. Undigested samples and digested residues were analyzed for 18 amino acids. Disappearance of total amino acids from bags was greater with the high roughage diet. The pattern of amino acid disappearance from SBM and ESBM appeared to be similar as in situ digestion time increased and was affected little by diet or extraction of the soluble protein fraction.

Results may be interpreted to indicate that dietary roughage level affects protein degradation in the rumen through alterations in ruminal retention time, pH and shifts in microbial species. Further, the SBM protein reaching the small intestine that has escaped microbial degradation in the rumen may not be extensively different in amino acid composition from that fed.

Introduction

Considerable effort has been expended in chemically or physically modifying protein in feedstuffs to increase their resistance to microbial degradation and loss in the rumen (Chalupa, 1975; Ferguson, 1975). Increased escape or bypass of dietary protein should increase productivity of ruminants under some conditions. Relatively less effort, however, has been expended in understanding the various factors which influence the ruminal environment and alter both protein degradation in the rumen and efficiency of microbial protein synthesis. Several workers have listed estimates of the extent of ruminal degradation of feedstuffs (Chalupa, 1975; Satter et al., 1977; Klopfenstein et al., 1982), though application to other feeding practices remains unclear. Recent work (Ganev et al., 1979; Zinn and Owens, 1983) suggests that protein degradation does not proceed at a constant rate but is subject to dietary influences.

Efficiency of microbial protein synthesis, likewise, is quite variable as indicated by recent reviews (Czerkawski, 1978; Stern et al., 1979). Some of this variation can be attributed to the analytical techniques employed in estimation, but animal and dietary influences probably are involved also (Johnson and Bergen, 1982; Van Soest, 1982).

In vivo experimentations on the influence of dietary roughage level on ruminal protein degradation and efficiency of microbial protein synthesis are essential to improve the predictive capabilities of the various metabolizable protein models as discussed in a recent symposium (Owens, 1982). Furthermore, such models eventually must be

refined from total non-ammonia nitrogen to metabolizable amino acid supply to the small intestine. This will require more detailed information concerning the amino acid composition of feed protein escaping destruction in the rumen and reaching the small intestine.

The objectives of these three studies were to evaluate the influence of dietary roughage level on protein degradation in the rumen and efficiency of microbial protein synthesis. The dacron bag procedure was used to examine the in situ change in amino acid composition of undigested SBM protein when fed in conjunction with concentrate and roughage diets.

Materials and Methods

Exp 1

The effect of dietary roughage level on disappearance of protein from vegetable and animal sources was studied in dacron bags suspended in the rumen of three Hereford steers (635 kg) equipped with ruminal cannulas. Each steer was fed a diet of either high concentrate (C), high roughage (R) or a mixture of the two (M; table 1). Daily intake of dry matter was limited to approximately 1.8% of body weight. Animals were fed every 12 h. Duplicate dacron bags containing approximately 1 g of soybean meal (SBM) or meat meal (MM) were suspended in the rumen for 12 or 24 h for each of the three diets. Bag construction and procedures are described by Weakley et al. (1983b). After the bags were washed, dried for 24 h at 105 C and weighed, Kjeldahl nitrogen (AOAC, 1975) was determined on each bag plus its residue to determine nitrogen (N) disappearance.

Exp 2

Four dairy steers (225 kg), each equipped with a ruminal cannula and a T-cannula in the duodenum proximal to the bile duct, were used to measure the influence of dietary roughage level on SBM protein degradation in the rumen. Each animal received a high concentrate diet without (C2) or with added SBM (CSBM) or a high roughage diet without (R2) or with added SBM (RSBM) in a 4 x 4 latin square (table 2). Urea was added to diets not supplemented with SBM to avoid deficiency of ruminal ammonia for maximum microbial activity. Diet R2 could not be supplemented with a sufficient enough amount of urea to raise the dietary protein above the NRC (1976) recommended maintenance level due to ammonia toxicity in one animal even though the amount of urea added was below the toxicity level suggested by Hungate (1966). Chromic oxide was included as an indigestible marker.

The difference in nitrogen flow at the duodenum between diets with vs without added SBM at each roughage level was used as a measure of the proportion of SBM nitrogen escaping ruminal degradation. However, since SBM replaced corn in the diet, bypass of corn protein had to be assumed. It was assumed that the additional corn protein in the unsupplemented diets was 40% degraded in the rumen (Satter et al., 1977); however, the difference in protein attributable to added corn is only about 20% of the protein contributed by the added soybean meal. Daily intake of dry matter was limited to 1.6% of body weight to avoid unequal feed intakes which can confound SBM protein bypass calculations. Diets were offered every 12 h and were readily

consumed. In each of the four nine-day periods, the first five days were for adaptation to the diet.

On days 6 through 8, approximately 250 ml of duodenal digesta and 200 g wet feces were collected 2 and 8 h post-prandially. Samples from each animal within each period were composited on an equal wet basis and dried for 48 h in a 60 C oven. Feed samples were obtained prior to each sampling day and composited within each diet and period. All samples were ground in a Wiley mill fitted with a 1 mm screen and stored for future analysis. On the ninth day of each period, contents from the rumen were withdrawn 2 and 8 h post-prandially, strained through four layers of cheese cloth and 250 ml was saved for ammonia-N analysis. The pH was measured and 1 ml 20% H_2SO_4 was added per 50 ml strained fluid. During two of the periods, an additional 1500 ml of strained rumen fluid were collected in iced flasks 8 h post-prandially, for isolation of bacteria. This fluid was strained through 4 layers of cheesecloth, centrifuged at 200 g for 5 min to remove feed particles and protozoa and the supernatant fluid centrifuged at 30,000 g for 15 min to precipitate bacteria. The pellet was washed twice, once with .9% saline and once with water and recentrifuged at 30,000 g for 15 min after each washing. The pellet was then lyophilized and stored for nucleic acid analysis.

Feed, duodenal and fecal samples were analyzed for dry matter (DM; 105 C for 24 hr), ash (600 C for 12 hr), Kjeldahl nitrogen (N; AOAC, 1975), starch (MacRae and Armstrong, 1968), acid detergent fiber (Goering and Van Soest, 1970) and chromium (Fenton and Fenton, 1979). Ammonia N and pH measurements were obtained from the liquid rumen samples. Ammonia N also was measured on the dried, ground duodenal

samples. Ammonia N was measured with 40 ml rumen fluid distilled over magnesium oxide in a macro-Kjeldahl flask (AOAC, 1975). Lyophilized bacteria and dried duodenal samples were analyzed for nucleic acid-N by the procedure of Zinn and Owens (1982) for use as a bacterial marker to quantitate daily microbial nitrogen passage from the rumen. Calculations were based on comparisons of daily duodenal nucleic acid N flow with content of N and nucleic acid-N in bacterial samples collected in two of the four periods of feeding. Calculations of daily bacterial N flow in the remaining two periods were based on the average of the individual bacterial values (bacterial N=7.31% of bacterial DM; bacterial nucleic acid N=17.8% of total bacterial N). Daily amounts of non-microbial DM and N flowing past the duodenal cannula were calculated by subtracting the microbial contribution from the total. Daily duodenal organic matter (OM) flow, corrected for microbial contributions, was calculated from the corrected duodenal DM flow x duodenal OM percentage.

Means were compared by Duncan's multiple range test protected by a significant F test calculated from analysis of variance for a 4 x 4 latin square (Steel and Torrie, 1960). Orthogonal comparisons were also analyzed to determine overall effects by dietary roughage level or SBM supplementation.

Exp 3

The effect of dietary roughage level on the disappearance of amino acids from SBM suspended in dacron bags in the rumen was studied with four ruminally cannulated Hereford steers (635 kg). Two animals were fed a high concentrate diet (C3) while the other two were fed a

high roughage diet (R3; table 3). Diets were fed every 6 h at a daily dry matter intake equal to 1.8% of body weight.

Triplicate dacron bags containing SBM or .15 N NaCl extracted SBM (ESBM) were suspended in the rumen of each of 4 steers for 4, 14 or 24 h. Bag construction and procedures are described by Weakley et al. (1983b). After removal from the rumen, bags were washed and dried for 48 h at 55 C. The residues removed from duplicate bags as well as undigested substrates were analyzed for 18 amino acids. Seventeen of the amino acids were measured by automatic amino acid analysis while tryptophan was measured by bioassay. Relative and total disappearance of amino acids were calculated from composition of the undigested substrates and digested residues. The SBM employed was derived from a bulk supply screened to have a particle size between 50 um and 2 mm. Half of this was extracted for 6 h in 39 C, .15 N NaCl solution to remove soluble protein. Relative amino acid disappearance from SBM and ESBM should reflect the contribution of the soluble protein fraction. In addition, certain residues of ESBM remaining after 4 or 14 h ruminal exposure were subjected to nucleic acid analysis (Zinn and Owens, 1982) to assess the degree of contamination of residues by attached bacteria.

The additional duplicate bags containing SBM or ESBM, concurrently exposed in the rumen of the same animals for the same exposure periods, were washed, dried for 24 h at 105 C and the bag and contents analyzed for N by macro-Kjeldahl (AOAC, 1975) and DM and N disappearance rates were calculated.

To determine the nature of the ruminal environment of the steers used in the in situ study, rumen contents were sampled 1 and 3 h

post-prandially and strained through 4 layers of cheese cloth, pH was measured and samples acidified (1 ml 20% H_2SO_4 per 50 ml fluid). Frozen samples were later analyzed for ammonia N by distillation over magnesium oxide (AOAC, 1975).

Means were compared by Duncan's multiple range test protected by a significant F test calculated from analysis of variance for a 4 x 4 latin square (Steel and Torrie, 1960). Orthogonal comparisons were also analyzed to determine overall effects by dietary roughage level or substrate type. Residues were additionally analyzed for linear or quadratic effects by exposure time on amino acid composition.

Results and Discussion

Exp 1

Disappearance of SBM N from dacron bags placed in the rumen for 12 or 24 h appeared greater with steers fed roughage (R), least with concentrate (C) fed steers and intermediate with steers receiving a mixed diet (table 4). Though disappearance ranked similarly with the three diets for meat meal (table 4), differences due to diet were only about one-fifth as great. Ruminal pH for steers fed diets R, M and C were 6.71, 6.53 and 6.41, respectively.

The differences in ruminal degradation due to diet may be associated with specific microbial or chemical characteristics of the ruminal fermentation. Rate of degradation of vegetable protein sources placed in dacron bags was greater in the rumen of sheep fed grass than sheep fed barley (Ganev et al., 1979). But, when fish meal was tested, no effect of the sheep diet on rate of in situ degradation

was seen. This led the authors to propose that fiber barriers may limit vegetable protein degradation in the rumen. A similar effect was observed in diary cows, where changing the diet from 25 to 40% roughage increased the extent of disappearance of SBM N from dacron bags suspended in the rumen (Weakley et al., 1983b).

Since roughage level appears to affect the disappearance of vegetable but not animal sources of protein from dacron bags (Exp 1; Ganev et al., 1979), it has been suggested that increased cellulolytic activity observed with higher roughage diets (Stewart, 1977) may be exposing more fiber bound protein for microbial degradation. This seems reasonable since DM and N disappearance from feedstuffs suspended in bags in the rumen have been shown to be highly correlated (Nocek et al., 1979; Lindberg, 1981).

Addition of roughage to diets usually increases ruminal pH, another factor which can influence protein degradation. Okeke et al. (1983b) suspended bags containing SBM in the rumen of steers fed high or medium concentrate diets supplemented with three levels of buffer to change ruminal pH. A strong positive correlation existed between ruminal pH 4 h after feeding and N disappearance from bags suspended in the rumen for 24 hr. In continuous culture, where pH was modified by infusion of artificial saliva and sodium carbonate, protease activity measured in bacteria maintained at pH 5 was only 22% of that of bacteria at pH 7 (Erfle et al., 1982). Wohlt et al. (1973) demonstrated the profound effect pH can have on protein solubility. Therefore, pH can influence the solubility of feed protein as well as ruminal capacity for microbial proteolysis.

The extent to which pH changes are responsible for roughage level effects on protein degradation in the rumen was investigated by Loerch et al. (1983). Two cows were fed NaOH treated corn or high moisture corn (HMC) at 20, 40, 60 or 80% of the DM of an ensiled corn stalk diet. The NaOH treated corn maintained a higher and more constant ruminal pH over all levels of inclusion while ruminal pH decreased with increasing HMC inclusion in the diet. Various protein sources were placed in dacron bags and exposed in the rumen. Protein solubility at various pH was also measured in vitro. The mean 12 h in situ N disappearance, averaged for all proteins and corn levels, was 54.5% with NaOH treated corn feeding and 47.7% with HMC feeding. Roughage level effects on SBM N disappearance were much less pronounced with the feeding of NaOH treated corn, which resulted in less obvious ruminal pH changes. Rates of N disappearance in situ from SBM and dehydrated alfalfa meal were affected more by corn treatment and level than N disappearance from blood meal, meat and bone meal (MBM) or corn gluten meal (CGM). Since both SBM solubility and in situ degradation were reduced at low pH, while with BM and CGM, solubility and in situ degradation were low and relatively unchanged by pH, these workers concluded that protein solubility had a greater impact on ruminal degradation than alterations in bacterial populations or proteolytic activity. The evidence to support this conclusion is circumstantial, however, since no direct evidence was demonstrated concerning the effect of roughage level on microbial activity in the absence of a pH change in the rumen. Unfortunately, microbial activity and pH are both intertwined with dietary roughage level.

Work by Stewart (1977) and Franklin et al. (1981) has demonstrated that addition of concentrates to diets depressed cellulolysis in vitro and in situ, respectively, only if pH was allowed to fall. Modifying pH and microbial activity independently is extremely difficult. So, absolute statements concerning the relative importance of various factors on ruminal protein degradation appears imprudent. Taxonomic evaluations of the ruminal microbiota in such studies should strengthen conclusions.

A change in pH could have indirect or direct effects on ruminal proteolysis. Franklin et al. (1981) recently illustrated that increasing the proportion of concentrate in the diet decreased the disappearance of bromegrass DM from nylon bags suspended in the rumen of sheep only when ruminal pH dropped below about 6.1. Altered microbial types or activities may alter proteolytic activity. As solubility of certain proteins in alfalfa and soybean meal decrease as pH decreases, rate of immediate loss of protein from dacron bags could be altered directly by pH as suggested by Loerch et al. (1983). In contrast, solubility of other protein sources such as meat meal and fish meal are less sensitive to pH, and solubility of protein from corn grain will increase as pH drops. Since the fraction of total protein lost in situ considerably exceeds the soluble fraction, it is unlikely that solubility differences alone can explain the total in situ effect.

An additional physical influence by roughage in the rumen may compliment the previously proposed ruminal factors and contribute to the effect of roughage on protein degradation in animal experiments. Sriskandarajah et al. (1981) noted a positive relationship between the

retention time of fiber and over-treated formaldehyde casein in the rumen of sheep. The formation of a fibrous mat in the rumen with roughage feeding may serve to retain particles in the rumen for further degradation.

Exp 2

The influence of feeding a high concentrate or high roughage diet with or without supplemental SBM on ruminal parameters is shown in table 5. Roughage feeding (R2 and RSBM) produced higher ruminal pH, while microbial proteolysis and deamination of supplemental SBM in diets CSBM and RSBM resulted in greater ruminal ammonia N concentrations.

Intake of OM was similar with all diets (table 6). The amount of total or non-microbial OM leaving the abomasum and rectum daily was greatest with R2, resulting in the least ($P<.05$) OM digestion in the rumen and total tract. This was probably due to the low concentration of ammonia N in the rumen (2.4 mg/dl) with this diet. Kropp et al. (1977b) fed cottonseed hull diets supplemented with SBM or various levels of urea. They found that the lowest concentration of ruminal ammonia (3.7 mg/dl) did not depress ruminal OM digestion but decreased total tract OM digestion. The use of greater amounts of concentrate in the present study may have increased the need for ruminal ammonia, since microbes may have approached closer to their maximum potential growth rates. Digestion of OM in the rumen, both adjusted and unadjusted for microbial OM, and in the total tract was greater ($P<.05$) with the concentrate diets (C2 and CSBM). Digestion of OM in the rumen and total tract was increased ($P<.05$) by SBM addition.

However, the proportion of total tract OM digestion occurring in the rumen was similar for all diets, with the exception of the unsupplemented roughage diet (R2). The 75 to 79% of total tract OM digestion apparently occurring in the rumen observed with the other three diets is larger than the 60 to 63% calculated from a summary of a number of experiments involving all forage to 90% concentrate diets (Johnson and Bergen, 1982). The extensively rolled form of the corn fed in Exp 2 may have increased ruminal digestion. Daily flow of chyme at the duodenum was greater with steers fed the higher roughage diets. This would parallel greater saliva flow and buffer input into the rumen, stabilizing pH.

Nitrogen intake was greater with the diets supplemented with SBM (table 7). Ruminal output of microbial N tended to be depressed with R2, even though efficiency of synthesis tended to be high, since a smaller amount of OM digestion occurred in the rumen with this diet. The ruminal ammonia concentration (2.4 mg/dl) observed with this diet is below the 5 mg/dl suggested as being required to achieve maximum microbial N production in vitro (Satter and Slyter, 1974). Duodenal N flow exceeded intake with the R2 diet. Ruminal N digestion, prior to adjustment for microbial N contributions, was low with all diets and negative with R2. Duodenal N passage often exceeds N intake especially, but not exclusively, with low crude protein rations (Hume et al., 1970a; Kropp et al., 1977b; Prigge et at al., 1978; Stern and Satter, 1982). When corrected for the bacterial N contribution, true ruminal N digestibility with all diets becomes positive. Digestion of N in the rumen and total tract was greater ($P<.01$) with the SBM supplementation and lower ($P<.01$) with higher roughage in the diet,

with an interaction ($P<.05$) existing between both factors. Kropp et al. (1977b) noted a similar increased ruminal and total tract N digestion when urea was added to a cottonseed hull diet. Unaccounted endogenous N losses should comprise a smaller proportion of the fecal and ruminal N output with higher N intakes, resulting in greater estimated digestibility of feed nitrogen.

Efficiency of microbial protein synthesis (table 6) was greater ($P<.10$) with the higher roughage diets. Similar relationships previously have been reported with sheep (Chamberlain and Thomas, 1979; Mathers and Miller, 1981) and steers (Cole et al., 1976b). Faster ruminal liquid dilution rates, which can result from increased salivation with roughage feeding, have been shown to support greater efficiency of microbial protein synthesis in vitro (Isaacson et al., 1975), though in the rumen from 50 to 80% of the bacteria are associated with particles, not fluid (Cheng and Costerton, 1980). Additional growth factors, removal of wall-adhering microbes, a more stable ruminal environment or decreased uncoupling might be involved, as well.

Intake of starch was greater with the higher concentrate diets (table 8). But the percentage of digestion of starch in the rumen was not affected by dietary roughage level or SBM addition. In the present study, the least amount of roughage addition was about 13 percent, which may be above the range where the greatest depression in ruminal starch digestion occurs. Even though digestion in the total tract was significantly affected by diet, differences were small. Total tract starch digestion was slightly depressed ($P<.10$) with

higher roughage in the diet and slightly increased ($P<.05$) by SBM addition.

Acid detergent fiber (ADF) digestion data are shown in table 9. Including as little as 8% corn in straw diets fed to sheep (Henning et al., 1980) or 33% barley in hay diets fed to sheep (MacRae and Armstrong, 1969) has depressed cellulose digestion in the rumen, previously. Hence, a depression in ADF digestion was expected with C2 and CSBM diets. However, ruminal ADF digestion was not significantly affected by any of the diets or roughage addition, in particular. Since the least amount of corn addition to any diet was 22%, ADF digestion may have been in the suboptimum range with all diets. Alternately, at this relatively low level of feed intake, ruminal pH was not depressed greatly by addition of grain (table 5). Only when pH dropped below about 6.1 has fiber digestion been depressed in situ and in vivo (Franklin et al., 1981).

Digestion of ADF, both in the rumen and total tract, was greater ($P<.05$) with the addition of SBM to either the high roughage or high concentrate diets. Kropp et al. (1977b) also observed an increase in ruminal digestion of cellulose when SBM was substituted for urea in cottonseed hull diets. One possible explanation relates to additional amino acids provided by the soybean meal. Peptides and amino acids have been demonstrated as precursors for branched-chain fatty acids required for growth of certain ruminal species of bacteria, particularly the cellulolytics (Bryant, 1973; Russell and Hespell, 1981). Since most of the protein in the unsupplemented diets was derived primarily from corn and urea, increased activity of

cellulolytic ruminal bacteria may have been due to amino acid derivatives from SBM.

From 81 to 100% of the total tract digestion of ADF occurred in the rumen in this trial (table 9). This compares with 80 to 97% of total cellulose digestion observed to occur in the rumen of high roughage fed steers (Kropp et al., 1977b) and 93 to 97% of total cellulose digestion observed to occur in the rumen of high concentrate fed steers (Cole et al., 1976a). These values add confidence that sampling and marker procedures were working satisfactorily in this experiment.

Based on the differences in N flow leaving the abomasum between diets not supplemented or supplemented with SBM and assuming that 60% of the additional corn protein in the unsupplemented diets bypassed ruminal destruction, 13.8 and 20.4% of the SBM protein intake escaped ruminal degradation with the high roughage and high concentrate diets, respectively. These values are lower than the 20% observed with cottonseed hull based diets (Kropp et al., 1977b) or 24 and 43% observed with 40 and 80% concentrate diets, respectively (Zinn and Owens, 1983b). The lower intake levels in the present study may have allowed for more extensive ruminal degradation.

Escape of SBM protein from ruminal degradation was, therefore, 48% greater with the higher concentrate diet. The results from Exp 1 and those of Ganev et al. (1979) discussed earlier demonstrated a similar effect by roughage level on in situ degradation of SBM protein. Other comparisons of roughage level on in vivo protein bypass have been made across different experiments (Zinn and Owens,

1983b). In those studies, increasing concentrate level from 40 to 80% increased bypass by 79% for SBM and 16% for cottonseed meal.

The influence of roughage level on ruminal protein degradation in vivo would reflect not only the factors influencing rate of ruminal proteolysis, such as pH and microbial species, but also time for proteolysis, as influenced by ruminal residence time. If one were to assume that rate of degradation in the rumen (k_d) and rate of passage (k_p) were independent, then extent of escape or bypass of protein (B) would fit the following equation described by Miller (1973): $B = k_p/(k_d+k_p)$. This equation would apply only to the fraction being degraded at an intermediate rate and not be applicable to either the immediately soluble or the nondigestible fraction. Figure 1 illustrates this relationship. As dilution rate increases, escape increases, but the magnitude of the effect of dilution rate on escape depends on the size of k_d.

Exp 3

The effect of feeding a high concentrate (C3) or high roughage diet (R3) on ruminal pH and ammonia concentration in steers in which dacron bags were exposed is shown in table 10. Both ammonia N concentration and pH were elevated by roughage feeding. Before subjecting the in situ digested residues from SBM and .15 N NaCl extracted SBM (ESBM) to amino acid analysis, selected samples of ESBM, obtained before and after digestion, were analyzed for nucleic acids to determine the extent of microbial N contamination. Results in table 11 show that residues remaining after 14 h in situ digestion demonstrated only a slight increase in nucleic acid content over the

initial basal amount in ESBM. This suggests that microbial N contamination of ESBM may be only as high as 6% of the feed N (assuming 20% of bacterial N is nucleic acid N; Smith, 1969). Mathers and Aitchison (1981) found that microbial contamination of another protein source, fish meal, after 24 h exposure in situ was less than 2% of the residual N, but nearly 20% with alfalfa. Extent of washing of the bags and feed residues following in situ digestion will certainly affect the degree of contamination with bacteria.

Disappearance of DM, N and amino acids from SBM and ESBM placed in dacron bags and suspended in the rumen of steers fed diets of concentrate (C3) or roughage (R3) is shown in table 12. The amino acid disappearance values in table 12 were calculated as the total of the 18 amino acids analyzed.

This information is represented graphically in figures 2 and 3. As observed in Exp 1, disappearance of DM, N and amino acids from SBM placed in dacron bags and suspended in the rumen was greater with the higher roughage diet (R3). Dietary influences on DM, N and amino acid disappearance were small before 4 h digestion, but increased in magnitude until 14 h digestion. This occurred even after the soluble N fraction had been removed (ESBM) and suggested that with higher roughage diets, increased protein degradation is due to factors other than, or in addition to, pH modification of protein solubility alone. If disulfide linkages hinder microbial attack as suggested by Mahadevan et al. (1980), protein structure could control degradation independent of pH. Figure 4 illustrates the close relationship between DM and amino acid disappearance in the rumen ($R=.98$; $N=24$; $P<.01$). This supports the suggestion that losses of amino acids and

DM from dacron bags are related. Whether this relationship exists for protein supplements in the dynamic rumen is unknown.

The essential and non-essential amino acid composition (expressed as percentage of total amino acids) of SBM and ESBM, shown in figures 5 and 6, respectively, appears little influenced by extraction of the soluble N component, suggesting that the more complete ruminal digestion of soluble protein from soybean meal may not necessarily contribute to changes in the amino acid profile of the remaining residue. Previously, MacGregor et al. (1978) observed a marked difference between the amino acid profile of the total and insoluble protein fraction among 19 feedstuffs. In soybean meal, however, all of the amino acids were divided to the same extent between the two protein fractions, emphasizing the possibility that the patterns in amino acid disappearance observed in Exp 3 with SBM, may not pertain to a broad class of feedstuffs. Nevertheless, if only a small fraction is solubilized, its amino acid pattern may have little impact on the composition of the insoluble fraction.

Figures 7 through 10 represent the essential and nonessential amino acid survival from ruminal degradation as SBM and ESBM were exposed in dacron bags in the rumen of steers fed high concentrate (figures 7 and 8) or high roughage (figures 9 and 10) diets. The outer ring of these figures represents the undigested material or 100% survival. Each ring progressing inward represents an increased in situ residence time and amino acid degradation. The rings became closer to the center when roughages were fed (figures 9 and 10) than when concentrates were fed (figures 7 and 8), demonstrating greater extent of degradation. Amino acid survival of SBM and ESBM are

compared on the same figure with each diet, so that the influence of soluble N extraction on amino acid degradation can be assessed. Overall, degradation of the insoluble fraction (ESBM) was less than with intact SBM since the insoluble fraction represents the fraction generally considered to be less rapidly degraded in the rumen. Removal of the soluble N fraction accounted for a majority of the earlier (4 h) disappearance and compositional changes, especially when steers were fed a concentrate diet, as evidenced by the close proximity of the 0 and 4 h exposure time rings with ESBM in figures 7 and 8. A high correlation of protein solubility in .15 M NaCl with 2 h in situ disappearance was reported by Crawford et al (1978).

Concentricity among rings reflect similarity in amino acid composition as digestion occurs, while nonconcentricity reflects a shift in amino acid composition. With the possible exception of lysine, cystine and tryptophan, rings are generally concentric for both SBM and ESBM with both diets. Cystine and tryptophan survival appeared slightly higher (11 and 22%) than the mean while lysine survival was 6% below the mean.

In order to assess this statistically, analysis of variance was performed on the amino acid profile of the undigested SBM and ESBM and their digested residues. Changes in each amino acid's proportion of the total amino acids, will reflect any selective susceptibility or resistance to ruminal degradation. Significant differences were identified by Duncan's multiple range test and presented in tables 13 and 14 for SBM and tables 15 and 16 for ESBM with a concentrate or roughage diet, respectively. Immediately apparent is the low variability, as evidenced by low SE, which greatly increases the

opportunity for identifying many significant differences, regardless of their biological importance. Preferential disappearance of glutamic acid may contribute to the relative increases in some of the other amino acids. Differences in lysine and tryptophan survival, apparent in the figures, were established in the early times of digestion.

Extraction of the soluble N fraction (ESBM) did not appear to influence these relationships, supporting the earlier contention that, with SBM, more complete ruminal digestion of the more soluble protein fraction has little impact on the amino acid composition of ruminally digested residues.

The averages of the values in tables 13 through 16 are presented in table 17, along with evaluations for effects by roughage, substrate or time. Both dietary roughage level and substrate type influenced the average essential and non-essential composition of residues ($P<.05$). In addition, linear and quadratic effects by in situ digestion time were also present ($P<.05$). Differences were small and may not be biologically important.

Work by others have observed little evidence of selective amino acid degradation from 16 h in situ degradation of soybean meal in cows fed mixed diets (Weakley et al., 1983b) or from 9 h in situ degradation of soybean meal, groundnut meal or sunflower meal in sheep fed barley or dried grass (Ganev et al., 1979). In another experiment, however, changes in amino acid profiles after in situ digestion of SBM, cottonseed meal, fishmeal, linseed meal, distillers dried grains, wheat bran or alfalfa meal were apparent but quite variable among feedstuffs (Crooker et al., 1981). Threonine and

leucine tended to resist ruminal degradation more than other amino acids in all feedstuffs tested in their study. Similar effects were detected in our study.

Whether these relationships exist in the dynamic rumen is difficult to ascertain. Synthesis of microbial amino acids complicated the analysis. In many cases, the amino acid profile of protein flowing to the small intestine was relatively constant despite feeding of a variety of protein sources (Hagemeister et al., 1976; Oldham et al., 1977; Ben-Ghedalia et al., 1978; MacRae and Reeds, 1980). However, digestion studies conducted with cattle and sheep have suggested that the degradation values for lysine, histidine and arginine in the rumen were 38, 21 and 22 percent more extensive than degradation of the mean of other amino acids (Stern and Satter, 1982). Such differences, even for soybean meal, were much greater than the in situ data would imply. In two other experiments with cows fed brome hay or mixed diets supplemented with four different protein sources (Arambel and Coon, 1981) or sheep fed a mixed diet supplemented with four levels of soybean meal (Laughren and Young, 1979), the essential to nonessential amino acid ratio was slightly greater at the duodenum than in the diet. In this experiment, the essentials as a percentage of total amino acids increased from 46 to 47% at 24 h, largely a result of a decrease in glutamic acid.

Results from these experiments demonstrate that protein bypass to the small intestine is greater with feeding of a high concentrate diet. The amino acid composition of bypassed SBM protein reaching the small intestine did not differ greatly from that fed. At what level of dietary concentrate extent of proteolysis drops and whether this

relationship exists at various levels of intake and with different types of diets merits further study.

TABLE 1

COMPOSITION OF DIETS USED
IN EXP 1

	Diet[a]		
	C	M	R
	Percentage of dry matter		
Dry rolled corn (IFN 4-02-931)	62	31	0
Ground prairie hay (IFN 1-03-185)	0	41	83
Cottonseed hulls (IFN 1-01-599)	14	7	0
Soybean meal (IFN 5-04-604)	10	13	15
Ground alfalfa hay (IFN 1-00-063)	6	3	0
Molasses (IFN 4-04-696)	6	3	0
Trace mineralized salt[b]	.5	.5	.5
Dicalcium phosphate (IFN 6-01-080)	.5	.5	.5
Limestone (IFN 6-02-632)	.5	.5	.5
Crude protein, %[c]	12.5	12.5	12.5

[a] C=concentrate diet, M=mixed diet, R=roughage diet.
[b] Morton Salt Co., Chicago, IL 60606.
[c] Dry matter basis.

TABLE 2

COMPOSITION OF DIETS USED
IN EXP 2

	Diets[a]			
	C2	CSBM	R2	RSBM
	Percentage of dry matter			
Dry rolled corn (IFN 4-02-931)	82.1	61.6	41.5	22.3
Chopped prairie hay (IFN 1-03-185)	8.1	7.9	48.3	48.0
Soybean meal (IFN 5-04-604)		21.2		20.9
Supplement				
Cottonseed hulls (IFN 1-01-599)	5.16	5.21	5.68	4.93
Dicalcium phosphate (IFN 6-01-080)	1.23	1.24	1.22	1.17
Limestone (IFN 6-02-632)	.47	.48	.47	.45
Urea	.60	0	.51	0
KCl	.58	.58	.57	.55
Na_2SO_4	.50	.50	.49	.48
Trace Mineralized Salt[b]	.50	.50	.49	.48
Molasses (IFN 4-04-696)	.50	.51	.50	.48
Chromic oxide	.25	.25	.25	.24
Vitamin A[c]	.01	.01	.01	.01
Vitamin D[d]	.002	.002	.002	.002
Crude protein, total %[e]	10.3	17.3	8.4	15.2
From soybean meal, % of total	0	59.7	0	66.5

[a] C2=concentrate diet, CSBM=concentrate diet plus soybean meal
R2=roughage diet, RSBM=roughage diet plus soybean meal.
[b] Morton Salt Co., Chicago, IL 60606.
[c] Vitamin A=30,000 USP/g.
[d] Vitamin D=15,000 IU/g.
[e] Dry matter basis.

TABLE 3

COMPOSITION OF DIETS USED
IN EXP 3

	Diet[a]	
	C3	R3
	Percentage of dry matter	
Dry rolled corn (IFN 4-02-931)	62	
Ground prairie hay (IFN 1-03-185)		83
Cottonseed hulls (IFN 1-01-599)	14	
Soybean meal (IFN 5-04-604)	10	16
Ground alfalfa hay (IFN 1-00-063)	6	
Molasses (IFN 4-04-696)	6	
Trace mineralized salt[b]	.5	.3
Dicalcium phosphate (IFN 6-01-080)	.5	.3
Limestone (IFN 6-02-632)	.5	.3
Crude protein, %[c]	13.1	13.6

[a] C3=concentrate diet, R3=roughage diet.
[b] Morton Salt Co., Chicago, IL 60606.
[c] Dry matter basis.

TABLE 4

DISAPPEARANCE OF DRY MATTER (DM) AND NITROGEN (N) FROM SOYBEAN MEAL (SBM) OR MEAT MEAL (MM) PLACED IN DACRON BAGS AND SUSPENDED IN THE RUMEN OF STEERS FED A CONCENTRATE (C), MIXED (M) OR ROUGHAGE (R) DIET (EXP 1)

		Substrate and diet						
		SBM			MM			
	Exposure time, h	C	M	R	C	M	R	SE
Dry matter disappearance, %[a]	12	64	71	78	39	39	41	7.2
	24	74	89	93	42	41	42	9.9
Nitrogen disappearance, %[a]	12	60	67	80	50	53	56	4.5
	24	64	88	95	52	56	55	7.5

[a] Each value is the average of 2 observations

TABLE 5

RUMINAL PARAMETERS IN STEERS FED A HIGH CONCENTRATE DIET WITHOUT (C2) OR WITH ADDED SOYBEAN MEAL (CSBM) OR A HIGH ROUGHAGE DIET WITHOUT (R2) OR WITH ADDED SOYBEAN MEAL (RSBM) (EXP 2)

	Diets				
Item	C2	CSBM	R2	RSBM	SE
Ammonia-N, mg/dl	5.6[c]	18.9[a]	2.4[c]	12.8[b]	.95
pH	6.55[c]	6.44[c]	6.93[a]	6.77[b]	.04

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

TABLE 6

ORGANIC MATTER DIGESTION IN STEERS FED A HIGH CONCENTRATE DIET WITHOUT (C2) OR WITH ADDED SOYBEAN MEAL (CSBM) OR A HIGH ROUGHAGE DIET WITHOUT (R2) OR WITH ADDED SOYBEAN MEAL (RSBM) (EXP 2)

	Diets				
Item	C2	CSBM	R2	RSBM	SE
Intake	3410	3403	3397	3410	7.6
Leaving abomasum, g/d					
Total	1307^{bc}	1231^{c}	1861^{a}	1435^{b}	93.7
Non-microbial	1017^{c}	882^{c}	1621^{a}	1191^{b}	71.0
chyme, 1/d	26^{c}	27^{c}	40^{a}	35^{b}	1.1
Ruminal digestion					
% unadjusted	62^{a}	64^{a}	45^{b}	58^{a}	2.7
% adjusted[e]	70^{ab}	74^{a}	52^{c}	65^{b}	2.0
Ruminal digestion, % of total	78^{ab}	75^{ab}	66^{b}	79^{a}	3.4
Feces, g/d	705^{c}	516^{d}	1068^{a}	909^{b}	43.1
Post-ruminal digestion, % of entering	45^{b}	58^{a}	41^{b}	36^{b}	2.4
Total tract digestion, %	79^{b}	85^{a}	69^{d}	73^{c}	1.2

^{abcd} Means in a row with different superscripts differ statistically ($P<.05$).

[e] Adjusted for microbial organic matter.

TABLE 7

NITROGEN (N) DIGESTION IN STEERS FED A HIGH CONCENTRATE DIET WITHOUT (C2) OR WITH ADDED SOYBEAN MEAL (CSBM) OR A HIGH ROUGHAGE DIET WITHOUT (R2) OR WITH ADDED SOYBEAN MEAL (RSBM) (EXP 2)

	Diets				
Item	C2	CSBM	R2	RSBM	SE
Intake, g/d	59[c]	100[a]	49[d]	90[b]	1.4
From SBM, g/d	0	59.7	0	59.9	
From corn, g/d	46.9	35.7	24.0	13.2	
Leaving abomasum, g/d					
Total N	53[b]	61[ab]	57[ab]	62[a]	2.3
Microbial N	23[ab]	25[a]	21[b]	23[ab]	.82
Non-ammonia non-microbial	27[b]	33[ab]	33[ab]	35[a]	1.7
Corn adjusted escape, total g/d[e]	20.3	32.6	26.5	34.6	
Ruminal digestion, %					
% unadjusted	10[b]	39[a]	-16[c]	31[a]	2.9
% adjusted[f]	54[b]	67[a]	33[c]	62[a]	1.9
SBM escape, %		20.7		13.8	
Microbial efficiency, g microbial N/kg OM truly digested in rumen	9.6[b]	9.9[ab]	13[a]	11[ab]	.87
Ruminal digestion, % of total	15[b]	48[a]	-29[c]	41[a]	5.0
Feces, g/d	20[bc]	19[c]	22[ab]	23[a]	.59
Post-ruminal digestion, % of entering	62[b]	68[a]	61[b]	63[b]	.67
Total tract digestion, %	66[c]	81[a]	55[d]	75[b]	.92

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

[e] Assumes 60% of corn protein escapes ruminal degradation.

[f] Adjusted for microbial and ammonia nitrogen.

TABLE 8

STARCH DIGESTION IN STEERS FED A HIGH CONCENTRATE DIET WITHOUT (C2) OR WITH ADDED SOYBEAN MEAL (CSBM) OR A HIGH ROUGHAGE DIET WITHOUT (R2) OR WITH ADDED SOYBEAN MEAL (RSBM) (EXP 2)

	Diets				
Item	C2	CSBM	R2	RSBM	SE
Intake, g/d	2148[a]	1679[b]	1136[c]	679[d]	13.7
Leaving abomasum, g/d	374[a]	319[a]	279[a]	131[b]	41.4
Apparent ruminal digestion, %	83	81	76	81	2.5
Ruminal digestion, % of total	89[a]	84[b]	83[ab]	85[b]	2.8
Feces, g/d	140[a]	54[b]	98[ab]	37[b]	17.6
Post-ruminal digestion, % of entering	59[ab]	84[a]	60[b]	70[ab]	7.8
Total tract digestion, %	94[ab]	97[a]	91[b]	94[ab]	1.2

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

TABLE 9

ACID DETERGENT FIBER DIGESTION IN STEERS FED A HIGH CONCENTRATE DIET WITHOUT (C2) OR WITH ADDED SOYBEAN MEAL (CSBM) OR A HIGH ROUGHAGE DIET WITHOUT (R2) OR WITH ADDED SOYBEAN MEAL (RSBM) (EXP 2)

	Diets				
Item	C2	CSBM	R2	RSBM	SE
Intake, g/d	425[b]	447[b]	1088[a]	1115[a]	9.7
Leaving abomasum, g/d	215[c]	169[c]	587[a]	431[b]	44.9
Apparent ruminal digestion, %	50	62	46	62	4.8
Ruminal digestion, % of total	94[b]	98[b]	81[a]	100[a]	7.0
Feces, g/d	199[b]	166[b]	481[a]	432[a]	20.5
Post-ruminal digestion, % of entering	6.7	1.8	18	-0.6	6.5
Total tract digestion, %	53[b]	63[a]	56[ab]	62[ab]	2.6

[abc] Means in a row with different superscripts differ statistically (P</05).

TABLE 10

RUMINAL PARAMETERS IN STEERS FED HIGH CONCENTRATE (C3) OR HIGH ROUGHAGE (R3) DIETS (EXP 3)

	Diet		
	C3	R3	SE
Ammonia-N, mg/dl	10.94	12.30	1.28
pH	5.98	6.42	.15

TABLE 11

NUCLEIC ACID-N CONTENT OF NACL EXTRACTED SOYBEAN MEAL AND RESIDUES FOLLOWING DIGESTION 4 OR 14 HR IN DACRON BAGS (EXP 3)

	Digestion time in situ, hr			
	0	4	14	SE
Nucleic acid-N, % of dry matter	.099	.102	.192	.04
Nucleic acid-N, % of total nitrogen	1.12	1.12	2.34	.32

TABLE 12

DISAPPEARANCE OF SOYBEAN MEAL (SBM) OR .15 N NACL EXTRACTED SOYBEAN (ESBM) FROM DACRON BAGS PLACED IN THE RUMEN OF STEERS FED HIGH CONCENTRATE (C3) OR HIGH ROUGHAGE (R3) DIETS (EXP 3)

		Substrate and diet				
		SBM		ESBM		
	Exposure time, hr	C3	R3	C3	R3	SE
Dry matter disappearance, %[a]	4	38	40	10	17	4.0
	14	54	80	31	64	5.5
	24	64	92	44	85	6.1
Nitrogen disappearance, %[b]	4	23	26	18	24	1.1
	14	42	79	31	64	5.1
	24	48	95	41	89	6.2
Amino acid disappearance, %[c]	4	28	33	8	16	3.8
	14	41	80	22	69	8.8
	24	56	96	34	88	9.5

[a] Each value is the average of 6 observations.
[b] Each value is the average of 4 observations.
[c] Each value is the average of 2 observations.

TABLE 13

AMINO ACID COMPOSITION OF SOYBEAN MEAL BEFORE OR 4, 14 OR 24 HR AFTER RUMINAL DIGESTION IN DACRON BAGS PLACED IN THE RUMEN OF STEERS FED A CONCENTRATE DIET (EXP 3)

	In situ exposure time, hr				
Amino acid	0	4	14	24	SE
	Percentage of total amino acids				
Essential (EAA)					
Lysine	6.37^{a}	5.99^{b}	6.00^{b}	5.96^{b}	.024
Methionine	1.24^{b}	1.31^{ab}	1.44^{a}	1.44^{a}	.040
Arginine	7.19^{a}	6.75^{b}	6.49^{c}	6.36^{d}	.028
Histidine	2.53^{a}	2.49^{ab}	2.37^{bc}	2.35^{c}	.026
Isoleucine	4.80^{c}	5.05^{b}	5.29^{a}	5.36^{a}	.031
Leucine	7.73^{c}	8.15^{b}	8.46^{a}	8.57^{a}	.031
Phenyalanine	4.86^{c}	5.07^{b}	5.21^{a}	5.26^{a}	.016
Threonine	4.01^{c}	4.10^{bc}	4.25^{ab}	4.28^{a}	.035
Valine	5.22^{c}	5.63^{b}	5.90^{a}	5.95^{a}	.040
Trytptophan	1.23^{c}	1.33^{bc}	1.49^{ab}	1.63^{a}	.042
Total	45.2^{b}	45.9^{b}	46.9^{a}	47.2^{a}	.057
Non-essential (NEAA)					
Cystine	.90	.95	1.07	1.13	.088
Tyrosine	3.70	3.56	3.76	3.63	.044
Aspartic acid	11.4^{a}	11.2^{b}	11.2^{b}	11.2^{b}	.044
Serine	5.18^{b}	5.19^{b}	5.26^{ab}	5.32^{a}	.023
Glutamic acid	19.1^{a}	18.1^{b}	16.8^{c}	16.5^{c}	.187
Proline	5.86^{ab}	6.20^{a}	5.79^{b}	5.72^{b}	.077
Glycine	4.35^{b}	4.41^{b}	4.52^{a}	4.60^{a}	.020
Alanine	4.27^{b}	4.47^{b}	4.70^{a}	4.73^{a}	.047
Total	54.8^{a}	54.1^{a}	53.1^{b}	52.8^{b}	.064
EAA/NEAA, %	82.5^{b}	84.8^{b}	88.4^{a}	89.3^{a}	.153

^{abcd} Means in a row with different superscripts differ statistically ($P<.05$).

TABLE 14

AMINO ACID COMPOSITION OF SOYBEAN MEAL BEFORE OR 4, 14 OR 24 HR AFTER RUMINAL DIGESTION IN DACRON BAGS PLACED IN THE RUMEN OF STEERS FED A ROUGHAGE DIET (EXP 3)

	In situ exposure time, hr				
Amino acid	0	4	14	24	SE
	Percentage of total amino acids				
Essential (EAA)					
Lysine	6.37	5.80	5.68	6.18	.197
Methionine	1.24[a]	1.31[a]	1.29[a]	1.11[b]	.019
Arginine	7.19[a]	6.68[b]	6.01[c]	5.26[d]	.059
Histidine	2.53[ab]	2.53[ab]	2.43[b]	2.54[a]	.022
Isoleucine	4.80[c]	5.08[b]	5.23[a]	5.04[b]	.010
Leucine	7.73[c]	8.24[b]	8.51[a]	8.47[a]	.024
Phenyalanine	4.86[b]	5.10[a]	5.13[a]	4.86[b]	.014
Threonine	4.01[b]	4.16[ab]	4.34[a]	4.31[a]	.040
Valine	5.22[c]	5.73[b]	5.96[a]	6.01[a]	.035
Tryptophan	1.23	1.57	1.52	1.56[e]	.119
Total	45.2	46.2	46.1	45.4	.113
Non-essential (NEAA)					
Cystine	.90[a]	.97[a]	1.04[a]	.40[b]	.058
Tyrosine	3.70[b]	3.45[c]	3.76[b]	4.24[a]	.039
Aspartic acid	11.4[a]	11.2[a]	11.3[a]	10.5[b]	.101
Serine	5.18[b]	5.25[b]	5.44[ab]	5.69[a]	.061
Glutamic acid	19.1[a]	18.0[b]	16.8[c]	15.9[d]	.138
Proline	5.86[b]	5.95[b]	5.91[b]	6.80[a]	.048
Glycine	4.35[c]	4.45[c]	4.85[b]	6.13[a]	.077
Alanine	4.27[d]	4.50[c]	4.78[b]	5.03[a]	.038
Total	54.8	53.8	53.9	54.6	.127
EAA/NEAA, %	82.5	85.9	85.6	83.0	.288

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

[e] Assumed value due to inadequate amount of sample.

TABLE 15

AMINO ACID COMPOSITION OF .15 N NACL EXTRACTED SOYBEAN MEAL BEFORE OR 4, 14 OR 24 HR AFTER RUMINAL DIGESTION IN DACRON BAGS PLACED IN THE RUMEN OF STEERS FED A CONCENTRATE DIET (EXP 3)

	In situ exposure time, hr				
Amino acid	0	4	14	24	SE
	Percentage of total amino acids				
Essential (EAA)					
Lysine	6.26^{a}	6.27^{a}	5.99^{b}	6.07^{b}	.021
Methionine	1.34	1.34	1.47	1.53	.048
Arginine	6.92^{a}	6.62^{b}	6.33^{c}	6.23^{c}	.029
Histidine	2.53^{a}	2.55^{a}	2.47^{ab}	2.39^{b}	.025
Isoleucine	4.99^{d}	5.14^{c}	5.33^{b}	5.39^{a}	.009
Leucine	8.13^{b}	8.29^{b}	8.54^{a}	8.63^{a}	.044
Phenyalanine	4.99	5.08	5.17	5.21	.048
Threonine	4.16^{c}	4.28^{bc}	4.38^{ab}	4.40^{a}	.027
Valine	5.54^{c}	5.75^{b}	6.00^{a}	6.04^{a}	.010
Tryptophan	1.30^{b}	1.47^{ab}	1.62^{a}	1.56^{a}	.042
Total	46.1^{b}	46.8^{ab}	47.3^{a}	47.5^{a}	.052
Non-essential (NEAA)					
Cystine	1.01	1.11	1.04	1.19	.084
Tyrosine	3.69	3.51	3.78	3.90	.167
Aspartic acid	11.1	11.0	11.1	11.0	.030
Serine	5.28^{ab}	5.23^{b}	5.29^{ab}	5.35^{a}	.023
Glutamic acid	18.0^{a}	17.1^{ab}	16.3^{bc}	15.9^{c}	.211
Proline	5.93^{ab}	6.04^{a}	5.74^{b}	5.70^{b}	.058
Glycine	4.35	4.46	4.58	4.59	.062
Alanine	4.48^{c}	4.66^{b}	4.84^{a}	4.85^{a}	.032
Total	53.8^{a}	53.2^{ab}	52.7^{b}	52.5^{b}	.059
EAA/NEAA, %	85.7^{b}	87.9^{ab}	89.8^{a}	90.4^{a}	.141

^{abcd} Means in a row with different superscripts differ statistically ($P<.05$).

TABLE 16

AMINO ACID COMPOSITION OF .15 N NACL EXTRACTED SOYBEAN MEAL BEFORE OR 4, 14 OR 24 HR AFTER RUMINAL DIGESTION IN DACRON BAGS PLACED IN THE RUMEN OF STEERS FED A ROUGHAGE DIET (EXP 3)

	In situ exposure time, hr				
Amino acid	0	4	14	24	SE
	Percentage of total amino acids				
Essential (EAA)					
Lysine	6.26$_{b}$	6.13$_{b}$	6.12$_{a}$	6.06$_{b}$	.055
Methionine	1.34^{b}	1.33^{b}	1.48^{a}	1.30^{d}	.028
Arginine	6.92^{a}	6.47^{b}	5.97^{c}	5.28^{d}	.050
Histidine	2.53$_{c}$	2.52$_{b}$	2.52$_{a}$	2.53$_{a}$	.010
Isoleucine	4.99^{c}	5.18^{b}	5.33^{a}	5.37^{a}	.031
Leucine	8.13^{c}	8.39^{b}	8.63^{a}	8.70^{a}	.035
Phenyalanine	4.99$_{b}$	5.04$_{ab}$	5.08$_{a}$	5.09$_{a}$	.035
Threonine	4.16^{b}	4.33^{ab}	4.51^{a}	4.53^{a}	.049
Valine	5.54^{c}	5.82^{b}	6.10^{a}	6.25^{a}	.047
Tryptophan	1.30^{b}	1.63^{a}	1.58^{ab}	1.75^{a}	.066
Total	46.1^{c}	46.8^{b}	47.3^{a}	46.8^{b}	.024
Non-essential (NEAA)					
Cystine	1.01	1.02	1.08	.99	.078
Tyrosine	3.69	3.67	4.00	3.83	.118
Aspartic acid	11.1$_{b}$	11.1$_{b}$	11.0$_{ab}$	11.0$_{a}$	.055
Serine	5.28^{b}	5.24^{b}	5.39^{ab}	5.58^{a}	.061
Glutamic acid	18.0^{a}	16.9^{b}	15.7^{c}	15.4^{c}	.127
Proline	5.93$_{b}$	6.13$_{a}$	5.81$_{b}$	5.88$_{a}$	.089
Glycine	4.35^{c}	4.42^{c}	4.79^{b}	5.34^{a}	.038
Alanine	4.48^{c}	4.70^{b}	4.94^{a}	5.09^{a}	.040
Total	53.8^{a}	53.2^{b}	52.7^{c}	53.1^{b}	.026
EAA/NEAA, %	85.7^{c}	88.1^{b}	89.8^{a}	88.2^{b}	.062

^{abc} Means in a row with different superscripts differ statistically ($P<.05$).

TABLE 17

AMINO ACID COMPOSITION OF SOYBEAN MEAL AND .15 N NACL EXTRACTED SOYBEAN MEAL BEFORE OR 4, 14 OR 24 HR AFTER RUMINAL DIGESTION IN DACRON BAGS PLACED IN THE RUMEN OF STEERS FED CONCENTRATE AND ROUGHAGE DIETS (EXP 3)

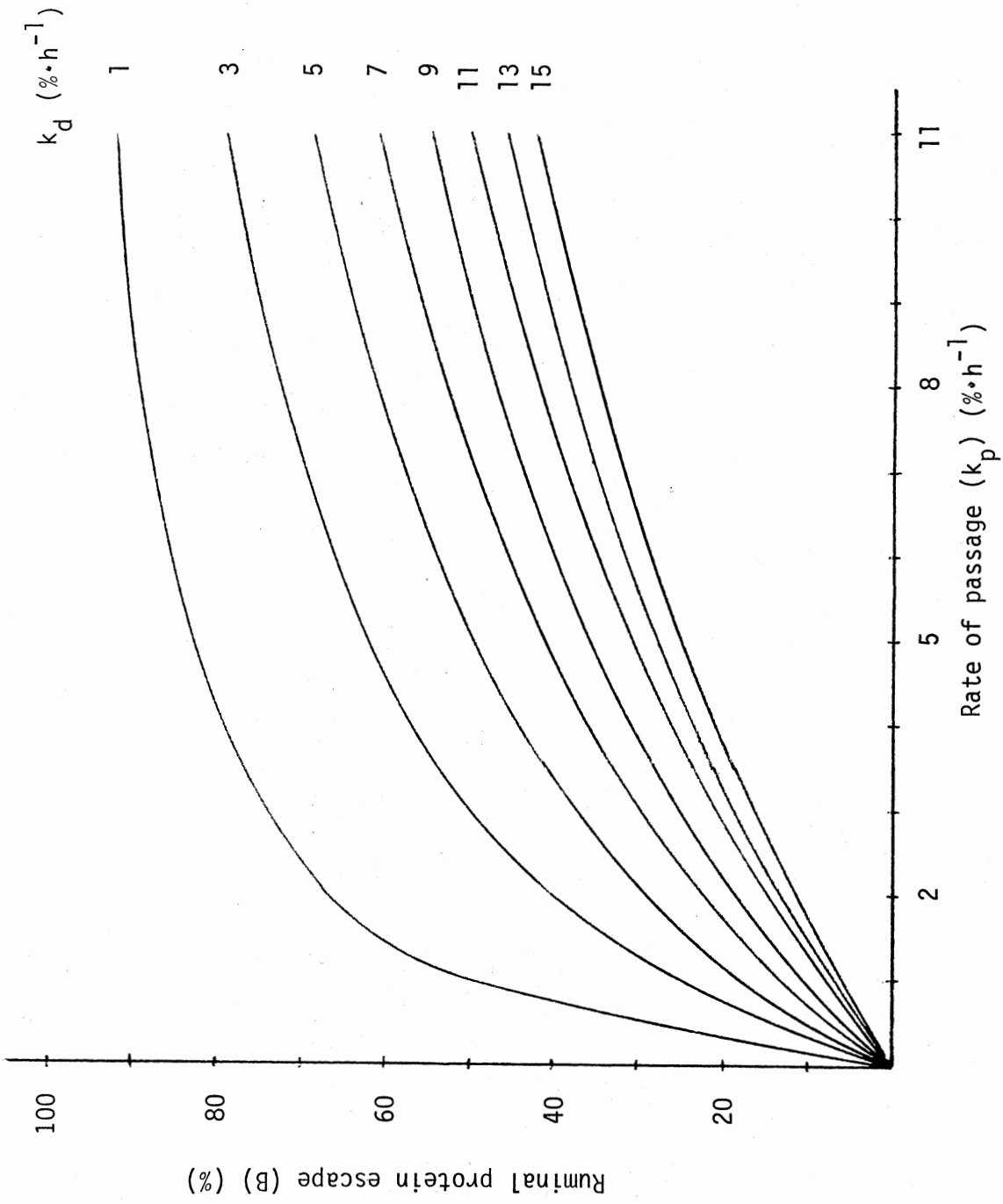
	In situ exposure time, hr					
Amino acid	0	4	14	24	SE	Effect[e]
	Percentage of total amino acids					
Essential (EAA)						
Lysine	6.31[a]	6.05[b]	5.95[b]	6.09[b]	.063	LQ
Methionine	1.29[b]	1.32[b]	1.42[a]	1.35[ab]	.029	RS Q
Arginine	7.05[a]	6.63[b]	6.20[c]	5.80[d]	.078	R L
Histidine	2.53[a]	2.52[a]	2.45[b]	2.46[b]	.020	R L
Isoleucine	4.90[c]	5.11[b]	5.29[a]	5.31[a]	.022	S LQ
Leucine	7.93[b]	8.27[c]	8.54[b]	8.63[a]	.031	S LQ
Phenyalanine	4.93[b]	5.07[a]	5.15[a]	5.12[a]	.030	R LQ
Threonine	4.08[c]	4.22[b]	4.37[a]	4.40[a]	.020	RS LQ
Valine	5.38[d]	5.73[c]	5.99[b]	6.09[a]	.029	RS LQ
Tryptophan	1.26	1.50	1.55	1.24	.133	LQ
Total	45.7[b]	46.4[a]	46.9[a]	46.7[a]	.053	RS LQ
Non-essential (NEAA)						
Cystine	.95[bc]	1.01[c]	1.06[ab]	.93[a]	.062	RS
Tyrosine	3.70[a]	3.55[a]	3.82[a]	3.92[b]	.067	L
Aspartic acid	11.2[a]	11.1[a]	11.1[a]	11.0[b]	.053	L
Serine	5.23[c]	5.23[c]	5.34[b]	5.51[a]	.036	R L
Glutamic acid	18.6[a]	17.6[b]	16.4[c]	16.0[d]	.107	RS LQ
Proline	5.90[b]	6.08[b]	5.81[b]	6.05[a]	.096	
Glycine	4.35[d]	4.44[c]	4.68[b]	5.19[a]	.114	R L
Alanine	4.38[d]	4.58[c]	4.82[b]	4.95[a]	.034	RS LQ
Total	54.3[a]	53.6[b]	53.1[b]	53.3[b]	.059	RS LQ
EAA/NEAA, %	84.1[b]	86.7[a]	88.4[a]	87.7[a]	.136	RS LQ

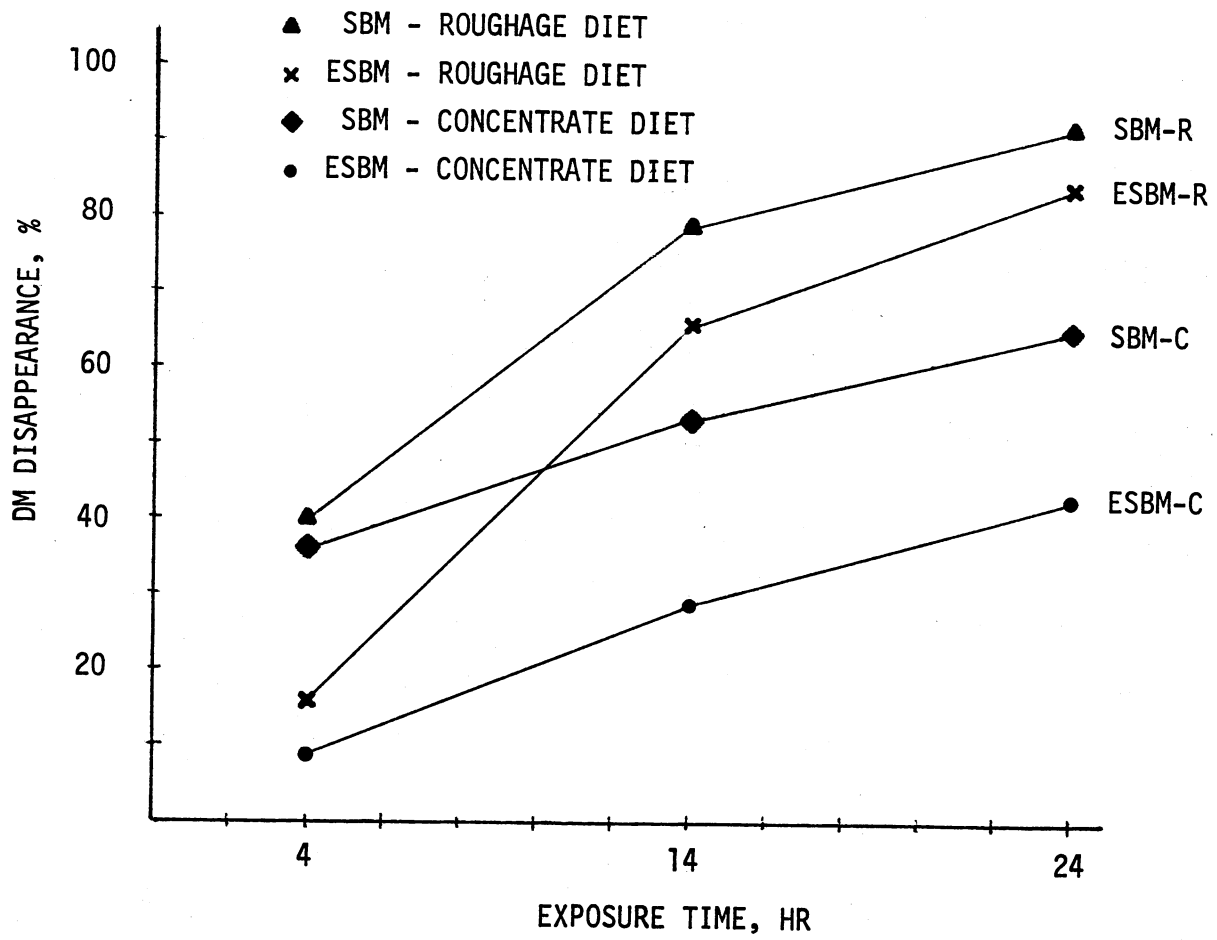
[abcd] Means in a row with different superscripts differ statistically ($P<.05$).

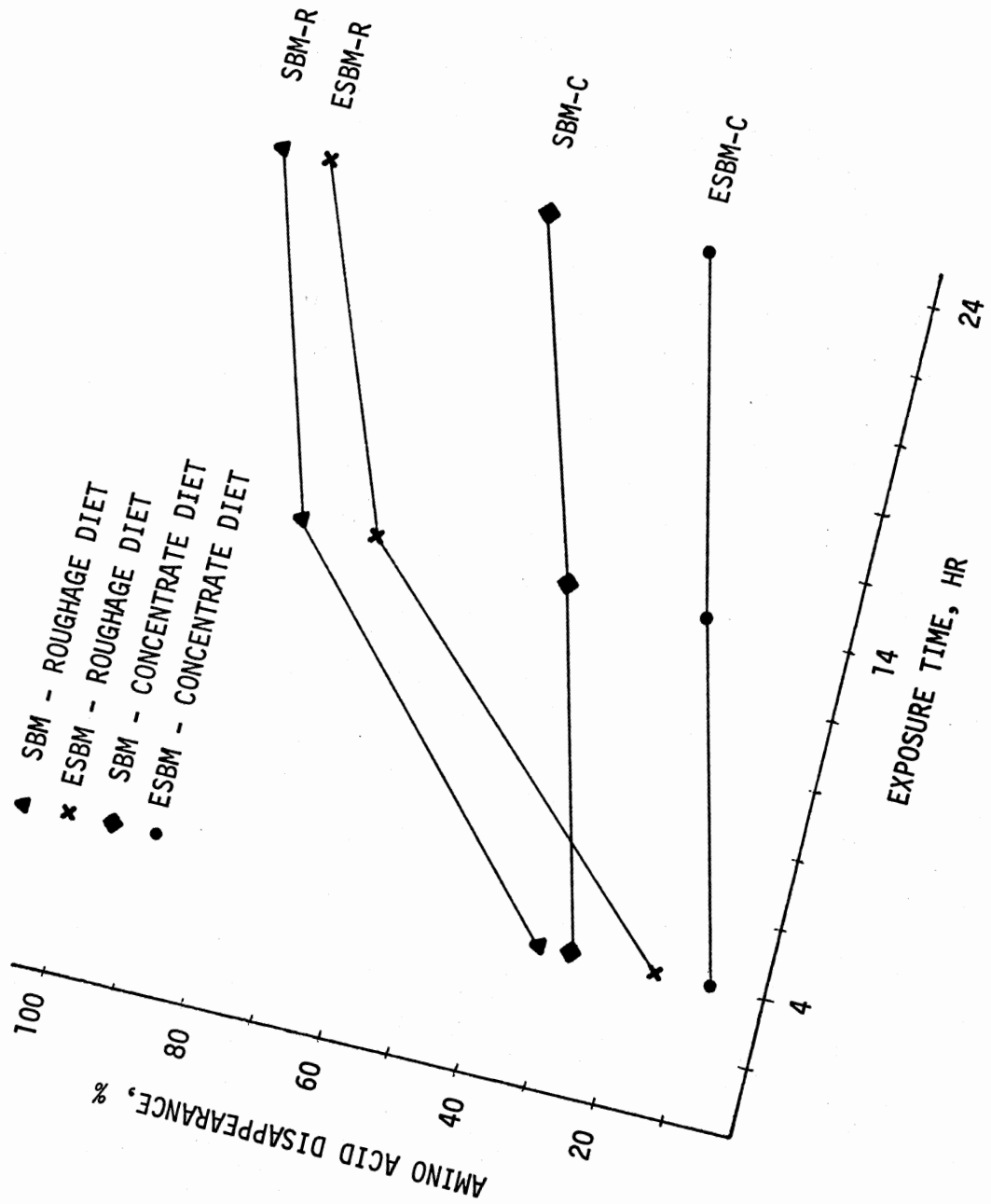
[e] Means in a row statistically ($P<.05$) affected: by dietary roughage level (R), by substrate (S), linearly by time (L), quadratically by time (Q) (no significant R x S interaction).

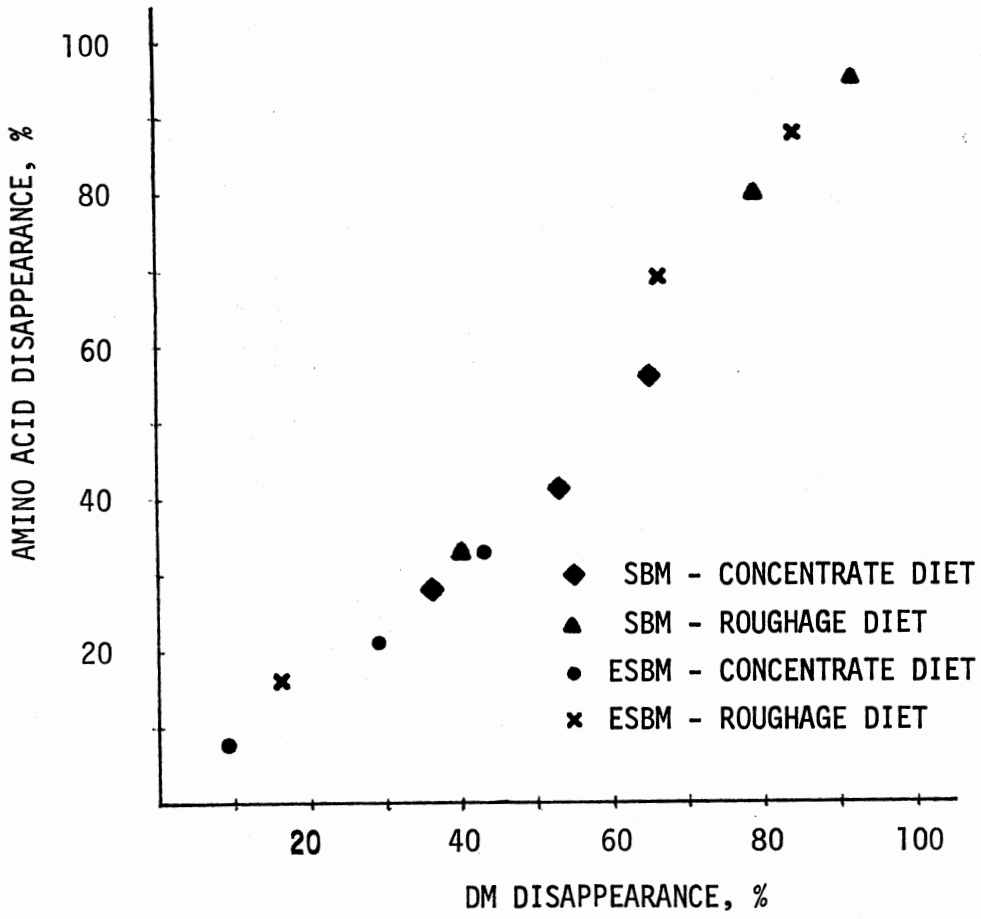
List of figures

List of figures (continued)

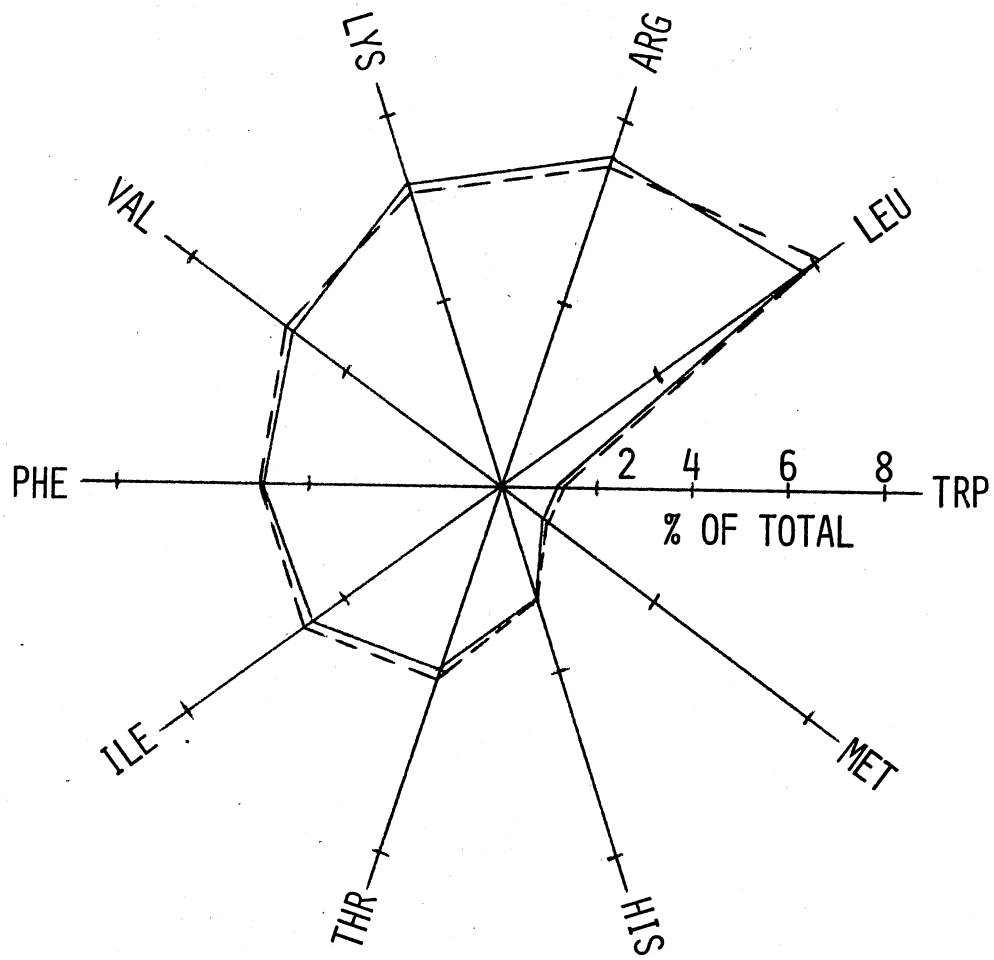

k_d (%·h^{-1})
1
3
5
7
9
11
13
15
Ruminal protein escape (B) (%)
100
80
60
40
20
2
5
8
11
Rate of passage (k_p) (%·h^{-1})


SBM - ROUGHAGE DIET
ESBM - ROUGHAGE DIET
SBM - CONCENTRATE DIET
ESBM - CONCENTRATE DIET
SBM-R
ESBM-R
SBM-C
ESBM-C
DM DISAPPEARANCE, %
100
80
60
40
20
4
14
24
EXPOSURE TIME, HR

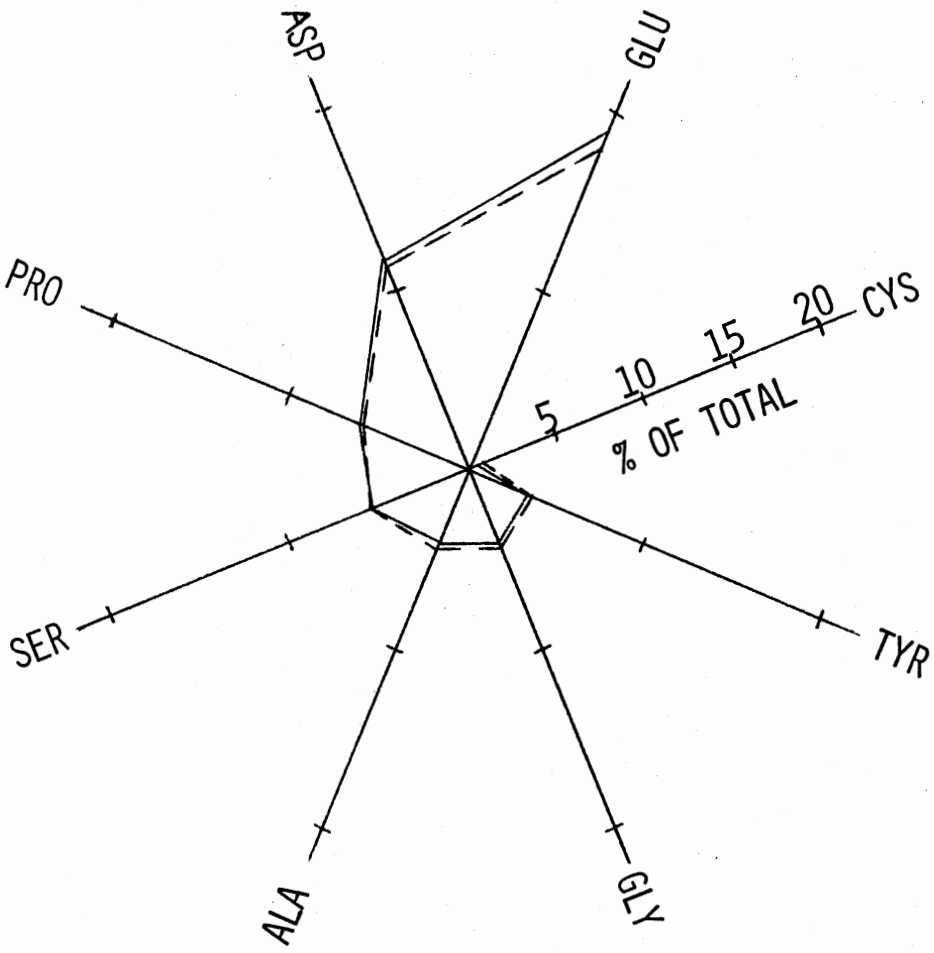

SBM - ROUGHAGE DIET
ESBM - ROUGHAGE DIET
SBM - CONCENTRATE DIET
ESBM - CONCENTRATE DIET
SBM-R
ESBM-R
SBM-C
ESBM-C
AMINO ACID DISAPPEARANCE, %
100
80
60
40
20
EXPOSURE TIME, HR
4
14
24


AMINO ACID DISAPPEARANCE, %
100
80
60
40
20
20
40
60
80
100
DM DISAPPEARANCE, %
SBM - CONCENTRATE DIET
SBM - ROUGHAGE DIET
ESBM - CONCENTRATE DIET
ESBM - ROUGHAGE DIET

AMINO ACID COMPOSITION
(% OF TOTAL)



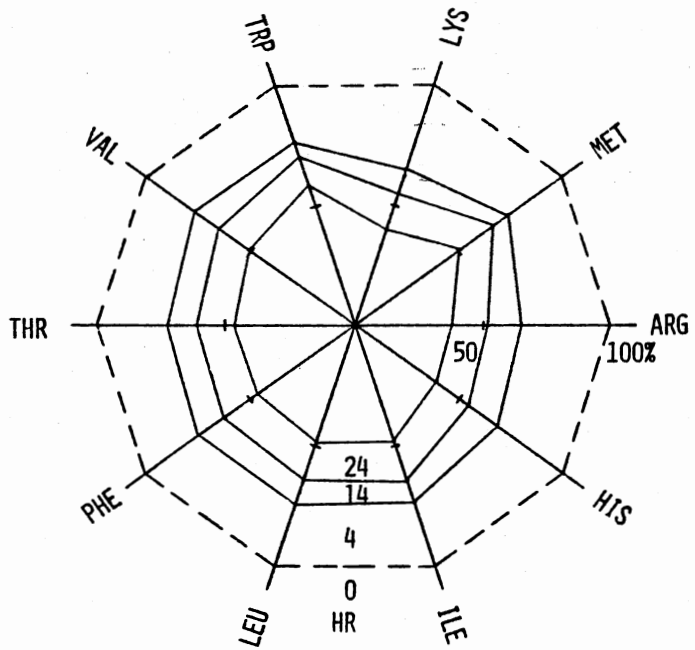
AMINO ACID COMPOSITION
(% OF TOTAL)



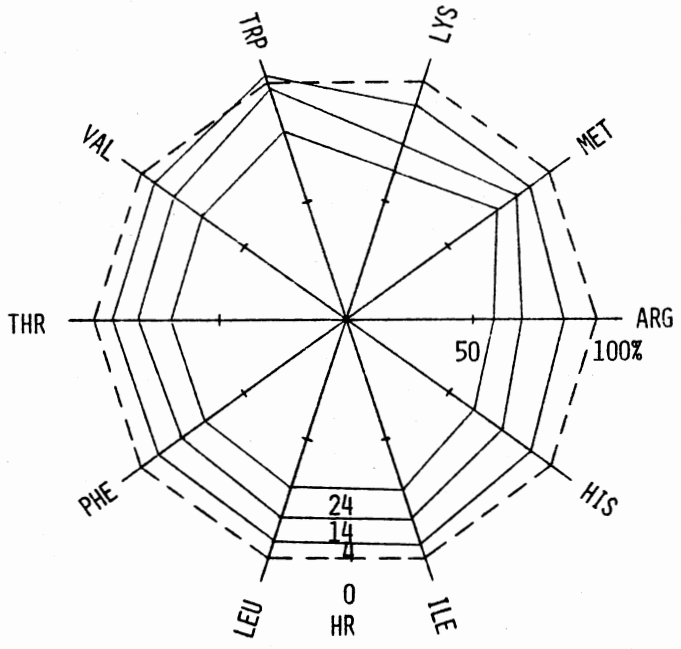
——— SBM

– – – – NaCL EXTRACTED SBM

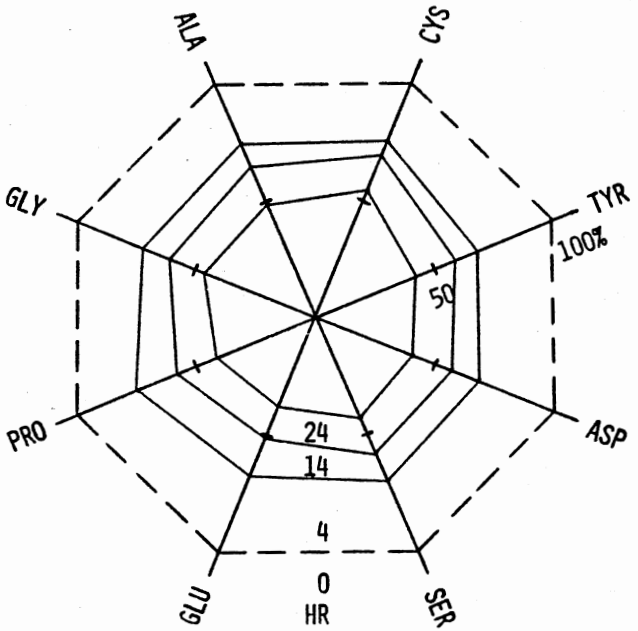
SBM
IN SITU AMINO ACID SURVIVAL, %
INITIAL, 4, 14, 24 HR
HIGH CONCENTRATE DIET



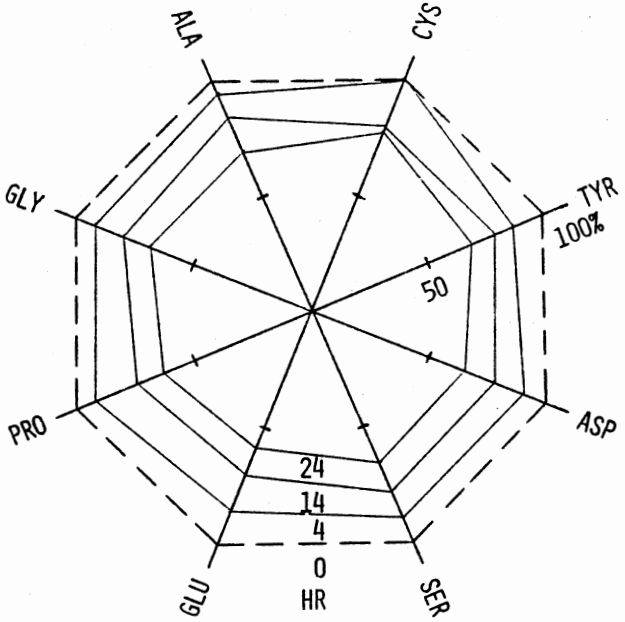
NaCL EXTRACTED SBM
IN SITU AMINO ACID SURVIVAL, %
INSOLUBLE, 4, 14, 24 HR
HIGH CONCENTRATE DIET



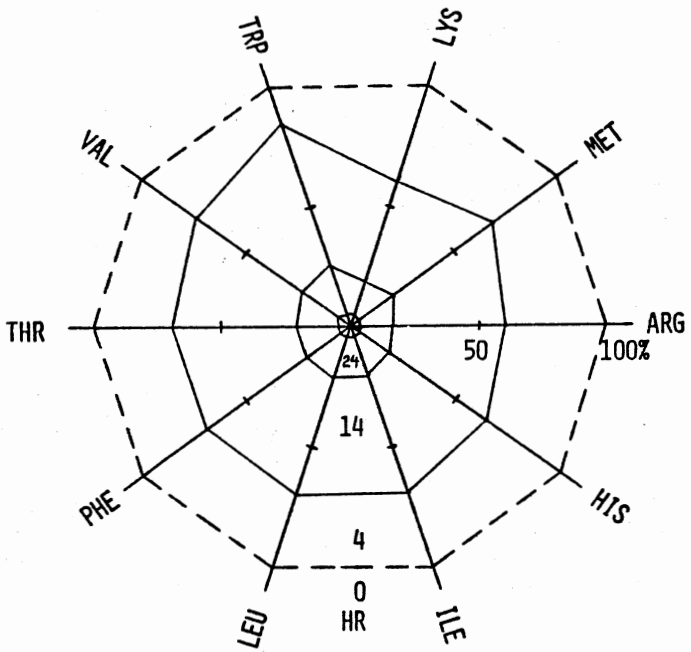
SBM
IN SITU AMINO ACID SURVIVAL, %
INITIAL, 4, 14, 24 HR
HIGH CONCENTRATE DIET



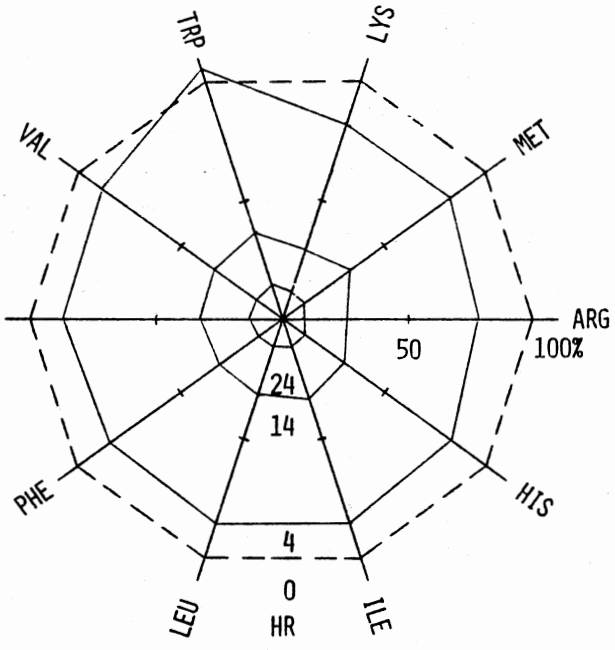
NaCL EXTRACTED SBM
IN SITU AMINO ACID SURVIVAL, %
INSOLUBLE, 4, 14, 24 HR
HIGH CONCENTRATE DIET



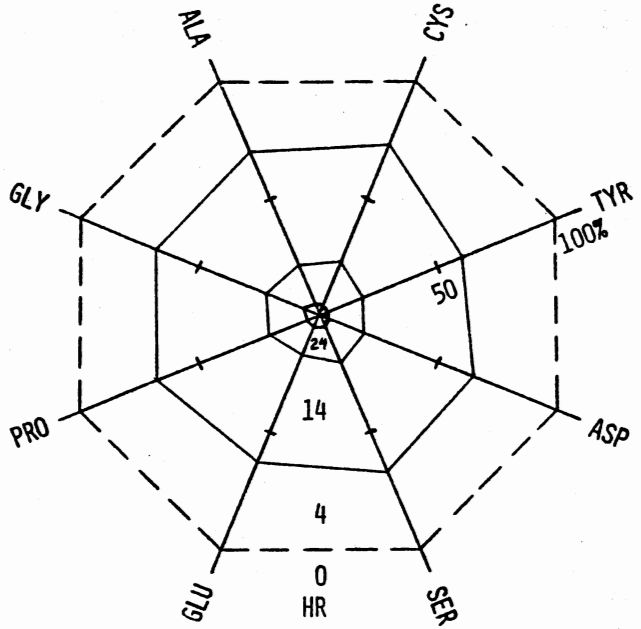
SBM
IN SITU AMINO ACID SURVIVAL, %
INITIAL, 4, 14, 24 HR
HIGH ROUGHAGE DIET



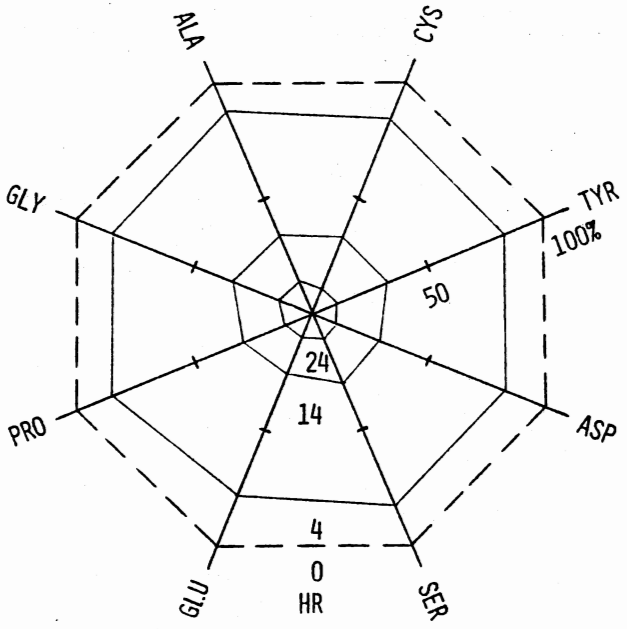
NaCL EXTRACTED SBM
IN SITU AMINO ACID SURVIVAL, %
INSOLUBLE, 4, 14, 24 HR
HIGH ROUGHAGE DIET



SBM
IN SITU AMINO ACID SURVIVAL, %
INITIAL, 4, 14, 24 HR
HIGH ROUGHAGE DIET



NaCL EXTRACTED SBM
IN SITU AMINO ACID SURVIVAL, %
INSOLUBLE, 4, 14, 24 HR
HIGH ROUGHAGE DIET



CHAPTER IV

INFLUENCE OF AMMONIA CONCENTRATION AND ORGANIC ACIDS ON MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN OF STEERS

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Summary

Two experiments were conducted to determine the influence of ruminal ammonia concentration or organic acid addition on organic matter digestion and efficiency of microbial protein synthesis in the rumen of steers fed high concentrate diets. In Exp 1, four Angus steers (490 kg) equipped with ruminal and duodenal T-cannulas were fed a ground corn based diet supplemented with 0, 1, 2 or 3% additional crude protein equivalent from a mixture of ammonium acetate and urea. Ruminal ammonia concentrations above 3 mg/dl rumen fluid did not increase efficiency of microbial protein synthesis ($P<.10$).

In Exp 2, the influence of acid addition to a high concentrate diet (1.9% lactic and 2% acetic from ammonium acetate) on microbial protein synthesis was studied with 520 kg steers. Average ruminal pH was only slightly reduced by the addition of a total of 3.9% of these acids to the diet (6.27 vs 6.36), demonstrating the strong buffering capacity of ruminal contents. Acid addition had little influence on

ruminal or total tract digestion of organic matter, nitrogen, starch or efficiency of microbial protein synthesis.

Combined data from both experiments indicated little influence of ruminal pH (5.8 to 6.7) or ammonia concentration (1 to 22 mg/dl) on efficiency of microbial protein synthesis. However, ruminal organic matter digestion increased with increasing ruminal ammonia concentrations to about 10 mg/dl ($P<.10$). Ruminal organic matter digestion also peaked at approximately pH 6.3 and declined above or below this pH.

Results can be interpreted to suggest that low ruminal ammonia concentrations (3 mg/dl rumen fluid) appeared adequate to maximize efficiency of microbial protein synthesis with a high concentrate diet, but higher concentrations increased organic matter digestion in the rumen. Furthermore, altering pH up or down from 6.3 with a high concentrate diet may reduce ruminal organic matter digestion, but had little effect on efficiency of microbial protein synthesis. Ruminal residence time of particles and dilution rate of liquids may be involved.

Introduction

The amount of ruminal ammonia required to maximize efficiency or total production of microbial protein has been estimated using in vitro trials (Satter and Slyter, 1974; Schaefer et al., 1980) or organic matter digestion in situ (Mehrez et al., 1977). Estimated required levels (mg/dl ruminal fluid) from these three studies are 5, 1.4 and 23.5, respectively. While few estimates of efficiency of microbial protein synthesis with various ruminal ammonia

concentrations exist with natural diets, one trial reported that efficiency of microbial protein synthesis increased with ammonia concentrations (Leibholz, 1980) while other studies have reported no change (Redman et al., 1980; Veira et al., 1980). Knowledge of the minimum concentrations of ammonia required to maximize efficiency of microbial protein synthesis and organic matter digestion in the rumen would greatly aid attempts to predict supply and requirements for N in ruminants. Ideally, the estimate should be based on in vivo measurement of microbial production with a variety of diets.

Ruminal pH has been shown to influence ruminal fiber digestion (Stewart, 1977; Slyter, 1981), bacterial growth rate in vitro (Russell et al., 1979), production and concentrations of volatile fatty acids (Esdale and Satter, 1972), protein solubility (Wohlt et al., 1973) and bacterial protease activity (Erfle et al., 1982). Most of this work has been in vitro since it is difficult to change and maintain pH in vivo, while maintaining other ruminal conditions constant. However, the role of pH in vivo in bacterial competition and protein degradation in the rumen can only be answered through carefully controlled animal experiments.

The objectives of these two experiments were to determine the influence of ruminal pH and ammonia concentration on organic matter digestion and efficiency of microbial protein synthesis in the rumen of steers fed high concentrate diets.

Materials and Methods

Exp 1

Four Angus steers (490 kg), equipped with ruminal cannulas and T-cannulas in the duodenum proximal to the bile duct were fed a ground corn based diet (8.3% crude protein) supplemented with 0, 1, 2 or 3% supplemental crude protein equivalent (CPE) from non-protein nitrogen (NPN) in a 4 x 4 Latin square (table 1). A mixture of ammonium acetate and urea was used as the NPN source to avoid the elevation in ruminal pH often seen with urea supplementation. In this manner, ruminal ammonia levels could be altered without causing major changes in ruminal pH.

Diets were fed every 6 h at a daily level of dry matter intake equal to 1.2% of body weight. This level of intake was equivalent to the ad libitum intake of the steer eating the least. Level of feed intake has been shown to alter efficiency of microbial protein synthesis (Zinn and Owens, 1983a) and would be expected to alter the required amount but not the required concentration of ammonia in the rumen. Data were not collected from one animal in one period. A general linear model program (SAS, 1979) was used to estimate missing values based on the intakes and quantities of digesta flow measured with the other animals.

Exp 2

The same four Angus steers (520 kg) used in Exp 1, were used to measure the influence of addition of acids to the diet on efficiency of microbial protein synthesis in the rumen. Ground corn based diets

supplemented with isonitrogenous amounts of either urea or ammonium acetate plus lactic acid (NHAc) were fed to the steers in a crossover design (table 2). Urea or ammonium acetate plus lactic acid were used as sources of NPN in order to alter ruminal pH while maintaining constant ruminal ammonia concentrations. Two animals were fed each diet every 6 h at a daily level of dry matter intake equal to 1.1 percent of body weight, equivalent to the ad libitum intake of the steer eating the least amount of feed.

Sampling

After 5 days of adaptation to the diet, approximately 250 ml of duodenal digesta and 200 g wet feces were collected 2 h post-prandially twice daily for three days. Compositing and processing of samples prior to analysis were described by Weakley and Owens (1983c). On the ninth day, approximately 500 ml of contents from the rumen were withdrawn twice, two h post-prandially, and strained through four layers of cheese cloth. The pH was measured and 250 ml were saved for ammonia-N analysis. For two of the periods in Exp 1 and both periods in Exp 2, on day 9 of sampling, 1500 ml of strained rumen fluid were collected in iced flasks 2 h post-prandially for bacterial isolation and nucleic acid determination. Isolation procedures were described by Weakley and Owens (1983c).

Analytical Procedures

Feed, duodenal and fecal samples were analyzed for dry matter (DM; 105 C for 24 h), ash (600 C for 12 h), Kjeldahl nitrogen (N; AOAC, 1975), starch (MacRae and Armstrong, 1968), acid detergent fiber (Goering and Van Soest, 1970) and chromium (Fenton and Fenton, 1979).

Ammonia N and pH measurements were obtained from the liquid rumen samples. Ammonia N was also measured on the dried, ground duodenal samples. Ammonia N was measured with 40 ml rumen fluid distilled over magnesium oxide in a macro-Kjeldahl flask (AOAC, 1975). Lyophilized bacteria and dried duodenal samples were analyzed for nucleic acid N by the procedure of Zinn and Owens (1982) for quantitation of daily microbial nitrogen flow from the rumen. Calculations of bacterial N production and daily amounts of organic matter (OM) and nitrogen (N), corrected for microbial contributions, flowing past the duodenal cannula were described by Weakley and Owens (1983c). For the two periods in Exp 1, where bacteria were not isolated, average bacterial values obtained from the other two periods were used for calculations.

Statistically significant differences were located with Duncan's multiple range test protected by a significant F test in an analysis of variance for a 4 x 4 Latin square (Steel and Torrie, 1960). Comparisons of ruminal pH and ammonia concentration with ruminal organic matter digestion and efficiency of microbial protein synthesis were analyzed for linear and quadratic relationships.

Results

Chemical compositions of bacteria isolated in Exp 1 and 2 are shown in table 3. Values are slightly lower than with bacteria obtained from animals consuming higher roughage diets (Weakley and Owens, 1983c). Consumption of higher concentrate diets can result in accumulation of polysaccharide by bacteria (Smith, 1975), possibly decreasing bacterial proportions of other components. The mean

bacterial composition values were used only where individual values from each animal and diet combination were unavailable.

Exp 1

Ruminal ammonia N levels increased in response to added increments of NPN (table 4). Individual values ranged from 1.2 to 13.1 mg ammonia N/dl rumen fluid. Ruminal pH values with each diet were not greatly different with added NPN. This should help avoid confounding of effects of pH with effects of ruminal ammonia on ruminal metabolism.

Organic matter (OM) intake was slightly lower with the 0 and 3% diets due to partial feed refusal by one animal (table 5). Ruminal OM digestion, both unadjusted and adjusted for microbial OM contributions, was not significantly changed by added NPN. However, added NPN tended to reduce ruminal OM digestion. This was recovered post-ruminally so that digestion in the total tract was influenced little by added NPN except for a drop at the highest level of addition. This reduction is presumed to be a result of higher ruminal ammonia concentrations since concentrations as great as 57 mM have not inhibited bacterial growth in vitro (Harrison and McAllan, 1980). Reasons for depressed OM digestion with higher concentrations of ruminal ammonia in this study are unknown. The proportion of total apparent OM digestion occurring in the rumen is similar to the 63% calculated from a summary of a number of experiments involving 50 to 90% concentrate diets (Johnson and Bergen, 1982). Flow of total chyme to the small intestine tended to increase with added NPN (table 5), possibly due to a stimulatory effect on salivary flow.

Nitrogen intake increased with increasing NPN supplementation (table 6). Although not significantly affected by ruminal ammonia concentration, microbial N passage to the duodenum tended to be depressed with the lowest concentration of ammonia in the rumen. Efficiency of microbial protein synthesis was least ($P<.10$) with the lowest concentration of ruminal ammonia, reaching a plateau between 2.7 and 3.7 mg ammonia N/dl rumen fluid. The lowest concentration of ruminal ammonia N (2.7 mg/dl) is below the 5 mg/dl suggested as being required for maximum microbial N production in vitro (Satter and Slyter, 1974). This concentration also was similar to the lowest level observed in another experiment with a higher roughage diet (2.4 mg/dl) that was associated with a tendency for depressed microbial N production (Weakley and Owens, 1983c).

Total N leaving the rumen daily (table 6) exceeded N intake resulting in negative ruminal digestibility. This would reflect microbial utilization of up to 25 g of N recycled back to the rumen with these low crude protein, high concentrate diets. Similar observations have been made even with higher protein diets fed to dairy cows (Stern and Satter, 1982). Amounts of N recycled to the rumen according to the recent calculations of Satter (personal communication) (% of N intake = $121.7-12.01xCP+.3235xCP^2$) would be up to 38% of N intake with the 9.3% protein diet or 32 g per day.

Digestibility of N in the rumen and total tract tended to increase with increasing total N intake, due in part to dilution of unaccounted endogenous N loss. For comparison, digestibility of N, calculated from the standard relationship (percent digestible protein = .9 x percent crude protein -3; NRC, 1976) is also presented in table

6. This close check of values illustrates that apparent digestibility changed with protein intake as expected. Correlations of fecal N output with organic matter intake, protein intake and fecal organic matter output were .24, .26 and .75, respectively.

Starch digestion in the rumen, although variable, was not significantly affected by ruminal ammonia concentration. Post-ruminal and total tract digestion of starch was slightly depressed with the greatest ruminal ammonia concentration, paralleling depression observed in OM digestion. Altered starch digestion can explain many of the alterations in organic matter digestion.

Exp 2

Although individual ruminal pH values among animals with both diets differed (5.9 to 6.7), the mean ruminal pH was not significantly changed by added acid (table 8). The fact that addition of almost four percent acid to the diet caused only a slight pH change illustrates the strong buffering capacity of ruminal contents. Diet had little influence on ruminal or total tract digestibility of OM (table 9), N (table 10), starch (table 11) or efficiency of microbial protein synthesis (table 10). Total tract digestion of starch in both experiments (tables 7 and 11) was very high. Organic matter digestion in the rumen and total tract tended to be slightly greater with NHAc (table 9) which also produced slightly higher ruminal ammonia concentrations (table 8).

Discussion

Individual measurements from Exp 1 and 2 were combined to examine ruminal OM digestion and efficiency of microbial protein synthesis over a wider range of ruminal pH and ammonia N concentrations. The data are shown in figures 1 through 4. Linear and quadratic relationships were analyzed after removal of experiment and animal effects. Where a significant relationship was detected, the equation was plotted on the graph.

No significant relationship between ruminal ammonia concentration and efficiency of microbial protein synthesis was observed with both experiments, although an effect ($P<.10$) was observed in Exp 1 as discussed previously (figure 1). Similarly, no relationship was detected between ruminal pH and efficiency of microbial protein synthesis (figure 2).

A quadratic relationship ($P<.08$) was detected between ruminal ammonia concentration and ruminal OM digestion (figure 3). This is derived primarily from the trend in Exp 1 for ruminal OM digestion to decrease as ruminal ammonia concentrations increased to approximately 10 mg/dl. With ruminal ammonia concentrations above 5 mg/dl, however, ruminal OM digestion tended to increase. This would indicate that although 3 mg ammonia N/dl rumen fluid was adequate to maximize efficiency of microbial protein synthesis, greater concentrations may increase OM digestion in the rumen.

The ammonia requirement (23.5 mg/dl) observed by Mehrez et al. (1977) to maximize rate of digestion in situ is greater than most estimates of ammonia concentrations required to maximize microbial

yield or efficiency (Satter and Slyter, 1974; Slyter et al., 1979; Schaefer et al., 1980) and requirements for both functions may not necessarily be equal. Higher ammonia concentrations may be necessary to adequately perfuse bacteria adhering to feed particles to support maximum OM digestion while only a lower level is needed for freely floating bacteria.

A single concentration of ruminal ammonia may not support maximum growth under all conditions. While some have observed improvements in efficiency or total protein production by microbes in vivo with greater concentrations of ruminal ammonia (Okarie et al., 1977; Slyter et al., 1979; Kang-Meznarich and Broderick, 1980), others have not (Redman et al., 1980; Veira et al., 1980). Hespell (1979) suggested that ruminal ammonia concentration cannot be affecting bacterial growth directly since concentrations as low as .25 mM do not reduce bacterial growth rate in vitro. He suggested that beneficial effects of higher ammonia concentrations observed in vivo on cell yields and OM digestibility may be due to indirect effects of ammonia on other factors such as ruminal pH. Smith (1979) suggested that bacteria may accumulate a labile N reserve to buffer against fluctuations in N supply. He further questioned whether ruminal ammonia supply becomes limiting in vivo with daily feeding. Since a considerable amount of ammonia is trapped in the rumen in the ammonium form at normal ruminal pH and will be absorbed only after digesta leaves the rumen, quite high concentrations of ammonia may be retained in the rumen and not necessarily be limiting or wasted.

Depression in ruminal OM digestion was observed at extremes in pH ranging from 5.8 to approximately 6.7 (figure 4). Ruminal OM

digestion and pH were quadratically related ($P<.10$), with a tendency for maximum ruminal OM digestion at a ruminal pH of approximately 6.3. Streptococcus bovis, one of the more prominent ruminal species with high starch diets, has been observed to demonstrate its greatest growth rate in pure culture at a pH of approximately 6.4 (Kistner et al., 1979), agreeing well with the effect of pH on the ruminal digestion of ground corn observed in the present two experiments.

The study of pH effects on ruminal digestion in vivo is difficult, as demonstrated by Exp 2. Not only is ruminal pH difficult to manipulate, uncertainty exists concerning possible effects on other ruminal parameters. Therefore, most work has been conducted in vitro (Russell et al., 1979) or in situ (Erfle et al., 1982; Loerch et al., 1983; Okeke et al., 1983a, 1983b). Protein degradation in the rumen is greater with high roughage than high concentrate diets (Weakley and Owens, 1983c; Zinn and Owens, 1983b). The involvement of ruminal pH in this relationship has been discussed by Weakley and Owens (1983c).

These studies indicate that with high concentrate diets ruminal pH and ammonia concentration appear to have a greater influence on OM digestion in the rumen than on efficiency of microbial protein synthesis. Results from Exp 1, however, indicate that ammonia concentrations below 3 mg/dl appear inadequate to support maximum efficiency or total production of microbial protein with high concentrate diets. Ruminal infusions of larger amounts of acidic or basic solutions may be required to obtain the information needed about the influence of pH on ruminal digestion in vivo.

TABLE 1

COMPOSITION OF DIETS USED
IN EXP 1

	Diet[a]			
	0	1	2	3
	Percentage of dry matter			
Ground corn (IFN 4-02-931)	81.2	80.6	80.0	79.3
Cottonseed hulls (IFN 1-01-599)	15.1	15.1	15.1	15.1
Molasses (IFN 4-04-696)	.45	.45	.45	.45
Chromic Oxide	.22	.22	.22	.22
Supplement				
Ammonium acetate		.53	1.05	1.57
Urea		.14	.28	.41
Dicalcium phosphate (IFN 6-01-080)	.55	.53	.53	.53
Limestone (IFN 6-02-632)	.95	.91	.91	.90
Trace mineralized salt[b]	.47	.45	.45	.44
Na_2SO_4	.47	.45	.45	.44
KCl	.56	.54	.54	.54
Vitamin A[c]	.01	.01	.01	.01
Vitamin D[d]	.002	.002	.002	.002
Crude protein, %[e]	8.3	9.3	10.5	11.5
Starch, %[e]	58.1	58.2	57.5	57.8

[a] Basal diet supplemented with 0, 1, 2 or 3% crude protein equivalent (CPE) from a mixture of ammonium acetate and urea.
[b] Morton Salt Co., Chicago, IL 60606.
[c] Vitamin A=30,000 USP/g.
[d] Vitamin D=15,000 IU/g.
[e] Dry matter basis.

TABLE 2

COMPOSITION OF DIETS USED
IN EXP 2

	Diet[a]	
	Urea	NHAc
	Percentage of dry matter	
Ground corn (IFN 4-02-931)	80.2	76.7
Cottonseed hulls (IFN 1-01-599)	15.2	15.2
Molasses (IFN 4-04-696)	.46	.46
Chromic oxide	.20	.20
Supplement		
Urea	1.04	
Ammonium acetate		2.75
Lactic acid		1.90
Dicalcium phosphate (IFN 6-01-080)	.53	.50
Limestone (IFN 6-02-632)	.91	.86
Trace mineralized salt[b]	.45	.43
Na_2SO_4	.45	.43
KCl	.54	.51
Vitamin A[c]	.009	.008
Vitamin D[d]	.002	.002
Crude protein, %[e]	12.1	12.7
Starch, %[e]	58.4	57.3

[a] Basal diet supplemented with isonitrogenous amounts of urea or ammonium acetate (NHAc).
[b] Morton Salt Co., Chicago, IL 60606.
[c] Vitamin A=30,000 USP/g.
[d] Vitamin D=15,000 IU/g.
[e] Dry matter basis.

TABLE 3

COMPOSITION OF BACTERIA HARVESTED
FROM RUMEN FLUID IN EXP 1
AND EXP 2

	Experiment	
	1	2
Bacterial nitrogen, % of dry matter	6.14	5.39
Bacterial RNA-nitrogen, % of total nitrogen	13.2	15.8

TABLE 4

RUMINAL PARAMETERS IN STEERS FED A GROUND CORN BASAL DIET SUPPLEMENTED WITH 0, 1, 2 OR 3 PERCENT CRUDE PROTEIN EQUIVALENT FROM A MIXTURE OF AMMONIUM ACETATE AND UREA (EXP 1)

	Diet				
Item	0	1	2	3	SE
Ammonia-nitrogen, mg/dl	2.7^{c}	3.7^{c}	5.8^{b}	9.2^{a}	.58
pH	6.40^{ab}	6.43^{a}	6.16^{c}	6.20^{bc}	.04

^{abc} Means in a row with different superscripts differ statistically ($P<.05$).

TABLE 5

ORGANIC MATTER DIGESTION IN STEERS FED A GROUND CORN BASAL DIET SUPPLEMENTED WITH 0, 1, 2 OR 3 PERCENT CRUDE PROTEIN EQUIVALENT FROM A MIXTURE OF AMMONIUM ACETATE AND UREA (EXP 1)

	Diet				
Item	0	1	2	3	SE
Intake, g/d	5046	5353	5337	5193	175
Leaving abomasum, g/d					
Total	2461	3140	2867	2735	374
Non-microbial	1851	2431	2156	1985	318
Chyme, l/d	42	48	44	50	4.6
Ruminal digestion,%					
Unadjusted	52	42	46	47	6.5
Adjusted[c]	64	55	60	61	5.7
Ruminal digestion, % of total	67	53	58	63	7.5
Feces, g/d	1148	1167	1076	1358	88
Post-ruminal digestion, % of input	52[ab]	61[a]	60[a]	50[b]	4.5
Total tract digestion, %	77[ab]	78[a]	80[a]	74[b]	1.3

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

[c] Adjusted for microbial organic matter.

TABLE 6

NITROGEN (N) DIGESTION IN STEERS FED A GROUND CORN BASAL DIET SUPPLEMENTED WITH 0, 1, 2 OR 3 PERCENT CRUDE PROTEIN EQUIVALENT FROM A MIXTURE OF AMMONIUM ACETATE AND UREA (EXP 1)

	Diet				
Item	0	1	2	3	SE
Intake, g/d	70[c]	85[b]	95[a]	102[a]	2.7
Ruminal Ammonia-N, mg/dl	2.7[c]	3.7[c]	5.8[b]	9.2[a]	.58
Leaving abomasum, g/d					
Total N	88	110	108	109	9.1
Microbial N	39	50	52	51	4.7
Non-ammonia, non-microbial	46	56	52	53	5.4
Ruminal digestion, %					
Unadjusted	-26	-29	-13	-7.2	7.8
Adjusted[f]	35	35	46	48	5.7
Microbial efficiency, g microbial N/kg OM truly digested in rumen	12[d]	18[e]	17[e]	16[e]	1.9
Ruminal digestion, % of total	-56	-50	-22	-15	12
Feces, g/d	35	36	37	38	2.0
Post-ruminal digestion, % of input	60	66	65	63	2.9
Total tract digestion, %	49[b]	57[ab]	61[a]	62[a]	2.3
Expected total digestion, %[g]	54	58	61	64	

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

[de] Means in a row with different superscripts differ statistically ($P<.10$).

[f] Adjusted for microbial and ammonia nitrogen.

[g] Calculated from % digestible protein = .9 (% crude protein) - 3.

TABLE 7

STARCH DIGESTION IN STEERS FED A GROUND CORN BASAL DIET SUPPLEMENTED WITH 0, 1, 2 OR 3 PERCENT CRUDE PROTEIN EQUIVALENT FROM A MIXTURE OF AMMONIUM ACETATE AND UREA (EXP 1)

	Diet				
Item	0	1	2	3	SE
Intake, g/d	3119	3308	3271	3200	116
Leaving abomasum, g/d	780	1203	968	785	173
Apparent ruminal digestion, %	75	64	71	75	5.1
Ruminal digestion, % of total	77_{b}	66_{ab}	72_{b}	79_{a}	5.0
Feces, g/d	72^{b}	132^{ab}	65^{b}	148^{a}	20
Post-ruminal digestion, % of input	91^{a}	89^{ab}	91^{a}	81^{b}	2.5
Total tract digestion, %	98^{a}	96^{ab}	98^{a}	95^{b}	.6

^{ab} Means in a row with different superscripts differ statistically ($P<.05$).

TABLE 8

RUMINAL PARAMETERS IN STEERS FED A GROUND CORN BASED DIET SUPPLEMENTED WITH ISONITROGENOUS AMOUNTS OF UREA OR AMMONIUM ACETATE (NHAc) (EXP 2)

	Diet		
Item	Urea	NHAc	SE
Ammonia-nitrogen, mg/dl	14.3	16.9	.90
pH	6.36	6.27	.10

TABLE 9

ORGANIC MATTER DIGESTION IN STEERS FED A GROUND CORN BASED DIET SUPPLEMENTED WITH ISONITROGENOUS AMOUNTS OF UREA OR AMMONIUM ACETATE (NHAc) (EXP 2)

Item	Diet		SE
	Urea	NHAc	
Intake, g/d	5564	5543	1.5
Leaving abomasum, g/d			
Total	2272	2264	74
Non-microbial	1719	1620	63
Chyme, l/d	35	38	.87
Ruminal digestion, %			
Unadjusted	59	59	1.4
Adjusted[a]	69	71	1.1
Ruminal digestion, % of total	71	70	1.5
Feces, g/d	920	841	20
Postruminal digestion, % of input	60	62	1.5
Total tract digestion, %	83	85	.36

[a] Adjusted for microbial organic matter.

TABLE 10

NITROGEN (N) DIGESTION IN STEERS FED A GROUND CORN BASED DIET SUPPLEMENTED WITH ISONITROGENOUS AMOUNTS OF UREA OR AMMONIUM ACETATE (NHAc) (EXP 2)

Item	Diet		SE
	Urea	NHAc	
Intake, g/d	114[b]	118[a]	.02
Leaving abomasum, g/d			
Total N	85	84	1.1
Microbial N	34	36	.88
Non-ammonia, non-microbial	43[a]	41[b]	.30
Ruminal digestion, %			
Unadjusted	26	29	.89
Adjusted[c]	62[b]	66[a]	.30
Microbial efficiency, g N/kg OM truly digested in rumen	8.8	9.1	.18
Ruminal digestion, % of total	35	37	.99
Feces, g/d	29	27	.58
Postruminal digestion, % of input	66	68	.46
Total tract digestion,%	75	77	.52

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

[c] Adjusted for microbial and ammonia nitrogen.

TABLE 11

STARCH DIGESTION IN STEERS FED A GROUND CORN BASED DIET SUPPLEMENTED WITH ISONITROGENOUS AMOUNTS OF UREA OR AMMONIUM ACETATE (NHAc) (EXP 2)

Item	Diet		SE
	Urea	NHAc	
Intake, g/d	3416	3342	2.7
Leaving abomasum, g/d	835	814	55
Apparent ruminal digestion, %	75	76	1.6
Ruminal digestion, % of total	78	77	1.8
Feces, g/d	99	57	16
Post-ruminal digestion, % of input	88	93	2.2
Total tract digestion, %	97	98	.48

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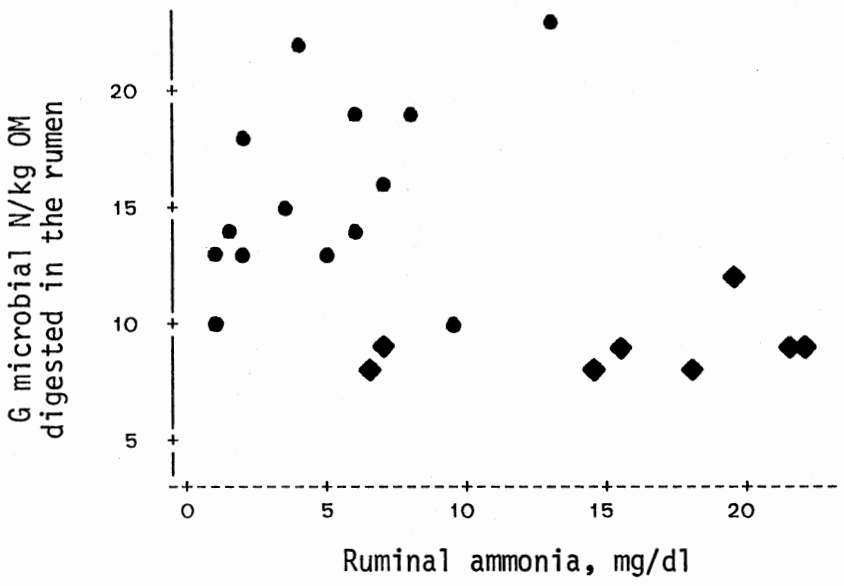
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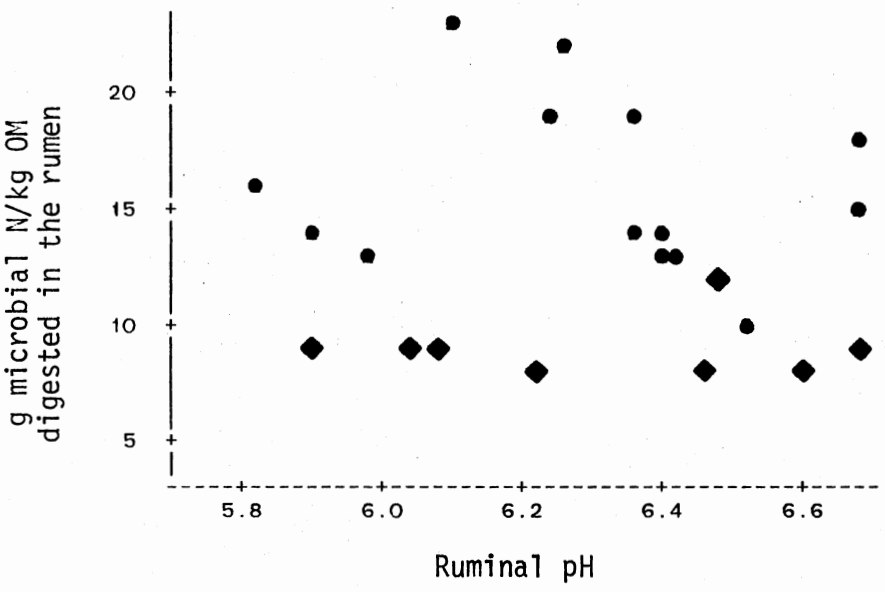
15
10
5
0
5
10
15
20
Ruminal ammonia, mg/dl


g microbial N/kg OM
digested in the rumen
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15
10
5
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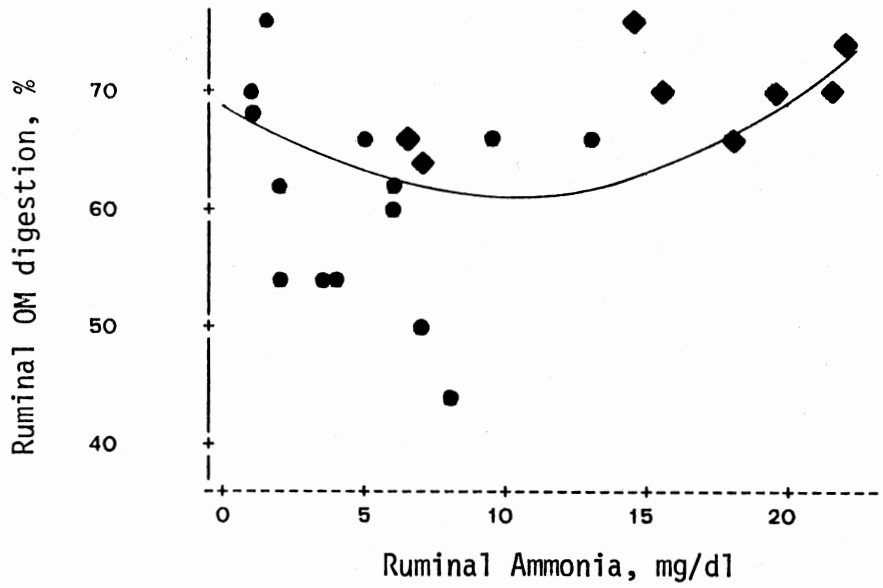
6.4
6.6
Ruminal pH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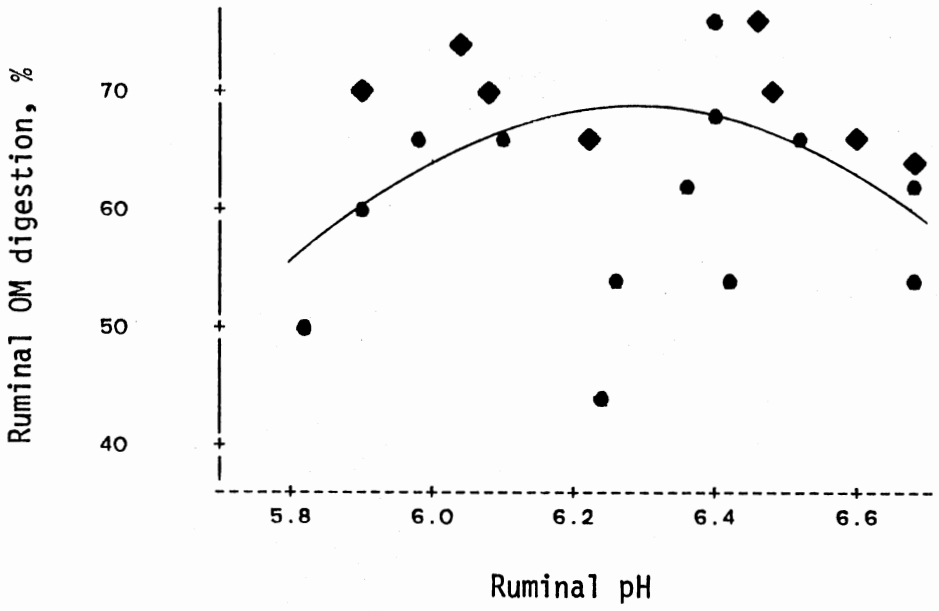
50
40
0
5
10
15
20
Ruminal Ammonia, mg/dl


Ruminal OM digestion, %
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60
50
40
5.8
6.0
6.2
6.4
6.6
Ruminal pH

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APPENDIX

EQUATIONS USED TO CALCULATE DAILY DIGESTA FLOW AND DIGESTIBILITY TO VARIOUS LOCATIONS ALONG THE GASTROINTESTINAL TRACT OF ANIMALS WHEN USING MARKERS

Introduction

The basic approach in calculating parameters in animal experiments using markers is to first calculate daily flow of all nutrients flowing past all sampling sites, rather than first attempting to calculate digestibility directly through manipulation of marker ratios. This allows an opportunity to correct daily digesta flow leaving the rumen for microbial organic matter and microbial and ammonia nitrogen prior to calculating digestibility. Digestibility measurements based on digesta flow leaving the rumen, corrected for microbial contributions, will represent true feed protein digestion in the rumen.

This method is not the only approach to calculations, but permits uncomplicated accounting of intermediate values. A description of terms and computer printout (SAS, 1979) of equations follows. Nutrient composition of samples are entered as decimal, rather than percentage, values for direct application in calculations. Unless stated otherwise, nutrient composition values are expressed on a dry matter basis. Strict attention must be maintained in keeping units of daily nutrient flow consistent (e.g. g/d). It is also advisable to convert all values to a dry matter basis to avoid errors associated with an inconsistent accounting of sample moisture.

Description of terms

PER=experimental design period
ANIM=animal designation
DIET=diet designation
CRNIN=daily corn intake (as fed basis)
SBMIN=daily SBM intake (as fed basis)
SUPPIN=daily supplement intake (as fed basis)
HAYIN=daily hay intake (as fed basis)
SUPPDM=supplement dry matter composition
SUPPOM=supplement organic matter composition
DUODM=duodenal sample dry matter composition
DUOOM=duodenal sample organic matter composition
FECDM=fecal sample dry matter composition
FECOM=fecal sample organic matter composition
SUPPN=supplement nitrogen composition
DUON=duodenal sample nitrogen composition
FECN=fecal sample nitrogen composition
DUONHN=duodenal sample ammonia-N composition
DUOST=duodenal sample starch composition
FECST=fecal sample starch composition
SUPPCR=supplement chromic oxide composition
DUOCR=duodenal sample chromium composition
FECCR=fecal sample chromium composition
DUORNAN=duodenal sample nucleic acid composition
BRNAN=bacterial nucleic acid composition
BACTN=bacterial nitrogen composition
RUMNHN=ruminal liquid ammonia-N concentration, mg/dl
RUMPH=ruminal liquid pH
SUPPFB=supplement fiber composition
DUOFB=duodenal sample fiber composition
FECFB=fecal sample fiber composition

The above terms are unique to each animal-period-diet combination and are entered in an input statement (statement lines 1 - 9). Dietary components, or any other parameter, constant among all animals within a period, may have their composition values entered in IF-THEN statements to avoid repetitious entry on data cards (statement lines 10 - 45). If a total mixed ration is fed, rather than combining feed components at feeding time, entry of nutrient intake is less complicated than shown here. However, if feed refusals occur, an accounting of this must be made in calculating nutrient intake. If treatments are arranged in a factorial arrangement of treatments,

statement lines 46 - 57 may be entered to test for orthogonality. Statement lines 58 - 82 calculate daily nutrient intake. Multiplication by 2 represents twice daily feeding. Statement lines 83 - 104 calculate daily nutrient flow past sampling sites. Bacterial nitrogen and dry matter flow are calculated in statement lines 99 - 100. Statement lines 97 - 98 enter a default average bacterial composition value to compensate for periods when bacteria are not collected (although it may be advisable to collect bacteria in all periods if drastically different diets are tested). The remaining statements calculate digestibility in various segments of the gastrointestinal tract by manipulating daily nutrient flow.

A detailed description of all terms used in statement lines 10 - 130 is unnecessary since all are built from a basic set of key abbreviations:

HAY=hay
CRN=corn
DM=dry matter
N=nitrogen
FB=fiber
IN=meal intake
FL=daily flow
CR=chromium
NH=ammonia
B=bacterial
DMD=dry matter digestibility
ND=nitrogen digestibility
FBD=fiber digestibility
SBM=soybean meal
SUPP=supplement
OM=organic matter
ST=starch
D=DRY
DUO=duodenal
FEC=fecal
LQ=chyme
RNA=nucleic acids
BACT=bacterial
OMD=organic matter digestibility
STD=starch digestibility
A (preceding digestibility)=apparent digestibility
C (preceding digestibility)=digestibility corrected for microbial contributions
EFF=efficiency of microbial protein synthesis (g N/kg OM truly digested in the rumen)
USEFF=efficiency of microbial protein synthesis (g protein/100 g OM truly digested in the rumen)
PR=post-ruminal digestion (% of entering)
RTF=percentage of total digestion occurring in the rumen
BYEPASS=feed nitrogen escaping ruminal digestion

```
1          DATA BYPASS;
2          INPUT PER 1-2 ANIM $4 DIET $6 CRNIN 8-11 SBMIN 13-15 SUPPIN 17-19 HAYIN 21-24
3          SUPPDM 26-29 4 SUPPOM 31-34 4 DUODM 36-39 4 DUOOM 41-44 4 FECDM 46-49 4
4          FECOM 51-54 4 SUPPN 56-59 5 DUON 61-64 5 FECN 66-69 5 DUONHN 71-74 6
5          #2 PER 1 ANIM $3 DIET $5 DUOST 7-10 4 FECST 12-15 4 SUPPCR 17-20 5 DUOCR 22-25 6
6
7          FECCR 27-31 6 DUORNAN 33-36 5 BRNAN 38-41 4 BACTN 43-45 4 RUMNHN 47-50 2
8          RUMPH 52-54 2
9          #3 PER 1 ANIM $3 DIET $5 SUPPFB 7-10 4 DUOFB 12-15 4 FECFB 17-20 4;
10         IF PER=1 THEN HAYDM=.9049; IF PER=1 THEN HAYOM=.9383; IF PER=1 THEN HAYN=.00878;
11         IF PER=1 THEN SBMDM=.9123; IF PER=1 THEN SBMOM=.9329; IF PER=1 THEN SBMN=.08471;
12         IF PER=1 THEN CRNDM=.8478; IF PER=1 THEN CRNOM=.9852; IF PER=1 THEN CRNN=.01651;
13         IF PER=1 THEN HAYST=.0175;
14         IF PER=1 THEN SBMST=.0261;
15         IF PER=1 THEN CRNST=.7229;
16         IF PER=1 THEN CRNFB=.0437;
17         IF PER=1 THEN HAYFB=.4928;
18         IF PER=1 THEN SBMFB=.0741;
19         IF PER=2 THEN HAYDM=.9249; IF PER=2 THEN HAYOM=.9353; IF PER=2 THEN HAYN=.00820;
20         IF PER=2 THEN SBMDM=.9200; IF PER=2 THEN SBMOM=.9302; IF PER=2 THEN SBMN=.07821;
21         IF PER=2 THEN CRNDM=.8713; IF PER=2 THEN CRNOM=.9853; IF PER=2 THEN CRNN=.01555;
22         IF PER=2 THEN HAYST=.0183;
23         IF PER=2 THEN SBMST=.0271;
24         IF PER=2 THEN CRNST=.7056;
25         IF PER=2 THEN CRNFB=.0433;
26         IF PER=2 THEN HAYFB=.4957;
27         IF PER=2 THEN SBMFB=.0790;
28         IF PER=3 THEN HAYDM=.9018; IF PER=3 THEN HAYOM=.9255; IF PER=3 THEN HAYN=.00821;
29         IF PER=3 THEN SBMDM=.9053; IF PER=3 THEN SBMOM=.9305; IF PER=3 THEN SBMN=.07764;
30         IF PER=3 THEN CRNDM=.8748; IF PER=3 THEN CRNOM=.9808; IF PER=3 THEN CRNN=.01669;
31         IF PER=3 THEN HAYST=.0214;
32         IF PER=3 THEN SBMST=.0620;
33         IF PER=3 THEN CRNST=.7495;
34         IF PER=3 THEN CRNFB=.0404;
35         IF PER=3 THEN HAYFB=.4579;
36         IF PER=3 THEN SBMFB=.0974;
37         IF PER=4 THEN HAYDM=.9088; IF PER=4 THEN HAYOM=.9373; IF PER=4 THEN HAYN=.00715;
38         IF PER=4 THEN SBMDM=.9032; IF PER=4 THEN SBMOM=.9314; IF PER=4 THEN SBMN=.07398;
39         IF PER=4 THEN CRNDM=.8610; IF PER=4 THEN CRNOM=.9859; IF PER=4 THEN CRNN=.01520;
40         IF PER=4 THEN HAYST=.0231;
41         IF PER=4 THEN SBMST=.0916;
42         IF PER=4 THEN CRNST=.7456;
43         IF PER=4 THEN CRNFB=.0437;
44         IF PER=4 THEN HAYFB=.4938;
45         IF PER=4 THEN SBMFB=.1092;
46         IF DIET='A' THEN SBM=0;
47         IF DIET='B' THEN SBM=1;
48         IF DIET='C' THEN SBM=0;
49         IF DIET='D' THEN SBM=1;
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50          IF DIET='A' THEN R=0;
51          IF DIET='B' THEN R=0;
52          IF DIET='C' THEN R=1;
53          IF DIET='D' THEN R=1;

54          IF DIET='A' THEN IA=0;
55          IF DIET='B' THEN IA=1;
56          IF DIET='C' THEN IA=1;
57          IF DIET='D' THEN IA=0;
58          DCRNIN=2*CRNIN*CRNDM;
59          DSBMIN=2*SBMIN*SBMDM;
60          DHAYIN=2*HAYIN*HAYDM;
61          OMCRNIN=DCRNIN*CRNOM;
62          OMSBMIN=DSBMIN*SBMOM;
63          OMHAYIN=DHAYIN*HAYOM;
64          NCRNIN=DCRNIN*CRNN;
65          NSBMIN=DSBMIN*SBMN;
66          NHAYIN=DHAYIN*HAYN;
67          STCRNIN=DCRNIN*CRNST;
68          STSBMIN=DSBMIN*SBMST;
69          STHAYIN=DHAYIN*HAYST;
70          DSUPPIN=2*SUPPIN*SUPPDM;
71          OMSUPPIN=DSUPPIN*SUPPOM;
72          NSUPPIN=DSUPPIN*SUPPN;
73          FBCRNIN=DCRNIN*CRNFB;
74          FBHAYIN=DHAYIN*HAYFB;
75          FBSUPPIN=DSUPPIN*SUPPFB;
76          FBSBMIN=DSBMIN*SBMFB;
77          DMIN=DCRNIN+DSBMIN+DHAYIN+DSUPPIN;
78          OMIN=OMCRNIN+OMSBMIN+OMHAYIN+OMSUPPIN;
79          NIN=NCRNIN+NSBMIN+NHAYIN+NSUPPIN;
80          STIN=STCRNIN+STSBMIN+STHAYIN;
81          FBIN=FBCRNIN+FBHAYIN+FBSUPPIN+FBSBMIN;
82          CRIN=DSUPPIN*SUPPCR;
83          DUODMFL=CRIN/DUOCR;
84          DUOOMFL=DUODMFL*DUOOM;
85          DUOLQFL=DUODMFL/DUODM;
86          DUONFL=DUODMFL*DUON;
87          DUONHFL=DUODMFL*DUONHN;
88          DUOSTFL=DUODMFL*DUOST;
89          DUOFBFL=DUODMFL*DUOFB;
90          DUORNAFL=DUONFL*DUORNAN;
91          FECDMFL=CRIN/FECCR;
92          FECOMFL=FECDMFL*FECOM;
93          FECLQFL=FECDMFL/FECDM;
94          FECNFL=FECDMFL*FECN;
95          FECSTFL=FECDMFL*FECST;
96          FECFBFL=FECDMFL*FECFB;
97          IF BRNAN=. THEN BRNAN=.1779;
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98          IF BACTN=. THEN BACTN=.07314;
99          DUOBNFL=DUORNAFL/BRNAN;
100         DUOBDMFL=DUOBNFL/BACTN;
101         DUONCONC=DUONFL/DUOLQFL;
102         CDUODMFL=DUODMFL-DUOBDMFL;
103         CDUOOMFL=CDUODMFL*DUOOM;
104         CDUONFL=DUONFL-DUONHFL-DUOBNFL;
105         ARUMDMD=((DMIN-DUODMFL)/DMIN)*100;
106         CRUMDMD=((DMIN-(DUODMFL-DUOBDMFL))/DMIN)*100;
107         ARUMOMD=((OMIN-DUOOMFL)/OMIN)*100;
108         CRUMOMD=((OMIN-CDUOOMFL)/OMIN)*100;
109         ARUMND=((NIN-DUONFL)/NIN)*100;
110         CRUMND=((NIN-(DUONFL-(DUONHFL+DUOBNFL)))/NIN)*100;
111         ARUMSTD=((STIN-DUOSTFL)/STIN)*100;
112         ARUMFBD=((FBIN-DUOFBFL)/FBIN)*100;
113         EFF=DUOBNFL/((OMIN-CDUOOMFL)/1000);
114         USEFF=DUOBNFL*6.25/((OMIN-CDUOOMFL)/100);
115         AFECDMD=((DMIN-FECDMFL)/DMIN)*100;
116         AFECOMD=((OMIN-FECOMFL)/OMIN)*100;
117         AFECND=((NIN-FECNFL)/NIN)*100;
118         AFECSTD=((STIN-FECSTFL)/STIN)*100;
119         AFECFBD=((FBIN-FECFBFL)/FBIN)*100;
120         APRDMD=((DUODMFL-FECDMFL)/DUODMFL)*100;
121         APROMD=((DUOOMFL-FECOMFL)/DUOOMFL)*100;
122         APRND=((DUONFL-FECNFL)/DUONFL)*100;
123         APRSTD=((DUOSTFL-FECSTFL)/DUOSTFL)*100;
124         APRFBD=((DUOFBFL-FECFBFL)/DUOFBFL)*100;
125         RTFDMD=(ARUMDMD/AFECDMD)*100;
126         RTFOMD=(ARUMOMD/AFECOMD)*100;
127         RTFND=(ARUMND/AFECND)*100;
128         RTFSTD=(ARUMSTD/AFECSTD)*100;
129         RTFFBD=(ARUMFBD/AFECFBD)*100;
130         BYEPASS=(CDUONFL/NIN)*100;
131         CARDS;
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VITA 2

David Craig Weakley

Candidate for the Degree of

Doctor of Philosophy

Thesis: INFLUENCE OF ROUGHAGE LEVEL, RUMINAL PH AND AMMONIA CONCENTRATION ON RUMINAL PROTEIN DEGRADATION AND MICROBIAL PROTEIN SYNTHESIS IN CATTLE

Major Field: Animal Nutrition

Biographical:

Personal Data: Born in Pryor, Oklahoma, July 6, 1956, the son of Martin and Norma Weakley.

Education: Graduated from Pryor High School, Pryor, Oklahoma, in May, 1974; received Bachelor of Science Degree in Animal Science from Oklahoma State University in May, 1978; received Master of Science degree in Dairy Science from The University of Wisconsin in August, 1980; completed requirements for the Doctor of Philosophy degree in Animal Nutrition at Oklahoma State University in December, 1983.

Professional Experience: Raised and worked on a commercial Angus cow-calf operation in northeastern Oklahoma; Laboratory assistant in the Animal Science Department, Oklahoma State University, 1975-1978; Research assistant in the Department of Dairy Science, University of Wisconsin, 1978-1980; Graduate assistant in the Animal Science Department, Oklahoma State University, 1980-1983.

Professional Organizations: American Society of Animal Science, American Dairy Science Association.