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Limin Kung, Jr.

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J. T. Huber
Major professor

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MILK PRODUCTION AND NITROGEN METABOLISM OF HIGH PRODUCING COWS EARLY IN LACTATION FED NON-PROTEIN NITROGEN AND RUMEN UNDEGRADABLE PROTEIN

By

Limin Kung, Jr.

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ABSTRACT

MILK PRODUCTION AND NITROGEN METABOLISM OF HIGH PRODUCING COWS EARLY IN LACTATION FED NON-PROTEIN NITROGEN AND RUMEN UNDEGRADABLE PROTEIN

By

Limin Kung, Jr.

The objective of this research was to increase the efficiency of nitrogen utilization in high producing dairy cows early in lactation.

Experiment 1

Data from these experiments showed that formaldehyde treatment autoclaving, and dry heat treatment of soybean meal (SBM) decreased nitrogen degradability in the rumen. Compared to untreated SBM the treated exhibited less *in vitro* ammonia release, less nitrogen disappearance from rumen suspended nylon bags and less nitrogen disappearance during protease incubation.

Experiment 2

Eighty four high producing multiparous cows were placed on pretrial ration immediately after calving until day 21 postpartum. Cows were placed on one of the following rations from days 22 to 91 postpartum; 1) 11% crude protein (CP), corn silage (CS) and soybean meal (SBM); 2) 14% CP ammonia-treated corn silage (AS) and

heated soybean meal (HS); 3) 14% CP, CS-HS; 4) 14% CP CS-SBM; 5) 17% CP AS-HS; 6) 17% CP, CS-HS; 7) 17% CP, CS-SBM.

Dry matter intake and adjusted milk production increased with protein level. Milk production was greater for cows fed rations containing AS and/or HS when compared to SBM controls. Cows fed 17% CP AS-HS were most productive and profitable.

Interpretation of results from this experiment suggests feeding for maximum peak milk production even though return over feed costs may be less for productive rations early in lactation, contradicting the well accepted idea to feed for maximum profit in early lactation. The substitution of natural protein with ammonia added to corn silage was compatable with high milk yields at both 14 and 17% dietary protein.

Experiment 3

Four lactating cows fitted with rumen fistula, and duodenal and ilieal cannula were used to measure flow and digestion of nitrogenous compound in the digestive tract. Diets were 17% CP and similar to Experiment 2. Flow of dry matter to the duodenum appeared to be grossly overestimated when lanthanum was the marker, resulting in high estimates for microbial protein efficiencies at the duodenum. Estimates made with lignin were similar to accepted values and were deemed more appropriate.

No significant differences in digesta flow or digestion were but trends between diets were apparent. Non ammonia nitrogen (NAN) flow was greatest for cows fed heated soybean meal CS-HS and AS-HS.

Digestion of NAN in the small intestine was equal for all treatments. This suggests that availbility of heated soybean meal was not different in the lower gut even though rumen degradability was decreased.

This thesis is dedicated to my family,

Limin Kung
Myrtle Kung
Lani Bushnell
Mann-Chi Kung
Linza Kung

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INTRODUCTION

Valid estimates of protein digestion and amino acid absorption in the ruminant are complicated by rumen microbial fermentation. In monogastrics, feed and endogenous nitrogen are the two main sources of protein and amino acids reaching the small intestine for digestion and absorption. Microbial protein is a third and important contribution to this source in ruminants. Although energetically inefficient, microbial fermentation does allow the cow to use fibrous feeds via the volatile fatty acids and 2) nonprotein nitrogen which is incorporated into microbial protein. Thus, ruminants have the ability of utilizing cellulose material which is not digestible by direct competition for the same feed sources by the two types of animals.

There has recently been much attention given to refining our knowledge of protein metabolism in ruminants. This is especially true for factors which alter rumen fermentation and increase quantity and/or quality of protein reaching the small intestine for digestion and absorption.

Dairy cattle early in lactation are unable to consume sufficient dry matter to support maximal milk production (Bines, 1976; Clark and Davis, 1980; Kuber and Kung, 1981) and body fat is mobilized to provide significant amounts of energy for productive purposes during this period. However, mobilization of body protein is minimal and its rapid depletion occurs during periods of negative nitrogen balance (Botts et al., 1979; Paquay et al., 1972; Swick and Benevenga,

1977). Thus, quantity and/or quality of protein reaching the small intestine might reduce peak milk yields in early lactation.

Peak milk and persistency of production are influenced by nutrient intake and body stores. If persistency is normal, peak milk production is the major determinant of total milk yield for the lactation. Broster and Strickland(1977) showed that every kg increase in peak milk production results in 200 kg increase in total milk yield during the entire lactation. Assuming cows calve in good body condition and a balanced ration is fed, protein becomes the most limiting nutrient in early lactation. Increasing protein early in lactation has not always increased milk production, suggesting that amount and type of protein as well as genetic ability of cows and other factors might govern responsiveness.

Systems have been identified by Huber and Kung (1981) which quantitatively and qualitatively define nutrients for lactating dairy cows. Amino acid requirements for ruminants are unknown due to extensive rumen degradation of dietary protein and the contribution of microbial protein at the duodenum.

Oldham and Tamminga (1980) point out that increasing the supply of amino acids to the duodenum is only important if there is a concomitant increase of amino acids at the tissue and a change in animal performance. Huber and Kung (1981) also stated that quality as well as quantity of amino acids reaching tissue of high yielding cows must be improved. The quantitative requirements of protein and amino acids for high milk production are unknown due to problems in sample collection and partitioning of microbial and feed protein at the duodenum.

Sutton and Oldham (1977) reported a 10% animal variation in duodenal digesta flow between animals. These workers suggested that in order to obtain a significant difference ($P < 0.05$) between animals in two out of three experiments, the following was required: four animals/treatment (4 x 4 latin square) for a 20% difference and six animals/treatment (6 x 6 latin square) for a 15% difference. Problems in partitioning microbial and feed proteins at the duodenum have been thoroughly reviewed elsewhere (Hume, 1976).

Rumen bacteria can supply considerable quantities of microbial protein to meet the cows' protein requirement. Since these bacteria cannot distinguish the source of ammonia nitrogen needed for their protein synthesis, it is logical to feed bacteria inexpensive sources of nitrogen (from nonprotein nitrogen) while maximizing the amount of plant protein reaching the small intestine.

The results of this study describe:

1) methods to decrease rumen nitrogen degradation

2) milk production from high producing cows fed nonprotein nitrogen and rumen undegradable protein

3) the digestion and flow of nutrients in various segments of the digestive tract when cows are fed nonprotein nitrogen and rumen undegradable protein.

LITERATURE REVIEW

Protein Digestion in the Rumen

Digestion of protein to peptides and amino acids is prerequisite to utilization by animals and microorganisms. In the rumen, protein digestion occurs primarily via bacterial proteolysis and protozoal engulfment. Proteolytic activity in the rumen was first described by Sym (1938). Casein was rapidly degraded although he found little protease in rumen fluid. Blackburn and colleagues (1965) have also studied rumen proteolytic activity under *in vitro* and in vivo conditions and reported rapid hydrolysis of casein and other proteins within 48 hr. These workers showed that B. amylophilus discharged 20% of its total proteolytic activity into an *in vitro* medium. Although extracellular proteases are able to hydrolyze protein, proteolytic activity in cell-free rumen fluid is low (Isaacson and Owens, 1971). Allison (1969) suggested that this was not surprising since extracellular enzymes are primarily associated with gram positive bacteria and rumen bacteria are predominantly gram positive.

Bacterial proteases are cell bound, but are closely associated with the cell surface thus providing easy access to substrates. Since contact between enzyme and substrate is necessary, surface exposure may alter protein digestion. Thus, rupture of the herbage cell membrane or ingestion of large quantities of feed protein may limit protease activity for a few hours. It is generally agreed that rumen

proteases are not subject to metabolic control, and are constitutive enzymes (Allison, 1970; Hogan and Hemsley, 1976). Proteins, peptides or amino acids have no effect on production of proteases. Proteolytic activity, however, is directly related to cell mass, making it difficult to distinguish effects on the microbes or on their proteases. For example, Hume (1974) and Nugent and Mangan (1978) did show an increase in proteolytic in the rumen when protein in the diet increased. Nikolic et al. (1975) reported that urea spared protein from ruminal degradation, but others suggested that it did not (Orskov, 1979).

Temperature and pH can alter protease activity, but tend to be relatively constant in the rumen. Blackburn and Hobson (1960) reported optimal proteolytic activity between pH 6 and 7. Hence, activity is primarily related to enzyme and substrate concentration, time of association, and inherent differences in protein degradability. In terms of the latter, rate of protein digestion by rumen bacteria was positively correlated with protein solubility in salt solutions (Hendrickx, 1963).

Peptides and amino acids are products of protease action. Rumen concentrations of these moieties tend to be low. Allison (1970) estimated the pool of free amino acids to be 2.6 to 65 $x10^{-5}$M. Bacteria appear to preferentially take up peptides over free amino acids via a translocase or permease system. Di- and oligopeptide translocases both require a free carboxyl group while the latter can transport peptides lacking carboxyl groups (Hogan and

Hemsley, 1976). Prins et al. (1979) reported that the net rate of amino acid disappearance from the rumen was increased when peptides rather than free amino acids were used. The translocase system also allows inexpensive nitrogen uptake compared to active transport required for amino acid uptake. Although peptides and some amino acids are taken up by bacterial cells, most of the nitrogen is returned to the rumen fluid as ammonia nitrogen prior to incorporation into microbial protein (Nolan et al., 1973). The added cost of amino acid synthesis from ammonia and carbohydrates has been reported to be relatively small (Forest and Walker, 1971).

Within the bacterial cells, peptides are extensively attacked by a wide range of peptidases, resulting in release of amino acids. Upon completion of peptidase reactions, amino acids may be subject to deamination. Extensive amino acid degradation may be due to the absence of a transport mechanism for amino acids from cytoplasm to the external media (Pittman et al., 1967). The optimal pH for deaminase activity appears to be between 4.5 (Lewis and Emery, 1962) and 7.2 (Chalmers, 1969).

In _in vivo_ and _in vitro_ studies by Chalupa (1976) free amino acids were utilized to establish apparent degradation rates of some essential amino acids. Prins et al. (1979) demonstrated that net rates of _in vitro_ disappearance of amino acids by mixed rumen microorganisms was dependent on diet of the inoculum donor, form of amino acids (free or peptides), and presence or absence of energy sources in the incubation media. High rates of net disappearance of glycine,

methionine, valine, and histidine from peptides were found, but Vmax values were low for free amino acids, suggesting that peptides were preferred. Deamidases have also been found in rumen bacteria and can liberate ammonia from amides (Warner, 1956).

Like bacteria, protozoa do not excrete extracellular proteases. Protozoa can engulf protein and peptides and are able to degrade protein to ammonia at a pH optimum of 6.5 to 7.0 (Hogan and Hemsley, 1976). Little if any amino acid uptake occurs in protozoa (Coleman, 1967). Warner (1956) suggested that protozoa could contribute 50% of the rumen proteolytic activity. Deamination of amino acids by protozoa is likely because rumen ammonia levels are higher in animals containing protozoa, compared to defaunted controls (Purser and Moir, 1966).

It is uncertain whether proteolysis or deamination is the rate-limiting step in rumen protein degradation. Data show an increase in free amino acids after a meal, which suggests that proteolysis occurs faster than use of free amino acids (Leibholz, 1969). Degradation of protein within the rumen is random, and reasons for extensive breakdown are unknown (Tamminga, 1979). Prins (1977) described a strain of rumen bacteria requiring amino acids as a source of energy and suggested that ATP may be generated. However, under anaerobic conditions, proteolysis cannot yield ATP (Tamminga, 1979).

Another degradation route which may be more important is the deamination of amino acids, followed by decarboxylation of the alpha-keto acid which yields one ATP per decarboxylation (Prins, 1977). Lack of an amino acid transport mechanism from cytoplasm to media

has already been mentioned.

Recent estimates of protein degradation in ruminants show considerable variation between feedstuffs. For example, the protein in soybean meal and haylage is 65-75% degraded in the rumen, but that from corn gluten meal is only 45%. Protein degradation will be discussed in greater detail in a later section.

Factors Affecting Microbial Growth and Production

The full benefit of microbial fermentation can only be realized when growth and turnover of the microbial population are maximized. This can be achieved when requirements for carbon, energy, nitrogen, and other limiting elements are met. Energy and carbon are provided from fermentation of carbohydrates resulting in ATP, while ammonia, and to a lesser extent amino acids, provide nitrogen for protein synthesis. Energy may be the first limitation to microbial growth in the rumen since anaerobic fermentation limits ATP yield to 3.5-4.5 moles(m) per fermented hexose equivalent (Baldwin, 1970). Using batch cultures, Bauchop and Elsden (1960) related microbial ATP production and growth in defining YATP as the grams of microbial dry matter produced per mole of ATP. These workers suggested that YATP was a constant 10.8. However, Hespell and Bryant (1979) have calculated ATP expenditures for synthesis of microbial cells from preformed monomers and suggest theoretical values for YATP of 27 to 32. Indeed, Stouthamer and Bettenhauser (1973) showed that the efficiency of ATP utilization for growth in continuous culture systems at steady state was determined by specific growth rate and maintenance requirements. Observed YATP

values determined in pure bacteria and mixed cultures of rumen bacteria range from 8 to 23 (Satter and Slyter, 1974; Isaacson and Owens, 1975; Hespell and Bryant, 1979). Hespell and Bryant (1979) suggest changes in cell composition, nutrient availability and transport cost account for the lower than theoretical values of YATP found in most studies.

In order to maximize microbial synthesis, energy from rumen fermentable organic matter must be supplied at a rate which parallels the synthetic abilities of rumen microbes (Oldham et al., 1977). Readily available carbohydrates have been more effective than structural carbohydrates in increasing uptake of degraded nitrogen, in vivo (Offer et al., 1978) and in vitro (Stern et al., 1978). Russell and Baldwin (1978) showed that rumen bacteria have marked preferences for sugars as carbohydrate sources. McAllan and Smith (1976) found that starch supplied the greatest amount of energy for bacteria, and that high starch diets induced greater bacterial polysaccharide accumulation than soluble sugars (McAllan and Smith, 1974). Better conversion of urea to microbial protein with starch may then be explained by a gradual release of polysaccharides (McAllan and Smith, 1974). The source of starch also appears to affect efficiency of microbial growth as Oldham et al. (1981) found 30% more bacterial nitrogen per kg organic matter digested when barley was substituted for corn as an energy source.

McMeniman (1975) reported that efficiency of microbial protein synthesis was 33 g nitrogen/kg rumen digested organic matter (RDOM) in a forage-type diet vs 22 g N/kg RDOM for animals fed high grain.

These results differ from those discussed. However, rumen dilution rate tends to be faster on forage-type diets, which might partially explain these findings.

Microbial growth becomes more efficient as dilution (D) or turnover rate increases. The rumen half-life of a solid material may range from 30 minutes for casein to 48 hours for low quality forage (Sutherland, 1976). On the other hand, the half-life for liquid turnover may be 5-6 hours for sheep on pasture, or 18-20 hours for sheep fed all concentrate diets (Sutherland, 1976). As D increases, specific growth rate (U) also increases, resulting in less ATP used for maintenance and/or efficient microbial growth. Prins and Clarke (1980) reported maximum specific growth rates (Umax) for various rumen bacteria ranging from 0.28 h^{-1} for Lactobacilis sp. to 2.04 h^{-1} for S. Bovis. However, only 24% of YATP was used for maintenance when D was increased to 0.12 h^{-1}. Using mixed rumen bacteria, Isaacson et al. (1975) altered D from 0.02, 0.06, and 0.12 h^{-1} and found respective YATPs to be 7.5, 11.6, and 16.7. Harrison and McAllan (1980) recalculated data of Isaacson et al. (1975) and showed that when D was 0.02, 65% of YATP was used for maintenance. By infusing artificial saliva intraruminally in sheep, Harrison et al. (1975) increased D from 0.38 to 0.98 h^{-1} with a 24% increase in YATP.

Microbial ecology of the rumen is also drastically changed by D. Hobson and Summers (1972) studied growth rates of various bacteria, and found that efficiency appeared to always be a function of D. Latham and Sharpe (1975) reported a predominant population

of selonnomads and bacterioides in sheep with a low D. An increase in D by addition of minerals, resulted in a decrease in bacterioides and increase in gram variable, chain cocci organisms.

At high rates of D, decreases in protozoa and microbial cell lysis may also contribute to the increased microbial efficiency. The slower growth rate of protozoa would limit their numbers at high rates of D (Leng, 1976). Sutherland (1976) reported that when D rate was increased, microbial crude protein was increased 20 to 35% in defaunted sheep due to a reduction in cell autolysis and recycling within the rumen.

Nitrogen Requirements for Microbial Growth

Assuming sufficient energy and carbon, nitrogen becomes the next major limiting factor for microbial growth. Ammonia is the central and preferred N compound for synthesis of microbial protein (Bryant, 1970). Tracer studies using N^{15} suggest 50 to 80% of microbial N goes through an ammonia pool (Nolan and Leng, 1972; Mathison and Milligan, 1971). Pilgrim et al. (1970) reported that 63% of bacterial N and 37% of protozoal N was derived from an ammonia pool.

The concentrations of ammonia needed for optimal microbial growth has been controversial. Allison (1970) reported maximal growth until ammonia was less than 4.6 mM. These findings are similar to those found *in vitro* in mixed cultures by Satter and Slyter (1974).

Roffler and Satter (1975) presented *in vivo* evidence that rumen

ammonia concentration of 3.6 mM was sufficient to obtain maximum yields of cell protein. Orskov (1973) could not increase the flow of protein from the rumen of sheep when ammonia was greater than 6.3mM. Hume et al. (1970) also found no increase in rumen protein concentration when rumen ammonia was in excess of 6.4 mM, although flow of protein from the rumen was not maximized until 9 mM . In rumen fermentors similar to those used by Slyter and Satter, Bull et al. (1975) showed an increase in microbial protein production until rumen ammonia reached 10 mM. Similarly, Kansas workers (Edwards et al., 1980) demonstrated increased microbial protein production with rumen ammonia as high as 52 mM. Using cannulated animals, Miller (1973) suggested maximum non ammonia nitrogen (NAN) flow at the duodenum when rumen ammonia was 17 mM . In an original approach, Mehrez et al. (1977) reported that maximum rates of fermentation did not occur until rumen ammonia reached 11.4 mM. However, Ortega et al. (1979) found no effect on fermentation rates above 4.0 mM ammonia. More recently, Tamminga (1979) reported that dietary protein of 13.4% was inadequate to sustain maximum microbial fermentation of crude fiber, however, no effect was observed on degradation of the nitrogen free extract.

In sheep fed semi-purified diets of concentrate, roughage and concentrate, or roughage, microbial protein production did not increase when rumen ammonia exceeded 2.8, 6.9, and 1.6 mM for the three rations (Pisulewski et al., 1981). Wallace (1979) reported similar findings to Mehrez when rumen ammonia was increased to levels considered by some (Satter and Slyter, 1974) as excess. As mean rumen ammonia

concentrations increased from 6.1 to 13.4 mM, a 90% increase in rate of degradation of rolled barley, and smaller increases in rates of protein and fiber degradation occurred. Despite low activity of alanine dehydrogenase and glutamine pyruvate-aminotransferase, alanine concentrations were increased with greater ammonia. Wallace suggested that bacteria which use ammonia by the alanine pathway require high ammonia for growth and these same bacteria may be responsible for plant degradation.

In vitro, Illinois workers (Schaefer et al., 1980) showed ammonia saturation constants of less that 50 μM indicating that microbial growth was 95% maximal when ammonia was 1 mM. These data are much lower than data just discussed. Hespell and Bryant (1979) suggested that ammonia level in the rumen should seldom limit growth of ammonia requiring bacteria based on these constants. They believe that responses to greater levels of ammonia may be indirect such as formation of ammonium bicarbonate causing a higher pH.

In practice, rumen ammonia levels may be misleading as a guide for achieving maximal microbial growth, due to normal daily fluctuations (Coppock et al., 1976) and formation of microcolonies where ammonia concentrations may differ greatly from that in the surrounding media (Cheng and Costerton, 1980).

Although ammonia is the predominant nitrogen source for rumen microbial protein synthesis, peptides and amino acids may also be stimulatory to microbial growth (Pittman and Bryant, 1964; Hungate, 1966), especially for cellulolytic bacteria (Hespell and Bryant, 1979). Sauer et al. (1975) reported that all amino acid carbon could be synthesized

by ferredoxin dependent reductive carboxylation to appropriate keto acids. However, in vivo tracer studies with N^{15} (Salter et al. 1979) indicated that rates of methionine and phenylalanine may limit microbial growth on protein-free diets. Isonitrogenous substitution of small amounts of amino acids for urea markedly stimulated rumen microbial growth (Maeng and Baldwin, 1976). Maeng and Baldwin (1976) suggested urea:amino acid N ratio of 3:1 for optimal microbial growth. In explaining these data, Hespell and Bryant (1979) suggested that amino acids may reduce the degree of energetic uncoupling. This is supported by an increase in the ratio of cell yield:VFA and YATP with amino acid additions.

Ammonia Fixation in the Rumen

Ammonia fixation in the rumen has been studied by many workers (Allison, 1969; Erfle et al., 1977; Schaefer et al., 1980). The primary reaction involves the addition of ammonia to an alpha-keto acid. Glutamic acid is formed by reductive addition of ammonia to alpha keto glutarate. The former can then be converted to essential or non essential amino acids by transamination reactions. Addition of ammonia to amino acids to form amides can also occur. Amidic NH can then transform keto acids to amino acids. Glutamine synthetase and glutamate dehydrogenase are the primary enzymes involved in rumen ammonia fixation. At low concentrations of ammonia, glutamine synthetase increases 10-fold and facilitates transfer of amide nitrogen from glutamine to alpha keto glutarate. It is of primary

importance when ammonia is low since its Km of 0.2 mM is relatively low, which suggests a high affinity for ammonia. Glutamate dehydrogenase becomes more active as ammonia concentrations increase above 5 to 6 mM.

One molecule of ATP is required for every molecule of ammonia fixed via the glutamine synthetase reaction. Schaefer et al. (1980) calculated that 14% of the ATP available for metabolism would be needed to fix all ammonia by this reaction and would therefore result in low cell yields. Enzymes involved in ammonia fixation in the rumen are presented in Table 1.

TABLE 1.

Km's FOR AMMONIA OF SOME AMMONIA-FIXING ENZYMES[a]

Enzyme	Source	Km	pH of Determination
Alanine dehydrogenase	B. subtilis	3.8×10^{-2}M	8.0
Glutamate dehydrogenase (NADPH)	Yeast	5.0×10^{-4}M	7.6
Aspartase	B. cadavaris	3.0×10^{-2}M	6.8
Asparagine synthetase	S. bovis	4.0×10^{-3}M	7.2
Glutamine synthetase	E. coli	1.8×10^{-3}M	7.0
Carbamoyl phosphate synthetase	E. coli	1.2×10^{-2}M	8.0

[a] Data taken from Barman (1969)

Other Essential Growth Factors

Branched chain fatty acids of four and five carbons have been reported as essential nutrients for rumen microorganisms. These acids stimulate growth of cellulolytic organisms resulting in increased fiber digestion (Bryant, 1973; Dehority et al., 1967; Allison, 1970). Rumen microbes are able to carboxylate fatty acids to form alpha-keto acid analogs of amino acids (Allison and Robinson, 1970). A deficiency of branched-chain fatty acids could occur on low protein diets since they result from normal protein degradation.

Sulfur has been suggested as an essential element for optimal fixation of ammonia by rumen microbes. The optimum nitrogen to sulfur ratio is between 12 and 15:1 (NRC, 1978). Other minerals have also been identified as essential elements (Durand and Kawashima, 1981).

Systems for Estimating Protein Requirements

Traditional methods to estimate protein requirements for cattle have used practical feeding trials in which response to increments of protein in the diet has been determined. This method provides direct answers applicable to particular conditions of the trial; however, variable responses occur due to varying management conditions, level of production and types of feed. Traditional systems for calculating nitrogen requirements have been based on digestible crude protein or available protein. Shortcomings of this approach

are many: 1) Extensive degradation of dietary protein in the rumen and incorporation into microbial protein invalidate employing classical protein systems used in non ruminants (e.g., Biological Value); 2) Fecal nitrogen contains large quantities of undigested microbial protein from the rumen and hind gut thus complicating simple calculations of digestibility; 3) There is an inability to differentiate between absorption of various nitrogen sources, such as amino acid nitrogen, ammonia nitrogen or nucleic acid nitrogen; 4) Relationships between energy intake, protein requirements and rumen fermentation are not considered; and 5) The value of non protein nitrogen (NPN) under various circumstances is not estimated.

In ruminant animals, the protein value of the diet is reflected by the total amount and composition of amino acids absorbed at the small intestine. Hence, there have been many attempts to develop a reliable system for protein evaluation for ruminants (Waldo and Glenn, 1982; Huber and Kung, 1981). These systems equate protein not degraded in the rumen and microbial protein synthesis with flow of amino acid protein reaching the small intestine for absorption. By determining microbial protein and duodenal NAN, one can estimate undegraded dietary protein. One difficulty is that methods for estimating microbial protein are tentative (Smith, 1975; Theurer, 1982). Thus, accuracy of estimating undegraded feed protein is also suspect. Despite variability in rumen undegraded protein and the varying contribution of endogenous

nitrogen to digesta flow, amino acid composition of duodenal digesta is relatively constant (Tamminga and Van Hellemond, 1977). This can be partly explained by the appreciable amount of microbial protein and its uniform composition.

Waldo and Glenn(1982) have discussed the assumptions made with various protein systems. Microbial crude protein produced per unit of fermented organic matter was similar for the four systems tested, however, digestibility of that protein was variable. The British and French systems assumed 0.56 digestibility, while the German and Danish systems were 0.68 and 0.63, respectively. Likewise, digestion of rumen undegraded dietary protein tended to be low for the British and French systems, compared to the German and Danish.

Verite et al. (1979) compared a number of systems in formulating diets for dairy cows. Crude protein content of a theoretical diet was calculated for different levels of milk production in each system using a nitrogen degradability of 0.66 (see Figure 1). The Burrough's system gave unacceptably low values. The French system was similar to NRC recommendations, except at high levels of milk production where intake was low (early lactation). At low levels of production, the model of Satter and Roffler appeared to underestimate protein needs as rations with 10% protein would limit overall rumen fermentation (Huber and Kung, 1981). The British system generally called for less protein which increased

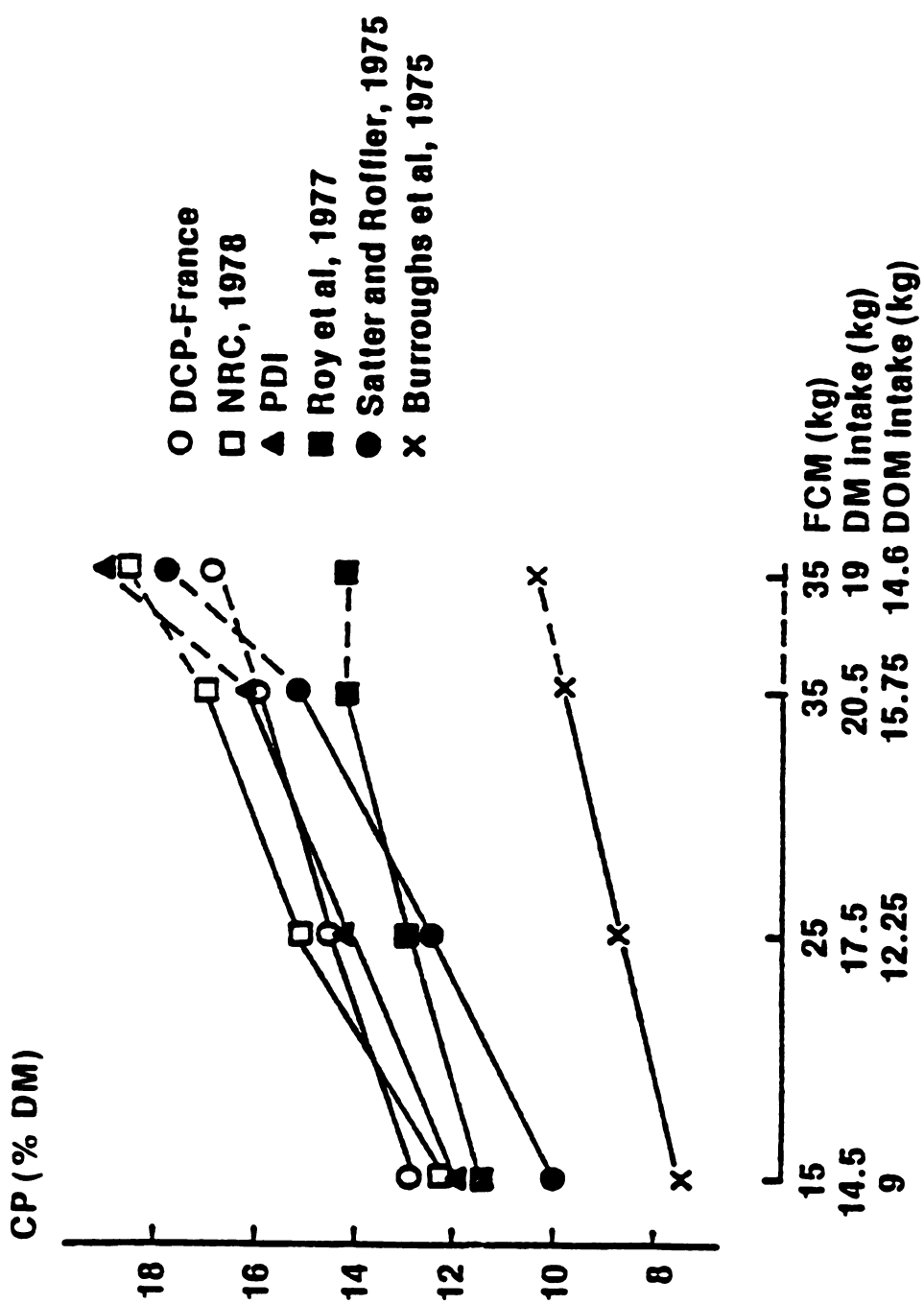


Figure 1. Crude protein of dairy rations according to different models with N degradability 0.66 (Verite et al., 1979).

to a lesser extent with increasing production. Requirements during high levels of production and energy deficits do not increase because they are dependent on microbial nitrogen requirements.

Waldo and Glenn (1982) have simulated typical rations of the various systems when nitrogen undegradability was optimized (Figure 2). Differences between systems at low production levels were small, 9 to 12.5% protein, and were all apparently below optimum requirements for rumen fermentation (Huber and Kung, 1981). Differences at high production levels ranged from 12 to 17% protein. The French system required the most protein because they assumed lowest dry matter intakes. On the other hand, British requirements were low because they assumed lower metabolizable protein requirements for maintenance and milk.

In all systems, degradability of dietary protein should be such that little or no ammonia nitrogen is wasted. Treacher (1979) has presented two figures relating this concept to requirements for production. Figure 3 depicts the relationship between milk yield and the proportion of dietary protein required as degradable (RDP) or undegradable (UDP). As production increases, percent UDP increases. Figure 4 presents a similar relationship between RDP, UDP, and milk produced throughout the entire lactation. Similar estimates for UDP have been made by Waldo and Glenn (1982) in Figure 5. The metabolizable protein system of Satter and Roffler (1982) is not included because it uses the point of ruminal ammonia accumulation as a benchmark for protein degradability and

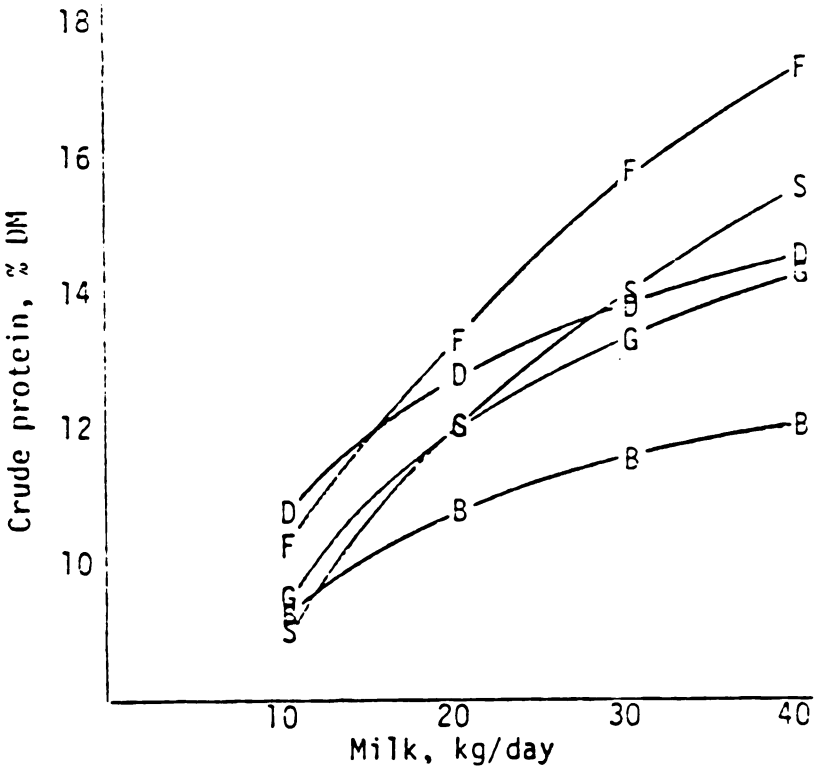


Figure 2. Crude protein (% of dietary DM) requirement as a function of milk production (kg/day) for five European systems with undegradability at optimum. B, British; D, Danish; F, French; G, German; and S, Swiss. (Waldo and Glenn, 1982).

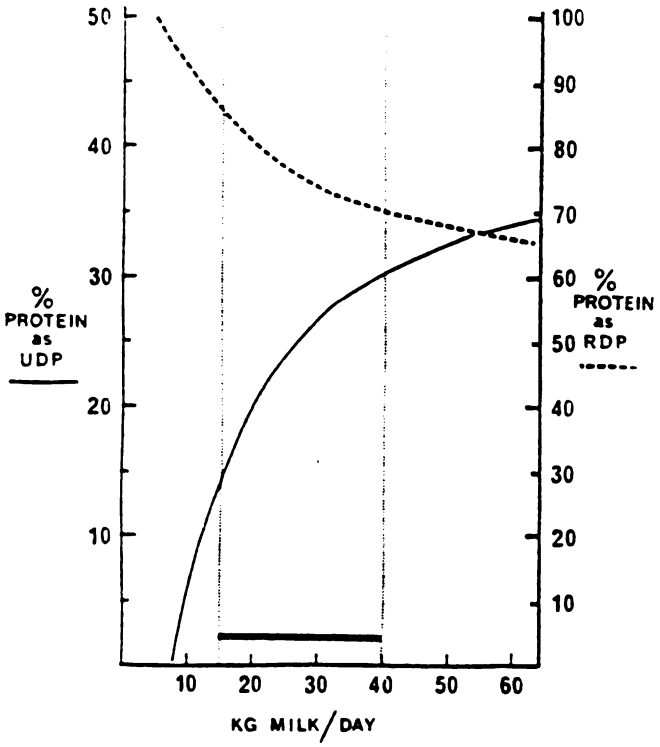


Figure 3. The relationship between milk yield and the proportion of dietary protein required as UDP and RDP, as predicted by the ARC model. Vertical lines indicate the commercial range of milk yield (Treacher, 1979).

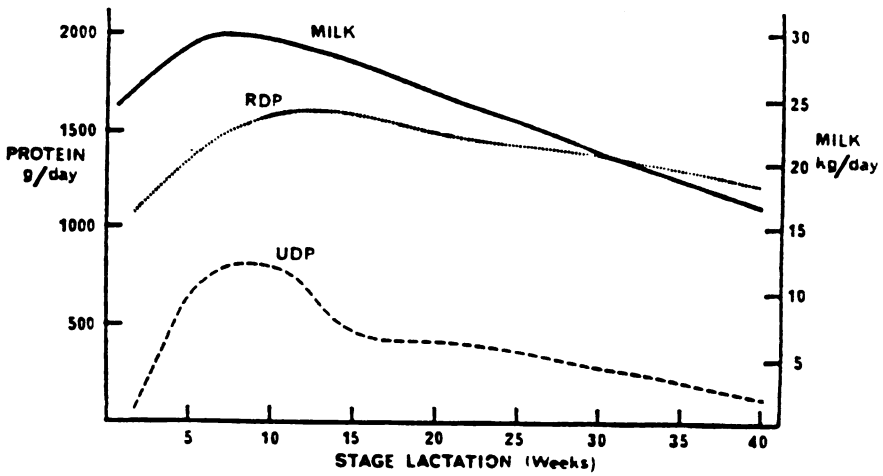


Figure 4. The dietary protein requirements over a complete lactation for a Friesian cow peaking at 30 kg milk per day, as predicted by the ARC model. The diagram takes account of body-weight changes and of restricted appetite in early lactation (Treacher, 1979).

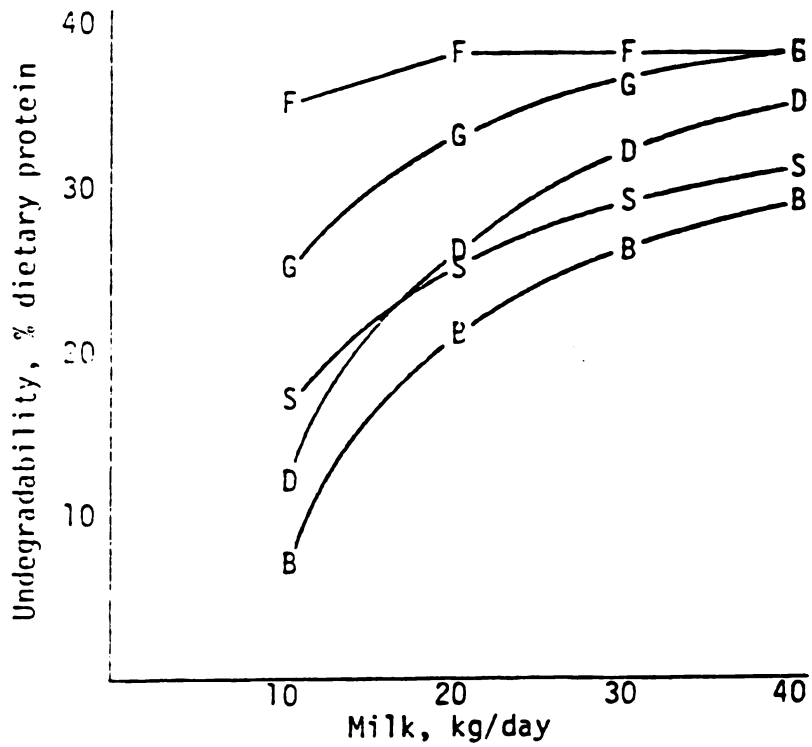


Figure 5. Undegradability (%) of dietary crude protein as a function of milk production (kg/day) for five European systems with crude protein at optimum. B, British; D, Danish; F, French; G, German; and S, Swiss (Waldo and Glenn, 1982).

microbial protein synthesis. Moreover, this system does not require determination of protein degradability on most low protein feeds and does not rely on an estimate of microbial protein synthesis.

Whitlow and Satter (1979) recently compared the ability of various systems to predict the flow of amino acids to the intestine, including those of Burroughs et al. (1975), Journet and Verite (1977), Kaufmann (1977), Roy (1977), and Satter and Roffler (1975). For most rations (low and high protein fed to sheep and cattle) Satter and Roffler best predicted actual flow of amino acid nitrogen to the small intestine. However, in rations fed only to cattle, the French system of Journet and Verite was most accurate (Table 2). Whitlow and Satter did point out that apparent over-

TABLE 2

ESTIMATES FOR AMINO ACID FLOW ACCORDING TO DIFFERENT MODELS
Rations Fed to Cattle (n = 26)

AAF[a]=					1093
AAF =	-144.09	+	1.50	AAF-Burroughs et al.	823
AAF =	28.24	+	0.97	AAF-Journet and Verite	1100
AAF =	-33.97	+	1.16	AAF-Kaufmann	971
AAF =	-194.77	+	1.42	AAF-Roy et al.	906
AAF =	26.45	+	0.96	AAF-Satter and Roffler (Constant Degradation)	1108
AAF =	30.04	+	0.96	AAF-Satter and Roffler (Variable Degradation)	1102
AAF =	22.14	+	0.84	Crude protein intake	1280

[a] Amino acid flow to small intestine measured in g/day.

or under-estimations of amino acid flow in certain systems were

probably compensated for by lower or higher efficiency of amino acid absorption and use by tissue.

Tamminga and Van Hellemond (1977) suggested it was easier to relate total amount of amino acids reaching the small intestine to readily determined characteristics of feed such as crude protein, digestible protein, organic matter, or digestible organic matter. These workers studied relationships where organic matter intake ranged from 4.7 to 14.6 kg/day and nitrogen intake varied from 140 to 430 g/day. They concluded that amino acid flow to the small intestine was more dependent on dietary supply of digestible organic matter than nitrogen or digestible crude protein. The current status of most protein systems is under intensive scrutiny. Overall, these systems better predict nitrogen needs compared to the crude protein or digestible crude protein approaches, but more information and field testing are needed before accurate prediction of nitrogen requirements of ruminants can be made.

Efficiency of Amino Acid Use

The absorption efficiency of amino acids from the small intestine is about 0.6 to 0.8 (Oldham, 1980). On the average a greater proportion of essential amino acids are absorbed. Values for early lactation are lacking but are probably closer to 0.8. Although it is difficult to quantitate amino acid absorption, Cripps and Williams (1975) and Weston (1979) have shown greater absorption in early lactation in rats and ewes. Oldham

(1980) speculated that absorption efficiency is 0.8 in early and 0.7 during the remainder of lactation. With an estimated transfer of absorbed amino acids to product of 0.60 to 0.85, he suggested the net transfer of duodenal amino acid to product was 0.60 for early and 0.46 during the remainder of lactation.

Amino Acid Requirements

In lactating animals the mammary gland extracts large amounts of amino acids for synthesis of milk protein. However, the animal still has a maintenance requirement that must be met which includes amino acids as precursors for gluconeogenesis.

Estimates of nitrogen requirements for maintenance are conflicting. When Verite et al. (1979) summarized maintenance requirements of the various systems, values ranged from 100 to 395 g of alpha-amino nitrogen x 6.25 needed to be absorbed daily from the intestine. Tamminga and Oldham (1980) stated these differences were due to differences in definition of "alpha-amino nitrogen absorbed" from the small intestine and much of the disparity was due to inclusion of metabolic fecal nitrogen and its definition.

A substantial proportion of amino acids required for maintenance are metabolized in the gut and the liver. There appears to be no estimates for dairy cows of amino acids metabolized in the gut wall. Tagari and Bergmann (1978) reported that a substantial part of most amino acids absorbed from the

small intestine of sheep were metabolized in the gut wall, but the former study showed no preference for either essential or non-essential amino acids. When a 15.6% protein diet was fed, 67 and 71% of the essential and non-essential amino acids were metabolized in the intestinal wall. Tamminga and Oldham (1980) calculated that increasing the amino acids absorbed from the small intestine of sheep from 2.8 to 4.5/kg $w^{0.75}$ per day caused a decrease in gut wall metabolism from 0.71 to 0.52. If dairy cattle absorbed 2 to 3 times this amount of amino acids, then a much smaller proportion would be metabolized in the gut wall.

Amino Acids as Energy Sources

Amino acids may be converted to glucose during either a shortage of glycogenic precursors, or a surplus of absorbed amino acids. A discussion of amino acids as energy sources was reported by Lindsay (1980). Clark (1975) showed preferential use of NEAA for gluconeogenesis, although essential amino acids could be used. Of the total amino acids present in the duodenal digesta, 16% are essential and glucogenic while 15% are essential and partially glucogenic (Tamminga, 1975). Since differences in amino acid absorption are relatively small, no more than 25% of amino acids are used for gluconeogenesis could be essential. Branched chain amino acids and lysine are not available for gluconeogenesis because they are ketogenic or partially ketogenic.

During early lactation when glucose is limiting, amino acids

may be used to meet glucose requirements. Bruckental (1980) suggested that in cows producing over 30 kg milk/day less than 5% of the glucose supply was from amino acids. Oldham (1978) presented evidence from dairy cows that protein conversion to end product was done 0.65 to 0.85 efficient; thus 0.15 to 0.35 of absorbed protein might be available for gluconeogenesis. Assuming a major part of surplus protein was oxidized and that 55 g of glucose was synthesized from 100 g of protein, high yielding cows would obtain less than 2% of their required glucose from protein.

After maintenance needs are met, protein is used for milk protein production with an efficiency of 0.60 to 0.75 (Tamminga and Oldham, 1980). Oldham (1980) reported that increased energy supply increased efficiency of protein utilization. Clark et al. (1978) calculated that amino acid nitrogen was utilized by the mammary gland with an efficiency of 0.91.

Part of the NEAA excreted in milk protein are synthesized de novo in the mammary gland. A major portion of the NEAA nitrogen is from essential amino acids, primarily arginine. Estimates for EAA nitrogen in milk range from 0.6 to 0.71. This would suggest that for a given output of EAA in milk, a surplus of about 50% must be extracted from the blood.

Protein Needs for Lactating Cows

Revisions in suggested protein allowances for lactation have recommended higher protein intake per unit of milk (NRC, 1971; NRC,

1978). These increases generally were supported by research results showing greater milk yields at higher protein percentages (Clay et al., 1978; Edwards et al., 1980; Gardner and Park, 1973; Grieve et al., 1974; Murdock and Hodgson, 1979; Sparrow et al., 1973; Van Horn and Zometa, 1978) but not always (Chandler et al., 1976; Foldager and Huber, 1979; Patton et al., 1970, Van Horn et al., 1976). Where milk yields were increased by increasing crude protein concentrations above 13 to 14% of the ration dry matter, total energy (Clay and Satter, 1979; Claypool et al., 1980; Cressman et al., 1980; Davis, 1978; Grieve et al., 1974; Murdock and Hogdson, 1979; Sparrow et al., 1973; Van Horn and Zometa, 1978) or concentrate (Edwards et al., 1980) intakes usually were improved.

Van Horn and Zometa (1978) summarized 13 experiments where soybean meal was used to increase protein in rations for lactating cows and concluded that higher protein had much of its effect through stimulation of energy intake. Foldager and Huber (1979) demonstrated that when high protein did not increase feed intakes, milk yields of early lactation cows (3 to 20 wks postpartum) fed 13% crude protein were equal to those fed 16%. Others have found little response in milk yields to dietary protein in excess of 13% when dry matter intakes were similar to lower protein control diets (Chandler et al., 1976; Clay et al., 1978; Van Horn et al., 1976).

Satter and Roffler (1975) recommended that average ability cows during the first 4 months after calving receive 16% protein of only

plant origin. A decrease to 12.5% was proposed for the remainder of the lactation during which NPN might be included.

As protein content of the ration increases, response in milk yield to higher protein diminishes (Claypool et al., 1980; Edwards et al., 1980; Satter et al., 1979). Satter et al. (1979) suggested that the amount of dietary protein for lactating cows should depend upon protein price relative to profit from the increased milk resulting from a given increment in ration protein. When the cost of soybean meal was 36¢, milk 26¢, and shelled corn 11¢ per kg, recommended crude protein for high producing cows early in lactation did not exceed 16%. Changes in prices of feed protein, feed energy, or milk will alter the percent of dietary protein that is most profitable, but several recent studies showed that income over feed costs was maximal for high yielding cows at 14 to 16% (Claypool et al., 1980; Satter et al., 1979). Satter's (1982) recommended protein requirements relative to feed costs are presented in Table 3. Higher levels of protein were not profitable due to the diminishing increase in milk yield with increase in ration protein. Similarly, Van Horn (1982) has suggested that 14% CP is optimum in dairy rations and that the likelihood of higher protein being needed was remote. As mentioned earlier, Broster (1977) has estimated a 200 kg increase in total milk yield for every 1kg increase in peak lactation. The typical response to increasing protein in the diet (Table 4) would be drastically different in mid to late lactation where nutrient needs

TABLE 3

RECOMMENDED LEVELS OF PROTEIN FEEDING FOR MAXIMUM PROFIT

Level of Milk Production for Total Lactation	Peak Daily Milk Production	Days of Lactation		
		0-100	100-200	200-305
(kg)	(kg)	(% of total ration dry matter)		
		Relatively low protein prices[2]		
<5,450	<27	14.5[3]	12.5[1]	12.5
5,450-6,800	31	16.0[3]	13.0	12.5
6,800-8,180	38	17.5[3]	14.5[3]	13.0
>8,180	>41	19.0[3]	16.0[3]	14.5[3]
		Relatively moderate protein prices		
<5,450	<27	13.0	12.5	12.5
5,450-6,800	31	14.5[3]	12.5	12.5
6,800-8,180	38	16.0[3]	13.0	12.5
>8,180	>41	17.5[3]	14.5[3]	13.0
		Relatively high protein prices		
<5,450	<27	13.0	12.5	12.5
5,450-6,800	31	13.0	12.5	12.5
6,800-8,180	38	14.5[3]	12.5	12.5
>8,180	>41	16.0[3]	13.0	12.5

[1]Underlined values mean that nonprotein nitrogen can be used to supply all or nearly all of the supplemental protein.

[2]As a crude guide to determine whether protein prices are relatively low, moderate, or high, use the following:

Take the difference between the price of 1 lb of soybean and 1 lb of shelled corn and subtract it from the price of 1 lb of milk:

If you obtain 6¢ or more, protein is relatively low in price;

If you obtain 2 and 6¢, protein is relatively moderate in price;

If you obtain less than 2¢, protein is relatively high in price.

Example: When milk is 14¢, soybean mean 15¢, and shelled corn 6¢ per lb, then subtracting the difference between soybean mean and shelled corn (15-6, or 9¢) from milk gives 5¢ (14-9 or 5¢). This is a moderate protein price.

[3]Situations where relatively resistant protein sources might be advantageous. Feeding resistant proteins would tend to lower the suggested requirement, assuming the resistant protein sources had comparable essential amino acid content. (from Satter, 1982).

TABLE 4

MARGINAL CHANGES IN MILK PRODUCTION AND DRY MATTER INTAKE AS A RESULT OF CHANGING RATION PROTEIN PERCENT[1]

Change in Protein % of Ration Dry Matter From:	kg/Day Increase In: Milk	DM Intake
10 to 11	1.9	1.4
11 to 12	1.5	0.7
12 to 13	1.0	0.4
13 to 14	0.8	0.3
14 to 15	0.6	0.2
15 to 16	0.5	0.1
16 to 17	0.4	0.1
17 to 18	0.3	0.1

[1]Soybean mean was the dominant protein supplement, and corn grain and corn silage were the major ration ingredients. Cows were in early lactation, and were capable of producing about 7000 kg of milk per lactation. (from Satter, 1982).

would be low. Indeed, Barney et al. (1981) showed no response to protein level. Switching cows from a basal ration of 16% CP producing 28 kg of milk 19 wks postpartum to either 12, 14, 16, or 18% CP had no effect on milk yields or dry matter intake. In light of these findings and those of Broster (1977), it is the opinion of this author that cows early in lactation should be fed for maximal milk production regardless of feed prices.

A differential response to protein was observed for multiparous cows and first calf cows (Cressman et al., 1980; Roffler et al., 1978). Roffler et al. (1978) reported an increase in milk yields of about 6 kg/day when dietary protein was raised from 12.2 to 16.2%, but no response was noted on first calf cows. Significantly greater dry matter intakes on high protein were noted for cows but not heifers. Second-calf cows and older responded similarly. Cressman et al. (1980) confirmed that mature cows and first calf heifers respond differently in milk yields when dietary protein increased above 12%, but age did not affect nitrogen utilization, digestabilities, or rumen or blood measurements.

The effect of level of milk production and extent of negative energy balance on net amino acid nitrogen need in cows is presented in Table 5. As milk production increases, microbial amino acid nitrogen cannot meet demands for high levels of production. Thus it is apparent that undegraded dietary protein must be increased as production increases.

Systems for establishing protein requirements have been

discussed earlier.

TABLE 5

EFFECT OF LEVEL OF MILK PRODUCTION AND EXTENT OF NEGATIVE ENERGY BALANCE ON THE NET AAN NEED IN COWS WEIGHING 600 kg[a]

Level of Production (kg FCM/d)	ME Requirement (MJ/d)	ME Intake (MJ/d)	Net AAN[b] Required (g/d)	Microbial Contribution (Net AAN g/d)	Net AAN[b] (g/MJ of ME)	Deficit (%)
0	61.9	61.9	9.7	32.8	0.16	—
10	110.5	110.5	65.7	58.6	0.59	11
20	159.1	159.1	121.7	84.3	0.76	31
40	256.3	256.3	232.7	135.8	0.91	42
40	256.3	207.8	232.7	110.1	1.12	53
60	353.5	353.5	346.7	187.4	0.98	46
60	353.5	256.3	346.7	135.8	1.35	61

[a] Orskor, 1980. [b] Amino Acid Nitrogen.

Amount of Protein

At excessive protein intakes, efficiency of utilization is reduced because more protein is available than the host can process physiologically (Broster, 1972). Inefficiencies arise from elimination of surplus urea that results from protein catabolism (Tyrell, 1970). Metabolic abnormalities also have been reported in cows fed more protein than needed (Gould, 1969; Jordan and Swanson, 1979; Julien et al., 1977). Julien et al. (1977) reported a high incidence of downer cow syndrome when dairy cows were fed excessive protein (15 vs 8% CP). These problems were compounded by more abortions, displaced abomasums, and milk fever.

Gould (1969) suggested consumption of too much protein tended to increase anestrus, decrease conception rate, and lower peak milk

production. Jordan and Swanson (1979) fed isocaloric rations of 12.7, 16.3, and 19.3% CP from 0 to 95 days postpartum. Highest protein produced fewest days to first observed estrus and most services per conception. Days open increased from 69 to 96 to 106 for low, medium, and high protein. Treacher et al. (1976) reported increased liver glutamic dehydrogenase and ornithine carbamyl transferase in plasma of cows fed excessive protein. Huber (unpublished data) recently summarized breeding data from 11 studies involving 1,109 cows fed varying protein levels in early lactation. The data in Table 6 would suggest no relationship between protein level and reproductive performance up to 18% CP. Cows fed 19 to 20% CP tended to have more services per conception than when less protein was fed, but also tended to have less days open. Recommendations never exceed 18% CP in dairy rations, which was reflected in only 3% of the animals fed in excess of this amount in the 11 studies. Caution should be taken because diets can contain extensive amounts of rumen degradable protein leading to high levels of plasma urea and ammonia which may be deleterious. Examples of such diets would be: 1) NPN-treated corn silage and soybean meal or 2) wet haylage and high moisture corn diets.

In ruminants, low protein intakes are utilized at relatively high efficiencies because of nitrogen recycling to the rumen as urea and reduced losses through the kidneys (Mercer and Annison, 1976). At the rumen level, increased retention time of nutrients, depressed intakes, and a lowered capacity to digest organic matter (Huber, 1978; Huber and Thomas, 1971) result from too low protein,

probably because microbial fermentation is curtailed (Orskov, 1976).

TABLE 6

EFFECT OF PROTEIN LEVEL ON REPRODUCTIVE PERFORMANCE OF DAIRY COWS[c]

% Protein	Number of Cows[a]	Services/ Conception[a]	Days Open	Settled First Service (%)[b]	Sold Open (%)[b]
9	7	2.10	114.0	----	----
11-13	306	2.14	123.2	35.4	16.7
14-15	259	2.09	121.9	38.8	13.9
16-18	328	1.92	119.3	41.3	13.7
19-20	35	2.34	103.8	----	----

[a] These data only include cows which settled in the trials.
[b] Number of cows averaged for protein levels (11-13, 14-15, 16-18) was 192, 188, and 256, respectively.
[c] From Huber (unpublished data).

Low protein also depresses milk production through decreased lactose synthesis and reduced mobilization of body fat (Orskov et al., 1977).

Protein Quality

It was thought that quality of protein was not critical in ruminant diets because of extensive modification and synthesis by rumen microbes (Brady, 1976). However, certain protein supplements such as distillers dried grains and soybean meal resulted in higher milk yields than others (Lossli et al., 1958; Lossli et al., 1961; Lossli et al., 1960; Warner et al., 1957), which authors attributed

to differences in energy and not protein. Delivery of protein or amino acids directly to the postruminal digestive tract to escape rumen breakdown enhanced milk and milk protein production (Clark, 1975; Schwab et al., 1976; Vik-Mo et al., 1974). Magnitude of response to abomasal infusion of casein varied with type of ration, protein content of the ration, and production by cows. In early lactation, cows producing over 25 kg/day of milk and fed adequate ration according to NRC (1971) increased milk protein production from infused casein 10 to 15%. Approximately 50% of the increase

TABLE 7

THE INFLUENCE OF CASEIN INFUSION ON THE MILK PRODUCTION AND MILK PROTEIN CONTENT OF DAIRY COWS[a]

Author	Daily Infusion (g)	Milk Yield (kg/day) control	Milk Yield (kg/day) infusion	Protein Content control	Protein Content infusion
Orskov et al. (1977)	300	14.4	17.0	3.07	3.51
	250	16.8[b]	19.8	2.52	2.84
	500	16.8[b]	21.6	2.52	2.96
	750	16.8[b]	21.4	2.52	3.15
Broderick et al. (1970)	800	30.4	32.0	3.14	3.34
Spires et al. (1973)		32.6	34.1	2.86	3.11
Derrig et al. (1974)	400	23.3	24.6	3.08	3.24
Tyrell et al. (1972)	860	≈24.0	≈27.0	----	----
Vik-Mo et al. (1974)	285	17.0	17.9	3.00	3.09
Schwab et al. (1976)	425	28.5	30.1	2.88	3.04

[a] Kaufmann, 1979.
[b] Glucose infusion.

was attributable to more milk and the remainder to higher percent protein in milk (Vik-Mo et al., 1974). Infusion of casein resulted in greater increases in milk protein production than isocaloric amounts of glucose, suggesting that milk protein synthesis was limited by both energy and amino acid shortage (Vik-Mo et al., 1974). The influence of casein infusion on milk production and milk protein content of dairy cows is presented in Table 7.

Specific amino acids identified in the lowest supply relative output in milk were methionine, lysine, threonine, and phenylalanine (Clark, 1975; Schwab et al., 1976; Vik-Mo et al., 1974). The former three (in that order) were first limiting amino acids of microbial protein to support nitrogen balance in prowing lambs (Hatfield, 1971). Wisconsin workers (Schwab et al., 1976) infused free amino acids in various combinations into the abomassum of lactating cows and showed that the combination of methionine and lysine gave the greatest response of production.

In contrast to plant proteins, the amino acid composition of rumen microbial protein is constant under a variety of nutritional regimes (Bergen et al., 1967,1968; Hungate, 1966; Purser and Buechler, 1966; Syvaoja and Kreula, 1979).

Protein Degradation in the Rumen

There has been a concerted effort to develop methods for minimizing degradation of protein in the rumen through a selection

of feedstuffs. Variation in distribution of amino acids in insoluble and soluble fractions of plant protein supplements (Macgregor et al., 1978) is an important finding because response to undegraded protein depends on the pattern of amino acids supplied for post ruminal absorption. Hence a protein source may contain an abundance of needed amino acid, but if that amino acid is degraded disproportionately compared to others in the rumen, post-ruminal absorption may be minimal. A simplified diagram of nitrogen metabolism is presented in Figure 6.

Form of Nitrogen

Dietary nitrogen often is divided into its protein and NPN components. The latter includes free amino acids, peptides, nucleic acids, free ammonia, ammonium salts, urea, biruet, and nitrates. Also, dietary nitrogen is separated into insoluble and soluble fractions. Soluble nitrogen is that extracted by a neutral buffer with low ionic strength (Wohlt et al., 1973) and the insoluble nitrogen often is calculated by subtraction of soluble from total nitrogen. Estimates of nitrogen solubility for various feeds have been reported (Crawford et al., 1978; Crooker et al., 1978; Wohlt et al., 1973). Even though nitrogen solubility of a protein and its degradability in the rumen are related (Hendrickx, 1975), they do not always equate to one another. For example, nitrogen solubility of soybean meal is about 15 to 20%. However, the nitrogen in soybean meal is probably 65 to 80% degraded in the

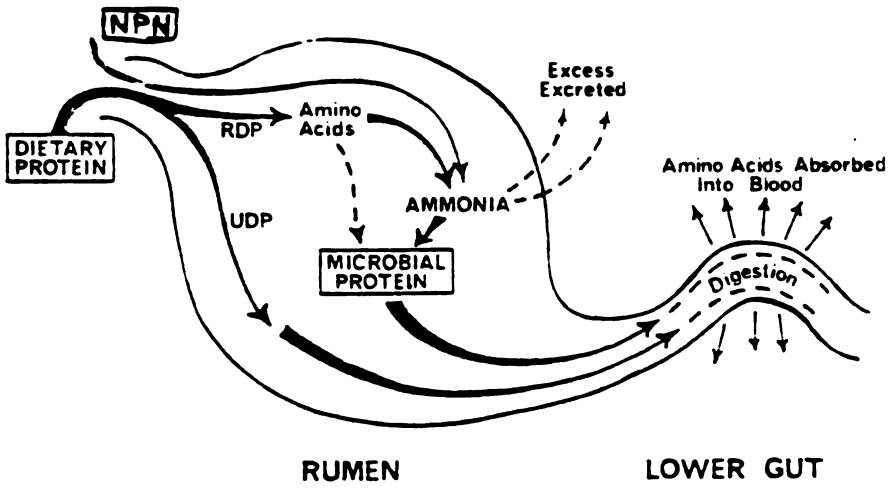


Figure 6. Simplified diagram of nitrogen pathways through the rumen. RDP = rumen degradable protein; UDP = undegraded dietary protein; NPN = non-protein nitrogen (Treacher, 1979).

rumen. Thus, it is apparent that solubility may not be the best way to estimate undegradable protein in dairy rations.

Regardless of the shortcomings of a nitrogen solubility index, its usefulness was demonstrated by significant increases in milk production when diets were formulated for lower protein solubility (Braund et al., 1978; Aitichison et al., 1976; Majdoub et al., 1978). Majdoub et al. (1978) fed two levels of protein (13 and 15%) and two levels of soluble protein (22 and 42%) to lactating cows. Milk, milk fat, milk protein, and solids not fat were greatest when cows were fed a diet that contained 15% CP with 22% soluble nitrogen. Of interest was the fact that cows fed 13% protein and 22% soluble nitrogen produced more milk than cows fed 15% protein but with a solubility of 42%.

After correlating several laboratory methods for predicting degradation of protein for nine classes of feeds with beef cattle grains, Poos et al. (1980) concluded that nitrogen solubility estimates were of questionable validity, that sampling time was critical for predicting from rumen dacron bag measurements, and that assay with fungal protease was the most accurate technique. The latter gave correlations with grain which exceeded 0.92 for all the times determined.

Feed Storage and Processing

Storage and processing of feeds greatly affects nitrogen utilization by ruminants. Ensiling increases soluble nitrogen to

40 to 60% of the total nitrogen in corn silage (Huber et al., 1973), to similar concentrations in low moisture haylage (Sutton and Vetter, 1971) and to over 70% in high moisture haylage. These soluble nitrogens are 2 to 3 times greater than in the fresh crop. Ensiled grains also increase in soluble nitrogen. Jones (1973) reported that 42% of the total nitrogen was soluble in high moistured shelled corn compared with 25% in dried corn. Additions of ammonia (Bergen et al., 1974; Buchanan-Smith, 1980; Huber et al., 1979; Huber et al., 1973; Waldo et al., 1980) and formaldehyde (Waldo et al., 1973) reduce proteolysis of ensiled forages. Harvesting too dry for good compaction, filling silos too slowly, or inadequate structures often causes excessive heating of forages (Thomas, 1982). Heat damage in forages measured by increased acid detergent insoluble nitrogen, results in less protein and energy for microbial and host needs. In excess of 40% of the nitrogen in haylage can be made unavailable due to excessive heating during storage.

Grinding, pelleting, rolling, cracking, and micronizing of feeds may affect protein utilization through altering rates of passage, degree of rumen degradation, and microbial protein synthesis (Osbourn et al., 1976; Thompson, 1972).

Heat Treatment

Controlled heating might decrease nitrogen solubility of forages (Beever et al., 1971, 1975) and grains (Glimp et al., 1967,

Nishimuta et al., 1972, 1974; Sherrod and Tillman, 1962; Tagari et al., 1962) without substantially diminishing overall protein a-variability. The heat causes carbonyl groups of surgars to combine with free amino groups of proteins in the Maillard reaction (Bjarnason and Carpenter, 1969). These linkages are more resistant than normal peptides to enzymatic hydrolysis. Even in the absence of sugars and carbohydrates, extensive heating causes unnatural amide bonds to form between the free amino groups of lysine and carbonyl groups of proteins (Bjarnason and Carpenter, 1969).

Rakes et al. (1972) roasted soybeans at 118°C for 3.5 minutes and noted slightly greater milk production for cows fed the roasted than raw beans, but the difference was not significant. Block et al. (1980) also reported that cows fed heated soybeans produced slightly more milk than those fed unheated beans. There was, however, a significant depression in milk fat for the group fed heated (2.52%) compared to unheated beans (3.50%).

A summary of trials feeding heat treated soybean or soybean products is presented in Table 8. Soybean meal (Ahrar and Schingoethe, 1979) or whole soybeans (Kenna and Schwab, 1979; Mielke and Schingoethe, 1980; Schwab et al., 1980; Smith et al., 1980) were heat processed in a cooker-extruder and fed as the main protein supplement to cows. For two early lactation studies (Mielke and Schingoethe, 1980; Smith et al., 1980), milk yields on extruded soy significantly exceeded controls (means of 32.9 vs 29.5 kg/day); but for the other trials, advantage for heat treated protein was

TABLE 8

SUMMARY OF EXPERIMENTS FEEDING HEAT TREATED SOYBEAN PRODUCTS TO LACTATING DAIRY COWS

Soy Product	Reference	Weeks in Lactation[a]	Normal Product milk/kg/day	Treated Product milk/kg/day	Comments
Soybeans	Smith et al., 1981	1	32.1	35.0	extruded
SBM flakes	Grummer & Clark, 1980	2	32.9	30.8	defatted flakes
Soybeans	Block et al., 1981	3	33.8	34.6	extruded beans, decreased milkfat
Soybeans	Kenna & Schwab, 1979	3	36.4	37.8	extruded beans, decreased milkfat
Soybeans	Schwab et al., 1980	3	33.6	35.0	extruded beans, decreased milkfat
SBM	Mielke & Schingoethe, 1979	3	26.8	30.8	extruded
Soybeans	Rakes et al., 1972	?	13.6	15.0	roasted
SBM[b]	Kung & Huber, 1982	3	34.2	35.4	dry heated
SBM	Netemeyer et al., 1982	6-15	23.0	23.7	flash heated
Soybeans	Mielke & Schingoethe, 1981	7	28.3	29.1	extruded
SBM	Arhar & Schingoethe, 1979	9	26.6	26.6	extruded
	AVERAGE	< 5	30.4	31.8	
		> 5	26.0	26.5	
		ALL TRIALS	29.2	30.4	

[a] Average weeks in lactation for cows when experiment started.
[b] Results from this dissertation.

slight.

Formaldehyde Treatment

Ferguson and coworkers (1967) showed that treatment of protein with formaldehyde (HCHO) retarded rumen degradation, allowing greater digestion and absorption in the small intestine. Initially, there is formation of methylol groups on the terminal amino group of protein chains and on the amino group of lysine (Ferguson, 1975). The methylol groups condense with primary amide groups of asparagine, glutamine, and arginine to form intra- and intermolecular methylene cross linkages between protein chains. Benefit of HCHO treatment is dependent upon reversibility of these reactions under acid conditions in the abomasum. Increases in alpha-amino nitrogen flowing to the duodenum has resulted with no reduction in overall digestion or absorption (Faichney, 1971; Barry, 1976). Formaldehyde treatment of high quality proteins such as casein (Flores et al., 1979; Stobbs et al., 1977) and whey protein concentrate (Muller et al., 1975) increased milk yields, but treatment of plant protein has not always proven beneficial (Clark et al., 1974; Hutjens and Schultz, 1971; Wachira et al., 1974). In several studies the amount of HCHO used approached the upper limit of application, so over-protection could have caused depressed protein utilization. The dramatic increase in milk yields (26.9 vs 23.7 kg/day) obtained by Muller et al. (1975) by applying 0.5% HCHO to whey protein concentrate was associated with greater mammary uptake of essential

amino acids. Thomas et al.(1981) reported slightly more milk production and significantly more dry matter intake when cows were fed ryegrass treated with formaldehyde and formalin. It was suggested by these workers that the increases in milk were mediated through changes in the supply of energy rather than protein. Milk yields of cows fed HCHO treated silages compared to untreated silages generally have been greater but results have not been consistent (Derbyshire et al., 1976; Waldo et al., 1973).

Tannin Treatment

Tannins contain phenolic-hydroxy groups capable of forming cross-linkages between proteins and other molecules. Naturally occurring tannins are located in organelles within the cytoplasm of plants and react with extracellular proteins upon rupturing of the cell wall (Ferguson, 1975). Driedger and Hatfield (1972) reported that addition of 10% tannin to soybean meal fed to lambs decreased in vitro deamination 90% and increased grains, feed efficiencies, and nitrogen retention. However, Nishimuta et al. (1974) found no benefit in steer rations when soybean meal was treated with 9% tannic acid. Experiments where tannin-protected protein was fed to lactating dairy cows were not found.

Measurments of Protein Degradation and Digestability

Measurment of protein flow to the small intestine requires 1) animals surgically prepared with cannula in the omasum, abomasum,

or proximal duodenum; 2) methods for estimating flow of digesta; 3) a marker for separating the microbial contribution from the total protein flow (Stern and Satter, 1982).

In vivo methods for estimating ruminal protein degradation are: 1) the regression technique which estimates the proportion of undegraded dietary protein from the relationship between duodenal protein flow and protein intake and 2) a direct method which measures dietary protein intake and the total flow of protein at the duodenum and determines degradability by difference. Total amino acid degradability in sheep and cattle determined by the regression technique was 65, 43, 34, and 32% for soybean meal, corn gluten meal, brewer's dried grain, and distiller's dried grains with solubles, respectively (Beardsly et al., 1977; Stern et al., 1979; Whitlow, 1979).

Merchen et al. (1981) and Prange et al. (1980) showed that 75 to 80% of the protein in alfalfa hay and low moisture alfalfa fed to sheep and cattle was degraded in the rumen. In these studies N solubility for hay and silage was 40 and 63%, respectively, but there were no differences in NAN flow to the duodenum, suggesting a poor relationship between solubility and degradability.

Using lactating cows, Wisconsin workers (Santos, 1980) compared amino acid flow to and absorption from the small intestine and showed greater flow and absorption for cows fed wet brewer's grain than soybean meal, even though amino acid intake was 400 g greater for the soybean meal ration. Extrusion of whole soybeans at 270 and

300°F resulted in a 10% increase in amino acid flow to and 17% greater apparent absorption from the small intestine, compared to raw soybeans.

In vitro methods for measuring rumen protein degradation such as the nylon bag technique, ammonia release, and protease assays were discussed in depth by Broderick (1982).

Amino Acid Supplementation

Addition of methionine hydroxy analog (MHA) to rations for lactating dairy cows increased milk yields and milk fat production in some studies (Bishop, 1971; Griel et al., 1968; Holter et al., 1972; Polan et al., 1968) but not others (Burgos and Olson, 1970; Chandler et al., 1976; Olson and Grubaugh, 1974; Rosser et al., 1971; Wallenius and Whitchurch, 1975; Williams et al., 1970). Milk production , stage of lactation during which the MHA was fed, and feeding systems may explain conflicting results.

Evidence suggests that MHA stimulates bacterial fermentation (Gill et al., 1973) and is degraded largely in the rumen with disappearance similar to that of DL-methionine (Emery, 1971; Papas et al., 1974). However, Belasco (1971) showed that MHA had greater stability than DL-methionine. More recently, Belasco (1980) showed that ^{14}C-MHA was degraded less in the rumen than labeled DL-methionine. He also found two or three times more radioactivity in the methionine of milk, blood, urine, kidney, and liver of cows fed MHA compared to DL-methionine. In addition to suggesting a

superiority of MHA over DL-methionine for ruminants, these data confirm biotransformation by the animal of MHA to methionine.

Nonprotein Nitrogen: Replacement of Dietary Protein

For the past two decades it has been profitable to include as much NPN in dairy rations as could be utilized without impairing production or health of cattle (Huber, 1978). Considerable controversy has developed as to the point at which NPN is not useful to ruminants (Chalupa, 1978; Huber, 1975). Others have even suggested against the use of NPN for feeding dairy cows early in lactation (Satter and Roffler, 1975; Clark and Davis, 1980).

In the past, quantity of NPN in ruminant rations was equated with added urea or other NPN compounds. Today it is recognized that many natural feeds, particularly silages, contribute NPN to the ration (Huber et al., 1973; Waldo, 1980) and these sources should be considered in calculating the overall NPN load. Moreover, highly degradable dietary proteins might release ammonia into the rumen more rapidly than certain forms of NPN (Waldo et al., 1980).

The maximum dietary protein at which NPN additions benefit dairy cattle probably is not over 15% of the ration dry matter even at high energy concentrations, providing proteins have not been treated or selected for low rumen degradability. At moderate production (up to 31 kg/day milk), numerous experiments have shown no differences in milk yields between rations of 14 to 15% protein

which compared limited NPN to natural protein supplementation (Holter et al., 1968; Huber et al., 1973; Huber et al., 1968; Kwan et al., 1977). Some of these studies (Huber et al., 1968; Kwan et al., 1977) included negative control groups supplemented with NPN. In contrast, Wohlt et al. (1978) reported decreased milk yields in cows fed 13.5 to 14.5% CP rations, in which urea furnished 50% of the supplemental nitrogen in the concentrate and soybean meal the remainder. These results might be partially explained by less concentrate fed the group receiving urea plus soybean meal compared to that supplemented with only soybean meal. In this study, concentrate was furnished at a ratio of milk yields, and intial milk production was lower for the urea plus soybean meal group. In other studies where urea depressed milk yields, NPN often was fed in excess of the recommended limit (Huber, 1978) which is 1.5% of the concentrate or 1.1% of the total ration (Huber et al., 1967; Polan et al., 1976; Van Horn et al. 1971; Van Horn et al., 1976). High yielding cows react more negatively to excessive NPN than low yielders (Huber, 1975; Huber et al., 1976). However, several studies suggest NPN feeding is compatible with high milk yields (Clay et al., 1979; Conrad and Hibbs, 1979; Foldager and Huber, 1979; Huber, 1975; Kwan et al., 1977; Murdock and Hodgson, 1979).

In a New Hampshire study (Holter et al., 1968), one-half the cows in a 58 cow herd were fed concentrate containing 1.5% urea for a complete lactation, and the others were fed equal nitrogen

from soybean meal. Lactation yields were about 8,000 kg and did not differ between rations. Crude protein fed the NPN group without the added urea would have been about 12%, too low for high yielding cows.

In four recent studies (Clay et al., 1978; Foldager and Huber, 1979; Kwan et al., 1979; Murdock and Hodgson, 1979), early lactation cows (starting 0 to 5 wks postpartum) were fed NPN or natural protein to increase the crude protein of low protein rations from 12 to 14 up to 15 to 18%. Treatment duration varied from 9 to 20 wks. Even though production differences were significant in only one study (Kwan et al., 1977), the pooled data (54 cows/treatment) showed average milk yields of 30.5 kg/day for low protein rations, 32.6 for increased natural protein, and 32.7 for urea rations. These data suggest that urea feeding is compatible with high milk production but do not prove its utilization because two of the studies used both soybean meal and urea to increase crude protein; hence, it is impossible to separate the effects of the two supplements.

Satter and Roffler (1975) and Clark and Davis (1980) suggest against the use of NPN in early lactation because they believe sufficient protein would be degraded in the rumen to furnish microbial needs and amino acids reaching the small intestine would not be limited. However, this idea is too simplistic in view of recent measurements of protein degradability. With considerable rations, protein low in rumen degradability (brewer's grain, corn gluten

meal, extruded soy) may actually be lacking in sufficient rumen ammonia for maximal rumen fermentation. The majority of trials that used NPN in early lactation or in high producing cows have also used soybean meal as the main protein source. Because soybean meal is highly degradable, it probably is not the best protein to be fed with NPN.

Roffler and Satter (1975) concluded that benefit from NPN ceased at about 12% CP. However, they used unadjusted milk yields without regard to pretreatment periods. Huber and Kung (1981) recalculated these data using adjusted milk yields at about 14% CP. This was approximately the same response point calculated for nine comparisons from the same studies which used natural protein increments.

Modified forms of urea and NPN sources have been developed that slow release ammonia. A more detailed description has been published by Huber and Kung (1981).

Urea and Ammonia Treatment of Silages

Silage has become widely used as a carrier for NPN in ruminant rations because intakes are distributed over the entire day, the undesirable taste of urea is masked, and ammonia is bound as the salt of organic acids, thus preventing volatilization of offensive odors (Huber, 1978; Huber et al., 1968). Numerous studies have shown successful feeding of urea-treated grain silages to lactating cows (see Huber and Kung, 1981). Studies comparing urea treated and

untreated silages in isonitrogenous rations have shown slightly greater milk yields for treated silages (Huber, 1975; Huber and Thomas, 1971; Huber et al., 1968), whereas others have shown no difference (Huber et al., 1968; Polan et al., 1968; Van Horn et al., 1967). When urea addition to silages was compared to urea addition to concentrates, milk yields were similar (Huber, 1972). Corn silage dry matter should range between 30 and 40% for urea additions to be most effective. Treatment of silage with less than 30% DM results in large losses through seepage (Huber et al., 1967). However, urea supplementation of silage above 42% DM adversely affects milk production (Huber et al., 1968; Van Horn et al., 1969).

Because ammonia was the most economical nitrogen source available for feeding ruminants and because the high concentrations of organic acids in grain silages bind ammonia as a salt, a program to evaluate ammonia additions to grain silages was initiated at Michigan State University in 1967. Previous attempts to add ammonia to layers of corn silage had been attempted by European workers (Abgarowicz et al., 1963), but these were unsuccessful because of an unequal distribution of ammonia to silages. A summary of seven experiments involving 169 cows showed milk yields averaged 0.7 kg/day greater for cows fed ammonia than control urea silages (Huber, 1978). The superiority of ammonia over urea-treated silages was supported by a study (Huber et al., 1980) in which cows fed ammonia silage and urea in concentrate out yielded those fed urea

in concentrate and urea-treated silage. No difference between groups fed the two silages was noted when the protein supplement of the concentrate was soybean meal.

Smith and Huber (1980) fed corn silage treated with $^{15}NH_3$ to cows in early lactation and determined ratios of the labeled N in feed and milk. Calculations suggested that nitrogen from the added ammonia converted to milk nitrogen about 82% as efficiently as the nitrogen from natural protein.

Compared to other silages, those treated with ammonia are higher in lactic acid (Huber et al., 1973), higher in water insoluble N (Huber et al., 1979; Huber et al., 1973; Goering et al., 1980), and less apt to heat and spoil when exposed to air (Britt and Huber, 1975; Soper and Owen, 1977). The increased lactic acid is from buffering of silage fermentation by the ammonia. The increased insoluble nitrogen results partly from binding of free ammonia onto water insoluble fraction of the forage and partly from a decrease in proteolysis of plant protein (Huber et al., 1979). The increased stability is caused by anti-fungal action of the ammonia and the ammonium salts (Britt and Huber, 1975).

Ammonia treatment of silages inhibits CO_2 production during early fermentation (Honig and Zimmer, 1975) resulting in a savings in energy in the ensiled crop. Goering and Waldo (1974) reported 5% greater dry matter and 8% greater energy recoveries with ammonia-treated than untreated silage. Despite greater lactic acid in ammonia-treated silages, sugars were also greater, reflecting lower CO_2 losses.

Problems With Feeding NPN

Urea has been suggested to cause poor feed intakes, reproductive failures, depressed milk fat, mastitis, metritis, milk fever, and a multitude of other problems which beset dairy cows (NRC, 1976). Excessive urea or NPN can be detrimental, but when it is fed whithin recommended limits and accoding to suggested methods of feeding, health of dairy cattle is not affected adversely (NRC, 1976; Huber, 1978).

Concentrates containing 1.5 to 2.0% urea might result in reduced feed intakes by dairy cows even though animals might be adapted to tolerate greater amounts of urea (Huber et al., 1967; Van Horn et al., 1967). Lowered intakes have resulted when silages contained over 1% urea as fed (Huber et al., 1968). Additions of urea to silage less than 30% or greater than 42% dry matter may also result in reduced intakes.

The mechanism of intake depression by urea is not understood. Data suggest that lowered consumption in cows eating moderate amounts of urea (up to 300 g daily) is from taste and not from ruminal or post-ruminal events (Huber et al., 1978). In these studies, the lowered intake was masked effectively by dissolving urea in molasses prior to feeding. However, Wilson et al. (1975) reported a marked decrease in dry matter consumption of a complete ration containing 2.3% urea (425 to 450 g/day) when the urea was fed orally or administered through a rumen fistula. Hence, large amounts of urea decrease intake by a mechanism other than taste. Chalupa et al. (1979)

demonstated that decreased intake of urea-containing rations is a learned response.

Additions of urea to corn silage reduces intake problems (Huber et al., 1968) probably because about 50% of the N is hydrolyzed to ammonia and consumed as ammonium salts, also because of the masking of the urea taste by silage acids.

Consumption of large amounts of urea (over 45 to 50 g/100 kg body weight) in a short period can be fatal to unadapted animals (Bartley et al., 1976; Word et al., 1969), but adapted cattle tolerate two to three times that amount (Huber, 1978). Bartley et al. (1976) observed muscle tetany at an average of 53 min after delivery of a toxic dose of urea (50 g/100 kg body weight) through a rumen annula. Rumen pH and blood ammonia were highly correlated with toxicity but rumen ammonia and blood urea were not. Carotid and juglar ammonia-N concentrations corresponding with initiation of muscle tetany were 1.46 and 0.95 mg/100 ml, respectively, suggesting the brain rapidly takes up ammonia.

Mistakes, such as cows breaking into urea supplies, inadvertantly spilling urea on feeds, or miscalculating feeding are often the cause of toxicity. Feeding NPN in complete feeds or mixing with grain silage negate dangers. Feeding urea with high moisture silages and grains which may contain high levels of soluble or NPN nitrogen is not recommended as is feeding more than one added source of NPN.

Feeding rations containing urea has been alleged to lower reproductive efficiency of cattle. To test this claim further,

85,000 individual lactation records from Michigan DHIA herds were analyzed for calving intervals during the 5 yrs of 1965 to 1969 (Ryder et al., 1972). The data show that about 55% of the herds were fed urea during 3 of the 5 years and were designated as NPN herds. Herds receiving NPN had an average calving interval of 380 days, identical to those fed no NPN. Little change occurred as NPN in the rations decreased. Percentage of cows culled because of sterility was about 2% and showed little relationship to feeding of NPN.

Erb et al. (1975) reported increased abortions (14% of 37 calvings) in heifers fed high urea (2% of the ration dry matter) during growth and gestation, whereas those fed less urea (1%) or soybean meal showed no abortions. In the same study cows fed high urea for four lactations did not abort and exhibited normal reproduction. Supporting a change in reproduction function of heifers from feeding NPN, the same laboratory (Garverick et al., 1971) reported lighter, softer, and more fragile corpora lutea. Upon in vitro incubation, corpora lutea from urea-fed heifers produced less progesterone than those from heifers fed soybean meal. In contrast, Illinois workers (Clark, 1978) fed similarly high levels of urea to young heifers and showed no increase in reproductive problems or abortions over those fed soybean meal. Rations continued through several lactations without any increase in sterility attributable to feeding high urea.

Huber (1981) recently summarized reproduction data from

trials using NPN as part of the protein supplement (Table 9). These data show no adverse effect on reproductive performance associated with NPN feeding.

TABLE 9

EFFECT OF FEEDING NONPROTEIN NITROGEN ON REPRODUCTIVE PERFORMANCE OF DAIRY COWS

Experiment Station	Fed NPN	Number of Cows On Trial	Number of Cows Settled	Services/ Conception	Days Open	Settled First Service (%)	Sold Open (%)
Morris, Minn.	No	66	59	1.96	98.5	45.4	10.6
	Yes	45	43	1.88	89.9	68.9	4.4
Urbana, IL	No	28	24	1.82	102.7	----	----
	Yes	19	18	1.65	109.0	----	----
East Lansing, MI	No	35	28	2.46	120.0	20.0	14.2
	Yes	35	25	1.80	101.1	42.8	20.1
East Lansing, MI	No	61	45	2.31	93.5	24.6	19.0
	Yes	25	14	2.07	88.6	32.0	32.0
MEANS	No	190	156(82)[a]	2.13	101.6	32.1	14.0
	Yes	124	100(81)[a]	1.85	96.0	51.4	16.0

[a] Percent cows on trial which settled.

Rumen Turnover and Marker Methodology

Significance of Turnover

The length of time ingested feed remains in the digestive tract will determine the extent of digestion of the potentially available nutrients. This process of turnover is affected by a multitude of factors and results in considerable alterations in the pattern of digestion. In ruminant animals the rumen is the first site of nutrient digestion and absorption. Its contribution to overall animal metabolism is vital due to fermentation by microbial organisms. Although the turnover and rate of passage of feed in any one digestive compartment may contribute significantly to nutrient utilization, this paper will deal primarily with determinants and consequences of altering rumen turnover.

In general, there are two types of material that turnover in the rumen: a solid particulate phase and a liquid phase. The latter is somewhat complicated by the passage of fine particulate matter with this phase. Although absorption of end products, such as VFA's, constitutes part of turnover, these factors will be discussed only in terms of consequential results of altering turnover and not their removal from the rumen.

The rumen is the first obligatory compartment of digestion and it is logical that any alteration of feed resident time may have profound effects on overall digestion. Sites of digestion may be altered as well as the overall digestibility of feed. Turnover of material from the rumen is important in that it provides

the ultimate flow of nutrients to the lower digestive tract for host animal digestion and absorption. The realization of any benefit of non-protein nitrogen (NPN) use by the ruminant as well as certain vitamin requirements is strongly dependent on the turnover of rumen digesta.

Rate of passage of material from the rumen cannot lag much behind intake if a specific level of intake is to be maintained. Altering rumen turnover will affect the amount of material bypassing the rumen and reaching the lower gut. In the case of protein material, this may be beneficial by increasing the supply of amino acids for absorption. On the other hand, increasing turnover of fibrous feeds decreases the potential degree of digestibility afforded by rumen microbes of the beta-linked glucose polymers.

Rate of passage and turnover are terms often used to describe movement of food. While they describe similar processes, they are not exactly the same. Turnover may be defined as the time required for placement of existing molecules in a pool by molecules entering the pool (Ellis et al., 1979). The rate of passage, as defined by Kotb and Luckey (1972), is the quantity of digesta that passes a point of the tract at a certain time.

Marker Methodology for Determination of Turnover and Digestibility

A complete critique of marker methodology used in quantitating rumen turnover is beyond the scope of this thesis. The reader is referred to excellent reviews by Balch (1965), Kotb and Luckey

(1972), MacRae (1974), Ellis et al. (1982), and other references cited throughout the text of this review.

The methods for studying rumen turnover have evolved around the use of markers and various sampling techniques. Markers have been used to indicate time that digesta passes from one point to another and as an inert reference material using concentration to measure digestibility, volume, or flow rates (MacRae, 1974). Markers have ranged from simple colored beads (Elliot and Smith, 1904) or stained hay particles (Balch, 1965), to the use of rare earth markers analyzed by neutron activation (Kennelly, 1980). External markers are those added to the diet or animal such as the rare earth elements, PEG, CrEDTA, stains, dyes, rubber pieces, or beads. Internal markers are those naturally occurring in the feedstuff such as lignin or acid insoluble ash. Markers have also been administered in numerous forms as pills, liquids, pellets, capsules, etc.

The choice of markers is critical in evaluating the obtained data. The criteria for an ideal marker is summarized below:

1. Marker should be inert
2. Non toxic
3. Non absorbable
4. Not metabolized in the digestive tract
5. Not affect or be affected by digestive tract secretions or microbes
6. Physically similar or closely associated with material it is marking
7. 100% recoverable
8. Specific quantitation with no interference with other analyses
9. No appreciable bulk
10. Uniform mix in feed and throughout digesta
11. No psychological or physiological affect on the animal

(Modified from Kotb and Luckey, 1972 and Faichney, 1975).

Different markers have been used to mark the solid and liquid phases of turnover. In chronological order, dyes, PEG, and CrEDTA have been most commonly used to mark the liquid phase. Problems associated with PEG have included non-specificity and interference when tannins were present in the diet (Downes and McDonald, 1964). PEG also accupies less fluid space in the rumen than other markers. For example, CrEDTA is associated with 99% of the water in feed while PEG is only associated with 92% of the water (Czerkawski and Breckenbridge, 1969). In support of this, Goodal and Kay (1973) found that on the average of 32 comparisons, PEG underestimated rumen volume in sheep when compared to CrEDTA (6.90 vs 7.95 liters, respectively).

Until the recent use of rare earth markers (Ellis and Huston, 1967; Gray and Vogt, 1974, Kennelly, 1980, Hartnell and Satter, 1979) a suitable marker for the particulate phase has been difficult to find. Lignin was suggested as an appropriate marker, however it has a variable digestibility and does change in composition as it travels the digestive tract. Balch and Campling (1965) and Ellis (1968) have discussed the limitation of using stained natural particles or artificial particles. Recovery of these markers is highly dependent on mesh size of screens used and disintegration of particles to fines are often not counted.

Most recently chromium and cerium mordanted plant cell walls have been used (Uden et al., 1979) and utilized with the nylon bag

technique to estimate protein degradation in the rumen (Orskov and McDonald, 1979; Ganev et al., 1979).

Failure of liquid or solid markers to withstand the test of time have been due to failure to meet one or more criteria previously set forth. This is especially true for rate of passage studies more so than digestibility studies. Since absolute measures of passage are needed for both liquid and particulate phases, which behave independently from each other, the respective markers must be in direct association with their labeled fractions.

To illustrate differences obtained with various markers, Drennan et al. (1970) found chromic oxide(Cr_2O_3) to estimate rumen dry matter disappearance from 76 to 36%, using lignin, estimates were 57 to 68%. Rumen starch digestibility with Cr_2O_3 ranged from 56 to 92% and with lignin estimates ranged from 98 to 96%.

The use of surgical alterations of the digestive tract and markers eliminates expensive slaughter methods, laborious total collections and/or controversial collections. The various alterations and sampling methods have been less controversial although they may have definite influences on experimental data collected. Cannulas in the proximal duodenum and/or terminal ileum have been used extensively. This has enabled researchers to estimate microbial protein, non ammonia nitrogen flow, amino acid flow to the small intestine, and apparent digestibility of various feed components in different sections of the digestive tract.

Re-entrant cannulas were quite popular in the 1960's and early

1970's. However, animal survival rate was low and much care was needed for these animals. MacRae (1975) indicated sheep living up to 18 months with cannulas of this type. In the past, sheep have been used intensively in turnover experiments. With the advent of using larger ruminants, T-cannulas have eliminated extensive surgery, post-operative complications, and blockage problems.

Hogan and Weston (1967) have discussed the difficulty in obtaining representative samples (liquid:particulate) through simple cannulas and suggested the use of a dual marker system. This system has since been used by many workers. ^{103}Ru-labeled ruthenium phenanthroline and ^{51}CrEDTA have been used to mark the particulate and liquid phases respectively (Faichney, 1975; Faichney, 1980; Tan et al., 1971). Hartnell and Satter (1979) have recently used the rare earth markers in a dual phase system. In practical use this system must be employed under steady state conditions to eliminate diurnal variations in flow. This can be achieved by continuous or frequent feeding and is best if the markers are fed with the diet (Offer et al., 1972). Use of non-radiolabeled rare earth elements has also eliminated problems associated with elimination of radioactive waste. The rare earths bind tenaciously by radiocolloidal properties at low concentrations. The efficacy of using a dual phase system has been established by Faichney (1980).

There has been some concern by researchers whether samples from T-cannulas accurately represent correct solid to flow ratios when compared to re-entrant cannulas. There has also been question as

to the effect of cannulas in general on tract motility. Unpublished data from MacRae (1975) show that the rate of passage and intake were the same for normal sheep, sheep with re-entrant cannulas, and sheep with T-cannulae. Stern et al. (1981) have also shown that CrEDTA:lanthanum ratio of feed, duodenal digesta, and fecal digesta were 5.62, 5.35, and 5.36 respectively. This would indicate that sampling from T-cannulae gave good representative samples of digesta flow.

Effect of Intake Level on Turnover

By our definition of turnover, any increase in dry matter intake sustained over time must be accompanied by an increase in turnover up to the physiological capacity of the rumen. It can therefore be speculated that voluntary intake may be limited by the capacity of the rumen and retention of feed in this compartment. Data of Owens et al. (1979) show an increase in rumen liquid turnover with increase level of feeding. At a level two time maintenance intake for steers, rumen liquid turnover increased by about 65%. The effect of intake level on turnover is complicated by factors such as plant quality, species, and processing (particle size). Using sheep and the stained particle technique, Blaxter et al. (1961) reported that intake increased and rumen transit time decreased as forage quality increased from poor, medium, to good. Thornton and Minson (1972) also demonstrated with sheep an inverse relationship between intake and mean rumen retention time of

roughages. These workers used 6 varieties of panicum known to vary in digestibility and intake. As organic matter intake ($g \cdot day^{-1} \cdot kg$ body weight$\cdot 0.75$) increased from 39.3 to 73.4, mean rumen retention time decreased from 26.3 to 14.2%/hr.

Greenhalgh and Reid (1973) compared forage quality and processing on turnover. Their general hypothesis was that ground and pelleted roughages pass through the digestive tract faster allowing greater intakes. Pelleting increased intake by 45% in sheep but only 11% in cattle. The increase was also greatest for the low quality forage. Owens et al. (1979) found no definite relationship between particle size and liquid rumen turnover. However, these workers used corn sizes (1/8 inch to whole kernels) to alter particle size which may not have been a wide enough difference. Castle et al. (1979) did not find that grass silage chopped short, medium, or long (9.4, 17.4, 72.0 mm) increased dry matter intake with decreasing length of chop. Dry matter intake for lactating cows was 6.97, 8.34, and 9.34 kg/day respectively. Using the stained particle technique, no difference in rumen retention time was found due to particle size or intake.

Pearce and Moir (1964) found that physical breakdown of fiber was very important in passage of digesta from the rumen. They inhibited rumination on low quality fiber diets and caused rumen distention and a marked decrease in rate of passage. In support of this Troelsen and Campbell (1968) using sheep and a seiving technique proposed that the reticulo-omasal orifice constituted a major

block for feed passage from the rumen. A critical size theory for particles leaving the rumen has been established by many workers (Poppi et al., 1980; Smith et al., 1967; Reid et al., 1977; Ellis et al., 1979). The upper limit of particle passage from the rumen seems to be about 1 mm. Data also suggest that little changes in particle size occur post-ruminal.

Effect of Altering Forage:Concentrate Ratio

Altering forage to concentrate ratios would have a similar effect as changing particle size. Cole et al. (1976) used steers and increased the amount of forage by replacing a basal diet of 90% whole shelled corn plus 10% premix with 0, 7, 14, or 21% cotton-seed hulls. Increasing roughage increased intake and resulted in an even more marked increase in rumen dilution rate. Hartnell and Satter (1979) found mean ruminal turnover rate of liquid, grain, and hay to be 8.1, 4.4, and 3.9% per hour in lactating dairy cows. However, they reported no difference in these rates when increasing the forage to concentrate ratio from 45:55 to 67:33. Prange et al. (1978) also reported similar data when the hay to grain ratio was changed from 83:17 to 29:71. Intakes were similar in all rations for this trial and no difference was found in rumen turnover or total mean retention time for liquid, grain, and hay. Although these data cannot fully be explained at this time, it is the opinion of this author that at levels of intake (4 times maintenance or more) which are high, turnover is so rapid that altering particle

size or forage to concentrate ratios have negligible overall effects.

Effect of Turnover on Rumen Microbes

The value of rumen fermentation is a consequence of microbial action and follows that altering their metabolism may be energetically advantageous. Increasing rumen dilution has been shown to increase the efficiency of microbial growth (Owens and Isaacson, 1977). The cause of this is due to a lesser proportion of energy being used for maintenance by the microbes. From the host standpoint, the ATP equivalent to support bacterial maintenance is less than 3% of the total energy required for the animal. Consequently, the energy saved by increasing dilution is negligible in terms of the entire animal economy. Increasing turnover may cause a redistribution of microbial population and may select against protozoa which are slow growing and which may have detrimental effects on available microbial protein (Sutherland, 1976). Probably of more importance is the increase in total cell yield and flow to the lower gut; cell yield almost doubles at high rates of dilution. This cell yield is also closely related to NPN use (Owens and Isaacson, 1977).

Coelho da Silva et al. (1972) fed 1.3 or 0.8 kg of organic matter to sheep and found a 50% increase in flow of microbial crude protein to the duodenum on the former ration. These findings may well be due to increases in OM fermented and increased turnover. Although confounded by intake, Cole et al. (1976) reported 359 g

vs 212 g of abomasal microbial protein on a diet with 21% cottonseed hulls vs a basal diet without the hulls.

Turnover and Protein Metabolism

There are few direct studies concerned with effect of turnover on protein metabolism. This is closely related to the previous section. The increase in cell yield flowing to the small intestine is beneficial due to increased use of NPN and increasing the amino acids available for absorption. If moderately degradable proteins are subject to an increase in rumen turnover, this quality protein may be spared from excessive microbial degradation. On the other hand, turnover may have minimal effects on proteins of high degradability. Data of Zinn (1978) show the direct effect of altered feeding level on rumen protein degradation. At increased turnover, proteins were less extensively degraded. It should be noted that the level of intake was moderately low in this trial.

Effect of Turnover on Carbohydrate Digestion

Most ideas on this topic are speculative in nature. There may be some effect of turnover on site of starch digestion. The hypothesis that suboptimal intestinal pH may alter site of digestion is implicated. Although increasing the amount of starch reaching the small intestine may be undesirable in normal feeding practices, there appears to be areas where it may be useful. In cases where alpha-linked glucose polymers are limiting in the diet

along with other potential glucose precursors (low quality roughage diets) an increase in post-ruminal absorption of glucose may be beneficial. This is especially true since carbohydrates may yield 11 to 30% more energy for production when digested post-ruminally (Owens and Isaacson, 1977). Preston (1976) has demonstrated this concept in Mexico feeding cattle sugarcane diets. This concept may also be of significance when feeding high producing dairy cows rations of readily fermentable energy (e.g., barley). Armstrong and Smithard (1979) presented data on glucose available for absorption in cattle fed barley or corn diets. Cows fed barley had only 19% of their total glucose requirement from absorption of glucose from the small intestine. This may be important energetically if 80% of the requirement must go through gluconeogenesis.

The amount of polysaccharide reaching the small intestine may also be affected by turnover of rumen microbes. McAllan and Smith (1976) estimated 110 g of alpha-linked glucose polymers/kg rumen bacteria which contributed 60 g/day of such polymers to digesta flow.

Digestibility of cellulose is also affected by rate of turnover. Mertens (1979) states that increaseing turnover decreases fiber digestibility in the rumen. Berger et al. (1980) showed increasing rumen turnover with NaOH decreased rate of fiber digestion. These workers also suggested these findings were due to possible dilution of rumen bacteria and a decreased substrate-enzyme contact.

Effect of Buffers on Turnover

Increases in rumen turnover are probably not associated with a simple increase in liquid intake. Harrison et al. (1975) infused up to 12 L/day of water into the rumen of sheep with little effect on rumen dilution. This indicated that the rumen wall probably absorbed a great deal of the infusate. However, infusion of 4 L of artificial saliva did increase dilution over water only (0.56 to 0.109 hr^{-1}). In addition PEG at levels of 4 or 8% with saliva increased rumen osmotic pressure and in turn dilution. These workers also showed a shift of fermentation from propionate to acetate. Grams microbial nitrogen/100 g OM was increased from 2.16 to 3.15% with saliva plus 4% PEG. YATP values were also higher for this treatment.

Data from Illinois (Davis, 1979) agree with these findings as addition of water had no significant effect on turnover. Addition of 0.5 or 1.0% NaCl did increase turnover to 7.1% compared to 5.9% per hour for controls. Potter et al. (1972) showed addition of 1.3% NaCl increased rumen dilution 32% on chaffed forage rations and 68% on the same ration if it was ground and pelleted. Chalupa (1980) altered forage to grain ratios with addition of NaCl. Although results were variable, the trends were similar to previous workers.

NaOH has increased rumen turnover time. Berger et al. (1980) reported that increasing NaOH from 0 to 8% in lambs fed corn cobs, significantly increased rumen turnover.

Davis (1979) has postulated that compounds such as magnesium oxide, bentonite, and magnesium carbonate may have effects on increasing turnover. This has been suggested since a negative correlation exists between turnover and rumen propionate (Hodgson and Thomas, 1972).

OBJECTIVES

The overall objective of this research was to increase the efficiency of nitrogen utilization of high producing dairy cattle early in lactation. Experiment one was designed to evaluate methods to decrease rumen protein degradability while retaining digestibility in the small intestine. Soybean meal was used as the test material since it is the most widely fed protein supplement in the midwestern United States. Experiment two, was to evaluate the use of rumen undegradable protein with and without non protein nitrogen as supplements for high producing dairy cows early in lactation where milk production is greatest relative to nutrient uptake. We also tested the level of protein which was most productive and profitable for producing milk. Finally, experiment three employed cannulated lactating cows fed diets similar to experiment two and quantified amino acid flow to the small intestine as well as apparent digestibility and absorption.

MATERIALS AND METHODS

Experiment 1

General Treatments

Commercial soybean meal(SBM) was chemically or physically treated to reduce nitrogen degradability in the rumen. Treatments were a) formaldehyde (HCHO), b) autoclaving, or c) dry heat.

Formaldehyde treated SBM was prepared by addition of 100 ml of dilute HCHO solutions to 1000 g of SBM resulting in concentrations of 0.4, 0.8, and 1.0 g HCHO per 100 g crude protein equivalent. Treated SBM was immediately sealed in glass containers for 48 hr, then allowed to air dry at room temperature. Other SBM was placed in pans, layered 3.5 cm deep and autoclaved for 5, 10, 15, 20, 30, or 40 minutes at sterilization temperatures of 121°C. Samples gained 2-3% moisture by autoclaving. For dry heat treatment, pans of SBM were layered 3.5 cm deep and placed in a laboratory forced draft oven for 2, 4, or 6 hrs at 149° C. All treated samples were allowed to equilibrate in air prior to storage and analysis.

Laboratory Evaluations

Normal and treated SBM were evaluated by laboratory and *in vitro* analyses. Acid detergent insoluble nitrogen was used as an index of heat damaged protein in autoclaved and heated samples (Goering and Van Soest, 1970). Nitrogen solubility was determined using dilute Wise Burrough's buffer (Crooker et al., 1978). A two stage

in vitro rumen fluid pepsin incubation (Goering and Van Soest, 1970) characterized potential dry matter disappearance of treated samples.

Nitrogen degradability was measured using a rumen-suspended nylon bag procedure and/or a ficin protease (Poos et al., 1980). Triplicate nylon bags (average mesh size 50 μm, 11.5 x 5.0 cm) containing 1-2 g of sample were suspended for 4, 8, 12, or 24 hours in the rumen of a fistulated heifer fed alfalfa hay. Upon removal from the rumen, bags were rinsed with water until clear and dried at 100°C for 24 hours. Nitrogen (N) residue was determined by Kjeldahl.

For the protease assay, samples were incubated with mineral buffer, then a ficin (ficus glabrata) enzyme solution. They then were filtered, washed, and residual N determined by Kjeldahl. Rock et al. (1981) reported that values for degradability obtained with this procedure were highly correlated (r = 0.9) with *in vivo* animal values.

In vitro ammonia release was also measured as an indicator of protein degradability. Rumen fluid was collected from a fistulated cow fed alfalfa hay one hour post feeding strained through four layers of cheesecloth, and placed in a water bath at 39°C for 1 hr prior to use. Twenty ml of a pre-warmed phosphate carbonate buffer and 24 ml of rumen fluid were added to 50 ml flasks containing 0.5 g of a soybean meal. Flasks were gassed with CO_2 capped with a bunsen valve stopper and incubated at 39°C with gentle agitation. Two flasks per treatment were removed at 0, 2.5, 4, 6, and 8 hr and

analyzed for ammonia-N (Kulasek, 1976).

A number of commercially available treated soybean and soybean meal products were also compared utilizing the nylon bag technique.

Data was analyzed by standard analysis of variance techniques.

Experiment 2

Animals, Treatments, and Feeds

Eighty-four multiparous Holstein cows were fed the normal herd ration containing 14% crude protein (CP) from days 0 to 21 postpartum. At 22 days cows were randomly allotted to one of seven treatments varying in amount and source of protein. Cows calved and were placed on treatment during the period of October 1979 through March 1981. An attempt was made to balance treatments on milk production and number of lactations. Animals developing mastitis or other metabolic problems were deleted from the experiment and replaced by the next available animal. Protein percentages were planned to be 11, 14, and 17 of the ration dry matter (DM). The different protein sources were normal corn silage (CS), ammonia-treated corn silage (AS), soybean meal (SBM), and heated soybean meal (HS). Only cows averaging over 26 kg milk from days 8 to 21-postpartum were selected for treatment. Treatment combinations designated by amount of protein, and silage and soybean meal source were: 11 CS-SBM, 14 AS-HS, 14 CS-HS, 14 CS-SBM, 17 AS-HS, 17 CS-HS, and 17 CS-SBM. Complete rations fed during the 70-day treatment period (22 to 91 days postpartum) consisting of 40% corn silage, 10%

ground alfalfa hay, and 50% concentrate (%DM) were offered ad libitum twice daily. High moisture ground ear corn or shelled corn was used as an energy source depending on availability. Calcium sulfate provided sulfur to obtain a N to sulfur ratio of 12:1. Other minerals and vitamins were added to meet NRC requirements (NRC, 1978). Feed refusals were measured daily. Feed samples were collected twice weekly, composited and analyzed for dry matter and N (AOAC, 1975) biweekly. Rations were rebalanced according to dry matter and N-analyses one to two times a month.

Ammonia was added to corn silage at ensiling and increased CP from 8 to 13.5% of DM. Ammonia was added to 30-35% DM corn silage as either: 1) an ammonia-molasses-mineral mix which contained 13.7% N and applied approximately 35 to 45 lb/ton; or 2) anhydrous ammonia applied at 7 to 9 lbs/ton. Treated corn silage was stored in 20'x 60' concrete stave silos. A large batch-dryer was used to heat 75 kg lots of soybean meal distributed in 3.5 cm layers for 2.5 hr at 140°C. Nitrogen degradability of each batch of heated and normal soybean meal was measured using the nylon bag technique as described for Experiment 1.

Collection and Preparation of Samples

Milk production was recorded twice daily. Composite milk samples from AM and PM milkings were analyzed biweekly for fat and protein by infrared analysis (AOAC, 1975) by Michigan DHIA testing lab. Cows were weighed within 12 hr after calving and at

biweekly intervals during treatment.

Rumen fluid was sampled by stomach tube and blood from the tail vein of all cows between weeks 10 and 13 of lactation. Samplings were at 0, 4, and 8 hr after the AM feeding. Rumen fluid was strained through four layers of cheesecloth and pH determined immediately. It was then stored at -20°C and subsequently analyzed for ammonia-N (Kulasek, 1976), and volatile fatty acids by gas chromatography. Blood was centrifuged at 3,000 x g and plasma was analyzed for urea-N (Kulasek, 1972). Animals developing mastitis or other metabolic problems were replaced on treatment, but were recorded to relate disease incidence to treatment.

Statistical Analysis

All data were subjected to analysis of variance. Milk production during treatment was covaried on pre-treament milk to obtain adjusted treatment means. Mean milk production and persistency of milk production (pretreatment milk/treatment milk (x 100)) was compared by non-orthogonal contrasts using Bonferroni T-test (Miller, 1966) while other contrasts were compared using Tukey's T-test (Gill, 1978).

Experiment 3

Animals and Treatments

Data collection for this experiment was done at the University of Wisconsin with the cooperation of Dr. Larry D. Satter.

Four lactating Holstein cows in mid to early lactation (less than 100 days in milk) were used as experimental animals in a 4 x 4 latin square. Cows were previously fitted with rumen fistula and duodenal and ileal t-cannula . Rations consisted of 50% grain (soybean meal and ground shelled corn), 40% corn silage, and 10% alfalfa hay, on a dry matter basis. Treatment combinations were 1) normal corn silage and normal soybean meal, CS-SBM; 2) normal corn silage and heated soybean meal, CS-HS; 3) ammonia-treated corn silage and normal soybean meal, AS-SBM; 4) ammonia-treated corn silage and heated soybean meal, AS-HS. Ammonia silage, similar to that fed in Experiment 2, was emptied from silos at the Michigan State University Dairy, packed in doubly-lined plastic bags, evacuated and sealed. This material and normal and heated soybean meal were transported by truck from Lansing, Michigan to Madison, Wisconsin. Heated soybean meal was prepared as described in Experiment 2. Normal corn silage and ground shelled corn were from local supplies at the University of Wisconsin.

Marker Preparation

Chromium-EDTA and lanthanum (LA) were sprayed on the grain mix to serve as dual markers for determination of apparent digestibility and flow of nutrients through the digestive tract. Ideally, the chromium to LA ratio in digesta and feces should be similar to that in feed. Variations in marker ratios would suggest disproportionate collection of either liquid or solids during sampling at the duodenum and ileum.

Collection and Preparation of Samples

Corn silage, grain, and hay were fed in four equal portions, at 4 AM, 10 AM, 4 PM, and 10 PM. Experimental periods were 14 days. Days 1 though 10 were for ration and marker equilibration. Duodenal, ileal, and fecal samples were collected at 8 hr intervals on days 11-14 for a total of 12 samples representing the odd numbered hours of the day. The sampling schedule with day and hour of sampling is given below:

Day 11	Day 12	Day 13	Day 14
1 AM	3 AM	5 AM	7 AM
9 AM	11 AM	1 PM	3 PM
5 PM	7 PM	7 PM	11 PM

Approximately 400 ml of duodenal contents, 300 ml of ileal, contents, and 500 g of feces were collected at each sampling. Samples were homogenized and composited prior to analyses. Total N and ammonia-N were analyzed on wet samples by Kjeldahl and the ammonia ion specific electrode. Non ammonia nitrogen (NAN) flow to the duodenum was calculated by difference. The following analyses were

performed on samples after being lyophilized and ground through a 1 mm mesh screen: acid detergent fiber (Goering and Van Soest, 1970), ash (AOAC, 1975), and marker concentration via neutron activation.

Rumen fluid was collected at 0, 2, 4, and 6 hr after the 10 AM feeding on day 14 and pH determined immediately. Fluid was frozen and stored at -20°C until analyses for rumen ammonia-N (Kulasek, 1976) and volatile fatty acids by gas chromatography. Two hundred ml of rumen fluid were also collected at each sampling, composited and centrifuged to isolate rumen bacteria. Bacteria were lyophilized and analyzed for total N by Kjeldahl and total nucleic acids (Zinn and Owens, 1982). Organic matter was determined by subtraction of ash content in feed, digesta, and feces.

Ration soluble nitrogen and protein degradability were determined as described in Experiment 1. Milk production was recorded on days 8 to 14 and composite milk samples were collected on day 14. Milkfat and protein were determined by infra-red analysis (AOAC, 1975) and total solids by drying 2 ml for 2 hr at 100°C.

Statistical Analysis

Data were analyzed by standard procedures of analysis of variance. One original animal was off feed for treatment AS-SBM and subsequently replaced. Data for the missing block in a latin square was estimated: $y = \frac{r(R + C + T) - 2G}{(r-1)(r-2)}$ where R,C,T,and G, respectively, are totals for periods, cows, and the treatment which include the missing

value and the grand total (Cochran and Cox, 1957). Treatment means were tested by Bonferonni's T-test (Miller, 1966).

Calculations

Estimates of digesta flow and digestibility were based on the following calculations:

A. crude protein (CP) = N x 6.25
B. Organic matter (OM) = dry matter(DM) - ash
C. Organic matter digested in rumen (uncorrected) = OM intake - OM reaching duodenum
D. Organic matter digested in rumen (corrected) = OM intake - (OM reaching duodenum - microbial OM)
E. Non ammonia nitrogen (NAN) = total nitrogen (N) - ammonia-N
F. Digesta flow:
   1) DM flow (g/day) = $\frac{\text{marker intake(g/day)}}{\text{marker/g digesta DM}}$
   2) Passage of digesta components (g/day) =

$$\frac{\text{DM intake x component(\%DMI)}}{\text{DM flow x component(\%digesta DM)}}$$

   3) Total N and NAN passage (g/day) =
      a) $\frac{\text{DM flow}}{\text{\% DM of wet digesta}}$ = total wet flow (TWF) 1/day

      Total N = (TWF) (% N of wet digesta)

      b) NAN = total N - (%NH_3-N content of digesta)(TWF)

Sample Calculations for Flow and Digestion

(1.84% lignin in diet)(13.22 kg DMI) = 0.2432 kg lignin intake

1) Flow:
   a) 0.2432 ÷ 3.62% duodenal lignin = 6.71 kg DM flow to duodenum.
   b) 0.2432 ÷ 6.59% ileal lignin = 3.69 kg DM flow to ileum.
   c) 0.2432 ÷ 7.44% fecal lignin = 3.27 kg DM flow to feces.

2) Disappearance:
   a) 13.22 DMI - 6.71 = 6.51 kg disappearance up to duodenum.
   b) " " - 3.69 = 3.58 " " " " ileum.
   c) " " - 3.27 = 9.95 " " " " feces.

3) Digestion:
   a) $\frac{6.51}{13.22}$ (100) = 49.24% DM digestion in rumen/abomasum.

   b) $\frac{6.51 - 3.69}{6.51}$ (100) = 43.32% DM digestion in duodenum, % flow.

   c) $\frac{3.69 - 3.58}{3.69}$ (100) = 2.98% DM digestion in ileum, % flow.

   d) $\frac{9.95}{13.22}$ (100) = 75.26% DM digestion in total tract.

4) NAN Flow:

   a) $\frac{6.71 \text{ kg DM flow to duodenum}}{6.29\% \text{ DM}}$ = 106.52 l liquid flow.

   b) (106.52 l)(0.1979 % N)(1000) = 210.80 g N.
   c) (106.52 l)(3.62 mg% NH_3-N) = 3.90 g NH_3-N.
   d) 210.80 - 3.90 = 206.90 NAN flow.

Bacterial N and OM in Duodenal Digesta

1a) $\frac{\text{RNA, \% in bacteria}}{(\text{RNA, \% in duodenal digesta})(\text{N, \% in bacteria})}$ = % bacterial N in duodenal digesta.

b) $\frac{\text{\% bacterial N in duodenal digesta}}{\text{\% N of duodenal digesta}}$ = % of duodenal N that is of bacterial origin.

c) example:
   1. bacteria, % RNA = 11.459
   2. bacteria, % N = 8.48
   3. duodenal digesta, % RNA = 2.185
   4. duodenal digesta, % N (DMB) = 2.97
   5. $\frac{11.459}{(2.185)(8.48)}$ = 1.62 ; $\frac{1.62}{2.97}$ = 54.55% of N at duodenum is of bacterial origin.

2a) $$\frac{\text{RNA, \% in bacteria}}{(\text{OM, \% in bacteria})(\text{RNA, \% in duodenal digesta})} = \text{\% bacterial OM in duodenal digesta.}$$

b) $$\frac{\text{\% bacterial OM in duodenal digesta}}{\text{\% OM of duodenal digesta}} = \text{\% of duodenal OM that is of bacterial origin.}$$

RESULTS AND DISCUSSION

Experiment 1

Laboratory Studies

Autoclaving and formaldehyde treatments reduced *in vitro* release of ammonia-N (NH_3-N) from SBM (Table 10). Values are expressed as mg NH_3-N per dl and percent reduction was compared to controls. Ammonia-N production was calculated as NH_3-N concentration of incubated rumen fluid plus substrate minus NH_3-N of rumen fluid without substrate. Reduction in NH_3-N release was calculated as: $\frac{(1\text{-net } NH_3\text{-N produced for treated SBM})}{(\text{net } NH_3\text{-N produced for untreated SBM})} \times 100$. Reductions in ammonia release greater than 100 for all HCHO-treated SBM and SBM-autoclaved 30 to 40 mintes suggested that substrate was totally unavailable for microbial degradation and that microbes probably utilized ammonia already in the incubation media. Findings with HCHO-treated SBM were similar to those reported by others (Peter et al., 1971; Schmidt et al., 1973 ; Thomas et al., 1979). Schmidt et al. (1973b) reported ammonia reductions greater than 100% for SBM treated with more than 0.4% HCHO, but Phillips (1981) suggested at least 0.5% HCHO was needed to inhibit *in vitro* ammonia release. *In vitro* ammonia release has been criticized as a method for estimating degradation due to recycling of ammonia by bacteria. However, SBM has small amounts of fermentable carbohydrate compared relative to

TABLE 10.

In Vitro Ammonia Release of Formaldehyde (HCHO) and Autoclaved Soybean Meal. Experiment 1.[a]

SBM Treatment	Incubation Time, hr				Average
	2.5	4.0	6.0	8.0	
	mg %				
Control	30.18	38.46	48.53	60.46	44.41[b]
Min of Autoclaving					
5	28.88(38)[d]	33.11(48)	40.39(41)	45.04(51)	36.86[b]
10	29.76(12)	30.33(73)	33.25(77)	67.79(74)	32.78[c]
15	27.97(90)	27.47(99)	30.33(91)	32.40(91)	29.54[c]
20	27.12(91)	27.44(99)	29.06(91)	31.17(95)	28.70[c]
30	26.03(123)	26.95(103)	27.23(107)	29.06(102)	27.32[c]
40	26.84(99)	27.33(100)	26.84(109)	28.04(103)	27.41[c]
HCHO, % of Crude Protein					
.4	24.45(170)	25.19(119)	28.74(99)	27.19(108)	26.39[c]
.8	23.92(185)	23.88(131)	22.79(129)	23.11(121)	23.42[c]
1.0	24.09(180)	23.78(132)	23.85(143)	24.09(118)	23.92[c]

[a]Four observations within each treatment.

[b,c]Means with unlike superscripts differ significantly from control SBM ($P<.05$; standard error = 4.42).

[d]Values in parentheses represent % reduction from control SBM.

substrates like corn which would result in rapid microbial protein synthesis. Broderick and Craig (1982) suggested the use of hydrazine to prevent recycling of released ammonia and amino acids.

Dry matter and N disappearance of HCHO-treated SBM from nylon bags suspended in the rumen are presented in Figures 7 & 8. Nitrogen disappearance was 2-3 fold greater for untreated SBM than HCHO-treated SBM after 4-12 hr incubation in the rumen. Estimates of 76% of the N being degraded after 12 hr of incubation in the rumen are similar to *in vivo* estimates for SBM degradation (Zinn et al., 1981). Virtually all the N in untreated SBM was degraded after 24 hr of incubation. Data from autoclaved-SBM are shown in Figures 9 and 10 . After 12 hr of incubation, N disappearance for SBM autoclaved more than 10 minutes was similar to HCHO-treated SBM. However, N disappearance after 24 hr was greater for autoclaved SBM than HCHO-SBM.

In vitro dry matter disappearance was not substantially decreased by HCHO or autoclaving (Table 11). Forty minutes of autoclaving or 1% HCHO reduced disappearance 4.25 and 10.20% respectively compared to control SBM, suggesting that over all degradation was not drastically curtailed.

Heating SBM for 2 hr reduced solubility in Burrough's buffer from 24.7 to 7.6%, decreased IVDMD by 7%, and lowered N disappearance from nylon bags from 83.4 to 36.3% (Table 12). Treatment for 4 hr or 6 hr only minimally decreased degradation in the rumen below that observed at 2 hr, but increased ADIN to 9.4 and 16.3% of the total N. Hence, dry heat treatment of SBM for 2 hr or less at

Figure 7. Dry matter disappearance of soybean meal (SBM) treated with formaldehyde (HCHO) from nylon bags suspended in the rumen. Legend, 1, SBM, normal; 2, SBM, 0.4% HCHO; 3, SBM, 0.8% HCHO; 4, SBM, 1% HCHO.

Figure 7.

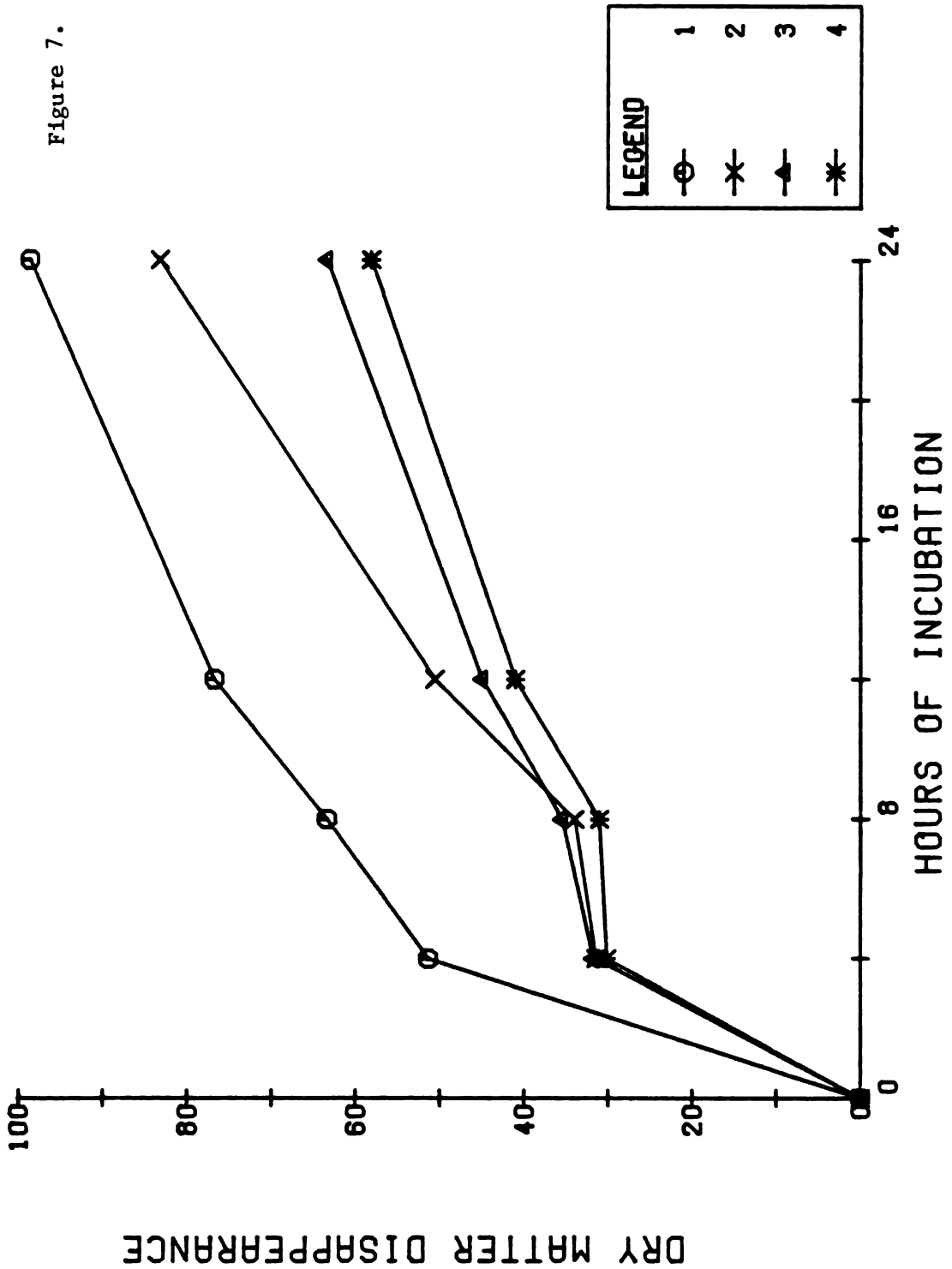

DRY MATTER DISAPPEARANCE
100
80
60
40
20
0
0
8
16
24
HOURS OF INCUBATION
LEGEND
1
2
3
4

Figure 8. Nitrogen disappearance of soybean meal (SBM) treated with formaldehyde (HCHO) from nylon bags suspended in the rumen. Legend, 1, SBM, normal; 2, SBM, 0.4% HCHO; 3, SBM, 0.8% HCHO; 4, SBM, 1% HCHO.

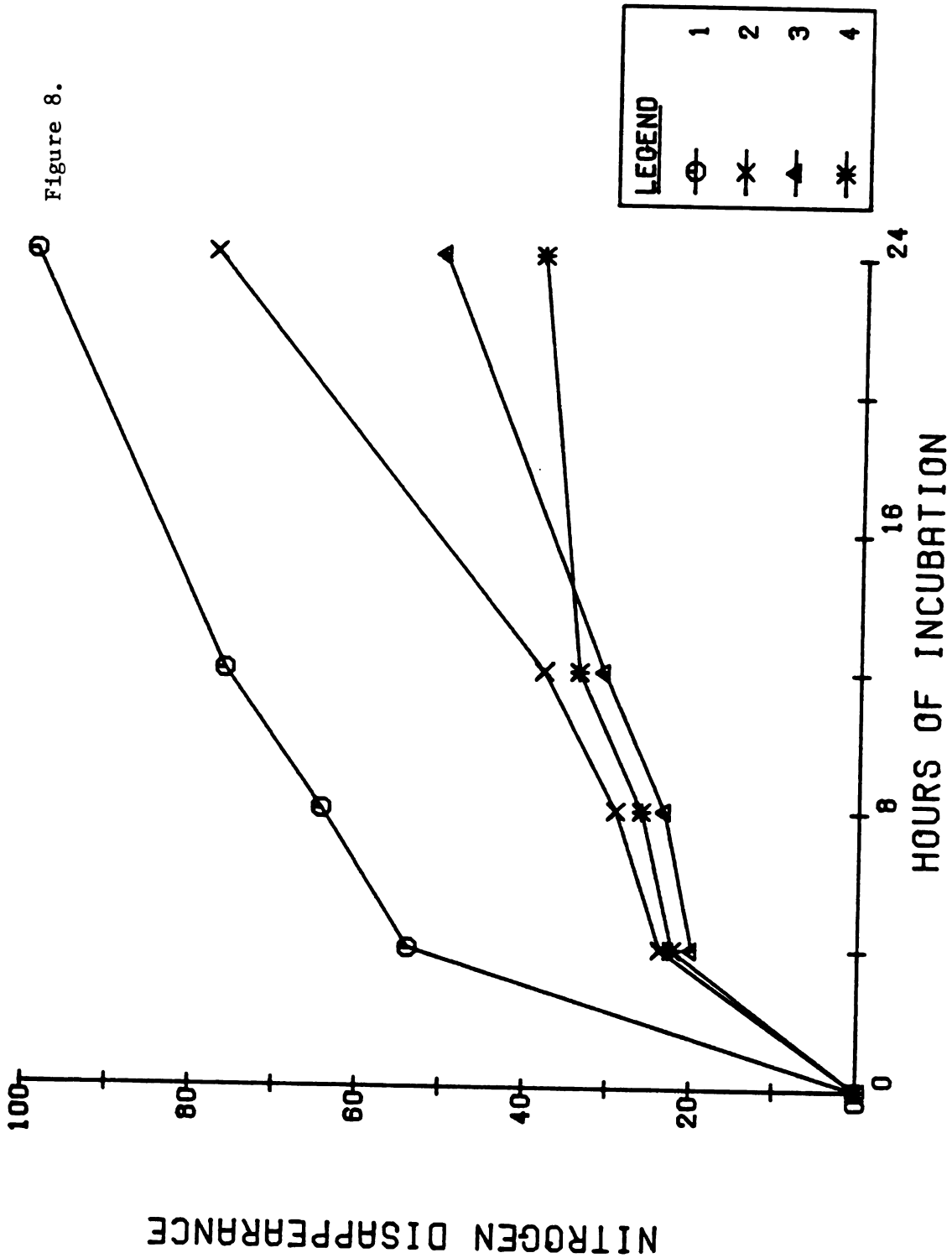
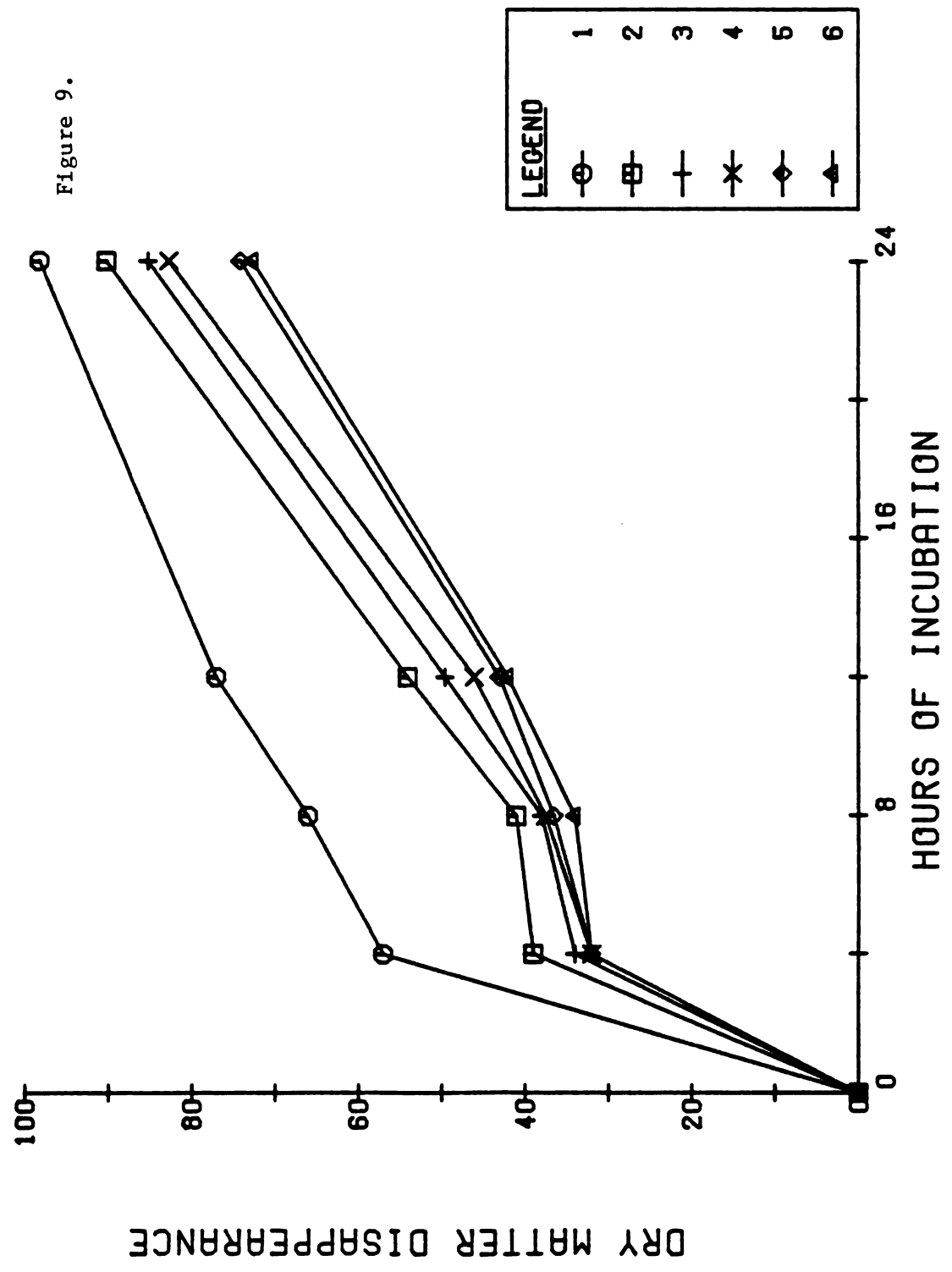

LEGEND
1
2
3
4
NITROGEN DISAPPEARANCE
HOURS OF INCUBATION
0
20
40
60
80
100
8
16
24

Figure 8.

Figure 9. Dry matter disappearance of soybean meal (SBM) autoclaved for various lengths of time from nylon bags suspended in the rumen. Legend, 1, SBM, normal; 2, SBM, autoclaved 10 min; 3, SBM, autoclaved 15 min; 4, SBM, autoclaved 20 min; 5, SBM, autoclaved 30 min; 6, SBM, autoclaved 40 min.


LEGEND
1
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DRY MATTER DISAPPEARANCE
HOURS OF INCUBATION
0
20
40
60
80
100
0
8
16
24

Figure 9.

Figure 10. Nitrogen disappearance of soybean meal (SBM) autoclaved for various lengths of time from nylon bags suspended in the rumen. Legend, 1, SBM, normal; 2, SBM, autoclaved 10 min; 3, SBM, autoclaved 15 min; 4, SBM, autoclaved 20 min; 5, SBM, autoclaved 30 min; 6, SBM, autoclaved 40 min.

Figure 10.

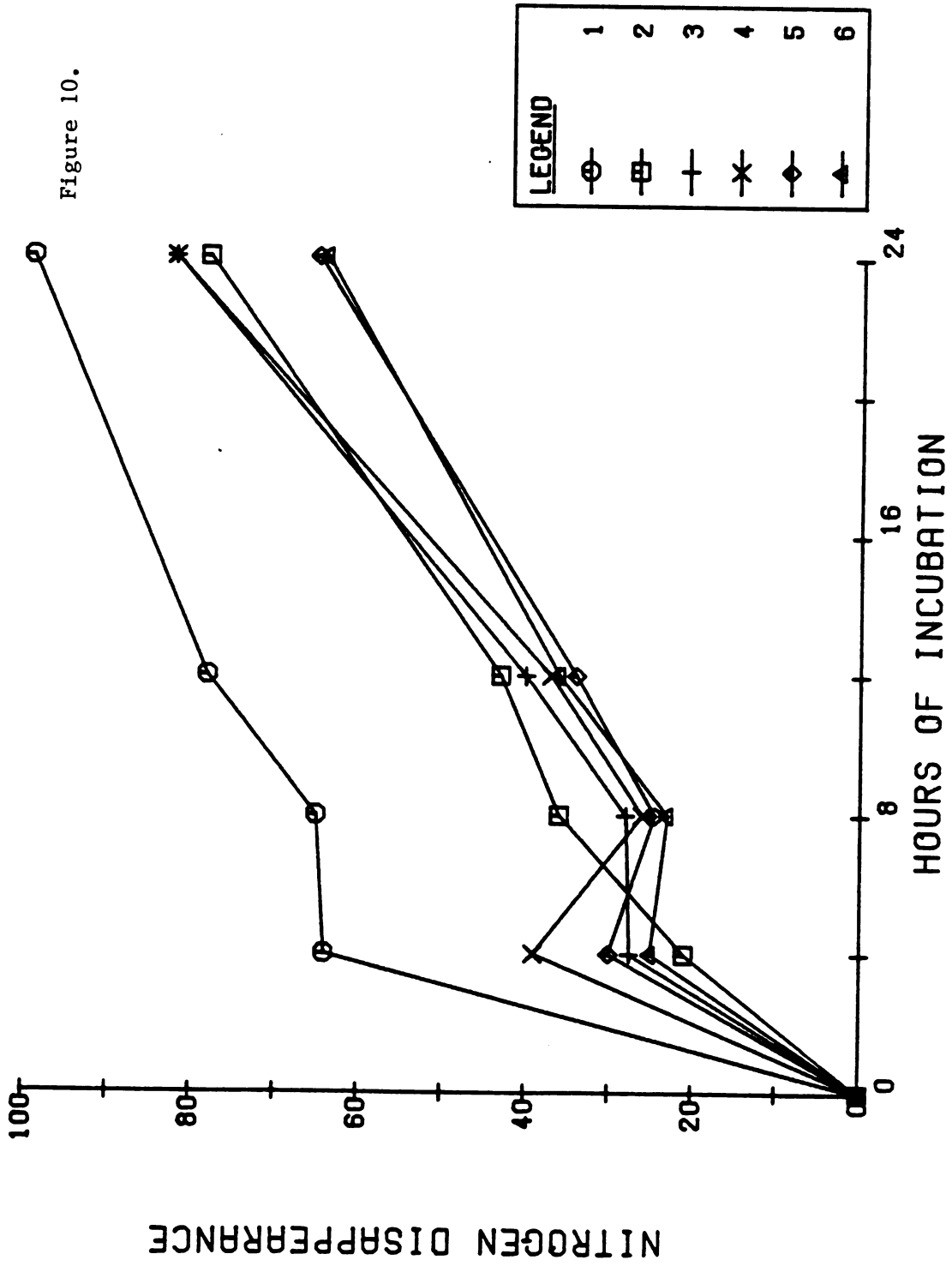
LEGEND
1
2
3
4
5
6
NITROGEN DISAPPEARANCE
100
80
60
40
20
0
0
8
16
24
HOURS OF INCUBATION

TABLE 11.

In Vitro Dry Matter Disappearance of Treated Soybean Meals. Experiment 1.[a]

Formaldehyde, % of Crude Protein				
0	.4	.8	1.0	SE
% of original DM				
89.19[b]	85.87[c]	82.22[b]	80.09[c]	2.63

Autoclaving, Minutes						
5	10	15	20	30	40	SE
% of original DM						
89.19[b]	89.58[b]	89.29[b]	86.86[b]	87.16[b]	85.40[c]	.84

[a]Each value is a mean of 3 replications.

[b,c]Means with unlike superscripts within a line differ significantly ($P<.05$).

TABLE 12.

Nitrogen Solubility, In Vitro Dry Matter Disappearance Acid Detergent Insoluble Nitrogen and Nitrogen Disappearance from Nylon Bags Suspended in the Rumen of Soybean Meal Subjected to 149°C for Varying Times. Experiment 1.[a]

Hours at 149 C	Nitrogen Solubility	IVDMD[b]	ADIN/N[c]	N Disappearance from Nylon Bags[d]
	%	%	%	%
0	24.7	94.6	.10	83.4
2	7.6	88.3	1.43	36.3
4	6.0	88.5	9.37	38.0
6	5.8	85.5	16.32	37.9

[a]Each value is an average of three replications performed at 2 to 4 different times.

[b]IVDMD = in vitro dry matter disappearance.

[c]ADIN/N = acid detergent insoluble nitrogen as a % of total nitrogen.

[d]12 hr rumen incubation.

149°C appeared optimal for reducing rumen nitrogen degradability with minimal heat damage which would lower digestion and absorption in the small intestine.

Poos et al. (1980) reported that N disappearance after 1 hr of incubation with bacterial protease correlated well with estimates of in vivo N degradability. In the current study, SBM was solubilized and degraded 69% after 1 hr of protease incubation (Table 13) which was similar to the value for SBM degraded 65% as reported by Poos et al. (1980). Degradabilities for SBM treated with heat for 2, 4, and 6 hrs were 50, 40, and 35% respectively.

Field Samples

Samples of raw, roasted, or extruded soybeans or SBM were collected from local farms. Dry matter and N disappearance from nylon bags from these are presented in Table 14. Raw soybeans degraded most after 12 hr of incubation. Disappearance of N for roasted beans was similar to SBM, while extruded beans were the most resistant to degradation. Roasted beans and soybean meal from different sources were similar in N degradability, although it is known that SBM can vary markedly in degradability, depending on source and processing.

Experiment 2

Ration Composition

Crude protein (of DM) for all diets was approximately 0.5 percentage points greater than planned (Table 15). Acid detergent

TABLE 13.

Nitrogen Degradation by FICIN Protease of Soybean Meal Subjected to 149°C for Varying Times. Experiment 1.[a,b]

Treatment	Hours of Incubation	Residual N	Degraded N	% Insoluble N Degraded
		% of original	% of original	
SBM	0	63.09	36.91[c]	
	.5	33.86	66.14	53.67
	1.0	30.58	69.42	48.47
	2.0	27.06	72.94	42.90
SBM, Heated 2 h[d]	0	88.85	11.15[c]	
	.5	60.36	39.64	67.93
	1.0	49.84	50.16	56.09
	2.0	45.28	54.72	50.96
SBM, Heated 4 h[d]	0	98.23	1.77[c]	
	.5	63.95	36.05	65.10
	1.0	59.53	40.47	60.60
	2.0	51.20	48.80	52.12
SBM, Heated 6 h[d]	0	93.73	6.27[c]	
	.5	67.26	32.74	71.76
	1.0	64.77	35.23	69.10
	2.0	57.10	42.90	60.92

[a]ENZELO(R) FICIN "D" 500 mcu, mg. Lot number FND 17-2779, Enzyme Development Corporation, 2 Penn Plaza, N.Y., N.Y. 10001.

[b]Values are means of duplicate incubations.

[c]Values at 0 time are indicate nitrogen solubility.

[d]149°C.

TABLE 14.

Nitrogen Disappearance of Soybean and Soybean Meal from Nylon Bags Suspended in the Rumen for Various Times. Experiment 1.[a]

	Hours Suspended			
	4	8	12	24
	---------- % N disappearance ----------			
Source 1				
Raw Beans	65.6	67.4	91.2	99.2
Roasted Beans	45.7	51.4	70.8	96.9
Extruded Beans	26.2	33.1	59.2	73.7
SBM	43.8	50.4	69.2	95.0
	Hours Suspended			
	6	12	18	24
Source 2				
Raw Beans	79.1	83.2	98.7	99.6
Roasted Beans	52.9	72.3	83.8	97.6
Source 3				
SBM	58.6	73.0	88.9	99.2

[a]Each value is a mean of 3 replicates.

TABLE 15.

Ingredient Composition of Rations Fed to Cows Receiving Varying Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	14 PRETRT	11 CS-SBM	14 AS-HS	14 CS-HS	14 CS-SBM	17 AS-HS	17 CS-HS	17 CS-SBM
	% DM							
Ingredients								
Corn Silage-CS	----	39.2	----	39.2	39.2	----	39.2	39.2
Corn Silage-AS	39.2	----	39.2	----	----	39.2	----	----
Alfalfa Hay[b]	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
HM Corn[c]	41.4	45.6	42.2	38.2	38.2	34.8	30.8	30.8
Soybean Meal-SBM	----	3.4	----	----	10.7	----	----	18.1
Soybean Meal-HS	----	----	6.9	10.7	----	14.2	18.1	----
44% Supplement	7.7	----	----	----	----	----	----	----
Vit.-Mineral Mix[d]	.5	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Ration Analysis								
Protein[e], %	14.1	11.3	14.4	14.6	14.6	17.5	17.7	17.7
ADF, %[e]	16.7	16.7	17.0	17.1	17.1	17.4	17.5	17.5
Soluble N, % of total N[f]	----	27.8	34.3	25.9	29.3	36.6	20.7	22.7
NE_l, Mcal/kg[g]	----	1.74	1.74	1.74	1.72	1.72	1.71	1.71

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]8 to 18% crude protein.

[c]High moisture ear or shelled corn.

[d]Included calcium carbonate, dicalcium phosphate, trace mineral mix, calcium sulfate, vitamin A (100,000 IU/day), vitamin D (5,000 IU/day).

[e]Average of 15 to 20 determinations with a range of approximately ±.5% crude protein units.

[f]Composite value of 5 to 10 determinations.

[g]Calculated from NRC (1978).

fiber content was similar for all treatments but slightly lower than recommended (NRC, 1978). Soluble nitrogen was greatest for AS-HS diets and least for the CS-17% protein diets.

Dry heated SBM was chosen primarily due to processing capacities available to us. Our preparation of dry heated SBM is not advocated due to production costs. In spite of its apparent impracticality, we used this product in order to standardize amino acid intake between degradable and undegradable protein sources. Variation in feedstuffs when selecting rations low in rumen N degradability has been a major criticism of studies comparing protein degradation. Standardized commercial sources of heat treated SBM were not available at the start of this experiment.

Normal and heated soybean meals were from similar commercial batches. Heating was in a batch dryer with a maximum attainable temperature of 140°C. Therefore, heating time was extended to 2.5 hr to compensate for a lower temperature than suggested by Experiment 1.

Throughout the trial, N disappearance from nylon bags after 12 hr incubation in the rumen ranged from 65-85% for SBM and from 30-45% for HS. Soybean meal was obtained from the Michigan State University feed mill.

Dry Matter Intake

Dry matter intakes are presented in Table 16. Dry matter intake for cows fed 11, 14, and 17% protein diets were 16.5, 19.1, and 20.8 kg/day. Huber and Kung (1981) reported little response

TABLE 16.

Dry Matter Intake of Cows Fed Varying Protein Amounts from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17			
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM	SE
Dry Matter Intake								
Diet Averages:								
kg/day	16.5[b]	19.6[cd]	18.4[bc]	19.4[cd]	20.7[d]	20.2[cd]	21.4[d]	.74
% BW	2.94	3.34	3.10	3.30	3.48	3.42	3.49	---
Averages for Dietory Protein Level								
kg/day	16.5		19.1			20.8		---

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[bcd]Means with unlike superscripts differ significantly ($P<.05$).

in milk yields to protein in excess of 13% when DMI were not stimulated by higher protein. Thus, a partial explanation for increased milk yields with increased dietary protein is increased energy consumption.

Milk Production

Pre-treatment milk production averaged 31.6 kg/day for all cows (Table 17). Adjusted treatment milk production and persistency was lowest for cows fed 11 CS-SBM and greatest for cows fed 17 AS-HS. Average percent milk fat, milk protein, and total solids were not significantly different among treatments. However, total output of all milk components corresponded with milk production. The low milk fat content of milk for all treatments was partly attributable to a marginal level of ADF and to grinding of hay in the rations.

Increasing the diet protein percent significantly ($p<0.01$) increased milk yields (Table 18). Cows fed 14.5 and 17.5% CP produced 3.8 and 5.2 kg more milk per day, than those fed 11.3%. The lesser increase (1.3 kg) obtained between 14.5 and 17.5% confirms a curvilinear response in milk yields with increasing protein (Claypool et al., 1980; Edwards et al., 1980; Oldham et al., 1981).

Replacement of SBM with HS resulted in 0.9 kg/day more milk (Table 18) suggesting that quantity and/or quality of absorbable protein reaching the small intestine of cows fed normal SBM limited milk production. Stern et al. (1980) used lactating cows fitted

TABLE 17.

Milk Production, Persistency and Milk Composition of Cows Fed Varying Amounts of Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17			
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM	SE
PRETRT Milk[b] kg/day	30.8	31.4	31.8	32.0	32.1	31.5	31.8	---
Treatment Milk[c] kg/day	30.7	34.4	35.1	34.0	37.3	35.6	34.9	---
Adjusted TRT[d] Milk, kg/day	31.6	34.7	34.9	33.7	36.7	35.8	34.7	.6
Persistancy, %[e]	99.6	109.4	110.4	106.4	116.1	113.2	109.9	2.1
Fat, %	2.90	2.94	2.98	3.02	2.95	3.00	3.08	.1
Protein, %	2.80	2.98	2.89	2.91	2.94	2.91	2.98	.1
Solids, %	11.36	11.84	11.70	11.60	11.58	11.76	11.73	1.5

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]PRETRT milk is the mean of days 8 to 21 postpartum.

[c]Treatment milk is the mean of days 22 to 91 postpartum.

[d]Adjusted TRT milk is obtained after treatment milk is covaried on pretreatment milk.

[e]$\frac{100 \times trt}{pretrt}$

TABLE 18.

Comparison of Main Effects of Milk Production Results of Cows Fed Varying Protein from Different Sources. Experiment 2.

Item	Milk Production (kg/day) Pre-Treatment	Treatment	Adjusted Treatment[a]	Persistency[b]
Protein, % (11 vs. 14 and 17, P .01; 14 vs. 17, P .05)[b]				
11	30.8	30.7	31.6	99.6
14[c]	31.7	34.5	34.4	108.7
17[c]	31.8	35.9	35.7	113.1
Type of Soybean Meal[de] (P<.05)				
CS-SBM	31.9	34.5	34.2	108.2
CS-HS	31.7	35.4	35.4	111.8
Type of Silage[de] (P>.15)				
CS-HS	31.7	35.4	35.4	111.8
AS-HS	31.8	35.9	35.7	112.8
Conventional vs. Unconventional Diet[def] (P<.10)				
CS-SBM	31.9	34.5	34.2	108.2
AS-HS	31.8	35.9	35.7	112.8

[a]Adjusted for pre-treatment milk production by covariate analysis.

[b]$\frac{100 \times trt}{pretrt}$

[c]Mean of CS-SBM, CS-HS and AS-SBM groups within each protein %.

[d]Mean of 14 and 17% CP groups.

[e]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[f]Conventional diet = CS-SBM; unconventional diet = AS-HS.

with duodenal and ileal cannulae and showed that feeding heat-extruded soybeans resulted in greater digestion of NAN and amino acids in the small intestine than raw soybeans of SBM. Similar to our findings, Schwab et al. (1980) and Smith et al. (1980) observed higher milk production in early lactation cows fed heat-extruded soybeans compared to SBM.

Others found no advantage in feeding protein resistant to rumen breakdown to cows in mid-lactation or to those past peak production (more than 7 wks post partum; Mielke and Shingoethe, 1981). Orskov et al. (1981) observed that the milk response to undegradable protein was greater when metabolizable energy intake was low. These workers point out that while consistent increases in milk protein have been observed when undegradable protein was fed or when protein was infused post-ruminally, increases in milk yield have been obtained mostly in experiments where cows were in negative energy balance because of intake restrictions. In their experiment there were no responses to changes in protein degradability when metabolizable energy intake exceeded 160 MJ/day; but at ME intakes less than 135 MJ/day, increases in supply of rumen undegradable protein increased milk production, milk protein production, and liveweight loss. Other studies have also shown increases in milk production when supply of undegradable protein was increased. Oldham et al. (1981) reported that cows fed fishmeal as their protein supplement produced more milk than those fed urea. Murdock et al. (1981) showed that cows fed wet brewers grains were more

productive than cows fed SBM as their protein source. However, absolute differences in amino acid supply in these two experiments could not be separated from effects of protein degradability. In general, ruminally protected protein appears to be more advantageous during early than late lactation when nutrient demands are greatest relative to milk production (Huber and Kung, 1981).

Partial replacement of HS nitrogen with ammonia added to silage did not significantly ($p>0.15$) affect milk yields (35.4 vs 35.9 kg/day; Table 18). In comparison, cows fed CS-HS at 14% CP had milk yields (35.1 kg/day) equal to those fed the conventional diet (CS-SBM) at 17% CP (34.9 kg/day). These findings suggest feeding HS resulted in more efficient use of nitrogen, since production was greater with less protein.

Since bacteria cannot distinguish the source of N for protein synthesis, it would be beneficial to supply bacteria with lower cost NPN sources while true protein of low rumen degradability would furnish amino acids to the small intestine for direct digestion and absorption (Journet and Remond, 1981). Some caution should be taken in feeding rations with large amounts of rumen undegradable protein since too little rumen ammonia might curtail optimal rumen fermentation.

In this study a combination of ammonia treated corn silage and rumen undegradable protein, an unconventional diet for high producing cows early in lactation resulted in greatest and most profitable milk production when protein was 17.5% (of DM). Nonprotein nitrogen

added through corn silage was equal to 2.2% CP. Some studies have shown that replacing NPN with preformed protein resulted in decreased milk production (Oldham et al., 1981; Treacher et al., 1979; Wohlt and Clark, 1978), while others (Holter et al., 1968; Kwan et al., 1977, Lundquist and Otterby, 1981; Teller et al., 1980) including the present, have shown NPN to be equal or better to other proteins. Clark and Davis (1980) suggested that high producing dairy cows should not be fed NPN in early lactation. Results of our study and others (Foldager and Huber, 1979; Kwan et al., 1977; Lundquist and Otterby, 1981; Sauer et al., 1980) contradict this view. The discrepancies between studies could be due to a number of reasons; these include insufficient nitrogen, a deficiency in energy, excessive NPN, or method of feeding. In support of the latter, Canadian investigators (Sauer et al., 1980) reported that feeding urea as part of a completely blended ration was more productive than a similar ration when urea was fed twice daily in the concentrate. Our current study incorporated ammonia treated corn silage as part of a completely blended diet and may in part have contributed to these findings.

Energy Intake and Body Weights

Calculated intakes and requirements for net energy (NE) lactation are presented in Table 19. Except cows fed 17 CS-SBM, average NE intake were less than requirements estimated to support the average milk produced during treatment. Cows fed 14 AS-HS and 17 CS-SBM

TABLE 19.

Estimated Net Energy Intake and Requirement and Body Weight and Efficiency of Milk Production of Cows Fed Various Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17		
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM
NE Intake[b] Mcal/day	28.7	34.1	32.0	33.4	35.6	34.5	36.6
NE Requirement[c] Mcal/day	31.8	34.9	35.5	34.6	37.2	35.8	35.7
NE Balance	-3.1	-.8	-3.5	-1.2	-1.6	-1.3	+.9
Body Weight[d] kg	562	587	594	588	595	590	613
Body Weight Loss[e], kg	-53	-6	-29	-22	-21	-15	-5
Milk/Dry Matter kg	1.86	1.76	1.91	1.75	1.80	1.76	1.63

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]NE = net energy.

[c]Calculated from NRC (1978). Based on average milk produced and body weight during treatment period.

[d]Average during treatment.

[e]Loss during treatment.

consumed close to NE requirements and tended to lose least weight during treatment. Body weight losses were greatest for 11 CS-SBM and least for 17 CS-SBM. Cows fed 17 AS-HS produced the most milk and lost the most weight of groups fed 17% protein. Orskov et al. (1977) have observed similar findings, and Robinson et al. (1974) suggested that at high energy intakes, increased protein may favor the partitioning of energy towards milk rather than tissue stores.

Production Efficiency

Because of low DM intakes for cows fed 11 CS-SBM ration, they were efficient in conversion of feed to milk; however, the 14 CS-HS group was most efficient, and 17 CS-SBM was the least.

Rumen and Blood Parameters

There were no significant ($p>0.10$) differences due to hour of sampling for rumen ammonia-nitrogen (RAN; Table 20). RAN increased ($p<0.05$) as protein percent increased. Cows fed 11 percent protein had RAN less than estimates needed for optimal microbial protein synthesis (Satter and Slyter, 1974). In general, cows fed 14 and 17% protein had RAN's comparable to suggested optimal concentrations (5 to 25 mg/dl). Within protein levels, cows fed CS-HS tended to have lowest levels of RAN, suggesting lower protein degradability in the rumen.

Rumen pH values were not significantly ($p>0.10$) affected by hour of sampling or treatment (Table 21). They were higher than

TABLE 20.

Rumen Ammonia-Nitrogen or Cows Fed Varying Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17			
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM	SE
	mg/dl							
Hours Post-Feeding								
0[b]	4.5	8.1	7.5	8.9	10.6	9.4	11.8	---
4[b]	3.8	6.2	6.0	6.4	12.5	9.3	12.2	---
8[b]	6.6	6.6	7.0	7.9	9.7	7.3	9.8	---
Average[c]	5.0[d]	7.0[d]	6.8[d]	7.7[d]	10.9[e]	8.7[d]	11.3[e]	1.5

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]Each value is the mean from 12 animals.

[c]Each value is the mean of 36 determinations.

[de]Means with unlike superscripts differ significantly ($P<.05$).

TABLE 21.

Rumen pH of Cows Fed Varying Protein from Different Sources. Experiment 2.[a]

Protein % Treatment	11	14			17			
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM	SE
Hours Post-Feeding								
0[b]	7.01	7.19	7.09	7.04	7.08	7.11	7.23	---
4[b]	6.93	6.95	7.05	6.90	6.81	6.90	6.99	---
8[b]	6.92	6.90	7.15	6.79	7.33	7.05	6.92	---
Average[c]	6.95	7.01	7.09	6.91	7.07	7.07	7.05	.14

[a]Differences were not significantly different (P>.10).

[b]Each value is the mean of 12 animals.

[c]Each value is the mean of 36 determinations.

expected and near neutral, probably due to salivary contamination during sampling.

Plasma urea nitrogen was also not affected ($p>0.10$) by hour of sampling but increased significantly ($p<0.05$) with increasing protein (Table 22).

Molar concentrations of volatile fatty acids were not different ($p>0.10$) between hour of sampling, thus, values presented in Table 23 are means of 0, 4, and 8 hrs. These data suggest that rumen fermentation was similar for all diets. Molar percentages of butyrate, iso-butyrate, and valerate tended to be slightly greater when cows were fed 17% protein and supports findings of others (Folman et al., 1981).

Economic Evaluation

Increased milk production when feeding greater amounts of protein is generally beneficial if income over feed costs is increased (Satter et al., 1979). Table 24 shows an economic evaluation of treatments based on treatment means. Cows fed 17 AS-HS were most productive and gave the greatest profits. The 14% protein groups ranked second, third, and fourth in profitability. Note that cows fed 17 CS-HS and CS-SBM produced more milk than 11 CS-SBM, but returned less income due to greater feed intakes and cost of additional protein. However, it is still more economical to feed greater amounts of protein if one considers projected milk production and differences in income returned over feed costs. For example, cows fed 17 CS-SBM

TABLE 22.

Plasma Urea-Nitrogen of Cows Fed Varying Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17			
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM	SE
	mg/dl							
Hours Post-Feeding								
0[b]	7.64	12.16	11.67	12.48	14.99	17.53	18.12	---
4[b]	7.81	10.88	11.33	12.02	15.61	17.60	16.26	---
8[b]	6.67	10.88	10.18	10.70	14.34	16.08	15.64	---
Average[c]	7.37[d]	11.30[e]	11.06[e]	11.73[e]	14.98[f]	17.07[f]	17.07[f]	.85

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]Each value is the mean from 12 animals.

[c]Each falue is the mean from 36 determinations.

[def]Means with unlike superscripts differ significantly ($P<.05$).

TABLE 23.

Rumen Volatile Fatty Acids of Cows Fed Varying Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11 CS-SBM	14 AS-HS	14 CS-HS	14 CS-SBM	17 AS-HS	17 CS-HS	17 CS-SBM	SE
VFA[b], Molar %								
Acetate	59.2	59.7	60.2	57.8	59.9	60.5	59.1	1.2
Propionate	28.2	26.6	26.4	27.9	25.9	24.7	24.9	1.4
Isobutyrate	1.2	1.2	1.3	1.2	1.2	1.2	1.6	.2
Butyrate	10.2	10.0	8.9	9.5	10.8	9.9	10.5	1.0
Isovalerate	1.1	1.1	1.1	1.3	1.2	1.7	1.7	.4
Valerate	1.8	1.6	1.7	2.1	1.4	2.0	2.0	.2

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]Mean of 0, 4 and 8 h post-feeding includes 36 values per mean. Differences were not significantly different (P>.10) for hour or treatment.

TABLE 24.

Economic Evaluation of Rations Containing Varying Amounts of Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17		
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM
Feed Cost ($/day)[b]	2.02	2.68	2.78	2.70	3.34	3.55	3.96
Milk Income ($/day)[c]	8.78	9.84	10.04	9.72	10.67	10.18	9.98
Income Over Feed Cost ($/day)	6.76	7.16	7.26	7.02	7.33	6.63	6.02
Relative Rank	5	3	2	4	1	6	7
Projected 305 Day Milk (kg)[d]	6967	7649	7805	7628	8176	7821	7782
Milk Income Over 11 CS-SBM ($/cow/ 305 day)	---	195	240	189	346	244	233

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

[b]Feed costs ($/ton): hay 60; corn silage-CS, 22; corn silage-AS, 24; high moisture ear corn (65% DM) 85; soybean meal-NS, 300; soybean meal-HS, 400.

[c]13¢/lb.

[d]Calculated according to McGilliard, 1965.

returned $.74 less per day in income over feed costs than cows fed 11 CS-SBM. However, cows fed the higher level of protein might be expected to produce 815 kg more milk in a 305 day production (McGilliard, 1965).

Assuming 50 milking cows, and milk priced at $.13/lb, cows fed 17 CS-SBM would yield $11,654 more per year than those fed 11 CS-SBM. The increased feed cost of $.74/cow/day would amount to $11,285 less in return over feed costs. Subtracting the increase in total milk income of $11,654 from increased feed costs of $11,285 results in a net gain of $369. Although this is not a significant amount of money, one must remember that the farmer would not feed a 17% protein ration for the entire lactation so that the net gain in this example would actually be more.

Reproductive Efficiency

A total of 14 cows were replaced due to various diseases and there was no relationship between treatments and incidence of mastitis or other metabolic problems. Breeding data are presented in Table 25. There were no apparent differences between treatments in services per conception, days open, or number of cows sold. Cows fed low protein (11 CS-SBM) had fewer cows settle on first service than other treatments which may have been due to a greater energy deficit . There were no apparent differences between treatments due to amount of protein (Table 26). A large percentage of cows in this experiment were sold open, but no apparent

TABLE 25.

Breeding Data of Cows Fed Varying Protein from Different Sources. Experiment 2.

Protein % Treatment[a]	11	14			17		
	CS-SBM	AS-HS	CS-HS	CS-SBM	AS-HS	CS-HS	CS-SBM
Item							
Services/ Conception	2.40	2.25	2.18	3.00	1.83	2.22	1.75
Days Open	108	87	95	100	91	87	80
Cows	11	8	11	8	6	9	8
Sold Open	3/13	4/13	2/12	3/12	5/12	2/12	4/13
Settled 1st Service, %	15	38	33	25	42	17	33

[a]CS-corn silage, SBM-soybean meal, AS-ammonia-corn silage, HS-heated soybean meal.

TABLE 26.

Breeding Data of Cows Fed Varying Protein from Different Sources Grouped by Protein Level. Experiment 2.

Protein, %	11	14	17
Services/Conception	2.40	2.50	2.04
Days Open	108	98	91
Cows	11	27	23
Sold Open	3/13	9/37	11/37
Settled 1st Service, %	15	32	24

reasons for this finding are evident.

Experiment 3

Feed Composition and Milk Production

Composition of feeds used is presented in Table 27. Ammonia-treated silage kept well in the plastic bags even after being emptied from a silo, transported to Wisconsin, and stored for 30-45 days. No heating was detected and only minimal mold at the tie of the bag was observed. These observations support those of Britt and Huber (1975) who also reported that ammonia-treated corn silage was less apt to heat and spoil. Vitamins and minerals were included in the grain mixes to meet NRC (1978) requirements. Diet composition and milk production are presented in Table 28. All diets were similar in dry matter, crude protein, and acid detergent fiber content. Milk production was not significantly ($p>0.10$) different between treatments although cows fed diets with ammonia-treated silage(AS) tended to be more productive. There were no significant differences ($p>0.10$) in milk composition between treatments.

Rumen Parameters

Nitrogen disappearance of soybean meals used in this study from nylon bags suspended in the rumen are presented in Figure 11. Estimated nitrogen degradability based on 12 hr incubation was 75% for SBM and 40% for HS.

Rumen ammonia nitrogen measured at 0, 2, 4, and 6 hrs after feeding is presented in Table 29. Peak RAN was observed 2

TABLE 27.

Composition of Feeds in Experiment 3.

Item[a]	DM, %	CP, %	ADF, %
Grain Mix[b]			
AS-HS	90.7	21.1	6.3
AS-SBM	88.9	21.7	6.2
CS-HS	90.2	23.9	6.0
CS-SBM	88.7	24.4	5.8
Corn Silage	35.1	8.1	29.3
Ammonia-Corn Silage	34.2	12.5	26.7
Alfalfa Hay	85.0	17.1	36.5
Complete Ration			
AS-HS	55.0	17.7	18.1
AS-SBM	54.4	17.8	18.1
CS-HS	54.6	17.0	18.0
CS-SBM	55.0	17.2	17.7

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Includes ground corn, limestone, dical, calcium sulfate, vitamins A--30-40,000 I.U./day, D--3,000-5,000 I.U./day, E--500 I.U./day.

TABLE 28.

Dry Matter Intake and Milk Production of Cows in Experiment 3.[a]

	Treatment[b]				
	AS-HS	AS-SBM	CH-HS	CS-SBM	SE
Dry Matter Intake kb/day	17.6	17.5	17.9	16.9	1.2
Milk, kg/day[c]	24.1	24.2	23.6	22.6	1.0
% BF	3.03	3.47	3.41	3.33	.24
% CP	2.69	2.91	2.81	3.08	.09
% Solids	11.00	11.21	11.41	11.48	.28

[a]None of the differences were significant (P>.10).

[b]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[c]Mean of days 8 through 14 averaged for 4 periods.

Figure 11. Nitrogen disappearance of normal soybean meal (SBM) and soybean meal heated for 2.5 hr at 140° C from nylon bags suspended in the rumen and fed in Experiment 3. Legend, 1, SBM, normal; 2, SBM, heated.

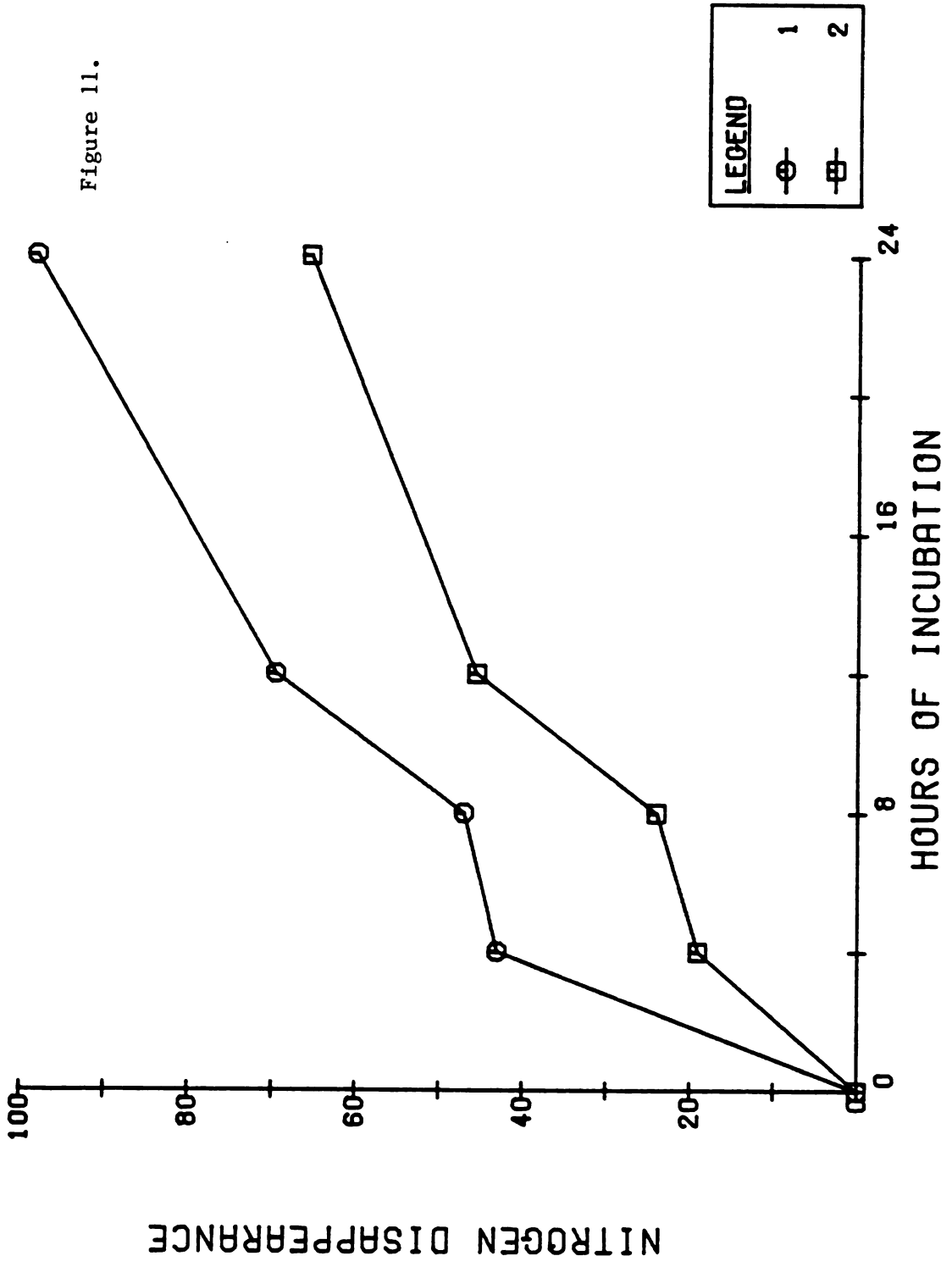
NITROGEN DISAPPEARANCE
100
80
60
40
20
0
8
16
24
HOURS OF INCUBATION
LEGEND
1
2

Figure 11.

TABLE 29.

Rumen Ammonia Nitrogen (ng/dl) of Cows in Experiment 3.[a]

Treatment[b]	Hours After Feeding[c] 0	2	4	6	$\bar{x}$[d]	SE
AS-HS	10.8	19.0	10.9	12.5	13.3[ef]	4.4
AS-SBM	14.9	25.1	13.1	18.7	17.9[e]	6.4
CS-HS	9.4	12.7	7.8	9.7	9.9[f]	3.9
CS-SBM	19.7	18.7	13.7	11.2	15.8[e]	5.7

[a]Rumen fluid collected via rumen fistula.

[b]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[c]Values are averages from 4 determinations.

[d]Values are averages from 16 determinations.

[ef]Means with unlike superscripts differ significantly ($P<.05$).

hr post feeding for all treatments. As expected, RAN was greatest for cows fed AS-SBM, intermediate for AS-HS and CS-SBM, and least for CS-HS. Rumen pH (Table 30) was not different ($p>0.10$) between treatments, but lower than in Experiment 2 since rumen fluid collection was made via a fistula in Experiment 3.

Molar percents of rumen volatile fatty acids (VFA) were not significantly ($p>0.10$) affected by treatments (Table 31). Propionate tended to be greatest for cows fed ammoniated silage (AS) while butyrate tended to be greatest for cows fed normal silage (CS).

Neutron Activation and Marker Analysis

Neutron activation was performed on triplicate samples at the University of Wisconsin Nuclear Reactor Laboratory (UWNRL). The marker ratios of chromium (Cr) and La in duodenal ileal, and fecal contents as percent of their ratios in feed ranged from 74 to 107. Calculated rumen digestibility of dry matter, nitrogen, and efficiencies of microbial protein synthesis were questionable when La was used to determine digestibility. Dry matter digestibility in the rumen was much lower than expected, and in some instances, these values were negative suggesting a net gain in dry matter at the duodenum. On the average, nitrogen and non ammonia nitrogen flow to the duodenum were 110 to 130% of nitrogen intake. Microbial efficiencies were

TABLE 30.

Rumen pH of Cows in Experiment 3.[ab]

Treatment	Hours After Feeding[c] 0	2	4	6	$\bar{x}$[d]	SE
AS-HS	6.39	6.20	6.24	6.38	6.30	.25
AS-SBM	6.49	6.34	6.30	6.54	6.42	.17
CS-HS	6.35	6.43	6.30	6.54	6.41	.23
CS-SBM	6.41	6.33	6.26	6.38	6.35	.17

[a]Rumen fluid collected via rumen fistula.

[b]Differences were not significant for treatment or hr after feeding.

[c]Values are averages from 4 determinatins.

[d]Values are averages from 16 determinations.

[e]Values were not significantly different (P>.10).

TABLE 31.

Rumen Volatile Fatty Acids of Cows in Experiment 3.[a]

	Treatment[b]				
	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
Molar, %					
Acetate	67.6	66.3	67.8	66.4	1.7
Propionate	21.1	20.1	17.4	18.9	1.2
Butyrate	9.8	9.9	11.3	10.6	.9
Total mmoles/dl	8.6	8.7	9.1	9.3	.7

[a]Average of 0, 2, 4 and 6 h post-feeding. Differences were not significantly different (P<.10).

also extremely high with some individual values over 100 g N/kg rumen fermented organic matter. These findings are disturbing but are similar to those found in a number of recent experiments also conducted at the University of Wisconsin using La as a marker. Close inspection of the data show no detectable error in analysis or computation of data. Wisconsin workers (L. D. Satter, personal communication) have suggested discrepancies in counting of rare earth elements at the UWNRL. Coefficient of variation (CV) for counting of La were larger (8 to10% CV) than from other rare earth elements (less than 5% CV). However, samples submitted over time show no statistical difference between activation runs. In one experiment, inspection of individual samples collected throughout the four-day collection periods showed marked variation of La at the duodenum but not at the ileum or feces. Statistical calculation resulted in theoretical rumen dry matter digestibilities of approximately -10 to 40% (D.K. Combs, personal communication). Possibilities which might explain these findings are: 1) La is not behaving ideally as an inert marker at the duodenum, 2) digesta flow is not in a steady state because of large dry matter intakes, 3) sampling of liquids and solids from the t-cannulae is not proportional to amounts present, 4) La is absorbed prior to the duodenum and is re-secreted into the lower tract.

Total tract dry matter digestibility appears normal when La was used as a marker. These and other data suggest that fecal La was accurately estimated digestibility. Personal communication with Wisconsin workers (L. Rode) indicate that use of lignin

results in rumen digestibility similar to accepted literature values with total tract digestibilities similar to those calculated with La.

In the present study, lignin was used as a digestibility marker after obtaining abnormal rumen digestion values with La. Coefficients of variation (CV) for lignin determination in hay and corn silage were less than 5%, while a CV for a grain sample of less than 20% was deemed acceptable since their lignin content of grain was less than 1%. Lignin was determined by the acid detergent lignin method (Goering and Van Soest, 1970).

Sample Collection

Visual observations of digesta flow from the duodenal cannula revealed marked variation in apparent liquid to dry matter content. To obtain unbiased samples, sample collection was as follows: 1) all solid material from the cannula was removed, 2) the first 100 ml of digesta were discarded, 3) complete collection of the next 400 ml of digesta were saved for compositing.

Ileal digesta flows were not always sufficient to obtain a representative sample for compositing. Ileal flows were poorest during the early morning hours (12 AM to 6 AM) suggesting the possibility of some diurnal variation in digesta flow.

Dry Matter Flow and Digestibility

When La was used as a flow marker, DM entering the duodenum was approximately 16 kg/day which resulted in low average rumen dry matter digestion (RDMD) (Table 32). Estimates for

TABLE 32.

Dry Matter Flow and Digestion in Various Segments of the Digestive Tract of Cows in Experiment 3.[a]

	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
Dry Matter Intake kg/day	17.62	17.50	17.87	16.90	1.16
Dry Matter Flow to: Duodenum, kg/day	12.13[b](18.0)[c]	10.95(16.2)	11.39(14.7)	10.93(15.1)	.81(.8)
Ileum, kg/day	7.67 (7.5)	7.51(7.8)	7.42(7.0)	6.65(7.2)	.46(.5)
Feces, kg/day	5.78 (5.6)	5.78(6.2)	5.79(6.3)	4.94(6.1)	.51(.4)
Apparent Dry Matter Digestion: Rumen/Abomasum, % intake	31.09 (-2.6)	37.49(7.9)	36.40(17.4)	37.88(10.0)	3.93(6.1)
Small Intestine, % entering[d]	34.65 (50.7)	32.40(50.9)	34.71(52.2)	39.99(52.3)	2.71(2.2)
Post Ileum, % entering[d]	27.54 (25.1)	30.82(17.9)	21.52(9.5)	23.71(14.3)	4.45(5.4)
Total Tract, % intake	67.31 (67.5)	66.45(64.0)	66.02(65.2)	71.76(64.5)	2.62(2.3)

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Calculated using lignin.

[c]Calculated using lanthanum.

[d]Digestibility of digesta entering.

DM flow were reduced 24% to 11 kg/day when lignin was used to calculate digesta flow. Average RDMD was 32.72% when estimated by lignin ratios and was much closer to literature values. Rumen dry matter digestion estimates with La suggest an inhibition of fermentation which was not corraborated by other data in this trial.

While some (Muntifering et al., 1981; Fahey et al., 1979) have cautioned against the use of lignin, it is apparent that in our study lignin resulted in more accurate estimates of RDMD than La. Muntifering et al. (1981) reported that lignin was digested in the rumen and intestine and that extent of digestion varied greatly with diet and method of lignin determination. Lambs fed Kenhy tall fescue had 35.2% of ingested lignin digested in the total tract. Of this, 97.2% and 7.3% were digested in the rumen and intestines, respectively, if the permanganate ($KMnO_4$) assay was used. By the acetyl bromide solubilization assay (ABSL), total tract digestion of lignin was 29.7% but, only 13.5% was digested ruminally and 86.5% in the small intestine. Lignin digestibility for the same diet was only -0.6 using acid detergent lignin procedure (ADL). Across three methods of determination and three diets, lignin digestibility in the rumen, intestine, and total tract were 13.1, 11.7, and 26.2%. Fahey et al. (1979) also fed various diets to lambs and compared lignin digestibility using the ADL and ABSL methods. They reported a negative correlation between the two methods of - 0.60 and suggested that the latter method was more

sensitive in detecting soluble lignin, which would be degraded by the ADL method.

Negative lignin digestibility may reflect artifact lignin formation due to heat damage (Morrison, 1972), incomplete removal of interfering materials during lignin analysis (Van Soest and Wine, 1968), or artifact lignin formed by postruminal formation of non-conjugated phenols that analyze as lignin (Allinson and Osbourn, 1970).

In the present study, heat was not used in preparation or analyses of ruminal, duodenal, or ileal contents so that artifact lignin formation due to heat damage did not exist. Moderate heat (55°C for 48 hr) was applied to feces and would only moderately increase heat damage.

Although Muntifering et al. (1981) showed lignin digestion in the intestine, our estimates of dry matter flow to the ileum and feces using lignin averaged within 10% of flows calculated with La. Because lignin gave lower estimates of DM flow to the duodenum but similar ileal flows, apparent DM digestibility in the small intestine was approximately 31% less with lignin than with La. Dry matter digestibilities in the total tract were similar for lignin and La.

Nitrogen Flow and Digestibility

Nitrogen intake and a comparison of N flow and digestibility calculated with lignin or La is presented in Table 33. As expected, N flow to the duodenum was 29% less with lignin than with

TABLE 33.

Nitrogen Flow and Digestion in Various Segments of the Digestive Tract of Cows in Experiment 3.[a]

	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
Nitrogen Intake g/day	498	497	492	474	13.71
Nitrogen Flow to: Duodenum, g/day	363[b](536)[c]	338(515)	402(515)	326(451)	32.73(28.7)
Ileum, g/day	170 (166)	151(159)	175(166)	155(160)	11.84(6.2)
Feces, g/day	139 (134)	135(149)	142(147)	114(141)	12.29(11.5)
Apparent Nitrogen Digestion in: Rumen/Abomasum, % intake	27.10(-5.6)	31.83(4.0)	17.83(4.6)	32.59(2.5)	5.99(3.9)
Small Intestine, % entering[d]	52.8 (68.7)	55.26(67.6)	55.85(67.6)	54.22(64.5)	1.53(1.5)
Post Ileum, % entering[d]	19.30(19.3)	12.45(6.4)	20.23(11.5)	21.10(10.3)	6.45(5.3)
Total Tract, % of intake	72.32(72.8)	73.00(69.9)	71.09(70.3)	76.17(69.7)	2.41(2.5)

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Calculated using lignin.

[c]Calculated using lanthanum.

[d]Digestibility of digesta entering.

La. However, N flow to the ileum and feces were very similar for the two markers, suggesting that the discrepancy in marker flow occurred only at the duodenum.

When based on La, apparent N digestion in the rumen/abomasum was negative for cows fed HS diets and only slightly positive (less than 5%) on SBM. Trends obtained with lignin were similar, except that disappearance of N between the rumen and duodenum was greater, averaging 27.3%. Others (see review by Theurer, 1979) found net increases of N flow at the abomasum/duodenum. Hume et al. (1970) observed large net increases in N in sheep fed on low N diets. In calves, Leiboholz (1980) reported a net disappearance of N at the duodenum when dietary protein exceeded 11.38%. Kaufmann and Hagemeister (1976) reviewed selected data and reported that a net loss in N occurred at the duodenum when dietary protein was 13% or greater while rations less than 13% CP had a net gain. The net increase in N on low protein diets is probably due to incorporation of dietary protein from recycled urea or to sloughing of mucosal cells and gastric secretions. Weston and Hogan (1967) and Clarke et al. (1966) estimated that 1 to 2 g/day of N was secreted into the abomasum of sheep. Although no estimates have been made for cows, van't Klooster and Boekholt (1972) suggested 15 to 30 g/day. Placement of the duodenal cannula may also affect degree of sample contamination from pancreatic and bile secretions.

A net loss of N at the abomasum/duodenum suggests wastage of N through absorption of ammonia released from deamination of amino

acids in the rumen. Ration protein in the present trial was greater than 17% CP and RAN values moderately high; thus, a net loss of N might be predicted. Estimates obtained with lignin but not La would support this prediction. In a summary of the literature, Theurer (1979) observed a tendency for a net loss from the rumen when lignin was used as the reference marker compared to N flows at the abomasum/duodenum exceeding 100% of intake in studies with chromium. Drennan et al. (1970) and Faichney (1975) suggested that lignin was superior to Cr_2O_3. In contrast, Isichei (1980) reported similar digesta flows with lignin or Cr_2O_3, and a net dietary N loss at the abomasum with both markers. Apparent N digestibilities in the present study were similar to those of Crickenberger et al. (1979) and Sachtleben (1980). Both found lignin a more satisfactory marker than Cr_2O_3. Santos (1980) and Stern (1980) also reported large net increases in N flow at the duodenum. These workers used La as a digestibility marker and reported estimates of rumen organic matter digestion of 30%. Although organic matter flow was low, efficiencies of microbial protein synthesis were normal.

Similar to findings with DM digestibilities, lignin resulted in lower estimates of apparent N digestibility in the small intestine than with La. However, apparent N digestibility in the post ileum tended to be greater with lignin. There were no differences between markers in total tract digestibilities.

NAN Flow and Digestion

Estimates of NAN flow to the duodenum averaged 344 g/day with lignin and 483 g/day with La (Table 34). With La, flow of NAN was greater for cows fed AS-HS (521 g/day) and CS-HS (502 g/day) than normal SBM (455 g/day), suggesting more N degradation in the rumen. A similar trend was observed when lignin was used to calculate NAN flow in that cows fed HS had greater NAN passage. With lignin, passage on CS-HS tended to be greater than on AS-HS, but the opposite was observed with La. However, differences between these rations were small.

The following observations were made based on lignin. When normal corn silage was fed, HS resulted in a 26% greater NAN flow to the duodenum than SBM, but a lesser increase when ammonia-treated corn silage was fed. Feeding HS increased NAN flow 17%, regardless of silage type. Rode (1981) reported similar findings to ours for NAN flow when he used lignin rather than La. Merchen (1981) also reported that NAN flows were 72 to 90% of N intake when based on La. However, Santos (1980) and Stern (1981) reported net N increase up to 140% or absolute gains of 100 g N/day at the duodenum.

Dietary N and Microbial N Flow

Estimates of dietary N at the duodenum also include endogenous and protozoal N (Table 34). Flow of dietary N to the duodenum showed trends similar to NAN flow. Microbial N flow to the duodenum was similar for all treatments. Microbial N flow was 16%

TABLE 34.

Flow and Digestion of Non-Ammonia Nitrogen (NAN) and Efficiency of Microbial Protein Production of Cows in Experiment 3.[a]

	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
Non-Ammonia Nitrogen Flow to: Duodenum (Total) g/day	352[b] (521)[c]	323(474)	391(502)	310(435)	33.00(28.7)
Duodenum (Bacteria) g/day	148 (226)	141(227)	170(222)	150(224)	14.35(27.6)
Duodenum (Dietary Endogenous, Protozoa) g/day	204 (295)	183(247)	222(280)	161(211)	28.08(15.8)
Ileum (Total) g/day	161 (156)	142(150)	166(157)	146(151)	11.50(5.7)
Microbial Ng/kg Rumen Digested Organic Matter	28 (110)	19(62)	26(36)	24(56)	2.59(20.7)
Microbial Ng/kg Rumen Digested Organic Matter (Corrected)	22	16	22	20	2.51
Dietary N Digested in Rumen/ Abomasum, % intake	58.84(40.5)	63.34(50.0)	55.05(43.6)	67.37(55.7)	5.08(2.6)
NAN Digestion in Small Intestine, g/day	192 (365)	182(324)	226(345)	165(285)	23.17(22.9)
% entering[d]	54.06(69.8)	56.03(68.1)	57.04(68.4)	54.81(65.4)	1.49(1.3)

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Calculated using lignin.

[c]Calculated using lanthanum.

[d]Digestibility of digesta entering.

greater for CS-HS than the average for all other treatments when lignin was used as a marker.

Microbial Protein Synthesis

Theurer (1982) summarized data from 12 experiments where RNA was used as a microbial marker and reported a mean of 24 g microbial protein/kg rumen fermented organic matter (RFOM). When lignin was used as a flow marker and RNA as a microbial marker, microbial efficiencies averaged 19.2 g/kg RFOM. Efficiencies of protein synthesis in our study averaged 24 g/kg RFOM and 20 g/kg RFOM when corrected for microbial organic matter, similar to the estimates obtained by Theurer (1982). Cows fed HS tended to have greatest efficiencies and low values obtained for cows fed AS-HS may be misleading, since estimates for a missing block on this treatment were low and reduced the overall average.

Using lignin as a marker, dietary N digested in the rumen was lowest for cows fed HS, and averaged 58.84, 63.34, 55.05, and 67.37% for the AS-HS, AS-SBM, CS-HS, and CS-SBM rations, respectively. Digestion of NAN in the small intestine was 192, 182, 226, and 165 g/day for the respective rations, and was related to total NAN flow but not availability because NAN digested as a % of flow was similar among treatments. These findings suggest that while protein from heated soybean meal was less degradable in the rumen, its availability in the small intestine did not decrease.

Organic Matter and Acid Detergent Fiber Digestion

Organic matter intake flow and digestibility are presented in Table 35. These values are based on lignin. No La values are presented since they would show trends similar to DM flows. There were no differences between treatments in organic matter (OM) flow to various segments of the digestive tract. Bacterial OM was subtracted from apparent total OM flow to the duodenum to obtain a corrected OM flow, which averaged 1.26 kg less than total flow. Estimates of OM digested in the rumen ($ROMD_c$) were 20% greater after correction for bacterial OM. Average $ROMD_c$ was 48.3% of OM intake for all treatments which is low compared to results of others obtained with cows (van't Klooster and Rogers, 1969; Watson et al., 1972; Tamminga, 1973). Tamminga (1975) observed rumen organic matter digestion similar to those in this trial. Dry matter intakes ranged between 14 and 16 kg/day in his trial and it was suggested that low estimates for ROMD may be due to higher feeding levels and an increased rate of digesta flow though the rumen.

Wisconsin workers (Santos, 1980; Stern, 1981; Rode, 1981) have also reported low rumen organic matter digestion of 30% in lactating cows consuming 14 to 17 kg of OM/day. These results were obtained with trials using lignin or La. However, Merchen (1981) fed haylage-based diets to cows and reported rumen organic matter digestion ranging from 59 to 70%.

In our study OM digestion in the small intestine was not different between treatments and ranged from 30.39% for AS-SBM to

TABLE 35.

Organic Matter Intake, Flow and Digestion in Various Segments of the Digestive Tract of Cows in Experiment 3.[ab]

	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
Organic Matter Intake, kg/day	16.47	16.46	17.22	16.03	.48
Organic Matter Flow to:					
Duodenum (uncorrected)[c] kg/day	10.64	9.40	9.68	9.57	.71
Duodenum (corrected)[d] kg/day	9.30	8.13	8.46	8.36	.69
Ileum, kg/day	6.79	6.63	6.23	5.69	.20
Feces, kg/day	5.18	5.29	5.41	4.41	.44
Organic Matter Digestion in:					
Rumen/Abomasum (uncovered)[c] % of intake	35.47	42.72	42.14	41.59	3.70
Rumen/Abomasum (corrected)[d] % of intake	43.53	50.56	49.82	49.30	3.65
Small Intestine, % entering[e]	34.20	30.39	34.79	41.20	3.27
Post Ileum, % entering[e]	24.66	19.80	13.32	20.43	3.89
Total Tract, % of intake	68.66	67.84	67.93	72.90	2.51

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Calculated using lignin.

[c]Includes bacterial OM.

[d]Does not include bacterial OM.

[e]Digestibility of digesta entering.

TABLE 36.

Acid Detergent Fiber (ADF) Intake and Digestion of Cows in Experiment 3.[ab]

	AS-HS	AS-SBM	CS-HS	CS-SBM	SE
ADF intake, kg/day	3.18	3.16	3.26	3.05	.09
ADF Flow to:					
Duodenum, kg/day	1.85	1.57	1.59	1.53	.11
Ileum, kg/day	1.77	1.72	1.74	1.64	.08
Feces, kg/day	1.66	1.63	1.60	1.40	.14
ADF Digestion:					
Rumen/Abomasum, % intake	42.12	46.58	51.09	50.20	3.33
Small Intestine, % entering[c]	4.25	-8.11	-15.16	-7.09	6.74
Post Ileum, % entering[c]	6.14	1.54	5.65	13.32	6.18
Total Tract, % intake	48.03	48.50	50.45	54.50	4.10

[a]AS-ammonia-corn silage, HS-heated soybean meal, SBM-soybean meal, CS-corn silage.

[b]Calculated using lignin.

[c]Digestibility of digesta entering.

41.20% for CS-SBM. These estimates are similar to those reported by Santos (1980) and Stern (1981).

Acid detergent fiber intake and digestibility are presented in Table 36. Digestibility of ADF tended to be lowest for cows fed ammonia treated silage (AS) but differences between treatments were not significant. Negative ADF digestibility would suggest some artifact-ADF formation but are low and could be within analytical error.

Summary

Experiment 1

Data from these experiments showed that HCHO, autoclaving, and dry heat treatment of SBM decreased nitrogen degradibility in the rumen. Compared to untreated SBM the treated exhibited less *in vitro* ammonia release, less N disappearance from rumen incubated nylon bags and less N disappearance during protease incubation. Commercial samples of roasted soybeans and extruded SBM showed protection from rumen degradation. Energy expended to treat SBM would be least for HCHO-SBM. However, HCHO is not approved in the U.S. as a feed additive so was not used in the subsequent studies with lactating cows.

Experiment 2

These data establish that high producing cows early in lactation yielded more milk and consumed more dry matter when protein

in the ration increased from 11.3 to 17.5% of the DM with a lesser increment as the level approached 17.5%. At 14.5 and 17.5% crude protein, heat treatment of soybean meal increased milk production over the untreated meal. The substitution of natural protein with ammonia added to corn silage was compatible with high milk yields at both 14.5 and 17.5% dietary protein. Moreover, greatest milk production was observed for the 17.5% ration containing heated soybean meal and ammonia-treated silage.

Projected 305 day milk yields suggested that farmers should feed for peak milk production even though return over feed costs may be less for productive rations early in lactation. This finding is in contrast to views of Satter et al. (1982) and Van Horn et al. (1982) who suggested feeding for maximum profit early in lactation but disregarded the effect of peak milk production on subsequent lactation curve and milk yields.

Experiment 3

Flow of dry matter to the duodenum appeared to be grossly overestimated when La was the marker, resulting in high estimates for microbial protein efficiencies and a net gain in N at the duodenum. Estimates made with lignin were similar to literature values and were deemed more appropriate for use in this experiment.

No significant differences in digesta flow or digestion were obtained between treatments, silage type, or protein type. This

was due to a missing block and large standard errors. Although differences were not significant, trends between diets were apparent. Dietary N was apparently degraded to a lesser extent in the rumen for diets containing HS with a greater flow of N and NAN to the duodenum. There were no treatment differences or trends for NAN digestion suggesting that NAN in all diets was equally digested and absorbed in the small intestine.

Increased NAN flow would support findings from Experiment 2 where cows fed rations containing HS were more productive than SBM controls.

CONCLUSIONS

Based on the data obtained in these studies, the following conclusions were made:

1) Formaldehyde and heat treatments effectively reduced nitrogen degradibility in the rumen.
2) High producing cows early in lactation were most productive and profitable when fed a ration of 17.5% CP which contained ammoniated corn silage and heated soybean meal.
3) Cows fed HS were more productive than those fed SBM.
4) Cows increased dry matter intake and milk production as ration crude protein increased from 11.3 to 14.5 to 17.5%.
5) The validity of using lanthanum as a digesta flow marker at the duodenum is questionable since rumen dry matter digestibilities were low and often negative.
6) Estimates of digesta flow and digestibility were closer to expected values when lignin was used as a reference marker.
7) Digesta flows to the ileum and feces were similar for estimates made with lanthanum or lignin, suggesting that the latter marker was valid.
8) Lactating cows had greater amounts of NAN flowing to the duodenum and more NAN digested in the small intestine when fed rations containing heated SBM (CS-HS and AS-HS) due to

less nitrogen degradation in the rumen.

9) Cows fed ammoniated corn silage and heated soybean meal (AS-HS) had greater amounts of NAN flow to the duodenum than cows fed ammoniated corn silage and soybean meal (AS-SBM).

10) Tailoring protein needs for dairy cows can result in optimum performance at economic return.

APPENDIX A

TABLE A1.

DRY MATTER AND CRUDE PROTEIN OF FEEDS FED IN EXPERIMENT 1

Year	Date	Hay		Corn		Corn Silage		Corn Silage P-AS[a]		Corn Silage A-AS[b]		SBM[c]		HS[d]	
		DM	CP	DM	CP	DM	CP	DM	N	DM	N	DM	N	DM	N
1979	9/11-10/7	79.8	14.9	64.0[e]	10.9	29.8	9.2	31.9	13.9						
	10/7-10/31	86.7	8.9	65.2[e]	10.2	31.1	9.0	31.7	14.5						
	10/31-11/12	85.2	----	66.5[e]	----	28.9	---	32.3	----						
	11/12-11/16	90.1	9.0	66.9[e]	10.3	30.6	9.0	31.8	11.8			87.1	52.0	95.3	57.7
	11/16-11/24	84.5	8.7	65.9[e]	10.1	30.3	8.4	32.1	13.5			88.3	50.9	94.8	51.4
	11/24-12/7	82.3	8.9	66.1[e]	10.1	31.8	9.1	33.8	14.5						
1980	12/7-1/4	87.3	11.4	----	----	30.1	8.8	34.4	13.2						
	1/4-1/14	----	----	66.3[e]	10.4	28.9	8.9	34.2	14.7						
	1/14-1/24	80.8	21.8	65.9[e]	9.6	29.0	8.8	33.9	14.7			85.8	52.4	95.6	53.9
	1/24-2/1	----	19.9	----	10.0	----	8.6	----	13.1			----	52.8	----	47.8
	2/1-2/18	83.6	18.1	66.0[e]	9.5	30.4	8.2	34.5	13.4						
	2/18-2/26	83.9	18.4	56.0[f]	9.3	35.4	8.7	28.6	13.4						
	2/26-3/10	81.6	17.6	55.2[f]	9.2	30.0	8.9	35.1	13.3						
	3/10-3/20	81.6	18.6	56.0[f]	9.6	30.1	9.5	35.5	13.0						
	3/20-3/31	----	9.5	----	----	----	8.3	----	13.2			----	48.2	----	48.8
	3/31-4/9	86.0	13.7	56.1[f]	9.9	32.3	8.1	38.3	12.9	32.0	11.6				
	4/9-4/15	84.8	16.2	53.3[f]	10.0	28.8	8.9	34.8	12.8	27.9	11.6				
	4/15-4/21	91.0	16.7	55.4[f]	9.9	31.0	8.9	37.4	12.7	32.3	12.4				
	4/21-5/7	93.1	14.0	54.6[f]	9.9	30.1	8.9	37.0	13.2	34.7	12.8				
	5/7-5/13	86.4	17.7	52.9[f]	9.7	26.8	8.8	34.2	13.4	33.8	13.4				
	5/13-5/26	91.6	15.6	50.8[f]	9.6	32.9	8.9	36.4	13.0	34.4	13.2				
	5/26-6/8	87.3	19.1	58.1[f]	9.1	31.2	8.5	36.1	13.5	34.0	14.5				
	6/8-6/20	76.9	17.2	60.6[f]	9.0	30.0	8.0	35.9	13.4	34.8	13.1				
	6/20-7/5	88.2	11.8	68.8[f]	10.1	28.8	8.2	37.0	11.8	35.8	----				
	7/5-7/21	88.0	12.5	69.1[f]	10.1	28.8	8.5	35.7	11.3	34.7	10.9				
	7/21-8/4	89.8	10.4	65.8[f]	10.5	28.5	9.4	41.1	11.5	36.5	11.1				
	8/4-8/18	84.8	17.2	89.1[g]	10.9	29.1	8.5			37.0	13.9				

Table A1 (cont'd)

Year	Date	Hay DM	Hay CP	Corn DM	Corn CP	Corn Silage DM	Corn Silage CP	Corn Silage P-AS[a] DM	Corn Silage P-AS[a] N	Corn Silage A-AS[b] DM	Corn Silage A-AS[b] N	SBM[c] DM	SBM[c] N	HS[d] DM	HS[d] N
	8/18-9/18	81.4	19.0	85.9[g]	10.2	32.0	6.9								
	9/18-10/16	83.7	18.1	48.4[f]	8.5	31.6	9.2	29.9	15.9						
	10/16-10/23			67.2[f]	9.3	35.1	9.0	33.1	13.8						
	10/23-11/5	76.8	---	65.0[f]	8.7	31.7	9.0	29.6	14.7						
	11/5-12/10	83.1	14.8	58.8[f]	7.8	29.9	---	34.3	----						
	1/3-1/10	83.6	18.7	53.7[f]	7.5	30.2	9.2	34.4	13.1						
1981	1/16-1/30	85.0	13.7	64.7[f]	8.8	32.5	8.7	36.9	11.5			87.6	47.1	98.8	51.8
	2/15-3/2	77.9	15.1	63.2[f]	9.5	31.4	9.2	34.2	13.1						
	3/2-3/20	85.6	13.2	64.4[f]	9.5	33.5	8.2	33.9	13.8						
	3/21-4/15	82.4	15.8	66.4[f]	9.3	29.2	8.3	35.8	13.6						

[a] P-AS-treated with Prosil.
[b] A-AS-treated with anhydrous ammonia.
[c] SBM-normal soybean meal.
[d] HS-treated soybean meal (2.5 hr, 140°C).
[e] High moisture shelled corn.
[f] High moisture ground ear corn.
[g] Dry shelled corn.

TABLE A2.

SELECTED DATA FOR COWS FED RATIONS CONTAINING VARYING AMOUNTS OF PROTEIN FROM DIFFERENT SOURCES. EXPERIMENT 2

Cow Number	Fresh Date	Services per Conception	Days Open	Pre-trt[a] Milk kg/day	Trt Milk[b] kg/day	Pre-Trt[c] DMI kg/day	Trt[d] DMI kg/day	Date Started
Treatment: 11 CS-SBM								
1511	10/18/79	1	49	33.5	99.7	-	18.6	11/14/79
1508	11/1/79	2	101	31.6	105.2	-	17.4	11/21/79
1365	1/3/80	4	142	35.4	93.4	16.8	18.2	2/4/80
1280	4/5/80	2	83	32.8	105.0	14.7	18.1	4/26/80
1561	5/12/80	2	82	35.8	99.4	13.4	16.1	6/1/80
1377	7/12/80	4	175	29.4	105.4	17.1	18.8	8/3/80
1580	10/10/80	-	-	29.8	99.1	19.6	18.1	11/2/80
1392	11/7/80	-	-	26.6	113.4	18.7	15.8	11/29/80
1628	12/13/80	3	98	27.9	84.5	16.3	11.6	1/4/81
1688	1/20/81	3	128	27.1	93.7	18.6	15.2	2/13/81
1683	1/31/81	-	-	30.0	98.8	18.1	15.8	2/22/81
1676	2/25/81	-	-	29.4	97.1	17.6	13.8	3/19/81
Treatment: 14 AS-HS								
1506	11/2/79	1	54	33.8	109.2	-	18.1	11/23/79
1275	11/26/79	-	-	34.4	109.0	19.4	20.0	12/18/79
1586	1/27/80	-	-	30.7	112.4	16.9	17.3	12/18/80
1619	4/14/80	6	154	33.5	108.7	20.0	22.3	4/26/80
1585	6/10/80	5	112	32.5	122.8	18.7	22.0	2/1/80
1547	9/25/80	-	-	27.8	109.4	21.8	22.9	10/17/80
1568	10/21/80	1	59	32.6	110.7	17.8	21.1	11/12/80
1650	12/3/80	2	75	31.0	111.3	18.4	17.1	12/25/80
1714	1/1/81	-	-	27.7	102.2	17.9	16.5	1/23/81
1602	1/25/81	-	-	26.6	111.3	20.4	22.6	2/13/81
1689	2/23/81	1	93	36.9	101.6	19.7	16.9	3/17/81
1486	3/1/81	1	90	29.7	104.4	16.4	18.9	3/23/81

Table A2 (cont'd)

Treatment: 14 CS-HS

Cow Number	Fresh Date	Services per Conception	Days Open	Pre-tmt[a] Milk kg/day	Trt[b] Milk kg/day	Pre-trt[c] DMI kg/day	Trt[d] DMI kg/day	Date Started
1232	9/30/79	2	67	31.9	108.8	--	21.0	
1392	11/11/79	1	60	30.6	118.6	--	21.1	
1408	12/26/79	1	90	31.7	112.3	16.2	19.5	
1631	3/26/80	2	65	31.8	110.1	17.0	17.4	
1571	4/9/80	1	53	33.1	104.5	19.3	20.3	
1487	7/3/80	5	141	28.6	119.2	12.5	16.6	
1680	8/26/80	-	---	28.3	102.1	14.9	16.9	
1684	11/8/80	3	175	28.3	104.6	18.5	15.3	
1359	12/13/80	-	---	30.1	129.9	18.3	19.9	
1627	1/21/81	2	60	38.7	110.1	18.6	17.0	
1583	2/29/81	1	90	37.5	104.8	18.4	17.7	
1712	3/17/81	2	91	31.8	99.7	19.9	19.4	
Treatment: 14 CS-SBM								
1579	10/7/79	1	46	30.2	107.9	17.9	20.6	
1580	11/6/79	1	57	32.1	110.3	--	22.9	
1522	12/8/79	-	---	28.2	112.1	17.4	16.9	
1465	3/21/80	7	192	37.1	97.8	18.8	20.0	
1490	5/16/80	5	151	37.4	104.3	15.0	16.1	
1494	8/4/80	3	135	26.7	110.8	12.3	20.6	
1493	8/11/80	1	55	29.6	106.8	15.4	19.2	
1587	9/5/80	4	90	31.6	94.0	20.0	22.7	
1519	11/29/80	2	70	32.6	110.1	17.2	18.4	
1675	1/25/81	-	---	33.9	104.4	17.2	20.3	
1702	2/14/81	-	---	28.6	109.8	17.5	17.0	
1469	3/7/81	-	---	36.6	108.5	18.0	18.2	
Treatment: 17 AS-HS								
1372	10/28/79	-	---	34.2	117.5	--	28.9	
1456	11/4/79	-	---	29.0	116.5	--	17.6	
1469	12/29/79	3	93	34.7	112.7	19.0	19.8	
1633	3/18/80	3	184	29.4	110.9	16.7	21.5	
1374	4/12/80	-	---	35.5	114.4	18.7	25.6	
1328	8/1/80	-	---	30.1	110.0	10.0	19.3	
1440	10/16/80	-	---	32.1	114.6	15.8	18.0	
1508	11/24/80	1	46	35.0	127.7	17.1	16.3	
1674	12/22/80	2	127	28.5	125.6	18.2	19.6	
1408	1/1/81	-	---	34.1	115.7	18.4	23.0	
1693	1/30/81	1	42	31.2	117.9	14.7	19.3	
1571	3/15/81	1	53	31.4	109.6	18.3	19.8	

Table A2. (cont'd)

Treatment: 17 CS-HS

Cow Number	Fresh Date	Services per Conception	Days Open	Pre-trt[a] Milk kg/day	Trt[b] Milk kg/day	Pre-trt[c] DMI kg/day	Trt[d] DMI kg/day	Date Started
1587	9/29/79	1	52	26.9	107.1	--	24.2	10/26/79
1628	11/18/79	3	106	29.9	110.0	--	17.3	
1386	11/28/79	4	120	32.7	122.3	17.8	28.8	
1486	3/23/80	1	68	31.8	107.2	14.7	22.3	
1617	4/1/80	-	-	33.1	103.0	16.7	18.7	
1605	7/9/80	2	106	34.7	110.4	17.7	20.8	
1663	12/8/80	2	72	28.4	110.2	19.7	18.8	
1662	12/24/80	-	-	31.4	118.5	18.4	18.6	
1669	12/27/80	2	71	33.7	116.0	19.1	20.5	
1544	1/28/81	2	64	32.5	111.1	19.8	15.8	
1631	2/18/81	-	-	35.2	107.7	17.9	17.3	
1530	3/17/81	3	138	27.4	135.4	16.6	19.4	
Treatment: 17 CS-SBM								
1547	9/30/79	1	52	30.7	106.5	--	26.0	
1518	11/3/79	-	-	32.5	121.2	--	21.5	
1376	11/26/79	2	127	32.7	111.0	21.4	28.0	
1540	3/30/80	-	-	36.5	103.3	17.6	19.9	
1579	8/27/80	-	-	29.7	105.4	16.5	20.6	
1232	9/7/80	2	103	28.1	101.8	19.6	25.6	
1708	11/24/80	-	-	30.8	107.8	20.3	22.0	
1499	11/25/80	-	-	33.6	113.7	16.8	19.5	
1618	12/28/80	1	81	27.2	109.9	21.0	19.7	
1679	1/29/81	2	53	30.4	120.1	19.4	20.2	
1706	2/14/81	1	66	29.5	106.8	16.9	15.9	
1561	5/10/81	4	107	39.3	111.7	17.2	18.2	

[a] Pretreatment milk, average of weeks 2 and 3 postpartum.
[b] Treatment milk, average of weeks 4 through 13 postpartum.
[c] Pretreatment dry matter intake, average of weeks 2 and 3 postpartum.
[d] Treatment dry matter intake, average of weeks 4 through 13 postpartum.

TABLE A3.

BACTERIAL COMPOSITION FROM EXPERIMENT 3

Cow	Period	Treatment	Nitrogen, %	Ash, %	RNA, mg/g
2470	1	CS-SBM	8.48	25.61	114.59
2578	1	AS-HS	8.37	26.30	109.89
2488	1	CS-HS	8.09	27.71	123.73
2497[a]	1	AS-SBM			
2470	2	AS-HS	8.80	24.01	101.51
2578	2	CS-SBM	5.24	50.89	179.95
2488	2	AS-SBM	8.74	23.34	125.96
2497	2	CS-HS	7.93	28.83	148.17
2470	3	AS-SBM	8.27	25.38	135.01
2578	3	CS-HS	8.15	26.11	122.16
2488	3	CS-SBM	8.84	24.78	123.09
2497	3	AS-HS	8.26	25.52	145.70
2470	4	CS-HS	8.17	29.35	116.98
2578	4	AS-SBM	8.12	26.39	133.95
2488	4	AS-HS	8.09	28.56	135.60
2497	4	CS-SBM	7.95	33.28	113.20

[a] Missing Value.

TABLE A4.

ILEAL COMPOSITION FROM EXPERIMENT 3

Cow	Period	Treatment	DM, %	N, % Wet Basis	ADF, % DMB	NH_3-N, mg/dl
2470	1	CS-SBM	10.12	0.2159	28.41	12.62
2578	1	AS-HS	11.66	0.2483	22.33	17.59
2488	1	CS-HS	11.44	0.2497	22.51	12.63
2497[a]	1	AS-SBM				
2470	2	AS-HS	11.52	0.2396	24.98	13.11
2578	2	CS-SBM	9.46	0.2407	22.76	12.79
2488	2	AS-SBM	11.84	0.2753	24.73	13.23
2497	2	CS-HS	11.58	0.2379	22.93	16.16
2470	3	AS-SBM	13.18	0.2731	22.40	15.25
2578	3	CS-HS	9.43	0.2595	25.10	14.50
2488	3	CS-SBM	11.59	0.2432	24.50	14.73
2497	3	AS-HS	11.56	0.2764	19.20	17.28
2470	4	CS-HS	11.83	0.2473	23.60	13.00
2578	4	AS-SBM	12.46	0.2615	22.80	17.19
2488	4	AS-HS	10.73	0.2455	25.00	15.57
2497	4	CS-SBM	10.85	0.2303	22.30	14.50

[a] Missing Value.

TABLE A5.

DUODENAL COMPOSITION FROM EXPERIMENT 3

Cow	Period	Treatment	DM, %	N, % Wet Basis	ADF, % DMB	NH_3-N, mg/dl	RNA, mg/g DM
2470	1	CS-SBM	6.66	0.1979	18.38	3.62	21.85
2578	1	AS-HS	7.12	0.2323	17.35	5.72	19.96
2488	1	CS-HS	6.96	0.2516	12.83	5.92	19.98
2497[a]	1	AS-SBM					
2470	2	AS-HS	8.90	0.2813	16.49	6.40	18.53
2578	2	CS-SBM	8.15	0.2567	13.69	10.34	27.10
2488	2	AS-SBM	9.01	0.2450	14.69	9.72	28.14
2497	2	CS-HS	6.79	0.2500	14.17	5.24	26.47
2470	3	AS-SBM	6.22	0.2046	12.87	8.54	23.09
2578	3	CS-HS	6.69	0.2326	13.82	8.70	25.50
2488	3	CS-SBM	7.04	0.2134	14.02	9.86	21.32
2497	3	AS-HS	7.25	0.2550	14.84	8.62	21.42
2470	4	CS-HS	8.36	0.2767	14.41	5.80	23.88
2578	4	AS-SBM	7.44	0.2149	14.79	10.54	20.78
2488	4	AS-HS	12.40	0.2904	12.84	9.30	13.36
2497	4	CS-SBM	7.98	0.2194	11.89	16.54	21.85

[a] Missing value.

TABLE A6.

FECAL COMPOSITION FROM EXPERIMENT 3

Cow	Period	Treatment	DM, %	N, % wet basis	ADF % DMB
2470	1	CS-SBM	19.27	0.4290	31.81
2578	1	AS-HS	19.01	0.4107	31.18
2488	1	CS-HS	19.80	0.4733	28.99
2497[a]	1	AS-SBM			
2470	2	AS-HS	19.70	0.4931	29.55
2578	2	CS-SBM	19.60	0.4298	28.08
2488	2	AS-SBM	21.00	0.4779	29.59
2497	2	CS-HS	19.37	0.5128	26.11
2470	3	AS-SBM	21.51	0.5079	25.60
2578	3	CS-HS	18.21	0.4393	29.54
2488	3	CS-SBM	19.31	0.4806	28.28
2497	3	AS-HS	20.13	0.5217	25.25
2470	4	CS-HS	20.39	0.4787	26.53
2578	4	AS-SBM	20.36	0.4780	29.00
2488	4	AS-HS	20.20	0.4679	28.14
2497	4	CS-SBM	20.58	0.4890	26.91

[a] Missing value.

TABLE A7.

LIGNIN (% DMB) CONTENT OF FEEDS, DIGESTA, AND FECES FROM EXPERIMENT 3

			Lignin, % DMB						
Cow	Period	Treatment	Silage[a]	Grain[b]	Hay[c]	Complete Ration	Duodenal Digesta[d]	Ileal Digesta[e]	Feces[f]
2470	1	CS-SBM	1.69	0.94	7.63	1.84	3.62	6.59	7.44
2578	1	AS-HS	3.17	1.09	7.63	2.60	3.99	5.43	6.74
2488	1	CS-HS	1.69	0.94	7.63	1.85	2.97	4.95	6.07
2497[g]	1	AS-SBM							
2470	2	AS-HS	3.12	0.95	6.74	2.37	3.56	5.38	6.19
2578	2	CS-SBM	2.44	0.74	6.74	2.03	2.78	4.41	6.26
2488	2	AS-SBM	3.12	0.98	6.74	2.41	3.48	5.37	6.73
2497	2	CS-HS	2.44	0.84	6.74	2.06	3.73	4.95	6.05
2470	3	AS-SBM	2.85	0.52	7.29	2.09	3.02	5.01	6.49
2578	3	CS-HS	2.25	0.65	7.29	2.08	3.73	5.39	7.24
2488	3	CS-SBM	2.25	0.41	7.29	1.82	3.14	5.27	7.42
2497	3	AS-HS	2.85	0.65	7.29	2.00	3.75	5.06	7.15
2470	4	CS-HS	2.46	0.73	6.82	1.99	2.44	4.32	4.76
2578	4	AS-SBM	3.08	0.59	6.82	2.16	3.21	4.71	6.41
2488	4	AS-HS	3.08	0.73	6.82	2.23	2.47	5.18	8.65
2497	4	CS-SBM	2.46	0.55	6.82	1.89	2.82	4.51	6.00

[a] Average coefficient of variation was 16.00.
[b] Average coefficient of variation was 0.90.
[c] Average coefficient of variation was 7.40.
[d] Average coefficient of variation was 6.06.
[e] Average coefficient of variation was 3.20.
[f] Average coefficient of variation was 3.20.
[g] Missing value.

TABLE A8.

CHROMIUM AND LANTHANUM CONCENTRATIONS IN FEED, DIGESTA, AND FECES FROM EXPERIMENT 3

			GRAIN			DUODENAL				ILEAL				FECES			
Cow	Period	Treatment	CRppm	LAppm	CR/LA	CRppm	LAppm	CR/LA	% of Grain	CRppm	LAppm	CR/LA	% of Grain	CRppm	LAppm	CR/LA	% of Grain
2470	1	CS-SBM	193.1	95.5	2.02	96.2	57.5	1.67	82.8	215.8	112.2	1.92	95.2	244.7	118.3	2.07	102.4
2578	1	AS-HS	204.3	104.8	1.95	69.6	48.2	1.44	74.1	172.2	93.6	1.84	94.4	199.1	111.8	1.78	91.3
2488	1	CS-HS	169.2	82.6	2.05	101.9	54.8	1.86	90.7	234.3	117.5	1.99	97.3	255.0	131.8	1.93	94.4
2497[a]	1	AS-SBM															
2470	2	AS-HS	163.9	76.7	2.14	71.1	40.4	1.76	82.2	198.4	99.9	1.99	93.0	267.5	129.2	2.07	96.7
2578	2	CS-SBM	230.7	97.1	2.38	126.5	66.5	1.90	79.9	308.4	138.9	2.22	93.3	296.7	147.5	2.01	84.5
2488	2	AS-SBM	186.2	75.0	2.48	79.5	36.9	2.15	86.9	221.7	95.0	2.33	94.1	258.5	113.3	2.28	92.0
2497	2	CS-HS	176.1	74.8	2.35	93.9	48.1	1.95	83.1	212.6	97.5	2.18	92.8	226.4	109.8	2.06	87.7
2470	3	AS-SBM	167.0	81.1	2.06	128.4	57.8	2.22	107.8	206.5	108.2	1.91	92.7	278.7	133.5	2.09	101.5
2578	3	CS-HS	204.8	101.9	2.01	112.4	65.6	1.71	85.2	300.5	138.6	2.17	107.9	292.3	142.6	2.05	102.0
2488	3	CS-SBM	174.8	79.8	2.19	95.3	46.8	2.04	93.0	242.9	103.7	2.34	107.0	313.6	133.1	2.36	107.6
2497	3	AS-HS	196.4	90.3	2.17	111.9	59.4	1.88	86.8	212.6	130.9	1.62	74.8	392.6	236.7	1.66	76.5
2470	4	CS-HS	183.1	80.5	2.27	108.0	49.5	2.18	96.1	210.4	92.5	2.27	100.0	241.7	112.0	2.16	95.1
2578	4	AS-SBM	216.4	99.7	2.17	125.9	57.9	2.17	100.0	242.8	119.8	2.03	93.4	283.1	146.8	1.92	88.5
2488	4	AS-HS	176.6	66.1	2.67	64.5	30.9	2.09	78.3	230.7	93.4	2.47	92.5	277.1	110.3	2.51	94.0
2497	4	CS-SBM	189.3	64.1	2.95	90.0	35.1	2.56	86.8	182.5	77.2	2.36	80.0	236.4	95.9	2.47	83.7

[a] Missing value.

APPENDIX B

PROCEDURE

Preparation Of CR-EDTA and Lanthanum

CR-EDTA

1. Requires about 1.5 g CR intake/cow/day. Calculate requirement.
2. 284 g $CRCl_3 \cdot 6H_2O$ dissolved in about 2 L dist. H_2O (volume is not critical; CR may not completely dissolve.) to give about 55 g CR.
3. Add 400 g Na_2EDTA dissolved in about 2-4 L dist. H_2O to step 2. ("Free acid" EDTA is less expensive: use 346 g).
4. Boil 2 to 3 hrs. Solution turns green to purple.
5. Check pH and precipitate in solution. pH should be >3-4. Solution should have no precipitate if pH is correct.
6. Add 80 ml 1 M $CaCl_2$.
7. pH can be adjusted with NaOH pellets. Add slowly! This addition causes violent boiling for a few seconds.
8. Precipitate will form if pH is too high. Use HCl to lower.

Lanthanum

1. Requires about 0.5 g La intake/cow/day. Calculate requirement. Use Lanthanum Oxide (89.67 % La).
2. Dissolve 27 g LaO (gives about 24 g La) in 96 ml conc. HCl. Boils violently.
3. Dilute with dist. H_2O to 624 ml H_2O.

PROCEDURE

Processing Rumen Fluid For Bacterial Isolate

1. A total of 800 to 1000 ml rumen fluid is needed to obtain 3 to 6 g of dry bacteria.
2. Collect rumen fluid on last two days of collection period at 0, 2, 4, and 6 hr after morning feeding; strain through cheesecloth.
3. Save 50 ml from each sample, acidify and freeze for analysis of VFA and ammonia.
4. Take remaining sample and add 1 ml 37% HCHO (∿0.9% NaCl) to every 4 ml of strained rumen fluid.
5. Pool samples at end of trial and make 1 composite per animal.
6. Centrifuge at 2,500 - 3,000 rpm (500 x g) for 5 minutes and discard pellet. Repeat.
7. Centrifuge at 20,000 x g for 20 minutes and discard supernatant.
8. Wash pellet with ∿15-20 ml of 0.9% NaCl.
9. Centrifuge at 20,000 x g for 20 minutes.
10. Resuspend pellet in distilled water.
11. Freeze until sample can be lyophilized.

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