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Microbial protein flow to the small intestine of cows fed different protein supplements

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MICROBIAL PROTEIN FLOW TO THE SMALL INTESTINE OF COWS FED DIFFERENT PROTEIN SUPPLEMENTS

by

Mohamad Shabir Sadik

A Thesis Submitted to the Faculty of the

COMMITTEE ON NUTRITIONAL SCIENCES (GRADUATE)

In Partial Fulfillment of the Requirements
For the Degree of

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In the Graduate College

THE UNIVERSITY OF ARIZONA

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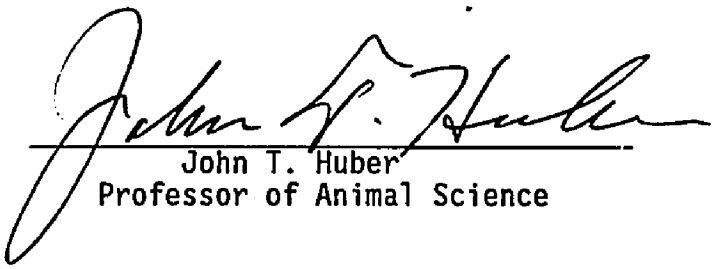

John T. Huber
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9/8/87
Date

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DEDICATION

This work is dedicated to my parents.

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ABSTRACT

Three duodenally cannulated lactating Holstein cows fed cottonseed meal (CSM), corn gluten meal (CGM) or blood meal (BM) as protein supplement were used in a 3 x 3 Latin Square experiment to determine microbial crude protein (MCP) in duodenal digesta. Diets, formulated to contain 15% crude protein (CP) on a dry matter basis, consisted of 60% concentrate, 31% corn silage and 9% alfalfa hay. Chromium oxide was employed as flow marker. Microbial protein fraction of digesta CP (MCP/DCP) was estimated by three microbial markers: ^{15}N, diaminopimelic acid (DAP) and ribonucleic acid (RNA). The isotopic method gave the most reliable results. Variability was higher with DAP and RNA. Results from RNA were lower ($P<.01$) and unreasonable. Based on ^{15}N, MCP/DCP differed among treatments ($P<.10$) with means of 61.5, 59.4 and 50.0% for CSM, CGM and BM, respectively, but differences were not significant for absolute amounts of total CP and MCP in duodenal digesta.

INTRODUCTION

The supply of protein to the small intestine of the ruminant is made up of dietary protein that escaped degradation in the rumen, microbial protein synthesized in the rumen, and endogenous protein. Of these, the microbial fraction usually makes the greatest contribution. However, the amount of undegraded feed protein bypassing the rumen may exceed the microbial contribution when protected supplements or protein sources resistant to rumen degradation are fed.

Relative to the requirements of the animal, the adequacy of protein nutrition is determined by the amount and balance of amino acids available for absorption from the digestive tract. Thus, the microbial protein supply to the small intestine has a major impact on the nitrogen nutrition of ruminants since it provides a sizeable source of protein of relatively high biological value. Moreover, this protein can be constituted from the nitrogen of nonprotein sources, such as urea, and from protein of relatively low nutritional quality. This capacity of the rumen microflora is probably not fully exploited even though much advantage is taken of it by incorporation of nonprotein nitrogen in modern feeding programs for ruminant livestock.

In today's dairy and beef industries, higher levels of production and increased growth rates, made possible by selection and breeding, increase the nutritional demands and place animals under severe nutrient stress. As efforts are intensified towards improved nitrogen nutrition, dietary protein sources resistant to ruminal degradation are assuming increasingly greater importance. Benefits from these supplements depend

on an increased rumen bypass of intestinally digestible feed protein without sacrificing the microbial supply. Thus, evaluation of the adequacy of protein nutrition for ruminant livestock requires accurate prediction of ruminal microbial protein synthesis and of dietary protein escaping degradation in the rumen. Further, true assessment of microbial protein is especially important when dietary nitrogen is not readily degradable since nitrogen availability in the rumen may limit maximal microbial synthesis.

One of the major problems in estimating the microbial nitrogen content of duodenal digesta is the choice of a convenient method that can be relied upon for reasonable accuracy. There are specific limitations associated with the different microbial markers used for this purpose. In many instances, results have been unrealistic. Some comparative studies indicated that inconsistencies between different estimation methods lead to markedly different conclusions. At present, no one method gives definitive results and, thus, it is advisable to use more than one method as a check on reliability of estimates.

This study was conducted primarily to determine the microbial protein supply to the small intestine in lactating dairy cows fed different protein sources of variable resistance to rumen degradation. Three microbial markers were used to obtain estimates of the proportion of microbial nitrogen in total non-ammonia nitrogen of duodenal digesta with the intent of adopting the most reliable and/or reasonable estimates for making diet inferences.

LITERATURE REVIEW

Rumen Nitrogen Metabolism and The Impact of Dietary Crude Protein

Brief Overview

The importance of rumen organisms in the digestion and utilization of dietary components ingested by the ruminant is well documented. In particular, fiber digestion and synthesis of microbial cells are pivotal to the special attributes of the multi-compartmented stomach of these versatile animals. Thus, ruminant livestock are endowed with the distinct ability to convert dietary raw materials, which have little nutritional value to their monogastric counterparts, into such useful products as meat, milk and wool. This unique capacity is dependent upon a symbiotic relationship between the host animal and a mixed population of teeming microorganisms in the rumen.

The nitrogen nutrition and metabolism of the ruminant represents an intricate network of complex pathways, and various aspects of this subject have been extensively reviewed over the years (Hutton, 1972; Armstrong, 1973; Broster, 1973; Chalupa, 1973; Miller, 1973; Nolan, et al., 1973; Thomas, 1973; Chalupa, 1975; Hogan, 1975; Satter and Roffler, 1975; Kennedy and Milligan, 1980; Huber and Kung, Jr., 1981; Owens and Bergen, 1983; Satter, 1986). Proteins in the rumen are subjected to varying degrees of degradation depending on a multitude of factors such as source, intake of protein, feed and energy, and other physical, chemical and physiological factors influencing microbial growth as well as rumen function. Degradation products resulting from rumen fermentation

include peptides, amino acids, volatile fatty acids (VFA) and ammonia. Of these, ammonia is of prime importance as an intermediate source of nitrogen for microbial cell synthesis.

In quantitative terms, ammonia-nitrogen, as an intermediate in bacterial synthesis, usually contributes the greater part of the nitrogen incorporated into ruminal microbial crude protein (Pilgrim et al., 1970; Al-Rabbat et al., 1971a, 1971b; Nolan and Leng, 1972). However, individual bacterial species vary in their dependence on ammonia. Some, especially cellulolytic types, have the ability to subsist entirely on ammonia, which may be an essential requirement, while others seem to have an absolute requirement for, or are stimulated by, organic nitrogen (Bryant and Robinson, 1961, 1962 and 1963). Hence, for the mixed rumen population, supplies of both organic nitrogen and ammonia are required for maximal protein synthesis. Beneficial effects of organic nitrogen on growth and function of mixed microbial populations have been demonstrated both in vivo (Hume, 1970) and in vitro (Maeng et al., 1976; Maeng and Baldwin, 1976a, 1976b).

Feeding Value of Nonprotein Nitrogen

The capacity of rumen bacteria to utilize ammonia to meet most of their nitrogen needs for protein synthesis establishes the basis for the incorporation of non-protein nitrogen supplements in rations for ruminant livestock, particularly the high-producing dairy cow. The economics of this practice makes it very attractive since it provides cheaper dietary nitrogen which can be transformed into bacterial protein of high nutritional value to the animal. However, the efficacy of this

strategy varies with different situations. Some pertinent factors and specific considerations regarding the use of non-protein nitrogen supplements have been examined and summarized in reviews (Chalupa, 1973; Huber and Kung, Jr., 1981). It is recognized that the effectiveness of added dietary non-protein nitrogen is especially dependent on an adequate supply of available energy and is also influenced by the level and nature of crude protein present in the diet.

Loosli et al. (1949) observed that lambs fed a purified diet containing 4% urea as the nitrogen source had an average daily gain of .23 lb as compared with .30 lb for lambs fed a ration containing casein. In an extended study spanning several years, Virtanen (1966) found that urea and ammonium salts as sole source of dietary nitrogen for cows supported maintenance and annual milk production ranging from 1932 to 4325 kg. These findings demonstrate that ruminants can subsist on the protein synthesized in the rumen on protein-free diets. Nevertheless, the adequacy of non-protein supplements as the sole source of dietary nitrogen for improved growth and production performance of ruminants is limited by the extent of microbial synthesis. Thus, non-protein nitrogen alone may be inadequate to meet the increased needs of the nutrient-stressed animal such as the modern day high-producing dairy cow.

Efficacy of Decreased Rumen Degradation of Dietary Protein

Research efforts have been directed towards increasing the total quantity of crude protein flowing to the small intestine. A feasible strategy for reaching this goal lies in increasing the quantity of feed protein and amino acids escaping degradation in the rumen, and can be

accomplished by increasing dietary crude protein intake (Barney et al., 1981; Zinn et al., 1981; Forster et al., 1983), pretreatment of supplements with heat (Schingoethe and Ahrar, 1979; Grummer and Clark, 1982) or chemicals (Crooker et al., 1983; Vicini et al., 1983), or by feeding protein sources naturally resistant to microbial degradation (Zinn et al., 1981; Erdman and Vandersall, 1983; Amos, 1986). The derived benefits from increased rumen bypass of ingested protein would depend on its digestibility in the small intestine, its nutritional value (i.e., amino acid composition) and the extent that available nitrogen in the rumen can meet microbial needs. The nitrogen stress relative to the production potential is a crucial determinant of the magnitude of positive response to improved nitrogen nutrition.

Variation of Ammonia Incorporation Into Bacterial Protein

Owing to the central role of ruminal ammonia, it is logical that the amount and degradability of dietary crude protein would have an impact on the relative contribution of ammonia-nitrogen to bacterial protein synthesis. Pilgrim et al. (1970) investigated the relative contribution of ammonia-nitrogen to microbial synthesis in a sheep fed either a low-nitrogen diet consisting largely of wheaten hay, or a lucerne hay diet of higher nitrogen content. They estimated that ammonia contribution to bacterial nitrogen was nearly 80% for the low nitrogen diet but was less than 65% for the high nitrogen diet. Siddons et al. (1985) also observed that the proportion of microbial nitrogen derived from the rumen ammonia pool tended to be lower in sheep fed high-nitrogen grass silage compared to a low-nitrogen dried grass diet. The nitrogen

degradability of the silage was estimated to be substantially higher than that of dried grass. These variations in proportion of bacterial nitrogen derived from ammonia are probably related to its supply relative to organic nitrogen in the rumen. A higher intake and/or increased degradability of dietary protein may increase the preformed amino acids directly incorporated into bacterial protein in preference to synthesis from carbon skeletons. Conversely, availability of preformed amino acids may be restricted at low protein intake, or when resistant protein is ingested, resulting in an increased dependence on ammonia for protein synthesis.

Variation in Recycling of Nitrogen to the Rumen

Depending on the magnitudes of ruminal influx and efflux, there may be either a net gain or loss of nitrogen, relative to dietary intake, in the upper digestive tract. For sheep consuming 19.5 and 11.0 g N daily, Siddons et al. (1985) estimated a net loss of 4.0 and a net gain of 5.5 g N/d, respectively, between the mouth and abomasum. This exemplifies the general pattern for high and low nitrogen intakes. The potential supply of nitrogen to the rumen through recycling is dependent on several factors. Hume et al. (1970) reported that at least 4 g of recycled nitrogen was utilized daily by microorganisms in sheep provided with 2 g of dietary nitrogen in the rumen while 16 g of casein-nitrogen was infused in the abomasum. This suggests that the amount of recycled nitrogen can be quite substantial when the available supply in the rumen is very low while there is a much higher postruminal supply. Such a situation may prevail when feeding protein that is very resistant to

rumen degradation but quite degradable in the small intestine. Accordingly, the lower rumen ammonia concentration resulting from slow degradation of dietary protein (Hume, 1970; Santos et al., 1984; Shqueir et al., 1984; Firkins et al., 1986) is a factor promoting greater recycling of nitrogen to the rumen.

If recycled nitrogen can compensate for any insufficiency of rumen nitrogen to meet microbial requirements, then diets containing slowly degraded protein provide obvious advantages by minimizing wasteful degradation of feed protein to ammonia. Moreover, it is feasible to combine a more readily degraded nitrogen source with the resistant protein to ensure that microbial needs are met.

Dietary Supply of Organic Nitrogen for Rumen Bacteria

Feeding of non-protein nitrogen in conjunction with resistant protein may provide adequate ammonia in the rumen but this combination should also be examined from the standpoint of meeting organic nitrogen requirement of rumen microorganisms. In vitro effects of amino acids on microbial growth (Maeng et al., 1976; Maeng and Baldwin, 1976a, 1976b) indicated marked increases in specific growth rates and efficiency of synthesis. Further, there were increased substrate utilization and production of volatile fatty acids (Maeng and Baldwin, 1976a). A 1:3 ratio of amino acid-nitrogen to urea-nitrogen was reported by Maeng et al. (1976) as being optimal for microbial synthesis. Extrapolating these findings to the in vivo situation, it becomes apparent that the amount and rate of release of amino acids relative to ruminal microbial needs could have significant effects, not only on bacterial cell yields,

but also on the digestion and utilization of dietary substrates and, consequently, on energy metabolism.

The availability of specific amino acids may also influence microbial synthesis. Salter et al. (1979) studied the relative extent to which different ruminal bacterial amino acids were synthesized or incorporated from the dietary pool in steers fed protein or urea-nitrogen. It was found that when an adequate supply of dietary preformed amino acids was available, proline, arginine, histidine, methionine and phenylalanine were derived as such to a greater extent than other amino acids. However, on the urea-containing diet, synthesis of proline, arginine and histidine increased, but that of methionine and phenylalanine did not. It appears, therefore, that maximal microbial synthesis in the rumen is dependent on a supply of preformed methionine and phenylalanine. Hume (1970) compared protein synthesis in the rumen of sheep on different sources of dietary protein in combination with urea. Microbial protein production for diets containing gelatin, casein, and zein were 91, 101 and 104 g/d, respectively. The yield from gelatin was comparable with that of a urea-VFA supplement, and was significantly less than on the other two diets. It was suggested that this was probably due to limited supply of specific amino acids, such as methionine, for which gelatin is deficient.

Combining slowly degraded protein and non-protein nitrogen in ruminant diets improved efficiency of conversion of feed and protein to gain (Stock et al., 1981) and resulted in greater nitrogen retention (Hume, 1970; Stock et al., 1981; Nelson et al., 1984). The nitrogen economy of these combinations is amply illustrated by Hume (1970) who

found that at least 7.5 g of recycled nitrogen was utilized in the rumen of sheep consuming a diet containing a zein-urea supplement, while only negligible amounts of nitrogen were recycled on diets containing urea-VFA, gelatin-urea, or casein-urea. The protein equivalent of the recycled nitrogen on the zein diet was sufficient to meet the nitrogen requirement of the sheep. In addition to the superior nitrogen retention, the resistant protein supplement supported microbial synthesis similar to that on the casein diet. These beneficial effects testify to the efficacy of increasing rumen bypass of intestinally degradable feed protein while at the same time providing for the nitrogen needs of the rumen microflora.

Excessively slow degradability of protein in the rumen might limit the availability of organic nitrogen to support maximal microbial synthesis even though availability of ammonia and energy may be favorable. It follows, therefore, that formulating a diet to provide increased rumen bypass of feed protein must be compromised with the needs of rumen microorganisms. For best results, this may require inclusion of two or more protein supplements of different degradabilities in conjunction with cheaper non-protein nitrogen. Stock et al. (1981) obtained superior growth in steer calves fed a corn gluten meal-meat meal-urea mixture compared with meat meal-urea in isonitrogenous diets. Further, these workers compared the same diets in a lamb metabolism trial and did not find any significant differences in daily fecal or urinary nitrogen, but there was a lower, though not statistically different, rumen ammonia concentration with the meat meal-urea diet 4 and 6 hours after feeding. These observations suggest that ruminal effects, at least in part, might account for the different responses between the two nitrogen supplements.

Nelson et al. (1985) found that a blood meal-corn gluten meal mixture added as a supplement to ammoniated corn cobs appeared to increase nitrogen utilization by bacteria but had minimal effects when incorporated into diets of untreated corn cobs. The positive effect on the treated cobs might have been due to an enhanced supply of amino acids since ammoniation decreased the content of most amino acids in the cobs.

Variation in Nitrogen Flow From the Rumen

Rumen degradability of dietary protein impacts duodenal flow of total nitrogen and the relative amounts contributed by the different protein fractions (Cummins et al., 1983; Santos et al., 1984; Firkins et al., 1986; Koeln and Paterson, 1986). Decreasing ruminal degradability of dietary crude protein often resulted in increased quantities of undegraded feed nitrogen, microbial nitrogen and total nitrogen flowing to the duodenum (Cummins et al.. 1983; Firkins et al., 1986). The positive effect on microbial crude protein flow was probably due to increased efficiency of conversion of ruminal nitrogen into bacterial crude protein as a consequence of lower rumen ammonia concentration. Cummins et al. (1983) illustrated that synthesis of microbial nitrogen, as a percent of degraded nitrogen, decreased when dietary nitrogen degradability is increased above the point where microbial synthesis and nitrogen degradation are in balance. It was further suggested that the greatest utilization of recycled nitrogen is likely to coincide with the peak outflow of microbial plus feed nitrogen from the rumen.

Moderation of Protein Effects on Microbial Synthesis by Other Factors

Microbial synthesis is energy-dependent and, therefore, one of the foremost considerations in relation to efficiency of ruminal synthesis is the compatibility of energy and nitrogen availability in the rumen. Depending on specific conditions, either of these essential factors might be limiting for maximal synthesis. Theoretically, the most favorable situation is obtained when energy release and nitrogen availability match each other, given that no other factor is limiting. Such synchronization can be expected to maximize bacterial protein synthesis while minimizing wastage of energy or nitrogen. In vitro data of Satter and Slyter (1974) lend support to this concept. These workers investigated the effect of ammonia concentration on microbial protein production in continuous-culture fermenters charged with ruminal contents obtained from steers fed different diets. Ammonia concentration was varied by infusion of urea into the fermenters. It was found that under nitrogen-limiting conditions, microbial protein yield increased linearly with supplementary urea until ammonia started to accumulate. The point of accumulation was determined by the amount of fermentable energy.

Factors such as feed intake (Robinson et al., 1985; Merchen et al., 1986), forage level (Mathers and Miller, 1981; Rode et al., 1985; Merchen et al., 1986; Wanderly et al., 1987), and particle size (Rode et al., 1985) also affect microbial synthesis. These variables influence rumen turnover, rate of passage and ruminal digestion of organic matter which, in turn, exert effects on bacterial growth rate and maintenance

requirements. Efficiency of bacterial synthesis is a function of intake of digestible organic matter (Zinn et al., 1981; Robinson et al., 1985; Merchen et al., 1986) and ruminal rate of passage (Zinn et al., 1981; Firkins et al., 1986). Therefore, effects of dietary nitrogen source per se on microbial synthesis and flow of protein to the duodenum are subject to alteration by a host of other factors. Hence, variation across different dietary regimes and animals can be expected.

Summary

Feeding of protein supplements that are slowly degraded in the rumen increases the supply of total crude protein flowing to the small intestine of ruminants. Increased nitrogen retention and more efficient utilization of dietary nitrogen are among positive effects reported. Microbial protein synthesis, being dependent on a supply of both organic nitrogen and ammonia, is likely to be affected by the nature of dietary protein supplement. Supply of preformed amino acids may be limiting if ruminal degradation of ingested protein is excessively slow. Ammonia requirements of the rumen microflora can be insured by inclusion of a nonprotein nitrogen source, such as urea, with the resistant protein supplement.

Microbial Markers

Direct estimation of the proportion of microbial nitrogen present in digesta flowing from the rumen to the small intestine requires the use of suitable microbial and digesta flow markers. Natural components of bacterial matter employed as markers for rumen microorganisms include total nucleic acids (Coelho Da Silva et al., 1972), ribonucleic acid

(McAllan and Smith, 1972; Ling and Buttery, 1978; Cockburn and Williams, 1984; Rode et al., 1984), diaminopimelic acid (Weller et al., 1958; Hutton et al., 1971; Dufva et al., 1982; Whitelaw et al., 1984), cytosine (Schelling and Byers, 1984), D-alanine (Garrett and Goodrich, 1984), and lysine and leucine (Rahnema and Theurer, 1986). Labelling with isotopes such as ^{15}N (Pilgrim et al., 1970; Al-Rabbat et al., 1971a; Salter et al., 1983; Kennedy et al., 1984), ^{35}S (Beever et al., 1972; Kennedy et al., 1984; Whitelaw et al., 1984) and ^{32}P (Van Nevel and Demeyer, 1977) has also been used. Aminoethylphosphonic acid (AEP) has been applied as a specific marker for protozoa (Ling and Buttery, 1978; Cockburn and Williams, 1984; Whitelaw et al., 1984).

A number of assumptions are made in connection with the use of the various markers. Ideally, the marker should be present exclusively in rumen bacteria and/or protozoa, and the ratio of marker:nitrogen should be the same for microbial isolates from both the rumen and duodenum without variation over time. This ratio provides the basis for calculating the fraction of microbial nitrogen in the digesta. However, each marker falls short, to varying degrees, of the ideal. Thus, there are inherent errors in the values obtained for the microbial fraction of digesta. Some specific merits or limitations of different markers have been comprehensively reviewed (Smith, 1975; Stern and Hoover, 1979). It is generally accepted that, owing to specific deficiencies and lack of verification, no one marker can be relied upon to give definitive results. At best, they provide a crude estimate of the microbial contribution to digesta crude protein.

Diaminopimelic Acid (DAP)

Diaminopimelic acid is probably the most commonly used bacterial marker for estimating ruminal microbial synthesis. It is usually assumed that this amino acid is present only in the cell walls of some bacteria, but there is evidence that this may not be true because DAP has been detected in a number of feedstuffs (Dufva et al., 1982; Rahnema and Theurer, 1986) and protozoa (Hutton et al., 1971; Ling and Buttery, 1978; Rahnema and Theurer, 1986). Such occurrence of DAP in feed and protozoa could be of bacterial origin, but Rahnema and Theurer (1986) claimed that their observed values for feed DAP-N were greater than could be accounted for by bacterial contamination. However, their relatively high values might have been due, in part, to an artifact, possibly allo-isoleucine. Nevertheless, these findings place the credibility of DAP as a specific bacterial marker in some doubt, and its non-bacterial occurrence needs further clarification.

Another pertinent consideration is the fate of DAP from the diet and in fragments of lysed bacteria in the rumen. Although DAP might be degraded in the hind gut (Mason and White, 1971), it is not established whether such degradation takes place in the rumen. If there is little or no ruminal degradation of extraneous DAP, then the elevated flow of this marker in the digesta would result in an overestimation of bacterial protein. Some impossibly high estimates for the bacterial nitrogen content in digesta have been obtained (Nikolic and Jovanovic, 1973; Mercer et al., 1980; Whitelaw et al., 1984). Thus, it may be necessary to make corrections for DAP in feed. Such has given more reasonable estimates compared with the use of uncorrected DAP values (Rahnema and

Theurer, 1986). A further refinement may entail adjustment for protozoal DAP, but estimating the protozoal fraction in digesta is now a bigger challenge in the light of reports that AEP, which has been used as a specific protozoal marker, is also present in bacteria (Ling and Buttery, 1978; Cockburn and Williams, 1984; Whitelaw et al., 1984) and dietary matter (Ling and Buttery, 1978). Moreover, some artifacts might have been identified as AEP (Rahnema and Theurer, 1986), which raises the question of analytical competence.

The ratio of DAP-N:total-N varies widely between different ruminal bacterial species (Dufva et al., 1982) and for mixed rumen bacteria on different diets (Dufva et al., 1982; Berger and Merchen, 1984). Moreover, there are indications that this ratio differs between bacteria attached to plant fibers and those in the fluid phase (Merry and McAllan, 1983; Klopfenstein and Britton, 1984). Hence, as with other methods, the need for a representative sample to determine the reference bacterial DAP-N:total-N ratio cannot be overemphasized. Additionally, short-term fluctuations in the composition of the rumen microbial population can become important, but Dufva et al. (1982) found that time of sampling in relation to feeding had no effect on DAP-N to total N ratio of bacteria when cows were fed either a high roughage or high concentrate diet. Hutton et al. (1971) also reported constancy of this ratio for bacteria in sheep kept on a constant dietary regime over a three-month period. However, it appears that animal variation exists (Berger and Merchen, 1984) and it is necessary to sample each animal in a given trial.

From the foregoing, it is apparent that DAP as a rumen microbial marker has limitations. Nonetheless, if it is assumed that feed,

protozoal and endogenous content of this amino acid are present in relatively low concentrations and insignificant compared to bacterial content, then DAP continues to have merit as a specific bacterial marker. For most reliable results, it is imperative that bacterial samples be as representative as possible and frequency of sampling be sufficient to insure against errors due to short-term changes in the relative proportions of different bacterial species.

Ribonucleic Acid (RNA)

The use of RNA as a microbial marker relies on the assumption that dietary RNA is degraded in the rumen and that nucleic acid exiting the forestomach is of microbial origin (Smith and McAllan, 1970; McAllan and Smith, 1973). There is some doubt as to the adequacy of this assumption, considering that variable quantities of dietary materials escape ruminal degradation. Ling and Buttery (1978) obtained an estimate greater than unity, based on RNA, for the proportion of microbial nitrogen in digesta of one sheep, and suggested that such elevated values might be due to RNA in undegraded feed material contributing to a higher concentration of the nucleic acid in duodenal digesta. Thus, correcting for non-microbial RNA might improve the estimate, but measurement of RNA in undegraded feed exiting the rumen is not feasible. Furthermore, endogenous RNA might also cause overestimation of microbial protein. However, isotopic labelling of bacterial RNA may prove useful as indicated by Smith et al. (1978) who, on the basis of results with ^{32}P-labelled RNA proposed an adjustment factor of .85 for the total RNA method to correct for overestimations. Such a factor may vary with different diets.

One of the often-quoted advantages of the RNA technique is that it measures the total microbial fraction, as opposed to DAP and AEP which conceptually assess only the bacterial and protozoal contributions, respectively. However, the RNA-N:total-N ratio of bacteria has been reported higher than protozoa (Ling and Buttery, 1978). Therefore, the microbial crude protein of digesta is underestimated when the bacterial ratio is used. The magnitude of this inherent error depends on the relative sizes of the bacterial and protozoal fractions, and on the difference in their RNA-N:total-N ratios.

As with the case of DAP, the RNA method is subject to variability of the marker-N:total N relationship. The RNA-N:total N ratio of mixed rumen bacteria is influenced by diet (Bates et al., 1985) and time relative to feeding (Smith and McAllan, 1974; Bates et al., 1985). Also, differences have been found between bacteria associated with the particles and those in the fluid phase of rumen contents (Merry and McAllan, 1983; Bates et al., 1985). The RNA content, relative to total protein, of particle-associated bacteria is lower (Merry and McAllan, 1983; Bates et al., 1985), and appears to be less subject to change relative to time of feeding (Bates et al., 1985), than bacteria in the liquid phase. Stage of growth of microbes (Bates et al., 1985) and availability of nutrients (Bates and Bergen, 1984) influence the relative content of RNA in bacteria and may account for differences between particle-associated and liquid-associated bacteria, as well as changes related to feeding time. From these considerations, it is probable that the RNA-N:total N ratio used for estimating microbial protein may not be totally accurate. However, representative samples of the entire

rumen microbial population obtained at frequent intervals should enhance the reliability of derived estimates.

^{15}N-Enriched Nitrogen

One advantage of ^{15}N is that it deals directly with nitrogen, and is useful not only as a microbial marker, but also as a tracer for studying nitrogen kinetics of ruminants (Al-Rabbat et al., 1971a; Nolan and Leng, 1972; Siddons et al., 1985). The marker is usually introduced into the microbial environment as an ^{15}N-enriched soluble ammonium salt such as ammonium sulphate (Pilgrim et al., 1970; Al-Rabbat et al., 1971a; Kennedy et al., 1984), ammonium chloride (Blake et al., 1983) or urea (Salter and Smith, 1984), which releases ammonia upon dissociation. The incorporation of labelled ammonia into microbial cells results in isotopic enrichment of duodenal non-ammonia-nitrogen due to its uptake by bacteria. However, endogenous nitrogen also becomes enriched due to absorption from the rumen and lower tract and, as mentioned by Siddons et al. (1985), small amounts of the label may be added by endogenous secretions thereby resulting in a slight overestimation of the microbial nitrogen at the small intestine. Another possible source of error relates to the ^{15}N enrichment of protozoa which is likely lower than that of the bacteria (Pilgrim et al., 1970). Thus, the ^{15}N technique is not perfect but errors due to isotopic enrichment of protozoal and endogenous nitrogen are relatively small.

Simultaneous Use of Different Markers

The fact that none of the current methods for estimating the proportion of microbial crude protein in digesta is free from error suggests a need for comparing different markers on the same samples as a check on the reliability of estimates. Hence, several studies (Nikolic and Jovanovic, 1973; Ling and Buttery, 1978; Smith et al., 1978; Mercer et al., 1980; Siddons et al., 1982; Kennedy et al., 1984; Rode et al., 1984; Rahnema and Theurer, 1986) have been conducted with two or more microbial markers. From such investigations an interesting, but somewhat confusing, array of results has emerged. The DAP method gave results approaching one extreme or the other relative to other methods. For example, values for the proportion of microbial nitrogen in digesta obtained with DAP were higher (Siddons et al., 1982), similar (Mercer et al., 1980; Rode et al., 1984) or lower (Ling and Buttery, 1978) compared to those based on ^{35}S as the marker. In some cases extremely high or impossible results were obtained (Nikolic and Jovanovic, 1973; Whitelaw et al., 1984). Additionally, DAP commonly shows more variation than other markers (Ling and Buttery, 1978; Mercer et al., 1980; McAllan and Smith, 1984; Rode et al., 1984). Compared to RNA, the DAP method has more commonly yielded lower estimates for the microbial nitrogen in digesta (Ling and Buttery, 1978; Smith et al., 1978; McAllan and Smith, 1984), but occasionally higher estimates have been obtained (Nikolic and Jovanovic, 1978; Ling and Buttery, 1978; Cockburn and Williams, 1984).

Different estimates may also be obtained between isotopic labels (Siddons et al., 1982; Kennedy et al., 1984) but it appears that these

markers provide the most reliable results (Ling and Buttery, 1978; Siddons et al., 1982; Whitelaw et al., 1984). Nonetheless, the other cheaper and more convenient methods are apparently of some value for comparative estimates since a number of studies have indicated similar ranking by different markers (Nikolic and Jovanovic, 1973; Ling and Buttery, 1978; McAllan and Smith, 1984; Rode et al., 1984). Agreement between the values obtained with different microbial markers on the same samples depends on a number of factors including the representativeness of bacterial samples, the nature and specific limitations of the markers, diet effects, animal effects, and errors associated with analytical techniques. Hence, some studies found significant differences between markers (Ling and Buttery, 1978; Siddons et al., 1982) but others have reported no differences (Mercer et al., 1980; Rode et al., 1984). In the midst of uncertainty, these are necessary considerations that must be borne in mind when attempts are made to assess the proportion of microbial nitrogen in digesta.

Source of Bacterial Marker-N:Total N Ratios

It is usual for the bacterial RNA-N:total N ratios, used as reference for estimation of microbial crude protein in duodenal digesta, to be derived from rumen isolates. In many cases, however, values reported in the literature have been employed rather than being obtained directly from the animals on the diets investigated. Also, some studies involved application of values from one animal or diet across other animals and diets. Considering the variation in these ratios in different animals or diets as reported by some workers, the reliability of the

estimates of microbial synthesis based on transferred bacterial ratios is questionable.

Within marker methods there may also be variation depending on whether the bacterial sample is isolated from the rumen or from the duodenum. Siddons et al. (1982) compared these sources of reference bacteria for estimates based on amino acid profile, or incorporation of ^{35}S or ^{15}N infused in the rumen of sheep fed different diets. The relative content of either isotope was lower in bacteria isolated from the duodenum compared to those from the rumen. Consequently, mean estimates of the proportion of microbial nitrogen in duodenal digesta nitrogen were higher when based on duodenal bacterial reference. Because of difference in amino acid profiles between bacteria from the two sampling sites, estimates based on the profiling method were higher with the duodenal bacterial reference. However, significant effects of these differences varied according to marker methods and diets. Similar trends in isotopic incorporation were also reported by Kennedy et al. (1984). Further, it appears that the ^{15}N labelling of bacteria is more susceptible to variation than ^{35}S due to site of bacterial sampling. These results suggest some modification of the initial rumen microbial population as it passes from the rumen to the duodenum. An important question arising from these finding is whether a rumen bacterial reference adequately represents the microbial population that reaches the duodenum.

Summary

Bacterial markers currently used for estimating microbial protein synthesis have specific limitations which affect the reliability of

results. Isotopic labelling seems to be the most dependable estimation method for more exacting purposes. For the RNA and DAP methods, a representative sample of the reference bacteria is especially critical, and it is advisable to sample at short intervals to insure against errors resulting from short-term fluctuations in marker content associated with changes in growth phase and species composition of the mixed population. Variations due to animal and diet effects on bacterial marker ratios suggest that, for accurate results, these ratios should be obtained from, and applied only to individual animals on a given diet. Bacteria from the rumen and the duodenum when used as references may yield different estimates of the microbial fraction of nitrogen in digesta.

MATERIALS AND METHODS

Three lactating Holstein cows, fitted with cannulae in the proximal duodenum, and three treatment diets were used in a 3 x 3 Latin Square design at the University of Arizona Agricultural Experiment Station. Cows were identified by numbers viz: 932, 958 and 41. The complete rations were formulated to contain 15% crude protein, on a dry matter basis, and were composed of 60% concentrate, 31% corn silage and 9% alfalfa hay. Based on the protein supplement incorporated into the concentrate mix, the diets were identified as cottonseed meal (CSM), corn gluten meal (CGM), and blood meal (BM). Table 1 gives the ingredient composition of the concentrate mixes while the mean chemical composition of the complete mixed rations is in Table 2. The BM diet had slightly higher crude protein than the others.

Cows were housed in separate stalls and had free access to water. They were fed ad libitum twice daily between 0700-0800 and 1700-1800 h. The experimental period on each ration consisted of adjustment for at least 14 days followed by a 4-day sampling period. However, due to intermittent health problems throughout the trial, two of the cows were taken off the treatment rations at least once and allowed to recuperate on more conventional feed made up predominantly of roughage such as corn silage, alfalfa hay and wheat straw. They were then gradually returned to the experimental diets before beginning the 14-day adjustment. The entire trial spanned 15 weeks during April to July, 1986, but individual cows completed the cross-over sequence of all rations within 8 to 10 weeks.

Table 1. Composition of concentrate mixes.

INGREDIENT	RATION CSM	CGM	BM
	----------%	----------	
Protein supplement	24.8	18.3	14.5
Corn,gr; shelled	69.4	63.9	66.0
Urea	0.5	0.5	0.5
Soybean hulls	---	7.6	7.8
Molasses	---	4.5	4.6
Minerals	5.2	5.3	6.6

Table 2. Chemical composition of complete rations.

INGREDIENT	RATION CSM	CGM	BM
	----------%		
Crude protein	14.9	14.5	16.4
Ether extract	3.5	2.5	2.2
ADF	17.0	17.1	14.9
NDF	25.8	26.4	29.3
Lignin	3.9	3.4	2.2
Cellulose	10.8	10.4	8.8

Beginning at least 7 days prior to, and continuing through the sampling period, a ground bread of chromium oxide (Cr_2O_3) was mixed into the ration at 0.07 lb per 10 lb of complete ration. The bread was prepared by thoroughly mixing 1 part of Cr_2O_3 with 4 parts of commercial whole wheat flour before water was incorporated to form a slushy dough. The dough was then oven-dried at 120-130°C after which it was ground in a Wiley Mill through a 2 mm screen. Also, during the sampling period, approximately 400 and 300 milligrams of ^{15}N were added to the feed. Earlier in the trial the larger amount of ^{15}N was used, but it was later elected to use the smaller quantity as a conservation measure. The isotope was administered by dissolving an appropriate amount of enriched ammonium sulphate in water to give about 200 ml of solution which was sprayed onto two pounds of corn silage previously minced in a Hobbart chopper. The sprayed corn silage was then mixed into the complete diet. Weights of feed offered were recorded and samples of the whole mixed ration were taken at each feeding. Orts were removed, weighed and sampled each morning before feeding. These samples were oven-dried at 55°C and then ground in a Wiley Mill through a 2 mm screen. For Cr_2O_3 analysis, samples were powdered by means of a cyclone grinder. Chemical analyses were performed on samples of morning feed composited for each period.

Cows were milked twice daily and, at each milking, milk weights were recorded and samples of milk were taken. Prior to the commencement of the sampling period, an indwelling urinary catheter was inserted into each cow and urine was collected into a 20 l plastic container. Each container contained 50 ml of concentrated sulphuric acid to prevent loss of ammonia. Total urine excretion was recorded, and samples were

taken daily. Fecal grab samples were taken from the rectum twice daily, over-dried at 55°C and ground in a Wiley Mill through 1 mm screen.

Duodenal digesta was sampled at intervals of 3 to 5 h for 72 h. As far as possible, this collection was done according to a preplanned schedule for the purpose of uniformity. However, due to the unsteady pattern in flow of digesta through the cannula, there were small fluctuations in time of sampling. Samples were stored in a refrigerator (8-10°C) for 4 to 48 hours while awaiting isolation of bacteria, but were processed as soon as facilities and time permitted. Two or three consecutive samples were composited to yield eight individual subsamples per cow for each period. These samples represented a time span of 7 to 12 hours. About two-thirds of each subsample was shaken in a Waring Blender at low speed for twenty seconds as an attempt to dislodge solid-associated bacteria before straining through four layers of cheesecloth. The strained fluid was centrifuged at 4000 x g to separate out feed particles and protozoa. The supernatant was further centrifuged at 27,000 x g to precipitate bacteria. After washing two times with distilled water, the bacterial pellet was retrieved and immediately placed in the oven to dry at 55°C. It was then ground into a fine powder in a hand mortar.

The remainder of the original digesta sample was also dried at 55°C. However, in most cases, this could not be done at the same time that bacteria was separated because of limited oven space. While awaiting drying, these samples were kept in a freezer at -4°C. After drying, samples were powdered in a cyclone grinder. In proportion to number of hours represented, weighted subportions of these digesta samples were

pooled to form a grand composite representing the entire sampling period for each cow on each diet.

Rumen contents were sampled randomly rather than according to a planned schedule. For two of the cows, without rumen fistulae, samples were taken via stomach tubes, but for the third cow samples were obtained through a rumen fistula. Bacterial samples were isolated from rumen contents in the same manner as duodenal sample. Where more than one rumen bacterial samples was isolated for a given period, these were pooled to a single composite representing that period.

For the purpose of studying ^{15}N "fall off" patterns, additional samples of milk, duodenal digesta and feces were taken beyond the regular sampling period. These samples were processed as described, but were not used for any other purpose.

Analytical Methods

Dry matter percent of feed, orts, digesta and feces were determined by drying at 100°C in a vacuum oven. Chemical analyses were performed on samples dried at 55°C and, by using appropriate conversion factors, values were expressed as fractions of the dry matter content. Acid-detergent fiber (ADF), neutral-detergent fiber (NDF), lignin and cellulose were analyzed according to Goering and Van Soest (1970) while ether extract was determined by the A.O.A.C. (1980) procedure. Milk compositional analysis was by the Arizona Dairy Herd Improvement laboratory. A modified Kjeldahl procedure (Lauro, 1931) was used for crude protein determination. This procedure was also the first step in processing samples for ^{15}N and Cr_2O_3 analysis.

For ^{15}N analysis, the acid digestion from the Kjeldahl procedure was steam distilled after which ^{15}N content was determined by mass spectrometry as described by Pessarakli (1981). For Cr_2O_3 analysis, 5 ml of deionized water and .4 to .5 g of periodic acid were mixed with the nitrogen digest and digestion continued for another four hours. Concentration of Cr_2O_3 was subsequently determined by atomic absorption spectrophotometry (Anonymous, 1980).

Ribonucleic acid content was determined by the method of Zinn and Owens (1980). For analysis of DAP, the procedure was as follows: 100 mg sample was hydrolysed by heating overnight in 25 ml of 6N hydrochloric acid. After hydrolysis, the HCl was removed by vacuum evaporation and 10 ml of sodium citrate buffer (pH 2.2) was added to the remaining hydrolysate mixture. This was followed by filtration through a Whatman #5 filter paper. Amino acid separation was accomplished by a modified procedure of Spackman et al. (1958) using a Beckman Model 121 automatic amino acid analyzer. Specifically, a two column gradient buffer system and a Beckman W-1 ion exchange resin were employed. The ninhydrin reaction was used for detection and quantification of amino acids. The column temperature was maintained at 55°C and flow rates were 70 and 35 ml/h for the buffer and ninhydrin, respectively. DAP was eluted on the long column between valine and methionine at a pH of about 3.3. A sample chromatogram is shown in Appendix A.

Calculations

Dry matter disappearance and flow were calculated on the basis of Cr_2O_3 as the digesta marker, assuming a steady state in marker passage existed during the sampling period. The marker concentration of ingested feed was adjusted for Cr_2O_3 of orts.

The microbial crude protein fraction of duodenal digesta crude protein (MCP/DCP) was determined by three different marker methods: namely, ^{15}N, RNA, and DAP. In the ^{15}N method, the value of this parameter was calculated as:

$$\frac{^{15}\text{N-enrichment of digesta}}{^{15}\text{N-enrichment of bacteria}}$$

Owing to low enrichment and the possibility of relatively large errors related to instrument sensitivity and variation, the first two pairs of digesta and bacterial samples within each period for each cow were not used in the calculation.

For the DAP and RNA methods, the respective formulas for calculating MCP/DCP were:

$$\frac{\text{Marker-N:total N of digesta}}{\text{Marker-N:total N of bacteria}}$$

It was assumed that RNA contained 14% nitrogen.

For all methods bacterial samples were matched with the subsamples of duodenal digesta from which they were isolated. Additionally, another set of estimates of MCP/DCP were obtained for the DAP and RNA methods by using a single ruminal bacterial value to compare with all the duodenal digesta samples taken within each period from each cow. The bacterial samples for this set of calculations were isolated form the rumen content of the cow. On the basis of the source of bacteria used, the methods

employed for estimating MCP/DCP are classified as duodenal bacteria methods and ruminal bacteria methods. Due to limited availability of rumen bacteria this source could not be applied to the ^{15}N technique.

The individual estimates of MCP/DCP within a period for each cow on each diet were used to calculate one mean value. A weighted mean was obtained by weighing each value within a given period according to the number of hours in the interval represented by the respective individual digesta subsamples. This weighted mean was used in the statistical analyses to assess treatment or animal effects of MCP/DCP. For the purpose of comparing variability between methods, unweighted means and associated coefficients of variation were used.

Organic matter content of bacterial dry matter was assumed to be 83% based on an ash content of 16.85% (Orskov, 1982). This facilitated calculation of true digestibility of organic matter in the rumen, and efficiency of microbial synthesis in terms of fermented organic matter.

Statistical Methods

Partitioning of variability was done by analysis of variance for a 3 x 3 Latin Square according to Cochran and Cox (1950). For testing effects of the marker methods on estimates of MCP/DCP, a split-plot design with Latin Square arrangements was assumed (main plots were cows on each diet and subplots were markers). The Duncan's multiple range test was used to separate means.

RESULTS AND DISCUSSION

Intake and Digestibility

Mean daily intakes and digestibilities of dry matter and crude protein for treatments are in Table 3. Analysis of variance did not reveal any significant effects ($P>.10$) of diet or animal on these parameters.

The relatively large standard errors associated with the digestibility data reflect the wide variation within treatments. It is possible that this variation could have obscured real differences due to protein source. Nevertheless, the data for true digestibility of crude protein (i.e. digestibility obtained by correcting for microbial crude protein in duodenal digesta) provide indices of protein degradability which are in accord with established trends for the three supplements. Further, the negative numerical values for apparent digestibility of crude protein indicate a net gain of nitrogen in the forestomachs when cows were fed these diets. On the BM ration, the average increase was more than 50% of the dietary nitrogen intake, but was considerably lower for CSM. On the basis of these results, it is inferred that slow degradability of the protein sources resulted in rumen ammonia levels low enough to promote a large influx of endogenous nitrogen. Recycling of nitrogen is increased with decreased rumen ammonia concentration (Kennedy and Milligan, 1978; Kennedy, 1980). It is inferred that rumen ammonia was lowest for the BM diet, even though ammonia was not measured.

Table 3. Intake and digestibilities of cows fed three protein sources.

ITEM	RATION CSM	CGM	BM	S.E
Intake (kg/d)				
Dry matter	16.78	15.90	14.38	1.57
Crude protein	2.51	2.32	2.36	.28
Upper tract digestibility (%)				
Dry matter[1]	46.74	54.41	52.35	6.15
Organic matter[1]	50.56	61.17	56.46	6.53
Crude protein[2]	-34.49	-39.04	-54.18	8.88
Crude protein[1]	48.38	43.29	31.20	4.08
Total tract apparent digestibility of crude protein (%)	52.85	50.10	34.11	8.62

[1] True digestibility calculated by correcting for bacterial fraction in duodenal digesta as estimated by the ^{15}N marker method.

[2] Apparent digestibility.

Milk Production and Composition

Average production and composition of milk for the different diets showed no significant (P>.10) treatment effects (Table 4) but there was a tendency towards lower milk fat on CSM which is not consistent with the higher intake and ether extract content. However, organic matter digestibility was numerically lower with this diet and might have reduced production and availability of acetate, a major precursor for milk fat synthesis. This, coupled with higher milk yields could have caused decreased fat in milk.

Microbial Protein Synthesis: Duodenal Bacteria Method

Unweighted means for the microbial protein fraction in duodenal digesta estimated by the three different marker methods for each cow on each diet are presented in Table 5. Considerable variation existed within each animal on each diet. Some of this variation could have been due to changes in microbial protein flowing with digesta at different times. However, marker methods introduced considerable error as evidenced by variabilities for the three techniques.

Variability was lowest with the ^{15}N method, which agrees with reports suggesting that isotopic bacterial markers are the most reliable method for estimating MCP/DCP (Ling and Buttery, 1978; Siddons et al., 1982). There was no consistent trend in differences of variability between the RNA and DAP methods, but the greater coefficient of variation for the RNA technique suggests more variability. This contrasts with greater variability observed for the DAP method (Ling and Buttery, 1978; Rode et al., 1984). The reason for this apparent discrepancy might be

Table 4. Effect of dietary protein supplement on milk production and composition.

ITEM	RATION CSM	CGM	BM	S.E
Milk (kg/d)	27.47	22.53	21.37	4.06
Fat %	3.38	3.79	3.86	.09
Protein %	2.76	2.98	2.92	.14
Lactose %	5.27	5.32	5.06	.07

Table 5. Unweighted means of estimates of the proportion (percent) of the microbial nitrogen in total non-ammonia nitrogen of duodenal digesta (MCP/DCP) by different marker methods.

			MICROBIAL MARKERS								
			^{15}N			DAP			RNA		
Cow	Period	Ration	Mean	S.D.	C.V.(%)	Mean	S.D.	C.V.(%)	Mean	S.D.	C.V.(%).
932	1	CSM	57.77	6.90	11.94	55.15	11.73	21.27	28.76	6.19	21.52
932	2	CGM	55.44	5.33	9.61	55.78	13.30	23.84	28.58	5.43	19.00
932	3	BM	49.88	4.63	9.28	65.30	13.82	21.16	32.67	5.10	15.61
958	1	CGM	60.93	9.19	15.08	72.00	14.31	19.88	42.45	9.25	21.79
958	2	BM	57.56	3.56	6.18	65.17	7.63	11.71	41.34	9.68	23.42
958	3	CSM	63.78	5.89	9.23	52.58	6.03	11.47	35.15	5.43	15.45
41	1	BM	58.50	5.10	8.72	52.23	4.92	9.42	45.15	10.76	23.83
41	2	CSM	62.15	5.54	8.91	65.40	8.96	13.70	39.06	5.73	14.67
41	3	CGM	62.50	4.62	7.39	50.68	8.86	17.48	36.13	4.87	13.48
MEAN					9.59			16.66			18.75

differences in methods of analysis and/or calculations. Also variability of marker-N:total N between bacteria and digesta samples (Tables 6 and 7) might account, at least in part, for the differences in variability in the estimates of MCP/DCP by the different methods. The digesta RNA-N:total N ratios were generally more variable than DAP-N:total N ratios, while the corresponding bacterial ratios showed a mixed pattern relative to each other but with quite similar overall mean variability.

Patterns of marker-N:total N ratios emphasize the array of factors influencing concentration and flow of the microbial markers and the caution necessary when results are interpreted. Variation in bacterial ratios could arise from short-term fluctuations in relative proportions of different bacterial species, or from changes in growth phase or nutrient supply. Fluctuations in digesta marker-N:total N ratios which do not parallel the variation in bacterial samples are due possibly to variable flow of the marker present in undegraded feed, microbial fragments and endogenous secretions.

The mean values for marker-N:total N ratios for the different diets are shown in Table 8. Partitioning of variability revealed no significant effects ($P>.10$) of diet or animal on digesta RNA-N:total N, digesta DAP-N:total N, or duodenal bacterial DAP-N:total N. Significant differences existed in bacterial RNA-N:total N ratios between diets and between animals. A lower ratio was obtained on BM which is difficult to explain. Bates and Bergen (1984) found that, for seven strains of predominant rumen bacteria, the RNA/protein ratio of stationary phase cultures was elevated, rather than depressed, when nitrogen was limiting

Table 6. Unweighted means of DAP-N:total N % of duodenal digesta and bacteria of cows fed three protein sources.

			DIGESTA			BACTERIA		
Cow	Period	Ration	Mean	S.D.	C.V.(%)	Mean	S.D.	C.V.(%)
932	1	CSM	.90	.08	8.89	1.68	.36	21.43
932	2	CGM	.74	.09	12.16	1.37	.27	19.71
932	3	BM	.83	.10	12.05	1.31	.23	17.56
958	1	CGM	.88	.11	12.50	1.25	.28	22.40
958	2	BM	.85	.06	7.06	1.31	.11	8.40
958	3	CSM	.68	.07	10.29	1.29	.10	7.75
41	1	BM	.91	.10	10.99	1.74	.11	6.32
41	2	CSM	.67	.06	8.96	1.03	.10	9.71
41	3	CGM	.82	.05	6.10	1.67	.33	19.76
MEAN					9.89			14.78

Table 7. Unweighted means of RNA-N:total N ratios of duodenal digesta and bacteria of cows fed three protein sources.

			DIGESTA			BACTERIA		
Cow	Period	Ration	Mean	S.D.	C.V.(%)	Mean	S.D.	C.V.(%)
932	1	CSM	.059	.007	11.86	.210	.033	15.71
932	2	CGM	.054	.007	12.96	.193	.029	15.03
932	3	BM	.056	.010	17.86	.173	.012	6.94
958	1	CGM	.066	.012	18.18	.157	.016	10.19
958	2	BM	.052	.005	9.62	.131	.036	27.48
958	3	CSM	.056	.007	12.50	.160	.022	13.75
41	1	BM	.063	.010	15.87	.143	.033	23.08
41	2	CSM	.062	.010	16.13	.158	.015	9.49
41	3	BM	.063	.006	9.52	.175	.015	8.57
MEAN					13.83			14.47

Table 8. Effect of protein supplement on DAP-N:total N and RNA-N:total N ratios (unweighted means) of duodenal digesta and bacteria of cows.

ITEM	RATION CSM	CGM	BM	S.E
DAP-N:total N (%)				
Bacteria	1.33	1.43	1.45	.19
Digesta	.75	.81	.86	.04
RNA-N:total N				
Bacteria	.176[a]	.175[a]	.149[b]	.005
Digesta	.059	.061	.057	.002

[a,b] Means within rows with different superscripts differ (P<.10).

for growth. Another possibility might be that the BM diet induced adaptive changes in the species composition of the microbial population. Loerch et al. (1983) observed that in situ nitrogen disappearance of soybean meal and blood meal from dacron bags was greater after 12 h of incubation in the rumen of steers fed the respective protein source and suggested adaptation to the protein sources. However, such a possibility seems remote in this study since there was not a concurrent decrease in the RNA-N:total N ratio in the digesta. Thus, experimental errors and/or inherent variability associated with the RNA technique, were probably responsible for the lower RNA-N:total N of bacteria for the BM treatment.

Mean Estimates of MCP/DCP

Weighted means of marker-N:total N ratios and MCP/DCP are presented in Table 9. For the ^{15}N and DAP methods, individual values derived from the pairs of subsamples of digesta and matched bacteria for each cow within a given period were within the range of two standard deviations of the respective unweighted means. On the other hand the RNA method yielded values that were more than two standard deviations from their respective unweighted period means and these were considered outlyers. Therefore, an adjusted weighted mean for the RNA method was determined by omitting outlyers within a period. Not all period means had to be adjusted, nor was there any case of more than one outlyer within any adjusted period. Both the adjusted and unadjusted treatment means derived from the RNA method are discussed relative to the estimates obtained by the DAP and ^{15}N methods.

Table 9. Weighted means of marker-N:total N ratios of duodenal bacteria and digesta, and of estimates of the proportion of nitrogen in total non-ammonia nitrogen of duodenal digesta (MCP/DCP) in cows fed three protein sources.

ITEM	RATION CSM	CGM	BM	S.E
DAP-N:total N (%)				
Bacteria	1.35	1.43	1.45	.19
Digesta	.75	.81	.87	.04
RNA-N:total N				
Bacteria	.176[a]	.176[a]	.150[b]	.004
Digesta	.059	.061	.057	.002
MCP/DCP (%)				
DAP	57.29	59.74	61.54	7.46
RNA	34.14	35.61	39.51	1.14
Adjusted RNA	33.13	35.61	39.96	1.22
^{15}N	61.52[a]	59.43[ab]	54.99[b]	1.05

[a,b] Means within rows with different superscripts differ (P<.05).

Significant animal and treatment effects ($P<.10$) were obtained with the ^{15}N method. The unadjusted RNA-based values revealed only an animal effect ($P<.05$) but the adjusted values also indicated a treatment effect. In sharp contrast, the DAP method did not show significant effects for diet or animal. These inconsistencies reflect limitations on credibility in use of microbial markers employed for comparing microbial synthesis across different diets and animals. Greater variability associated with the RNA and DAP methods could have obscured real effects of animals and/or treatment on MCP/DCP. Thus the ^{15}N method was considered to be the most reliable.

Based on the ^{15}N method, the BM diet resulted in a lower MCP/DCP than CSM, while there was no difference between CSM and CGM or between CGM and BM. By this method, there was no significant difference between treatments in absolute amounts of microbial protein in duodenal digesta. Thus differences in MCP/DCP must have been due mainly to differences in non-microbial protein present in digesta. It may be assumed that undegraded feed protein, rather than endogenous protein, was responsible for increase in non-microbial protein in duodenal digesta, and hence MCP/DCP should decrease with a decrease in degradability of dietary protein. Such trend has been shown previously (Ling and Buttery, 1978; Cockburn and Williams, 1984). The ^{15}N method provided treatment means for MCP/DCP which are in accord with the relative degradability values for the protein supplements. This further substantiates the greater reliability of the isotopic markers, considering that the other two methods gave values inconsistent with expectations. Due to small

number of observations, degradability of CGM was not sufficiently different from CSM and BM to produce a significant difference in MCP/DCP.

In contrast to the ^{15}N method, the RNA technique indicated an increase in MCP/DCP as ruminal degradability of dietary protein decreased. The apparent disparity in ranking of the diets in MCP/DCP might suggest that the significantly lower mean bacterial RNA-N:total N ratio obtained for BM, than for the other diets, was due to experimental error.

Although the RNA and ^{15}N methods did not give similar results for diet effects on MCP/DCP, they showed agreement with respect to animal effects. Both methods, showed that cow #932 had a lower MCP/DCP than the other animals. There was a significantly higher RNA-N:total N in duodenal bacteria of this cow which may account for the significant effect by RNA. Nevertheless, true animal effects are supported by the isotopic method.

Estimates of MCP/DCP from the DAP and ^{15}N methods are similar except that they tend to be in opposite directions with respect to treatment. In the case of RNA, values are significantly ($P<.01$) lower. This is difficult to explain since it is more common for RNA to overestimate MCP/DCP than DAP (Ling and Buttery, 1978; Smith et al., 1978; McAllan and Smith, 1984).

Mean values for duodenal bacteria RNA-N:total N (Tables 8 and 9) for the different diets are higher than the range of .064 to .139 commonly reported across several diets (Smith and McAllan, 1974; Ling and Buttery, 1978; Cockburn and Williams, 1984; McAllan and Smith, 1984). It is difficult to say whether this was due to experimental errors associated with analytical procedures or to effects of diet, but it is obvious

that there was a disproportionate effect on the corresponding ratios in matched digesta samples which accounted for the lower MCP/DCP estimates by RNA. Ideally, a tendency for bacterial marker content to increase or decrease should be accompanied by similar tendencies in corresponding digesta samples so that estimates of MCP/DCP are unaffected by changes not associated with the chemical composition of bacteria.

One may speculate that loss of bacterial RNA in digesta occurred during storage or subsequent processing. However, it seems unlikely that such losses would be appreciable. McAllan and Smith (1983) and Ha and Kennelly (1984) found that freezing of digesta samples did not affect RNA content of bacteria. Hence the reason for the low MCP/DCP values obtained by the RNA marker method remains obscure.

Microbial Protein Synthesis: Rumen Bacteria Method

Mean values of MCP/DCP for the treatments obtained by use of a rumen bacteria reference in the DAP and RNA methods are in Table 10. In this method a single rumen bacteria reference obtained for the individual cows in each period was used for estimating microbial protein of each digesta subsample within each period. This differs from the duodenal bacteria method in which there was matching of bacteria with the digesta sample from which it was isolated.

One rumen bacterial sample for CSM (cow #932) was lost and, thus, the corresponding marker N:total N (i.e. for RNA and DAP) ratios could not be obtained directly. These missing values were substituted by estimates obtained by the procedure described by Cochran and Cox (1950). The respective mean ratios for CSM, CGM and BM were DAP: 1.13, 1.13,

Table 10. Effect of dietary protein supplements on proportion of microbial protein in total protein of duodenal digesta (MCP/DCP) estimated by DAP and RNA marker methods using ruminal bacterial reference.

Ration	MCP/DCP			
	Unadjusted		Adjusted[c]	
	DAP	RNA	DAP	RNA
	----------%----------			
CSM	66.11	42.28[a]	66.11	42.28[b]
CGM	72.30	60.29[b]	71.94	59.03[b]
BM	68.61	42.39[a]	68.61	42.39[a]
S.E.	3.64	3.15	3.48	2.83

[a,b]Means within columns with different superscript differ (P<.10).

[c]Adjusted by eliminating outlyers from calculations.

1.28; RNA: 0.147, 0.104, 0.135. There were no significant differences between means for the respective markers. These values are somewhat lower than the corresponding mean values for the duodenal bacteria, and explain the increases in the MCP/DCP by this source of bacteria relative to those obtained by the duodenal bacteria reference. Estimates of MCP/DCP were significantly ($P<.01$) lower for RNA but there was a cow x marker interaction ($P<.10$).

Variability of the estimates of MCP/DCP by the rumen bacteria method logically parallel the patterns of variability of the respective marker-N:total N ratios of digesta samples since only a single bacterial ratio was applied within each period for each cow on each diet. Adjusted estimates were calculated on the same basis as for the RNA duodenal bacteria method. Number of outlying values were 1 and 3 for the DAP and RNA marker methods, respectively, and there was no more than one outlyer within any adjusted period.

There were no significant effects of treatment or animal on MCP/DCP by the DAP method. On the other hand, the RNA-based results indicate significant effects due to diet and animal. With the exception of the diet effect by the RNA method, these findings correspond to those of duodenal bacteria methods.

There was a marked contrast in the diet effect indicated by different sources of bacterial reference in the RNA technique. Whereas the duodenal reference indicated a tendency for MCP/DCP to be highest for the BM treatment, the rumen reference showed CGM to have higher ($P<.10$) MCP/DCP than the other two diets. This discrepancy comes from the differences of the mean bacterial RNA-N:total N between the two

sources. The rumen bacteria RNA-N:total N value was numerically lower for the CGM diet relative to the others, while the duodenal reference ratio was lower for the BM treatment.

It appears that the rumen reference does not give results that agree with those of the duodenal reference, especially for the RNA marker method. Differences between estimates derived from the two alternative sources of bacteria might be due to change in the microbial population as it passes from the rumen to the duodenum. However, while such changes could be partly responsible for the disparity of the RNA method, it is much more likely that experimental errors account for most of the observed difference in this study. The rumen bacterial samples were taken at random and errors could therefore be magnified due to the lack of uniformity between cows and diets. Any change in bacterial composition relative to time of feeding might significantly impact upon the results. Also it is unlikely that bacterial samples isolated from rumen contents obtained via a stomach tube would truly represent the total rumen bacterial population. Further, the number of samples taken per period per diet might have been two few (ranging from 1 to 3) to accommodate short term changes in the composition of the microbial population.

In view of the above, it is suggested that the results of the rumen bacteria method as conducted in this study are not conclusive. Nevertheless, they may be taken as indications of possible errors that could arise when a rumen reference is used for assessing the microbial nitrogen fraction of total non-ammonia nitrogen in duodenal digesta based on DAP or RNA as microbial markers.

Of the various markers investigated in this study, the ^{15}N method gave the most reasonable results for MCP/DCP and was therefore adopted for further calculations.

Flow of Dry Matter and Crude Protein at the Duodenum

Mean values for total and fractional flow of dry matter and crude protein at the duodenum for the different treatments are in Table 11. There were no significant differences in flow of dry matter, total crude protein, or microbial crude protein. However, there were significant differences in fractional composition of dry matter and of total crude protein. On the BM diet, percent crude protein in digesta dry matter was significantly ($P<.05$) greater than for CSM. This is consistent with the numerically lower flow of dry matter coupled with a slightly higher flow of crude protein for this ration.

The proportion of microbial crude protein in total digesta crude protein was significantly lower for the BM compared with the CSM treatment, as discussed earlier. However, when expressed as a fraction of total dry matter, microbial crude protein followed the reverse order of MCP/DCP, with BM and CGM having a significantly higher fraction than CSM.

These results demonstrate that, although the BM protein was the least degradable, there was no detrimental effect on absolute microbial yield. It is further implied that increased recycling of nitrogen was adequate to compensate for any tendency of BM to limit availability of nitrogen in the rumen. Thus, increased flow of undegraded feed protein from the BM diet resulted in a slight, but not significant, increase

Table 11. Effect of dietary protein supplement on flow of dry matter and crude protein at the duodenum of cows.

ITEM	RATION CSM	CGM	BM	S.E
Dry matter (DM) kg/d	13.57	11.67	11.30	.73
Crude protein (CP)				
as % of DM	25.00[a]	28.21[ab]	32.19[b]	.93
kg/d	3.33	3.24	3.65	.49
Microbial Crude Protein (based on ^{15}N method)				
as % of digesta CP	61.52[a]	59.43[ab]	54.99[b]	1.05
as % of digesta DM	15.43[a]	16.85[b]	17.73[b]	.20
kg/d	2.05	1.92	2.02	.32

[a,b]Means within rows with different superscript differ (P<.05).

in total protein at the duodenum, although average intake was slightly higher for CSM. A more pronounced difference in duodenal protein flow would be expected for equal intakes.

Efficiency of Microbial Synthesis

Mean values (and standard deviations) of efficiency of microbial synthesis expressed as gMCP/100g fermented organic matter (corrected for bacterial) were 27.38 (± 5.80), 21.71 (± 4.63) and 27.78 (± 6.90) for CSM, CGM and BM treatments, respectively. There were no significant differences between diets. Stern and Hoover (1979) listed efficiencies ranging from 6.3 to 31.6 g CP/100 g OM digested (apparently digested organic matter in most cases) for sheep and cattle across a wide variety of diets. The values from this study are in the upper region of this range and, hence, it is inferred that microbial efficiency was relatively high on the diets in this study. This might possibly be due to an enhanced synchronization of energy and nitrogen availability in the rumen. Increased efficiency of microbial synthesis with decreased degradability of dietary nitrogen has been observed in other studies (Cummins et al., 1983; Firkins et al., 1986). It is also possible that high rate of passage, facilitated by small particle size of the concentrate in the diets, contributed to high efficiency of microbial synthesis.

Nitrogen Balance

Mean output of nitrogen and nitrogen balance for the different treatments are in Table 12. There were no significant treatment effects on any for these measurements.

Table 12. Effect of dietary protein supplement on input, output and balance of nitrogen in cows.

ITEM	RATION CSM	CGM	BM	S.E
Nitrogen intake g/d	401.4	370.1	377.8	67.3
Nitrogen output g/d				
Urine	107.1	104.0	81.4	12.5
Feces	189.3	188.8	249.6	53.4
Milk	119.4	104.0	99.1	12.8
Nitrogen balance				
gN/d	-14.4	-26.7	-52.2	33.7
% of N intake	- 3.72	- 6.99	-13.56	8.76

Nitrogen balance was negative for all diets and tended to decrease with lower rumen degradability of protein supplements. The slightly greater nitrogen deficit for CGM compared to CSM was probably due to lower intake of protein for CGM. Intake of crude protein on the BM diet was comparable to CGM but nitrogen balance was much lower. The greater loss of nitrogen in feces on BM is consistent with a lower postruminal digestibility coefficient of CP for the BM diet (the respective values were 64.55, 64.34 and 57.52% for CSM, CGM and BM).

The negative N balances were somewhat surprising since some studies indicated greater nitrogen retention when diets contained protein supplements resistant to rumen degradation (Stock et al., 1981; Cummins et al., 1982; Nelson et al., 1984). However, negative N balances have also been reported for resistant protein supplements (Rogers et al., 1986). Postruminal N degradability is sensitive to heat damage to proteins and the apparently low digestibility observed in this study might have been due to excessive heat applied to BM during roller drying process. It is also possible that fecal nitrogen output was overestimated due to errors associated with the use of Cr_2O_3 as flow marker. Since fecal samples were taken periodically at intervals, non-uniformity in flow of the marker might introduce error in estimates of passage of fecal dry matter but should not bias results of any treatment more than another. Further, recovery of the flow marker was assumed to be 100% but actual recovery might have been less, which would also introduce error.

^{15}N Enrichment Patterns and Recovery:
^{15}N Enrichment

The ^{15}N enrichment patterns in bacteria, digesta, feces, urine and milk, averaged for all cows and diets, are in Figures 1 through 3. Average peak enrichment were in the order: bacteria > urine > digesta > feces > milk (Table 13).

Rate of enrichment was relatively high during the first two days (4 feedings of the isotope) and declined thereafter. It appeared that the enrichment was approaching a plateau by the end of the 72-h sampling period. Pilgrim et al. (1970) reported a fairly steady state in ^{15}N excess in rumen bacteria after 78 h of continuous infusion of the marker into the rumen of sheep. Thus, it is suggested that incorporation of ^{15}N in diets fed ad libitum mimics continuous infusion into the rumen. However, while feeding is a convenient alternative to infusion, loss of the marker in orts is an obvious economic disadvantage.

The patterns of enrichment of duodenal digesta and bacteria (Fig. 1) showed a marked parallelism. This is consistent with expectations for an ideal microbial marker and, thus, provide further testimony for the credibility of the isotopic method.

The presence of excess ^{15}N in feces and milk within 12 h after the addition to the diet reflects rapid nitrogen kinetics in ruminants. The first urine sample represented the first 24 h of collection and ^{15}N enrichment in urine probably began early in this period. Labelled urea resulting from labelled ammonia absorbed directly through the rumen wall or from deamination of absorbed microbial amino acids, accounted for ^{15}N enrichment of urine.

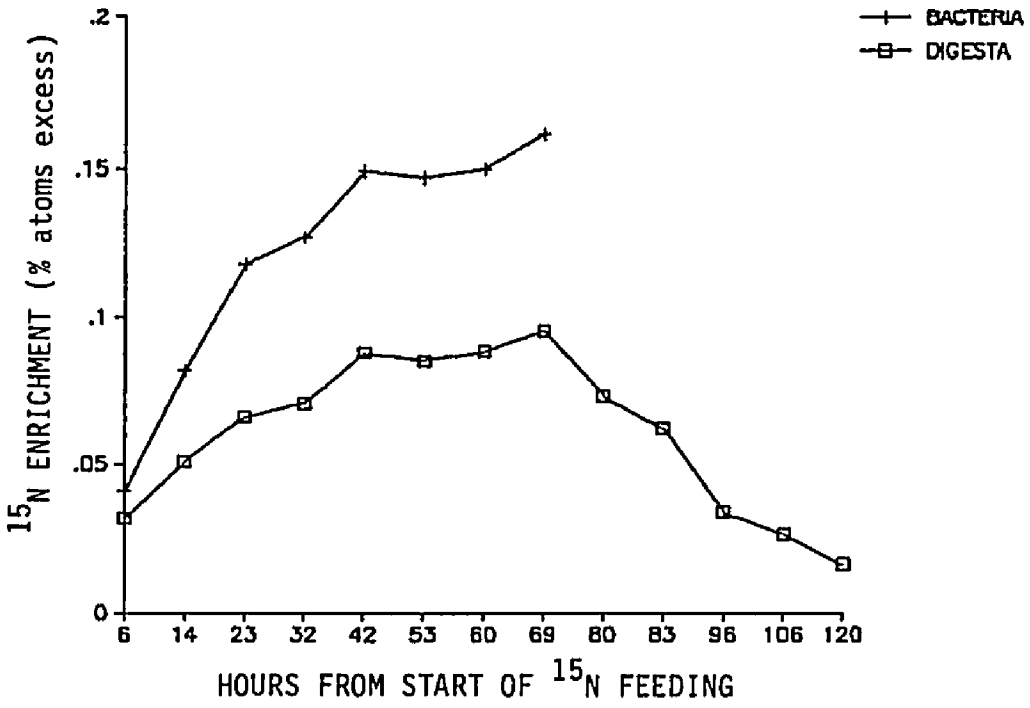


Fig. 1. Average ^{15}N enrichment of duodenal digesta and bacteria of cows fed different protein supplements.

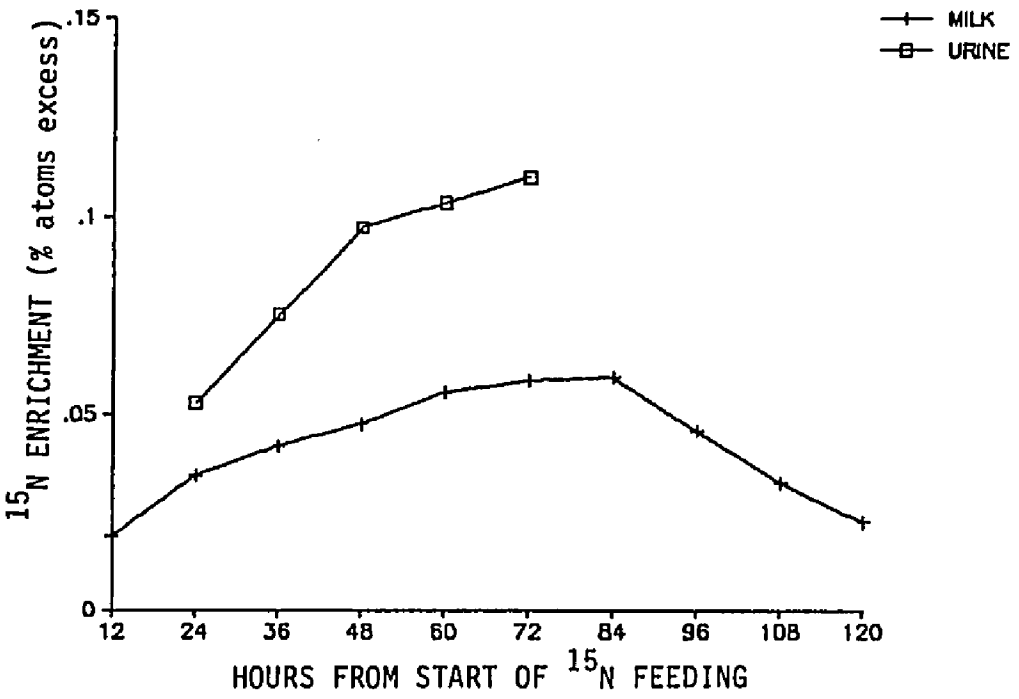


Fig. 2. Average ^{15}N enrichment of milk and urine of cows fed different protein supplements.

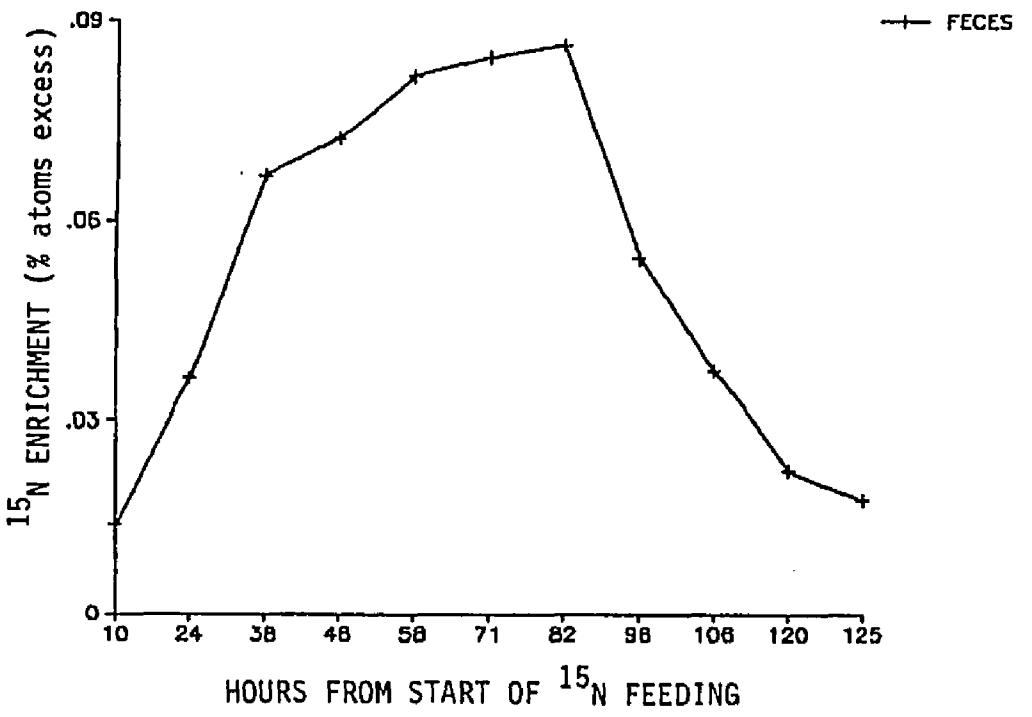


Fig. 3. Average ^{15}N enrichment of feces of cows fed different protein supplements.

Table 13. Peak ^{15}N enrichment in bacteria, digesta, urine, feces and milk of cows fed three protein sources.

Cow	Period	^{15}N enrichment (% atoms excess) Bacteria	Digesta	Urine	Feces	Milk
932	1	.1603	.0954	-----	-----	.0576
932	2	.2055	.1138	.1155	.1125	.0526
932	3	.2269	.1023	.1077	.0859	.0646
958	1	.1564	.1066	.0831	.0988	.0576
958	2	.1503	.0892	.1028	.1021	.0584
958	3	.1450	.0885	.1145	.0803	.0659
41	1	.1693	.1058	.1388	.0710	.0718
41	2	.1402	.0834	.1256	.0824	.0681
41	3	.1418	.0903	.1101	.0798	.0582
MEAN		.1662	.0973	.1123	.0891	.0616

The appearance of excess ^{15}N in milk suggests direct channelling of microbial nitrogen into milk protein but earlier enrichment could have resulted from incorporation of labelled non-essential amino acids synthesized in the liver from labelled ammonia absorbed through the rumen wall. Excess ^{15}N in feces would be due mainly to labelled, undigested microbial residues, but recycling of ^{15}N to the hind gut could also contribute. This recycling might result in enrichment of the lower tract even before labelled residues from the rumen appear in the feces.

After the 4-d sampling period, there was no further addition of ^{15}N to the feed. The consequent decline in enrichment in the various products was rapid and basal levels were approached within 72 h after cessation of feeding. This suggests that the endogenous pool of excess ^{15}N is quite labile and is rapidly depleted by recycling and loss in feces, milk and urine.

Recovery of Excess ^{15}N

Data for intake and recovery of excess ^{15}N during the 72-h sampling period are in Table 14. Intake was adjusted for loss of the marker in orts, assuming uniform distribution of added ^{15}N throughout the ration. Incorporation of the isotope into bacteria was 50 to 83% of intake, while total recovery in feces, urine and milk was 54 to 78%. Amounts of recovered ^{15}N in the different products were in the order: feces > urine > milk. Mean values these measurements for the different diets are in Table 15. There were no significant effects of treatments. However, the numerically higher proportion of ^{15}N incorporated into

Table 14. Intake, bacterial incorporation, and recovery of excess ^{15}N in cows fed three protein sources.

Item	Cow and Period 932 1	932 2	932 3	958 1	958 2	958 3	41 1	41 2	41 3
N-15 excess intake mg	1848	1905	1581	1500	1518	1669	1787	1512	1642
bacterial incorporation mg	1182	1394	1152	747	1171	1093	1475	1000	1311
% of intake	64.0	73.2	72.9	49.8	77.1	65.5	82.5	66.1	79.8
recovery (mg) urine	297	304	195	124	131	266	365	297	331
feces	441	566	321	211	476	340	423	283	417
milk	179	141	100	115	139	196	126	124	119
total	917	1011	616	450	746	802	914	704	867
total as %of intake	77.6	72.5	53.5	60.2	63.7	73.4	62.0	70.4	66.1
% recovery in urine	32.4	30.1	31.7	27.6	17.6	33.2	39.9	42.2	38.2
feces	48.1	56.0	52.1	46.9	63.8	42.4	46.3	40.2	48.1
milk	19.5	13.9	16.2	25.6	18.6	24.4	13.8	17.6	13.7

Table 15. Mean values for ^{15}N incorporation in rumen bacteria and output in urine, feces and milk during 72-h sampling period for cows fed three protein sources.

ITEM	RATION CSM	CGM	BM	S.E
% ^{15}N intake				
Incorporated into bacteria	65.2	67.6	77.5	6.2
Recovered in feces, urine and milk	73.8	66.3	59.7	4.5
% of total ^{15}N recovery				
In feces	43.6	50.3	54.1	3.6
In milk	20.5	17.7	16.2	1.4
In urine	35.9	32.0	29.7	2.7

bacteria when the BM diet was fed is consistent with a lower rumen ammonia concentration (Salter et al., 1979). A given intake of the marker will result in more enrichment of a smaller pool of ammonia. Thus, the higher enrichment of the rumen ammonia would result in greater incorporation of the label into bacteria. However, other factors could be involved. A lower level of rumen ammonia would decrease absorption across the rumen wall and increase recycling of urea. It is also possible that, since the BM diet was least degradable, organic nitrogen available for microbial synthesis was limited. Hence, there might have been a greater dependence on ammonia. These situations would increase the efficiency of incorporation of ^{15}N into microbial cells.

SUMMARY AND CONCLUSIONS

This study was conducted to determine microbial protein flow to the small intestine in cows fed cottonseed meal (CSM), corn gluten mean (CGM), or blood meal (BM) as protein supplements. Three lactating Holstein cows, fitted with cannulae in the proximal duodenum and the three treatment diets were used in a 3 x 3 Latin Square. Cows were offered feed ad libitum and fed twice daily. Experimental periods consisted of at least 14 d adjustment and 4 d sampling. Beginning at least 7 d prior to, and continuing through the sampling period, chromium oxide was mixed into the complete ration to serve as flow marker. During the sampling period, ^{15}N enriched ammonium sulphate was added to the diet for isotopic labelling of rumen bacteria. Duodenal digesta was sampled at intervals of 3 to 5 h over a 72-h period and consecutive samples were composited to give eight subsamples for each cow on each diet. From each of these subsamples, bacteria was isolated by differential centrifugation. Matched pairs of digesta and bacteria were used for calculating the proportion of microbial nitrogen in total non-ammonia nitrogen of duodenal digesta (MCP/DCP). Additionally single rumen bacterial references obtained for each cow on each diet, were used instead of the matched duodenal bacteria to yield another set of estimates of MCP/DCP.

Three microbial markers, namely: ^{15}N, DAP, and RNA were used to obtain estimates of MCP/DCP. Of these, the ^{15}N method (applied only for matched duodenal bacteria reference) gave the most reliable results and, therefore, inferences were based on the results of this method. Diets were ranked in the order of decreasing degradability with mean

values for MCP/DCP (%) being 61.5, 59.4 and 55.0 for CSM, CGM and BM, respectively. The BM diet was significantly ($P<.05$) lower than CSM. However, absolute flow of microbial crude protein (MCP) to the duodenum did not differ between treatments. Efficiency of microbial synthesis, expressed as g of MCP per 100 g of fermented organic matter (corrected for bacteria) were 27.38, 21.71 and 27.78 for CSM, CGM and BM, respectively, with no significant differences. These values suggest a relatively high efficiency of microbial synthesis on these diets.

The DAP and RNA methods were more variable, and ranked diets in the opposite order for MCP/DCP, compared to ^{15}N. Further, the RNA technique yielded significantly ($P<.01$) lower estimates of MCP/DCP than the other methods, probably due to experimental errors. Results obtained with the rumen bacteria references were not consistent with those obtained with the matched duodenal references. However, considering the small number of samples and many possibilities for error associated with the rumen bacteria, results using this reference were not surprising.

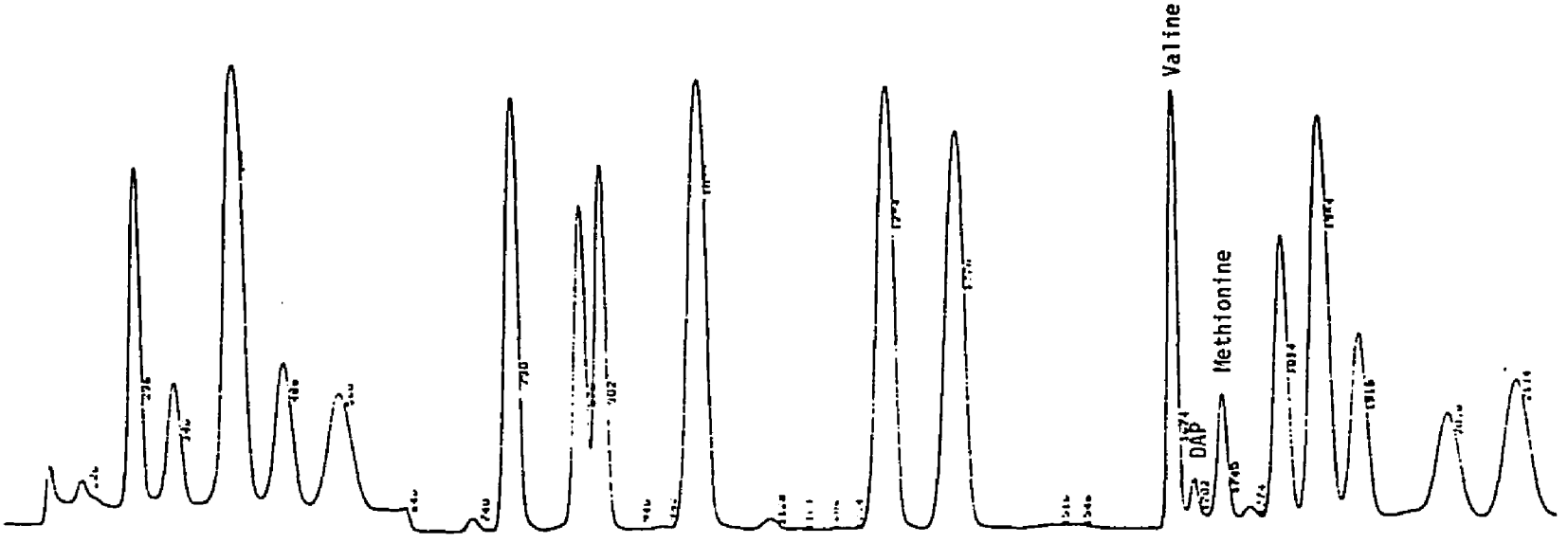
Intake and ruminal digestibility of dry matter and crude protein were not significantly different between diets. There was a net influx of nitrogen into the forestomachs which increased with decreased degradability on the protein supplement. There were no significant diet effects on milk production and composition but there was a tendency for lower milk fat on CSM. Nitrogen balance was negative for all treatments and the deficit was highest in BM which was attributed to lower N digestibility.

It is concluded that microbial crude protein synthesis was not affected by the sources of dietary protein tested. The recycling of

nitrogen to the rumen compensated for a possible insufficiency due to low degradability of BM. However, the higher nitrogen deficit (negative nitrogen balance) associated with this supplement suggests that it is inferior to the other protein sources for nitrogen nutrition of animals. A milder heat treatment during drying might improve feed value of BM and is worthy of investigation.

APPENDIX A

Appendix A. A sample chromatogrm showing elution of tiaminopimelic acid (DAP) between valine and methionine in amino acid analysis.



APPENDIX B

Table B.1 Intake and digestibilities for individual cows fed three protein sources.

Cows and Period	Ration	Intake		Upper Tract Digestibilities				[2]Lower tract digestibility of Crude Protein	[2]Total tract digestibility of Crude Protein
		Dry Matter	Crude Protein	[1]Dry Matter	[2]Crude Protein	[1]Crude Protein	[1]Organic Matter		
932 - 1	CSM	17.71	2.76	33.88	-22.46	49.64	36.13	60.36	51.45
2	CGM	16.93	2.35	37.33	-55.32	31.06	52.28	61.64	40.43
3	BM	14.50	2.30	55.59	-33.48	33.48	60.00	65.15	53.48
958 - 1	CGM	13.12	1.81	65.70	-25.97	49.72	68.63	68.86	60.77
2	BM	14.21	2.33	48.42	-51.93	35.62	50.15	48.87	22.32
3	CSM	18.61	2.70	52.82	-25.93	54.44	56.14	63.82	54.44
41 - 1	BM	14.44	2.45	53.05	-77.14	24.49	59.24	58.53	26.53
2	CSM	14.03	2.07	53.53	-55.07	41.06	59.41	69.47	52.66
3	CGM	17.66	2.79	60.19	-35.84	49.10	62.61	62.53	49.10

[1] True digestibility calculated by correcting for bacteria.

[2] Apparent digestibility.

Table B.2. Average milk production and composition for individual cows fed three protein sources.

Cow	Period	Ration	Milk Production kg/d	Fat %	Protein %	Lactose %
932	1	CSM	32.8	4.15	2.49	5.16
932	2	CGM	30.4	4.33	2.50	5.32
932	3	BM	17.9	4.99	3.04	4.93
958	1	CGM	20.7	3.05	3.05	5.11
958	2	BM	27.0	3.13	2.76	5.11
958	3	CSM	30.7	2.97	2.79	5.26
41	1	BM	19.2	3.45	2.96	5.14
41	2	CSM	18.9	3.02	2.99	5.39
41	3	CGM	16.5	4.00	3.39	5.53

Table B.3. Average flow of dry matter, total crude protein and microbial crube protein at the duodenum in individual cows fed three protein sources.

Cow	Period	Ration	Dry Matter (DM) kg/d	Total Crude Protein (CP) % of DM	Total Crude Protein (CP) kg/d	Microbial Crude Protein[1] % of DM	Microbial Crude Protein[1] kg/d
932	1	CSM	16.18	20.88	3.38	12.26	1.99
932	2	CGM	15.12	24.13	3.65	13.43	2.03
932	3	BM	9.91	31.00	3.07	15.56	1.54
958	1	CGM	7.91	28.83	2.28	17.35	1.37
958	2	BM	11.64	30.44	3.54	17.52	2.04
958	3	CSM	13.33	25.50	3.40	16.24	2.17
41	1	BM	12.36	35.13	4.34	20.12	2.49
41	2	CSM	11.21	28.63	3.21	17.79	1.99
41	3	CGM	11.97	31.67	3.79	19.97	2.37

Table B.4. Average intake, output and balance of nitrogen for individual cows fed three protein sources.

Cow	Period	Ration	Nitrogen intake g/d	Nitrogen Output (g/d) Urine	Feces	Milk	Nitrogen Balance g/d	% of intake
932	1	CSM	441.5	120.2	214.4	130.7	-23.8	- 5.39
932	2	CGM	375.7	117.6	224.0	121.6	-87.5	-23.29
932	3	BM	368.4	82.6	171.2	87.1	27.8	7.55
958	1	CGM	288.8	67.2	115.2	101.0	5.4	1.87
958	2	BM	372.9	53.0	289.6	119.2	-88.9	-23.84
958	3	CSM	431.7	99.3	196.8	137.0	- 1.4	- 0.32
41	1	BM	392.1	108.8	288.0	90.9	-95.6	-24.38
41	2	CSM	331.1	101.9	156.8	90.4	-18.0	- 5.44
41	3	CGM	445.9	127.2	227.2	89.5	2.0	0.45

APPENDIX C

Appendix C. Analysis of variance tables.

Parameter	Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F-value	Level of Significance
Dry matter intake	Total	8	32.56			
	Period	2	6.85	3.43	.47	N.S.
	Cow	2	2.15	1.08	.15	N.S.
	Treatment	2	8.85	4.43	.60	N.S.
	Error	2	14.71	7.36		
Crude protein intake	Total	8	.849			
	Period	2	.194	.097	.365	N.S.
	Cow	2	.062	.031	.117	N.S.
	Treatment	2	.062	.031	.117	N.S.
	Error	2	.531	.267		
Upper tract digestibility of dry matter (corrected for bacteria)	Total	8	821.90			
	Period	2	143.66	71.83	.63	N.S.
	Cow	2	356.54	178.27	1.57	N.S.
	Treatment	2	94.42	47.21	.42	N.S.
	Error	2	227.28	113.64		
Upper tract digestibility of organic matter (corrected for bacteria)	Total	8	684.19			
	Period	2	56.46	28.23	.22	N.S.
	Cow	2	202.46	101.23	.79	N.S.
	Treatment	2	169.70	84.85	.66	N.S.
	Error	2	255.57	127.79		

N.S. = non-significant

Appendix C. Analysis of variance tables (Continued).

Parameter	Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F-value	Level of Significance
Apparent upper tract digestibility of crube protein	Total	8	2686.29			
	Period	2	752.03	376.02	1.59	N.S.
	Cow	2	822.72	411.36	1.74	N.S.
	Treatment	2	637.94	318.97	1.35	N.S.
	Error	2	473.60	236.80		
Upper tract digestibility of crude protein (corrected for bacteria)	Total	8	853.78			
	Period	2	143.37	71.69	1.43	N.S.
	Cow	2	143.01	71.51	1.43	N.S.
	Treatment	2	467.47	233.74	4.67	P<.25
	Error	2	99.93	49.97		
Apparent lower tract digestibility of crude protein	Total	8	303.47			
	Period	2	23.02	11.51	.13	N.S.
	Cow	2	13.71	6.86	.08	N.S.
	Treatment	2	96.11	48.06	.56	N.S.
	Error	2	190.63	85.32		
Apparent total tract digestibility of crude protein	Total	8	1398.87			
	Period	2	289.99	145.00	.65	N.S.
	Cow	2	48.67	24.34	.11	N.S.
	Treatment	2	614.43	307.22	1.38	N.S.
	Error	2	445.88	222.94		
Milk production	Total	8	825.29			
	Period	2	21.80	10.90	.22	N.S.
	Cow	2	141.78	70.89	1.44	N.S.
	Treatment	2	62.91	31.46	.64	N.S.
	Error	2	98.80	49.40		

Appendix C. Analysis of variance tables (Continued).

Parameter	Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F-value	Level of Significance
Milk fat %	Total	8	4.154			
	Period	2	.437	.219	9.100	P<.10
	Cow	2	3.267	1.634	68.063	P.025
	Treatment	2	.402	.201	8.375	P<.15
	Error	2	.048	.024		
Milk protein %	Total	8	.652			
	Period	2	.169	.085	1.47	N.S.
	Cow	2	.288	.144	2.48	N.S.
	Treatment	2	.080	.040	.69	N.S.
	Error	2	.115	.058		
% lactose in milk	Total	8	.254			
	Period	2	.030	.015	1.15	N.S.
	Cow	2	.084	.042	3.23	P<.25
	Treatment	2	.114	.057	4.38	P<.25
	Error	2	.026	.013		
MCP/DCP % (^{15}N method)	Total	8	138.095			
	Period	2	.184	.092	.028	N.S.
	Cow	2	64.590	32.295	9.740	P<.10
	Treatment	2	66.691	33.346	10.059	P<.10
	Error	2	6.630	3.315		
MCP/DCP % (DAP duodenal bacteria method)	Total	8	493.34			
	Period	2	47.45	23.73	.14	N.S.
	Cow	2	84.66	42.33	.25	N.S.
	Treatment	2	27.26	13.63	.08	N.S.
	Error	2	333.97	166.99		

Appendix C. Analysis of variance tables (Continued).

Parameter	Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F-value	Level of Significance
MCP/DCP %	Total	8	259.95			
(RNA duodenal	Period	2	20.70	10.35	2.63	N.S.
bacteria	Cow	2	185.13	92.57	23.52	P<.05
method	Treatment	2	46.25	23.13	5.88	P<.25
- unadjusted)	Error	2	7.87	3.94		
MCP/DCP %	Total	8	253.204			
(RNA duodenal	Period	2	22.346	11.173	2.501	N.S.
bacteria	Cow	2	150.128	75.064	16.804	P<.10
method	Treatment	2	71.796	35.898	8.036	P<.15
- adjusted)	Error	2	8.934	4.467		
Efficiency of	Total	8	274.18			
microbial	Period	2	67.77	33.89	.58	N.S.
synthesis (g	Cow	2	20.39	10.20	.17	N.S.
MCP/100 g FOM	Treatment	2	69.03	34.52	.59	N.S.
corrected for	Error	2	116.99	58.50		
bacterial OM)						
Nitrogen	Total	8	17686.11			
balance	Period	2	8486.86	4243.43	1.25	N.S.
(g N/d)	Cow	2	167.16	83.58	.02	N.S.
	Treatment	2	2234.60	1117.30	.33	N.S.
	Error	2	6797.490	3398.75		
Nitrogen	Total	8	1235.90			
balance	Period	2	611.62	305.81	1.33	N.S.
(% of intake)	Cow	2	13.26	6.63	.03	N.S.
	Treatment	2	150.66	75.33	.33	N.S.
	Error	2	460.36	230.18		

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