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RUMEN MICROBIAL PROTEIN SYNTHESIS

IN COWS FED DRIED WHEY

By

PAUL M. WINDSCHITL

A thesis submitted  
in partial fulfillment of the requirement for the  
degree Master of Science  
Major in Dairy Science  
South Dakota State University  
1983

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RUMEN MICROBIAL PROTEIN SYNTHESIS

IN COWS FED DRIED WHEY

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

David J. Schingoethe  
Thesis Advisor

Date

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Date

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PMW

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## ABSTRACT

Two rumen fistulated Holstein cows, weighing approximately 550 kg, were used in a switchback design experiment to evaluate the effects of consuming large amounts (38% of total ration dry matter) of dried whey on rumen microbial protein synthesis. Cows were fed total mixed rations consisting of (dry matter basis) 45% corn silage, 10% alfalfa hay, and 45% concentrate mix. The concentrate mix was primarily corn and soybean meal (control) or 85% dried whole whey. Dry matter intakes averaged 16.4 and 15.3 kg/day for control and whey diets. Concentrations of bacteria and protozoa in rumen contents were estimated using diaminopimelic acid and aminoethylphosphonic acid, respectively, as markers. Diaminopimelic acid-N as percent of bacterial-N was similar for both diets (.61 and .63% for control and whey diets). Likewise, aminoethylphosphonic acid-N as percent of protozoal-N was similar for both diets (.17 and .19% for control and whey diets). For the control diet, total rumen-N was estimated to be 45% bacterial-N and 27% protozoal-N. Bacterial-N and protozoal-N, respectively, accounted for 52 and 22% of the total rumen-N in the cows fed the whey diet. Rumen fluid volume (33.8 and 39.2 liters for control and dried whey diets) and dilution rates (10.2 and 12.8%/h), as estimated with polyethylene glycol, were higher when fed dried whey. Rumen ammonia (5.0 and 3.4 mg/dl) was lower when fed dried whey. Butyrate (16.5 and 24.4 moles/100 moles total volatile fatty acids) was higher while propionate was lower (32.4

and 23.2 moles/100 moles total volatile fatty acids) when fed dried whey; concentrations of other volatile fatty acids were similar with both diets. Bacterial synthesis appeared to be increased when cows were fed a diet containing large amounts of dried whey.



## INTRODUCTION

Feeding adequate amounts of protein to lactating cows is one of the most expensive and important aspects of dairy cow nutrition. Protein requirements of these cows can be met by feeding both natural proteins and nonprotein nitrogen sources. Nonprotein nitrogen and some natural proteins are used as nitrogen sources for microbial protein production in the rumen. These microbes are then used by the host animal as a protein source (110). As more high quality plant proteins are used for direct human consumption, finding ways to maximize the conversion of nonprotein nitrogen and poor quality natural proteins to better quality microbial proteins may become increasingly important in ruminant protein nutrition. For maximum microbial protein synthesis, adequate nutrients, especially nitrogen and energy sources, must be available in the rumen (47, 55).

Whey is a nutritious by-product of the cheese industry. The dry matter of whey consists mainly of lactose, about 73%, which is easily fermented and may be an excellent soluble carbohydrate (energy) source for the microbes. Whey dry matter also contains about 14% highly soluble high quality protein, thus supplying a nitrogen source to the microbes. Whey is also quite high in sodium which may increase rumen fluid dilution rates. A faster fluid dilution rate, in turn, may increase the efficiency of microbial protein synthesis (24, 142, 146).

Previous experiments (72, 132, 133) suggested that microbial protein synthesis may be affected when dried whey was added to the

ration since rumen ammonia concentrations were consistently lower with the whey diet. Presumably, more ammonia was used for microbial synthesis. The objectives of this trial were: 1) to determine the effects of dried whole whey in the ration on microbial protein synthesis, and 2) to examine the effects of dried whey on rumen fluid dilution rates.

## LITERATURE REVIEW

Feeding Whey to Ruminants

Whey, the highly nutritious by-product of the cheese industry, can be utilized quite well as a foodstuff for ruminant animals. Whey can be fed as liquid whey (8, 79, 105, 120), condensed whey (156), fermented ammoniated condensed whey (41, 45, 53, 66), dried whey (72, 129, 130, 131, 132, 133), and dried whey products (18, 56, 121, 133, 134). The use of whey and whey products in animal feeding was recently reviewed (88, 128). Therefore, I will not extensively review all aspects of feeding whey, but will emphasize only those areas of whey feeding closely related to my research.

Fermented Ammoniated Condensed Whey

The basic idea with feeding fermented ammoniated condensed whey (FACW) is to ferment part of the lactose in whey to lactic acid, and by maintaining the pH with ammonia, produce ammonium lactate. The ammonium lactate would then serve as a nonprotein nitrogen source for ruminants (66). Palatability may be a problem with FACW; however, Welch et al. (156) showed that the acceptability of condensed whey by cows was improved substantially when it was mixed with equal amounts of molasses.

Huber et al. (53) fed FACW to lactating cows and showed that it was equal to soybean meal in maintaining milk yield when these sources furnished 27% of the nitrogen in lactating cows rations

containing about 13% crude protein. Essentially, a 13% crude protein ration is adequate during later lactation. Since FACW is a nonprotein nitrogen source, it is questionable as to whether or not FACW would equal soybean meal for cows in early lactation. Erdman et al. (41) showed that replacing 100% of the soybean meal with FACW in a 40% concentrate-60% corn silage diet resulted in reduced feed intakes and milk yields when fed to high producing (early lactation) cows.

Juenst (66) listed some advantages with using FACW as a ruminant feed. They include: 1) concentration of whey to 60 to 70% solids requires less energy than spray drying, 2) FACW has an elevated crude protein to about 45%, and 3) FACW is a superior source of non-protein nitrogen to that of urea because it contains an energy source and is less toxic than urea.

#### Dried Whey and Whey Products

Dried whole whey and whey products appear to be acceptable ruminant feedstuffs (128). An advantage of using dried whey is the ease of transportation compared to liquid whey. The cost of drying the liquid whey must be considered, however.

Adding dried whey or whey products to milk fat depressing diets (high grain-low roughage) will return milk fat percent back toward normal (54, 56, 121, 134). Whey minerals are most likely responsible for maintaining milk fat percent with these diets, although lactose may be responsible to a lesser extent (134). Rosser et al.

(121) suggested that whey and whey products may enhance triglyceride transport into the mammary gland, thus maintaining milk fat percent on high-concentrate diets. The increased rumen butyrate and acetate to propionate ratios, as well as the decreased rumen propionate observed when whey is fed may also be important in maintaining milk fat percent (56).

With rations containing normal amounts of roughage, actual milk production was similar or slightly lower when a concentrate containing 5% dried whole whey or dried whey product was fed to lactating cows (129, 131). However, 4% fat-corrected milk and fat percent were higher for cows receiving the whey diet. Milk protein percent was not affected by the whey ration. Adding small amounts of dried whey to the ration was not detrimental to milk production, although small amounts of dried whey did not have the stimulating "additive effect" often observed with nonruminants (128).

Feeding large amounts of dried whole whey (65% of concentrate mix) to lactating cows resulted in lower actual milk production, but 4% fat-corrected and solids-corrected milk were similar for control and whey fed cows (132). Steers consuming large amounts of dried whey (65 to 86% of concentrate mix) had dry matter intakes and weight gains similar to the control animals (72, 132). Cattle apparently have the capability to consume large amounts of dried whey without experiencing any serious health or production problems.

### Rumen Metabolism

Huber et al. (56) reported that pH of rumen contents was not significantly affected by type or level of whey fed. They do state, however, that partially delactosed whey may exhibit a buffering effect due to the high mineral content. Schingoethe et al. (134) also suggested that whey minerals play a role in maintaining rumen pH at an acceptable level. Fermented ammoniated condensed whey (FACW) generally does not elevate rumen pH (66). This fact may make FACW superior to urea as a nonprotein nitrogen source. Fermentation of lactose in the rumen caused a decreased pH in a trial done by Rayssiguier and Poncet (113). Lactate concentrations were elevated in these same animals. It should be pointed out that the lactose was fed as one meal in a short period of time. Thivend and Ehouinsou (145) also observed a decreased pH when lactose was fed to sheep.

Rumen molar percentage of butyrate was increased and propionate was generally decreased when whey or whey products were fed (56, 72, 121, 129, 132, 133, 134). Changes in acetate concentrations are not consistent when whey is fed. Schingoethe and Skyberg (132) noted decreased acetate when lactating cows were fed dried whole whey. In contrast, Schingoethe et al. (134) observed no change in rumen acetate when whey products were fed. King and Schingoethe (72) observed an increased molar percent of acetate when steers were fed dried whey.

The increased butyrate observed when whey or whey products are fed may be attributed to lactose fermentation (56). Data of Schingoethe et al. (134) also support this since butyrate was highest

in rations that contained the most lactose. Satter and Esdale (125) indicated that butyrate is the ultimate end product of lactate metabolism. The oxidation of lactate to pyruvate resulted in butyrate being synthesized from acetate in an attempt to maintain an oxidation-reduction balance. The production of butyrate rids the rumen of excess hydrogen ions which may be detrimental to rumen microorganisms. Essentially, the rumen microorganisms synthesize butyrate from acetate when threatened by low acidity, thus, reducing acidity by 50% by turning two acidic molecules into one. Total rumen volatile fatty acid (VFA) concentrations generally are not affected by diets containing whey or whey products (56, 129, 132, 133, 134).

Rumen ammonia concentrations tend to be lower in animals fed whey (72, 132, 133, 145). Thivend and Ehouinsou (145) observed a lowered rumen ammonia concentration when sheep were fed a urea-lactose diet. Since urea is easily hydrolyzed in the rumen, one might expect the ammonia concentration to be relatively high. The lowered ammonia concentration observed may be an indication of high bacterial activity since lactose is readily fermentable and is an excellent energy source for microbial synthesis. Poncet and Rayssiguier (107) also reported reduced rumen ammonia and blood urea when sheep were fed a lactose-hay diet. Presumably, more ammonia was being used for microbial synthesis.

Poncet and Rayssiguier (107) reported that total ration organic matter digestibility was improved when lactose was added to lucerne hay diets. There was, however, a decrease in acid detergent

fiber digestibility of the hay portion. Bowman and Huber (18) also reported a decreased crude fiber digestibility when lactose was included in the ration. Schingoethe et al. (133) reported higher digestibilities of energy and dry matter in steers fed diets containing 10 to 40% lactose either as lactose or as dried whole whey. Ration fiber digestibility was not changed. In an earlier trial, Schingoethe and Rook (130) found no increase in digestibility of dry matter, nitrogen, or energy when lactating cows were fed 5% dried whey product in the concentrate.

Absorption and retention of calcium, phosphorus, and magnesium were not affected by the addition of lactose (10 to 40% of the diet) or dried whey (5% of the concentrate) to the diet (130, 133). Schingoethe and Rook (130) concluded that small amounts of dried whey in ruminant rations will not improve mineral absorption and retention probably because the lactose is fermented in the rumen and is not available for aiding mineral absorption in the small intestine. In nonruminants, retention of minerals may be improved when small amounts of dried whey or lactose are fed (128). Rayssiguier and Poncet (113) reported that the addition of 400 g lactose to a 700 g hay diet fed to sheep significantly increased apparent absorption of magnesium, calcium, and phosphorus. Absorption of potassium was slightly decreased. They related these effects to changes in fermentation patterns.

Metzger et al. (85) studied the effects of whey products on rumen microbes in lactating cows fed high-concentrate, low-roughage diets. Concentrates were control, 14% dried whole whey, 5.9% high mineral whey,



11.8% demineralized whey product, and 9.8% lactose. Protozoal numbers in rumen contents were similar for the control, dried whole whey, high mineral whey, and lactose rations while numbers tended to be lower with the demineralized ration. Bacterial numbers increased during the high-grain feeding period. The number of lactose fermenters increased on all diets containing whey or whey products. No differences in starch digesters or proteolytic organisms were detected. Jouany et al. (65) studied the effects of different carbohydrates on protozoal populations. The Entodinium sp. was highest with starch and lactose diets and lowest with sucrose and cellulose diets. Isotricha sp. were highest with the lactose and starch diets. In agreement with Metzger et al. (85), Dasytricha populations were low with the lactose diet.

#### Measuring Microbial Synthesis

Markers used for the estimation of microbial protein production include diaminopimelic acid (DAP), aminoethylphosphonic acid (AEP), ribonucleic acid (RNA), adenosine triphosphate (ATP), and isotopes ( $^{35}\text{S}$ ,  $^{15}\text{N}$ ,  $^{32}\text{P}$ ). In order for a marker to be suitable it must meet two criteria. First, it must be absent or readily distinguishable from any dietary or endogenous constituents leaving the reticulorumen, and secondly, it must exist in a constant ratio with microbial-N under specific experimental conditions (12, 141).

Diaminopimelic acid is an amino acid found in the cell wall mucopeptide of most bacteria, with the exception of some gram-positive

cocci and lactobacilli (114, 160). Traces of DAP may also be found in some protozoa due to the ingestion of bacteria by protozoa (142). The DAP method involves estimating the ratio of DAP in rumen bacteria to the amount of DAP in digesta. Dufva et al. (37) concluded that to use DAP to predict bacterial production in the rumen accurately, one must correct for dietary effects on DAP content of rumen bacteria. The importance of this lies in the fact that DAP is absent in some species of rumen bacteria, for example, Streptococcus bovis (37).

A number of researchers have used DAP for estimating rumen bacterial protein synthesis (32, 40, 62, 77, 97). Methods for separation and determination of DAP were described by Hutton et al. (62), Dufva et al. (37), and Czerkawski (32). Most researchers (32, 37, 40, 62) found DAP to be a suitable marker for measuring bacterial growth. However, Nikolic and Jovanovic (97) had little success using DAP because the rumen digesta and mixed rumen bacteria contained similar amounts of DAP. Ling and Buttery (77) suggested unrepresentative sampling of rumen digesta or intraruminal degradation of bacteria, thus causing DAP to be released, as a cause of this. The presence of free DAP in rumen digesta does not agree with work done by Hutton et al. (62) who found no DAP in rumen fluid prepared by ultracentrifugation or dialysis.

Aminoethylphosphonic acid is an amino acid found mainly in the lipid fraction of protozoa (3, 73). It was the first compound isolated from biological material which has a covalent bond between

carbon and phosphorus (52, 73). This C-P bond is very resistant to acid hydrolysis (3). Aminoethylphosphonic acid content varies somewhat among protozoal species. Abou Akkada et al. (3) reported that Isotricha sp. contained greater quantities of AEP than Entodinium sp. These same researchers found no AEP in the ration components or in mixed rumen bacteria. They suggested the possibility of using AEP as a marker for determining protozoal growth in the rumen. Dufva et al. (36) studied the possibility of using AEP as a marker and concluded that to obtain accurate predictions of protozoal growth it is necessary to correct for dietary effects on AEP and chemical composition of protozoa.

Because AEP is mainly associated with the protozoal lipids, any diet that increases the lipid content of the protozoa may increase the AEP content. A diet that increases the Entodinium sp. population, such as a high grain diet, should increase the lipid and AEP content of mixed rumen protozoa. Smaller protozoa would have more total protozoal surface area than larger protozoa, and thus, more lipid because the majority of the lipids found in protozoa are in the cell membrane (36).

Although some researchers (3, 36) showed that AEP is not found in bacteria or feeds, Ling and Buttery (77) reported substantial quantities in dietary and bacterial material. Dufva et al. (36) indicated it is possible that these researchers (77) mistook other peaks for AEP since their eluting buffer pH was above 1.85, and Dufva et al. (36) could not separate AEP from unidentified peaks at a buffer

pH of greater than 1.85. Czerkawski (32) found AEP (determined as a phosphate) in mixed rumen bacteria. Possibly, outside phosphorus contamination could have accounted for this. More work needs to be done to determine the true reliability of AEP as a marker to estimate protozoal production in the rumen.

The use of RNA as a microbial marker was examined by various researchers (23, 90, 138, 139). This method relies on the assumption that nearly all dietary RNA is degraded in the rumen (90). This assumption may not be valid and, thus, the RNA method may tend to overestimate microbial production (23, 139). An advantage of the RNA method over the DAP method is that protozoa are included with the RNA method (141).

The use of ATP as a marker was reviewed by Stern (141). Variations in the efficiency of extraction of ATP from rumen contents and differences in the concentration of ATP in rumen microbes, together with the labile nature of ATP may limit its use as a marker (141).

Of all the isotopes used as microbial markers,  $^{35}\text{S}$  was used most frequently (70, 82, 96, 124, 151). Some methods utilizing  $^{35}\text{S}$  assume that virtually all microbial sulfur is derived from the rumen hydrogen sulfide pool. Error is introduced, however, due to the direct incorporation of dietary sulfur amino acids into microbial proteins (141). Walker and Nader (152) described an in vivo method for using  $^{35}\text{S}$  that is not dependent upon the previous assumption concerning the rumen sulfide pool. A disadvantage of using  $^{35}\text{S}$  is that

it creates relatively large quantities of radioactive wastes and it is expensive and laborious. A similar ratio of sulfur amino acids to total protein for microbial and dietary material must also be assumed (142).

The  $^{15}\text{N}$  method is based on the incorporation of nitrogen from ammonia into microbial protein and does not account for microbial protein synthesized directly from peptides or amino acids. The method is costly and complicated and has not been used extensively (142).

The use of  $^{32}\text{P}$  isotopes, amino acid profiles, and D-alanine as microbial markers was reviewed by Stern and Hoover (142). Limited knowledge of these potential markers make it difficult to evaluate them at present.

Each marker has its advantage and disadvantage. Continued research and improved experimental techniques may help in determining the relative suitability of each marker. It is important to note that all methods where mixed rumen microbes are separated from rumen fluid assume that these microbes are a representative sample of the total population. This assumption may not be totally true since some microbes adhere to feed particles while others are associated very closely to the rumen wall (26, 141). Smith (136) suggested that microbes found in the rumen fluid may be less metabolically active than those associated with feed particles or the rumen epithelium. Therefore, improved sampling techniques may result in more accurate estimations of microbial synthesis.

### Microbial Protein Synthesis

Microbial protein synthesized in the rumen is a relatively high quality protein source to the ruminant. Bacterial protein has a digestibility of about 80% and a biological value of around 81. Protozoal protein shows a digestibility of 86% and a biological value around 82 (29, 68).

Relative amounts of microbial protein synthesized in the rumen were determined by various researchers. Clark et al. (30) noted that the quantity of microbial protein synthesized in the rumen appears to provide sufficient absorbable amino acids to meet the requirements for maintenance, low milk production, and slow rates of growth when cows are fed a typical dairy ration. Satter and Roffler (126) estimated that microbial protein supplies about 60% of the absorbable amino acids reaching the lower gut of a dairy cow fed a typical ration of corn, soybean meal, alfalfa, and corn silage. The importance of microbial protein to the ruminant animal is illustrated in the fact that, in general, the amino acid composition of duodenal digesta usually reflects that of microbial protein except with diets containing high amounts of bypass proteins (141). For a 650 kg cow producing 25 to 45 kg of milk daily, microbial protein would contribute 42 to 56% of the total protein required by the animal when microbial synthesis in the rumen is about 15 g crude protein per 100 g organic matter apparently digested (141).

McAllan and Smith (91, 92) fed various concentrate diets to a protozoa-free calf and reported that microbial-N as a percent of total

non-ammonia-N in duodenal contents ranged from 50 to 79%. Results were similar when using either RNA or DAP as the marker, Prigge et al. (108) estimated microbial-N as being 35 to 42% of the abomasal-N in steers fed primarily a corn ration.

Nikolic and Jovanovic (97), using the nucleic acid method, estimated bacterial-N as being 61 to 99% of the total rumen-N at 3 h after feeding. Ibrahim and Ingalls (63) suggested that rumen microbes contributed 54 to 91% of the total amino acids in rumen digesta of a fistulated cow. They also indicated that 44% of the microbial-N was due to protozoal-N. Similarly, Harrison et al. (43) indicated that total protozoal amino-N was 45% of the total microbial amino-N (TMAN) in the rumen and 24% of the TMAN entering the duodenum. Weller et al. (159) indicated that protozoal protein in sheep fed hay diets could vary from 5% of the total microbial protein in the rumen 3 h after feeding to 40% 24 h after feeding.

Rumen-N was partitioned as being 26% plant-N, 46% bacterial-N, 21% protozoal-N, and 7% soluble-N in a study by Weller et al. (157). The extent of conversion of plant-N to microbial-N was estimated to be between 61 to 82%, with the value probably being closer to the upper limit. Diaminopimelic acid was used as a marker in this trial. Other research (104) indicated that the minimal extent of conversion of plant-N to microbial-N was 73% for a low-N diet and 59% for a high-N diet. Other studies using DAP as a marker indicated that 60 to 79% of the total rumen-N was bacterial-N (32, 40, 150).

The quantity of microbial protein synthesized in the rumen is commonly expressed as grams of microbial crude protein synthesized per 100 g of organic matter digested (OMD) in the rumen. Using DAP as a marker, values of 16.5 and 20.1 g CP/100 g OMD were obtained when sheep were fed a corn-soybean meal or corn-urea ration, respectively (142). The increased protein synthesis observed when urea was added to the diet may be due to the fact that most bacteria utilize ammonia as their principal nitrogen source (4).

Feeding cattle diets containing high-moisture corn, heated soybean meal, or regular soybean meal resulted in microbial protein production of 14.8, 18.1, and 15.6 g CP/100 g OMD, respectively (108, 139). Ribonucleic acid was used as a marker in these studies. Using DAP as a marker, 27.7 g CP/100 g OMD was synthesized in cattle fed corn silage, oat straw, and ground corn (142). Including an adequate amount of roughage in the diet appeared to be essential in maximizing microbial synthesis. Beaver et al. (15) fed a formaldehyde treated silage diet to sheep and obtained a value of 6.6 g CP/100 g OMD using  $^{35}\text{S}$  as a marker.

Stern and Hoover (142) summarized 64 trials in which microbial crude protein synthesized per 100 g OMD averaged 16.9 g. Likewise, Czerkawski (33) summarized various studies using DAP,  $^{35}\text{S}$ ,  $^{15}\text{N}$ , and nucleic acids as markers and reported values ranging from 14 to 18 g microbial CP/100 g OMD.



## Factors Affecting Microbial Synthesis

### Nitrogen Source

Ammonia appears to be the principal source of nitrogen for rumen microbes, especially the cellulolytic bacteria (21). Allison (4) stated that 82% of the bacterial strains isolated grew with ammonia as their main nitrogen source. Al-Rabbat et al. (7) and Pilgrim et al. (104) found similar results in their studies, indicating that 40 to 100% of microbial-N could be derived from ammonia-N. Dennis et al. (34) also showed that protozoal numbers were increased when urea (which is easily hydrolyzed to ammonia) was added to the diet. Ammonia and/or  $\text{NH}_4^+$  appears to be incorporated rapidly into rumen bacteria in the form of amide groups and used for amino acid synthesis (137). The most probable explanation for the failure of amino acids to compete with or inhibit the utilization of ammonia is the low activity, or absence of, systems for transport of amino acids into the rumen microbes (4).

For greatest economy, rumen ammonia should be present in the minimum concentration needed to maintain an adequate supply within bacterial cells. In vitro work by Satter and Slyter (127) indicated that maximum microbial synthesis is achieved when rumen ammonia-N concentration is about 5 mg/100 ml rumen fluid. Hume et al. (60) and Okorie et al. (101) reported that, in vivo, microbial synthesis was maximal when rumen ammonia-N concentrations were 5 to 9 mg/100 ml. In contrast to these studies, Miller (87) and Mehrez et al. (83)

suggested that maximal microbial synthesis occurred when rumen ammonia-N concentration was 20 to 29 mg/100 ml. Baldwin and Koong (11) suggested that all observations may be correct.

Two enzymes for utilization of rumen ammonia by microbes are known. They are glutamate dehydrogenase and glutamine synthetase. Glutamate dehydrogenase is a constituent enzyme with a low affinity for ammonia ( $K_m = 5$  mmol/liter) while glutamine synthetase is induced at low ammonia concentrations and has a higher affinity for ammonia ( $K_m = .2$  mmol/liter). At about 5 mg/100 ml ammonia concentrations, glutamine synthetase is saturated and velocity of this reaction approaches  $V_{max}$ , while ammonia concentrations of 20 to 30 mg/100 ml must be reached before the velocity of glutamate dehydrogenase approaches  $V_{max}$ . A major contribution is assumed from glutamine synthetase at low ammonia concentrations and an increasing dependence on glutamate dehydrogenase at higher ammonia concentrations. It is possible then, that by using different ammonia fixing mechanisms bacteria can utilize different concentrations of ammonia equally efficiently (11). Microbial growth does not appear to be inhibited by high concentrations of ammonia (44).

Although ammonia-N is the main source of nitrogen for rumen microbes, some require preformed amino acids and peptides for maximum protein synthesis. Peptides and amino acids are particularly important in low quality diets (high fiber, low protein) where up to 40% of the bacterial nitrogen did not come from ammonia (99). Allison

et al. (6) stated that peptides and amino acids are needed as precursors to produce branched-chain fatty acids which are growth factors for a number of bacterial species, particularly the cellulolytics. Non-availability of or insufficient amounts of amino acids, peptides, or branched-chain volatile fatty acids in the rumen can be considered as possible nutritional factors causing decreased microbial growth rates (46). Maeng and Baldwin (80) and Maeng et al. (81) indicated that adding small amounts of amino acids to urea diets will increase microbial yields. They found that 53% of the amino acids added to a urea ration were incorporated into the microbes. Maeng et al. (81) also indicated that the optimum ratio of nonprotein to amino acid nitrogen for microbial growth was 75% urea nitrogen and 25% amino acid nitrogen.

Hume (58) replaced 50% of the urea nitrogen in a diet fed to sheep with casein or zein and observed a marked increase in microbial production. Salter et al. (124) investigated the utilization of individual amino acids for microbial synthesis and found that preformed phenylalanine and methionine may be essential to maximize bacterial growth with diets high in nonprotein nitrogen and low in natural proteins. Peptide bound amino acids appear to be utilized more efficiently than free amino acids (109). Even though the addition of proteins or amino acids to the diet can improve microbial synthesis, Cotta and Russell (31) indicated that concentrations of these dietary proteins needed to maximize bacterial synthesis may not be associated

necessarily with the most efficient utilization of dietary protein in the ruminant animal.

The amount of dietary nitrogen or crude protein will have an effect on protein synthesis. McMeniman and Armstrong (94) indicated that 2.0 g of available N/100 g OMD is the minimum amount required for efficient microbial protein production with low-roughage diets. Satter and Roffler (126) reported that microbial synthesis will peak when typical dairy diets contain 12 to 13% crude protein. A similar value of 11% CP was found for sheep (58).

#### Energy Source

Readily fermentable carbohydrates are a very important requirement for maximizing microbial cell production. Inadequate amounts of soluble carbohydrates may lead to starvation and subsequent cell lysis of rumen bacteria (46, 48). Kropp et al. (74) stated that available energy appeared to limit microbial synthesis more than did the nitrogen availability. Bacterial protein synthesis is determined largely by the rate at which the organisms can release and utilize energy in the form of high energy compounds, especially as ATP (50, 141). In order to maximize protein synthesis, the energy from the fermentation of dietary organic matter must be supplied at a rate which matches the synthetic abilities of the microbes (102). Energy needed for amino acid biosynthesis by rumen microbes is derived from glycolytic reactions, and carbon needed for amino acid synthesis comes mainly from intermediates produced during carbohydrate

fermentation and from fermentation end products (4).

A major factor affecting the utilization of degraded dietary nitrogen is the type and rate of availability of the carbohydrate source present (142). Generally, carbohydrates such as sugars and starches are more effective than are other carbohydrates in increasing utilization of dietary nitrogen and increasing microbial protein synthesis (93, 100, 143). McAllan and Smith (91) further reported that starch diets are more efficient than sugar diets in converting urea to microbial protein. An explanation for this may be that the breakdown of starch is timed more closely with urea hydrolysis and release of ammonia, whereas sugars are degraded too rapidly and cellulose is degraded too slowly.

A recent study by Stern et al. (143) indicated that the amount of nonstructural carbohydrates in the diet will influence microbial growth. Microbial production was increased in continuous cultures when dietary nonstructural carbohydrates were increased, even though diets were isocaloric, and VFA production and dry matter digestibilities were similar. More work, however, needs to be done to determine the optimum rumen energy (or nonstructural carbohydrate) to nitrogen ratio for microbial synthesis.

#### Dilution Rate

Rumen dilution rate is positively correlated with

efficiency of microbial synthesis (43, 69, 109). An increased turnover rate should encourage selection of rumen microbes with fast growth rates since slower growing microbes will be washed out of the rumen (24). The maintenance energy requirement per unit of microbial mass becomes smaller as turnover rate is increased. Sufficient time must be permitted for organisms to reproduce and develop for maximum yields; however, cells must be removed before they either enter a "lag phase" in which they use up substrate for maintenance purposes without further growth, or before the cells are destroyed by autolysis (50). Nolan and Leng (98) reported up to 30% recycling of nitrogen by rumen microbes. An increased dilution rate will reduce this recycling and thus increase microbial production efficiency. Thomas (146) also stated that an increased dilution rate was beneficial to protein synthesis because it promoted bacterial growth rather than protozoal growth. Bacteria have a shorter generation time than protozoa and are, thus, more efficient in synthesizing protein. Generation times for bacteria and protozoa are 3 to 7 h and 16 to 24 h, respectively.

An increased turnover rate will cause the same rumen volume to turn out products at a faster rate, which supplies more fermentation products, including microbial cells, to the host (61). Presumably, fermentation rate is increased along with the increased dilution rate. Reasons why microbial synthesis is positively correlated with dilution rate may include: 1) reduced autolysis of bacteria when dilution rate is increased, 2) less bacteria are

engulfed by protozoa, probably because the increased rate of passage has a "sweeping out" effect on protozoa and thus reduces their numbers, and 3) slow generation microbes are washed out of the rumen, especially protozoa (28, 69).

Essentially, there are at least two major types of dilution rates associated with the rumen, that of the solids and that of the liquids (122). Feed particles (solids) turn over at slower rate (two to four times) than does the liquid fraction (61). Microorganisms can be associated with both the solid and liquid phases, thus, it is very important to determine which dilution rate (solid or liquid) is the most appropriate to use when relating microbial synthesis to rumen dilution rates (26, 109, 141). Russell and Hespell (122) indicated that measurements of solid or liquid dilution rates alone should not be used for quantitating microbial cell yields. Mercer et al. (84) suggested that assuming the microbes (especially bacteria) pass from the rumen with either the liquid phase or with the solid phase, two estimates of bacterial flow from the rumen may be obtained and regarded as the upper and lower limits, respectively, of the true situation.

The importance of either dilution rate to movement of microbial mass out of the rumen is still questionable. Owens and Isaacson (103) suggested that 75% of the ruminal protein in concentrate fed steers flows with the liquid. Harrison et al. (43) stated that bacteria generally leave the rumen in the liquid phase. Weller et al. (157) reported that 54 to 74% of the total rumen nitrogen is found in

the liquid phase. In contrast, Prins and Clarke (109) suggested that most bacteria as well as protozoa move with the solid phase, and thus, only a portion of the microbial population can be subjected to faster removal rates when liquid dilution rates are increased.

The turnover rate of liquids is a major factor influencing the function of the rumen ecosystem and outflow of nutrients because it is a determinant of nutrients which escape ruminal degradation and the distribution of microbial species (24). Owens and Isaacson (103) stated that fluid flow is important as it influences particulate and bacterial outflow. Protozoa, on the other hand, remain associated primarily with coarser fibers and have a slow dilution rate and outflow (1, 158). This may explain why protozoa make a proportionally smaller contribution to abomasal protein than their ruminal concentrations would suggest (103). In summary, increasing turnover rate of liquids and solids tends to increase the efficiency of bacterial synthesis, decreases protozoal synthesis, increases the bypass of dietary particulate proteins, increases bypass of feeds as a whole, slightly decreases fiber digestibility, and may cause reduced propionate, increased butyrate, and increased acetate production (49, 51, 103, 147).

Generally, dilution rate is expressed as the proportion of total rumen volume leaving the rumen per hour. This is referred to as volume percent (%/h). For liquid dilution rates, values obtained by various researchers ranged from 4.2 to 20.7%/h, depending upon the species of animal and type of ration fed (13, 117, 118, 119, 148, 153). The optimum dilution rate is difficult to determine. Kropp et al. (75)



indicated that microbial protein production reached a plateau at dilution rates of 5.5 to 6.0%/h in steers fed a soybean meal or urea diet.

Several markers are available for use in determining dilution rates. For fluid dilution rates, polyethylene glycol (PEG) and chromium-EDTA are used while chromic oxide, lignin, and heavy metals are used for solids dilution rate determinants (144, 154). Polyethylene glycol (MW<sub>v</sub>4000) is a water soluble marker that is not absorbed or metabolized in the rumen, making it a suitable marker. When using PEG, two assumptions must be made: 1) the volume of water in the rumen remains constant during the experiment, and 2) the rate of flow of water into and out of the rumen is continuous and constant. Fluid dilution rate and rumen fluid volume can be estimated by measuring the disappearance of PEG over time (148). Although some error may be introduced by the above assumptions, research (13, 123, 135, 148) has suggested that PEG is a valid marker for measuring fluid dilution rate and volume in the rumen.

Finally, rumen dilution rates may be affected by various factors, particularly the liquid dilution rate. Owens and Isaacson (103) listed fluids intake, salt intake, salivary flow, and rumen osmotic pressure as affecting fluid dilution rates. Particle size, particle density, and wettability or rate of density change are determinants of particulate turnover rates. Fluid dilution rate increased when additional salt (NaCl), minerals, artificial saliva, or sodium bicarbonate were added to the ration or infused into the rumen (109, 117, 118).

An increased proportion of roughage in the diet usually resulted in an increased rumen fluid dilution rate (22, 24). Presumably, this was due to the increased amount of saliva being secreted when animals consume forages. Liquid dilution rates were also higher in animals fed fresh vs. dried forage, and forage vs. concentrate diets (24). Again, an increased saliva secretion and osmotic pressure due to the amount of minerals in the fresh forage may have accounted for this. Harrison et al. (43) also noted a decreased dilution rate when animals were fed high grain diets. Increased feed intake usually resulted in increased fluid dilution rate (22, 89, 149). Also, feed intake appeared to influence liquid-turnover to a greater extent than it affected solids-turnover rate (149). Reducing average particle size will decrease liquid turnover, as will feeding monensin (22).

### Sulfur

Sulfur is needed by rumen microbes for methionine and cysteine synthesis (4, 142). Microbial biomass can contain as much as 8 g of sulfur per kg of dry matter, of which a large proportion is found in the protein (16). The rumen sulfide pool ( $H_2S$ ), inorganic sulfate, and sulfur amino acids may act as sources of sulfur for the rumen microbes (4, 95). McAllan (44) reviewed the literature and found that the ratio of protein-N:protein-S of mixed rumen bacteria averaged 18.5:1, with a range of 8.6:1 to 30.8:1 (n = 31). Rumen protozoa samples gave a similar value of 21.6:1 with

a range of 14:1 to 38:1 (n = 22). Assuming that the microbial requirement for sulfur is determined by its relationship with protein-N, the above data suggest that a ratio for rumen available-N:available-S of 20:1 should meet the microbial requirement.

In contrast, an in vivo study by Hume and Bird (59) suggested that a N:S ratio of 10:1 is optimal for microbial synthesis. When sheep were fed .61 g sulfur per day, 82 g of microbial protein was produced per day. Raising the dietary sulfur to 1.95 g/day increased the microbial production to 94 g/day. However, no further increase in microbial production was observed when the sulfur was raised to 3.42 g/day. The N:S ratios for each of the three sulfur diets were 34.3:1, 10.9:1, and 6.4:1, respectively. Protein production was not affected by form of sulfur.

The rate of ruminal release of feed sulfur and nitrogen may be an important factor determining microbial growth. A study (106) done with four different hays (lucerne, spear grass, stylo, and chloris) indicated that the rate of removal of feed sulfur in comparison to the rate of removal of feed nitrogen may vary among different feeds. The removal of sulfur from lucerne hay (N:S, 11.4:1) was similar to the nitrogen removal. However, the removal of sulfur from spear grass, stylo, and chloris (N:S, 9:1, 7:1, and 3.6:1, respectively) was at least 2.5 times faster than the nitrogen removal rate. Harrison and McAllan (44) concluded that, when formulating sulfur requirements on the basis of the N:S ratios in feeds, it is obvious that the total sulfur content (especially with forages) cannot be

taken as an indicator of the relative availability of nitrogen and sulfur. At present, insufficient data prevents researchers from formulating accurate sulfur requirements. Concerning the concentration of ruminal sulfide required for maximum microbial growth, a limiting level of 1 mg sulfide-S/liter of rumen fluid was suggested (20).

#### Other Factors Affecting Microbial Synthesis

Limited research has suggested that niacin may be important for optimum protein synthesis. Riddell et al. (115) observed that synthesis of microbial protein in the rumen was enhanced both in vivo and in vitro when niacin was added to the diet. The increased synthesis may be attributed to increased protozoa. Evidence for this lies in the fact that rumen ammonia and propionate were increased when niacin was added; and higher protozoal numbers are associated with increased ammonia and propionate concentrations (2, 27). Adding niacin to soybean meal rations resulted in increased microbial synthesis, especially when it was added to heated soybean meal diets (35, 116). Conventional heat-treatment of soybean meal may tie up niacin and destroy tryptophan. Bacteria can synthesize niacin from tryptophan while protozoa cannot, thus, the addition of niacin will usually affect protozoal synthesis mainly (35).

Nonavailability of or insufficient amounts of branched chain or higher VFA may cause decreased microbial growth rates (21, 46). Cellulolytic bacteria often require branched chain VFA for the

biosynthesis of amino acids (21). Hume (57) found that the addition of four- and five-carbon VFA to protein-free purified diets adequately supplied with nonprotein nitrogen increased the daily microbial protein production in sheep from 71 to 81 g. A negative correlation between molar proportion of acetate and protein production was also reported ( $r = -.62$ ).

Although limited data are available, fermentation patterns may affect microbial synthesis. Some evidence indicates that a butyrate fermentation pattern is less efficient than an acetate or propionate fermentation in promoting protein synthesis. A propionate fermentation appears to be the most efficient (44).

Protozoal growth may be inhibited by a number of factors not greatly affecting bacterial growth. A pH below 5.4 will lower protozoal numbers. High dietary salt content, grinding and pelleting of feeds, and an increased dry matter intake all tend to decrease protozoal production (5, 28, 61). This may be related to increased rumen dilution rates. Protozoa are, however, associated with high ammonia concentrations in the rumen (112). Although the effects of defaunation are still unclear, it is interesting to note that no consistent differences have been found between the estimates of microbial protein synthesis obtained with faunated and defaunated animals (17, 44).

Increased feeding frequency and cold exposure increased the efficiency of microbial production in the rumen (71). This may be indirectly related to dilution effects.

A limiting phosphorus supply tends to decrease microbial synthesis, since phosphorus is needed for nucleic acid synthesis (38, 44). High osmotic pressure in the rumen will lead to a depressed rumen fermentation which may have an effect on microbial synthesis. Optimum osmotic pressure for ciliates is around 260 mOs/kg. Above 400 mOs/kg is detrimental to microbial synthesis (29, 38).

Limited evidence indicated that low yields of microbial biomass may be caused by a partial "uncoupling" of the fermentation (energetic uncoupling), resulting in continued production of fermentation products without concomitant microbial growth (44). Data of Hume et al. (60) and Satter and Slyter (127) support this theory. The latter researchers found that ammonia concentrations of less than 3.6 mmol/liter depressed microbial growth but had no significant effect on VFA production, clearly showing that fermentation rate is not dependent upon microbial growth rate. Uncoupled fermentations have been attributed to deficiencies of available nitrogen and sulfur (44).

#### Chemical Composition of Rumen Microbes

Effects of diet on chemical composition of rumen microbes were studied by a number of researchers (10, 36, 37, 86, 111). Dufva et al. (37) examined the effect of diet on DAP content of various bacterial species and on mixed rumen bacteria. Among species there were significant differences in the ratio of DAP-N to total bacterial-N when

they were grown in media containing different carbohydrate concentrations. Diaminopimelic acid-N ranged from none in Streptococcus bovis to 1.61% in Ruminococcus flavefaciens. Because S. bovis is devoid of DAP, any high-starch or fermentable carbohydrate diets that cause S. bovis to proliferate would reduce the DAP content of mixed rumen bacteria. Mixed rumen bacteria from cattle fed a high-roughage diet contained more DAP-N to total-N (.65%) than those fed a high concentrate diet (.50%).

In another study, Dufva et al. (36) measured the AEP content of protozoa from cattle fed different diets. Protozoa from cattle fed a high-concentrate diet contained more AEP-N as a percent of total-N (.16%) than did those fed a high-roughage diet (.10%). Total-N was less in protozoa from cattle fed the high concentrate diet (7.8 vs. 8.4%) while the lipid content of these same protozoa was higher (6.2 vs. 3.0%). The authors suggested that the higher lipid content may be partially responsible for the higher AEP-N found in these protozoa.

Most researchers (10, 64, 86, 111) reported little effect of diet upon amino acid composition of rumen bacteria and protozoa. In contrast, Dufva et al. (36) reported that protozoa from cattle fed a high-grain (85%) diet had more threonine, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, and histidine while aspartic and glutamic acids were decreased. Differences in amino acid composition between bacterial and protozoal proteins were also

reported (86). Arambel et al. (10) noted no differences in amino acid composition between gram-positive and gram-negative bacteria in pure culture. These bacteria represented ones most often found in the rumen.

Nucleic acid and total-N content of rumen microbes may be affected by both diet and species of microbes. Arambel et al. (10) reported that deoxyribonucleic acid-N:total-N was lower in gram-positive bacteria, but no differences in the ratio of ribonucleic acid-N:total-N were observed. Total-N was lower in gram-positive bacteria. In agreement with (36), Arambel et al. (10) reported that total-N was lower in protozoa from cattle fed high-concentrate diets, but similar in bacteria for both high-concentrate and high-roughage diets. Deoxyribonucleic acid-N:total-N was lower in mixed rumen bacteria from cattle fed high-concentrate diets.



## MATERIALS AND METHODS

Two rumen fistulated Holstein cows (#3906 and #3819), weighing approximately 550 kg, were used in a switchback design experiment to evaluate the effects of consuming large amounts (greater than 10% of dry matter intake) of dried whey on rumen microbial protein synthesis. Experimental periods were 3 wk in length with a total of four periods. Cows were fed total mixed rations consisting of (dry matter basis) 45% corn silage, 10% alfalfa hay, and 45% concentrate mix. Composition of the concentrate mixes is given in Table 1.

TABLE 1. Composition of the concentrate mixes<sup>a</sup>.

	Control	Whey
	(%)	
Ground shelled corn	87.24	14.04
Soybean meal	9.54	---
Dried whole whey	---	85.50
Limestone	1.34	---
Dicalcium phosphate	1.43	---
Trace mineral salt	.45	.46

<sup>a</sup>Vitamin A added at a rate of 8800 IU/kg and vitamin D at a rate of 2200 IU/kg.

Dried whole whey comprised 38% of the total ration dry matter in the experimental diet.

Cows were fed once daily during the a.m. Dry matter intakes were recorded daily. Body weights were recorded at the beginning and

end of each period. Feed samples were taken once weekly and composited each period for analyses. Samples were oven dried at 57°C for 48 h then ground in a Wiley Mill through a 1 mm screen. Crude protein, ether extract, and ash were determined by the Association of Official Analytical Chemists (AOAC) method (9). Neutral detergent fiber and acid detergent fiber were determined by the methods outlined by Goering and Van Soest (42). Chemical composition of the feedstuffs is given in Table 2.

TABLE 2. Chemical composition of ration components.

	Alfalfa hay	Corn silage	Control concentrate	Whey concentrate
Dry matter, %	89.1	39.6	90.1	95.8
	( % of DM )			
Crude protein	15.8	8.3	13.7	12.2
Soluble nitrogen <sup>a</sup>	--	--	24.8	72.3
Acid detergent fiber	43.5	24.4	4.5	.8
Neutral detergent fiber	57.4	44.6	17.2	2.6
Ether extract	1.2	2.9	2.8	1.0
Ash	9.7	5.0	5.0	7.7

<sup>a</sup>Expressed as a percent of total nitrogen.

Soluble-N content of the concentrate mixes was determined by the procedure of Poos, Klopfenstein, and Britton (1979, personal communication) as described below. Samples of concentrate mixes

containing about 15 mg nitrogen were placed in polyethylene tubes. Samples were prewetted with 5 ml distilled water for 12 h before adding 10 ml of 1:1 (v/v) solution of in vitro rumen buffer (4 g  $\text{NH}_4\text{HCO}_3$  and 35 g  $\text{NaHCO}_3$  in 1 liter distilled  $\text{H}_2\text{O}$ ) and macromineral buffer (5.7 g  $\text{Na}_2\text{HPO}_4$ , 6.2 g  $\text{KH}_2\text{PO}_4$ , and .6 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in 1 liter distilled  $\text{H}_2\text{O}$ ) to each tube. One milliliter of 1% (v/v) sodium azide solution and .5 ml of 1% (v/v) Triton X-100 solution were then added. Tubes were stoppered and incubated in a water bath at  $39^\circ\text{C}$  for 2 h with frequent swirling. Tubes were removed, contents filtered, and Kjeldahl analyses done on the filter paper and residue to get an estimate of solubility (initial percent nitrogen in concentrate mix minus percent nitrogen in residue).

Samples of rumen contents were obtained 3 consecutive days near the end of each period. Samples were taken from a position immediately below the fistula approximately 3.5 h after feeding. Water was restricted from cows 2 h prior to sampling. Rumen fluid and semi-solid digesta were collected into the same insulated flasks. Rumen fluid ( $\sim$  5 liters) was obtained by both a suction strainer technique and by squeezing the fluid from the solid digesta. All unused rumen solids were returned to the rumen.

At the laboratory, approximately 4 liters of the fluid portion was strained thru three layers of cheesecloth. The remaining fluid and semi-solid digesta were frozen, lyophilized at  $-50^\circ\text{C}$ , and analyzed for total-N, DAP, and AEP. Two liters of strained rumen fluid were used for bacterial separation and 2 liters for protozoal separation.

Bacteria were separated by a differential centrifugation technique and protozoa were obtained by a gravimetric procedure (86). Bacteria and protozoa were frozen and later lyophilized at  $-50^{\circ}\text{C}$ .

At the same time of the above sampling, separate samples were collected into 250 ml jars containing .5 ml saturated mercuric chloride and used for pH, volatile fatty acid (VFA), and ammonia determinations. All samples were analyzed for pH and then strained thru four layers of cheesecloth. Two milliliters of 25% metaphosphoric acid were added to 10 ml of rumen fluid in centrifuge tubes. The mixture was centrifuged for 10 min at 1500 rpm and the supernatant used for VFA analysis. Volatile fatty acids were determined by gas-liquid chromatography using a stainless steel column containing neopentylglycol succinate as described by Baumgardt (14). An additional 10 ml of rumen fluid was centrifuged for 10 min at 1500 rpm. The supernatant was acidified with .5 ml of .1N HCl and analyzed for rumen ammonia by the procedure of Chaney and Marbach (25).

Proportions of bacterial-N and protozoal-N in the total rumen-N were estimated using DAP and AEP, respectively. Analyses of DAP and AEP in lyophilized rumen bacteria, protozoa, and rumen contents were done by the method of Czerkowski (32). In this method, AEP was determined as a phosphate. Total-N in bacteria, protozoa, and in rumen contents was determined by Kjeldahl (9).

Amino acid analyses were done on bacterial and protozoal composites from each period. A .5 g sample was hydrolyzed in 6 N HCl at

100°C for 24 h. Analysis was done on a Beckman 118 BL automatic amino acid analyzer using buffers with pH of 3.49, 4.12, and 6.40 (76).

Polyethylene glycol (PEG, MW ~4000) was used as a marker to measure rumen fluid volume and dilution rate. On the last day of each period, 50 g of PEG dissolved in 500 ml of water was added to the rumen via the fistula .5 h after the a.m. feeding. The rumen contents were then hand mixed. The first rumen fluid sample was taken from a position directly below the fistula 1 h after the addition of PEG to allow adequate time for proper mixing. Rumen fluid was then collected from the same area of the rumen for 8 consecutive h using a suction strainer. Polyethylene glycol analyses and calculations were done by the procedure outlined by Ulyatt (148). Measuring the disappearance of PEG over time would allow for the calculation of fluid dilution rate. Interpolation of the PEG concentration in the rumen fluid back to zero time would allow for the rumen fluid volume calculation.

Data, except for the fluid volume and dilution data, were analyzed as a four period switchback design by procedures outlined by Brandt (19) and Lucas (78). Rumen fluid volume and dilution rate data were analyzed by Student's t-test (140).

## RESULTS AND DISCUSSION

Fluid Volume and Dilution Rate

Rumen fluid volume and dilution rate data are presented in Table 3. Both fluid volume and dilution rate tended to be higher with the whey diet. Higher fluid volumes observed with the whey diet may, in part, be due to the higher mineral content (especially Na) of the whey diet. The high Na content may stimulate animals to drink more water (117, 119). The Na content of the whey ration expressed as a percent of dry matter was calculated to be .52% while that for the control diet was .13%.

TABLE 3. Rumen fluid volume and dilution rate in cows fed control and dried whey diets.

	Diet		SE
	Control	Whey	
Rumen fluid volume, liter	33.9	39.2	3.86
Dilution rate, %/h	10.2	12.8	1.08

Another factor contributing to the increased rumen fluid volume observed in cows consuming the whey diet may be differences in rumen fluid osmolarity. An increased amount of minerals, as in the whey diet, tends to increase rumen fluid osmolarity (38, 155). Due to increased osmolarity, more water or fluids from the blood and interstitial fluids may be drawn into the rumen in an effort to make

the rumen contents isotonic with body fluids. However, rumen fluid osmolarity (determined the last two periods) was similar for both diets in the present study (320 and 309 mOs/kg for control and whey diets, respectively). Low numbers of observations may have accounted for the lack of response. Also, Rogers and Davis (117) suggested that supplementation with mineral salts generally has only minor effects on rumen fluid osmolarity, as water intake increases to maintain water and electrolyte balance of the animals.

When expressed as a percent of body weight, rumen fluid volumes observed in the present study were similar to those observed by other researchers (13, 117, 118, 119). It should also be pointed out that the two cows used in this study had very similar body weights, thus, there was no need to make adjustments concerning differences in body size when comparing fluid volumes.

Rumen fluid dilution rates observed in this study were similar to those obtained by others (13, 117, 118, 119). The higher fluid dilution rate observed with the whey diet was probably a result of the higher mineral content (especially Na) of this diet. Infusing mineral salts into the rumen or feeding additional salts in the diet tended to increase rumen fluid dilution rates (117, 118, 119). Rogers et al. (119) indicated that infusion of NaCl may exert osmotic properties in the rumen, thereby increasing fluid dilution rate.

The higher rumen fluid dilution rate found with the whey diet should increase the outflow of soluble nutrients and particulate

matter that is associated with the fluid phase (24). Since some microbes are associated with the liquid phase (43, 61), a greater amount of these microbes should be carried to the lower gut when dilution rate is increased.

The determination of rumen fluid volume and dilution rate using PEG or other water soluble markers has some considerations that must be taken into account. Rogers et al. (118) indicated that manual mixing of rumen contents immediately after the addition of PEG may temporarily stimulate rumen fluid dilution rate. Rumen contents were hand mixed after the PEG addition in the present study. However, the effects of manual mixing of rumen contents on fluid dilution rate would probably be minimal. Warner and Stacy (155) indicated that large dilution effects due to ingested feed and water may lead to incorrect estimates of rumen fluid volume and dilution rate when sheep were fed only once daily. Although cows were fed once daily in the present study, rumen fluid data obtained in this study appeared to be within acceptable ranges when cow size is considered. Also, cows in the present study probably were not consuming all of their feed immediately but instead consumed it over several hours or over a 24 h period.

Rogers et al. (118) suggested that total rumen fluid outflow may be underestimated by water soluble marker techniques. During high feed consumption, a considerable amount of water consumed by the lactating cow may be shunted directly to the lower gut without



equilibrating with water in the rumen. Warner and Stacy (155) also reported that water soluble marker concentration in the rumen was always higher than would be expected after sheep consumed a measured quantity of water. Rogers et al. (118) found similar observations with cattle. Ellis et al. (39) indicated that PEG has some affinity for particulate matter. This would reduce its concentration in the liquid phase and may result in inaccurate estimates of rumen fluid kinetics.

Bauman et al. (13) found PEG to be an acceptable water soluble marker for measuring rumen fluid volume. No statistical differences were found between gravimetric measurements of rumen fluid volume and PEG measurements of rumen fluid volume. When using PEG as a water soluble marker for measuring rumen fluid volume and dilution rate, it is necessary to recognize potential sources of error in order to obtain accurate results. Polyethylene glycol may be best suited as a marker used for comparative purposes, as used in the present study, involving rumen fluid volume and dilution rate in animals fed different diets, but not necessarily for obtaining absolute values.

#### Distribution of Nitrogen in the Rumen

Total-N and DAP-N of rumen contents and mixed rumen bacteria are presented in Table 4. Total-N in rumen bacteria was similar for both diets. Values obtained in the present study are lower than the commonly accepted value of 10.5% N given by Hungate (61). However,

they are similar to values reported by some researchers (37, 62) and higher than that reported by Nikolic and Jovanovic (97).

TABLE 4. Total-N and diaminopimelic acid-N (DAP-N) of rumen contents and bacteria from cows fed control and dried whey diets.

	Diet		SE
	Control <sup>a</sup>	Whey <sup>a</sup>	
<b>Bacteria</b>			
Total-N, mg/g DM	87.6	82.3	4.07
DAP-N, mg/g DM	.53	.52	.036
$\frac{\text{DAP-N}}{\text{Total-N}} \times 100$	.61	.63	.035
<b>Rumen contents</b>			
Total-N, mg/g DM	39.6	39.4	1.44
DAP-N, mg/g DM	.11	.13	.065
$\frac{\text{DAP-N}}{\text{Total-N}} \times 100$	.28	.33	.15

<sup>a</sup>Each value is the mean of 12 observations.

Diaminopimelic acid-N content of rumen bacteria was similar for both diets. Since both total mixed rations contained the same ratio of forage to concentrate, one would not expect any wide variation in overall DAP-N content. Differences in DAP-N content of rumen bacteria may be observed when cattle are fed a high-concentrate versus a high-roughage diet (37). The lowered bacterial DAP content observed when a high-concentrate diet is fed is probably due to changes in proportions of bacterial species in the rumen. The *S. bovis* population may increase with high-grain feeding (61), thus, lowering the

DAP content of mixed rumen bacteria. Streptococcus bovis is devoid of DAP (37). There appeared to be some daily variation in DAP content of mixed rumen bacteria.

Diaminopimelic acid-N as a percent of total bacterial-N did not vary among diets. Values agreed with those obtained by previous researchers (37, 97, 157). There appeared to be some daily variation in the ratio of DAP-N to total bacteria-N. However, Dufva et al. (37) showed that sampling time in relation to feeding time had little influence on DAP-N to total bacterial-N.

Total-N of rumen contents was similar for both diets. Values tended to be higher than those reported by el-Shazly and Hungate (40). Differences in diet (roughage to concentrate ratio), the amount of microbial protein synthesized, and differences in sampling techniques may account for this. Diaminopimelic acid-N as a percent of total rumen-N tended to be higher with the whey diet. This may indicate that more bacterial cells were present in the rumen when cows were fed the whey diet, since DAP is generally found only in bacteria (37, 157).

Bacterial-N as a percent of total rumen-N tended to be higher with the whey diet (Table 5). Presumably, the higher amount of fermentable carbohydrate in the whey diet together with the increased rumen fluid dilution rate observed with the whey diet stimulated greater bacterial synthesis. Bacterial-N accounted for about one-half of the total rumen-N with either diet. Other researchers (40, 63, 157), feeding various diets, reported that bacterial-N accounted for one-half or more of the total rumen-N.

TABLE 5. Distribution of microbial nitrogen in the rumen of cows fed control and dried whey diets.

	Diet	
	Control	Whey
Bacterial-N as a % of rumen-N	45	52
Protozoal-N as a % of rumen-N	27	22
Total microbial-N as a % of rumen-N	72	74

Amino acid composition of mixed rumen bacteria is summarized in Table 6. Overall, diet appeared to have little effect on rumen bacteria amino acid composition. Other researchers (10, 86, 111) reported similar results. However, Purser and Buechler (111) indicated that some amino acids (valine, lysine, glutamic acid) may vary slightly among different bacterial strains. Overall, they concluded that the distribution of other amino acids in each of the organisms was extremely uniform, regardless of diet. Generally, the bacterial protein reaching the lower gut is relatively constant in its amino acid composition (86, 111). Furthermore, Meyer et al. (86) indicated that the amino acid makeup of rumen bacteria was remarkably similar for all animals tested.

Total-N and AEP-N data of mixed rumen protozoa and rumen contents are given in Table 7. Data presented in Table 7 were obtained from cow #3819 only because cow #3906 was defaunated three out of four periods, regardless of which diet she was fed. She had protozoa in

TABLE 6. Amino acid (AA) composition of mixed rumen bacteria from cows fed control and dried whey diets.

Amino acid	Diet	
	Control <sup>a</sup>	Whey <sup>a</sup>
	— (mg/100 mg total AA) —	
Aspartic acid	11.71	11.28
Threonine	5.11	5.04
Serine	4.34	4.08
Glutamic acid	12.66	13.07
Proline	3.44	3.56
Glycine	5.34	5.50
Alanine	7.40	7.57
Valine	6.80	6.95
Methionine	2.66	2.58
Isoleucine	5.22	5.18
Leucine	7.48	7.49
Tyrosine	5.48	5.55
Phenylalanine	5.04	5.44
Ammonia	2.38	2.36
Lysine	7.72	7.48
Histidine	2.02	1.87
Arginine	5.20	5.01

<sup>a</sup>Each value is the mean of four observations.

TABLE 7. Total-N and aminoethylphosphonic acid-N (AEP-N) of rumen contents and protozoa from cows fed control and dried whey diets.

	Diet	
	Control <sup>a</sup>	Whey <sup>a</sup>
Protozoa		
Total-N, mg/g DM <sup>b</sup>	79.70	89.40
AEP-N, mg/g DM	.14	.17
$\frac{\text{AEP-N}}{\text{Total-N}} \times 100$	.17	.19
Ether extract, % of DM	4.98	3.76
Rumen contents		
Total-N, mg/g DM	38.90	41.10
AEP-N, mg/g DM	.018	.018
$\frac{\text{AEP-N}}{\text{Total-N}} \times 100$	.046	.043

<sup>a</sup>Each value is the mean of six observations.

<sup>b</sup>Dry matter.

her rumen only during period three, while she was receiving the whey diet. Data obtained from her during that period were very similar to data in Table 7. However, only the complete set of data from cow #3819 were used for making numerical comparisons. No statistical analyses were applied to these data.

Total-N was higher in protozoa obtained when the whey diet was fed. The higher percent nitrogen in these protozoa may be associated with their lower ether extract percent. Dietary effects on percent nitrogen of these protozoa should be minimal since the roughage to concentrate ratio was similar for both diets. Previous research (10,36) showed that total-N in rumen protozoa was affected by the roughage to concentrate ratio.

Aminoethylphosphonic acid-N as a percent of protozoal-N was slightly higher with the whey diet. Similar values for AEP-N:total protozoal-N were observed by Czerkawski (32) while Dufva et al. (36) reported lower values. Dufva et al. (36) showed that this ratio was affected by the dietary ratio of roughage to concentrate, with a higher ratio being reported when a high-grain diet was fed. Less daily variation was observed with the AEP data than with the DAP data.

Previous research (36) suggested that the AEP content of rumen protozoa increased as the lipid content of the protozoa increased. Aminoethylphosphonic acid is known to be associated mainly with the lipid fraction of the protozoal cell (67). In the present study, ether extract was higher in protozoa obtained when the control

diet was fed. However, AEP content tended to be lower in these same protozoa, which is in contrast to (36). Aminoethylphosphonic acid-N as percent of total rumen-N tended to be higher with the control diet.

Protozoal-N as a percent of total rumen-N was higher with the control diet (Table 5). The decreased amount of protozoal-N in the cow fed the whey diet may be attributed to increased rumen fluid dilution rate or the higher Na content of the whey diet (61, 103).

Although some researchers (10, 86, 111) indicated that diet generally had little effect on amino acid composition of rumen protozoa, Dufva et al. (36) reported some differences when cattle were fed either a high-roughage or a high-concentrate diet. Results of the present study (Table 8) indicated that lysine was slightly higher and leucine was slightly lower in protozoa obtained from the whey fed cow. Concentrations of other amino acids were very similar for both diets.

When using DAP and AEP as microbial markers, it is critical that potential sources of error be recognized. Assuming fixed ratios of DAP-N to bacterial-N and AEP-N to protozoal-N may not be accurate assumptions. Dufva et al. (36, 37) showed that type of diet (high-concentrate vs. high-roughage) will affect these ratios. To get the most accurate estimates of these ratios, the DAP and AEP contents of rumen bacteria and protozoa, respectively, should be determined on an individual animal and diet basis. Bacterial and protozoal production should then be estimated for each individual animal and diet.



TABLE 8. Amino acid (AA) composition of mixed rumen protozoa from cows fed control and dried whey diets.

Amino acid	Diet	
	Control <sup>a</sup>	Whey <sup>a</sup>
	— (mg/100 mg total AA) —	
Aspartic acid	12.92	13.54
Threonine	4.74	4.68
Serine	4.12	3.89
Glutamic acid	14.13	14.23
Proline	3.50	3.36
Glycine	4.39	4.28
Alanine	4.40	3.82
Valine	4.70	4.38
Methionine	2.54	2.54
Isoleucine	5.75	6.04
Leucine	8.12	7.58
Tyrosine	5.30	5.35
Phenylalanine	5.74	5.76
Ammonia	1.97	1.86
Lysine	10.56	12.11
Histidine	2.10	1.94
Arginine	5.06	4.66

<sup>a</sup>Each value is the mean of two observations.

Unrepresentative sampling of rumen contents and rumen microbes may also lead to errors in estimating microbial production. More representative samples of rumen contents could be obtained by collecting small fractions from all areas of the rumen and then compositing them into presumably a more complete sample. Likewise, it may be best to collect bacteria (and possibly protozoa) from both the liquid and solid phases of the rumen, rather than just the liquid phase as is done in most studies.

Samples of rumen microbes and contents were obtained from a position immediately below the fistula in the present study. This was done in an attempt to eliminate any bias that might occur due to sampling. However, more complete representative samples of rumen contents could probably be obtained by sampling all parts of the rumen. In a sense, rumen bacteria and protozoa were collected from both the liquid and solid phase. Rumen fluid was collected by both a suction strainer technique and by squeezing the fluid from the solid digesta. It seems logical then, that some microbes associated with the solid phase would be collected. Also, samples obtained were relatively large in comparison to total rumen contents. This made it easier to get a good representative sample of rumen microbes and contents.

By combining the bacterial and protozoal data, an estimate of microbial-N as a percent of total rumen-N can be made. When cows were fed the control and whey diets, respectively, 72 and 74% of the total

rumen-N was derived from microbial-N (Table 5). Even though rumen total-N and microbial-N were similar for both diets, when the dilution rates were taken into account it appeared that more nitrogen (microbial-N) may have been carried to the lower gut when cows were fed the whey diet, since rumen fluid dilution rate was increased with the whey diet. Bacteria in the rumen of cows fed whey would probably have faster growth rates than bacteria found in cows fed the control diet. The increased fluid dilution rate observed in cows fed dried whey should promote organisms with faster growth rates. Slower growing, less efficient organisms, such as protozoa, would be flushed out of the rumen (24). The decreased protozoal-N found with the whey diet may be a reflection of this. The high level of soluble carbohydrate (lactose) in the whey ration should help to promote microbial growth. Lower rumen ammonia concentrations observed when whey was fed supported this idea.

A decrease in protozoal numbers often leads to increased bacterial numbers because of less protozoal predation on bacteria (28, 69). Results of this study suggested this to be true. Encouraged bacterial growth should be beneficial to the host animal as a protein source since bacteria are more efficient in synthesizing protein than are protozoa (146). Our results indicated that feeding whey appeared to stimulate total rumen microbial protein synthesis, particularly bacterial synthesis. The increased rumen fluid dilution rate observed with the whey diet may be an important factor affecting microbial synthesis.

### Rumen Metabolism

Rumen VFA, ammonia, and pH data are presented in Table 9. Total VFA concentration tended to be lower with the whey diet. Rogers et al. (119) and Rogers and Davis (117) suggested that higher rumen fluid dilution rates may increase the flow of substrate from the rumen, thereby decreasing the rate of fermentation which would result in decreased VFA production. The tendency for VFA concentrations to be lower with the whey diet may be partially attributed to the higher fluid dilution rate observed with this diet.

TABLE 9. Rumen volatile fatty acids (VFA), ammonia, and pH from cows fed control and dried whey diets.

	Diet		SE
	Control	Whey	
Total VFA, $\mu\text{m/ml}$	130.5	109.2	12.42
	————— (Mole %) —————		
VFA			
Acetate	43.0	43.5	1.85
Propionate	32.4	23.2**	1.75
Isobutyrate	.4	.6	--
Butyrate	16.5	24.4**	1.38
Isovalerate	2.6	2.6	.52
Valerate	5.9	6.0	.82
Ammonia, mg/100 ml	5.05	3.43	.94
pH	5.66	6.29**	.11

\*\*Different from control ( $P < .01$ ).

In agreement with others (129, 132, 133), acetate was not affected by the whey diet. In contrast, King and Schingoethe (72) reported an increased molar percent of acetate when steers consumed large amounts of dried whey. As in previous studies (18, 72, 133), molar percent of propionate was decreased with the whey diet ( $P < .01$ ). The lowered propionate may be attributed to decreased substrate availability in the rumen brought about by the increased rumen fluid dilution rate observed with the whey diet (24, 117, 118, 119).

Similar to previous studies (18, 56, 72, 132, 133, 134), molar percent of butyrate was higher with the whey diet ( $P < .01$ ). Presumably, this was due to the need to keep an oxidation-reduction balance in the rumen. The production of butyrate from acetate rids the rumen of excess hydrogen ions which may lower the pH and be detrimental to rumen microbes (125). Chalupa (24) also indicated that butyrate may be increased as the fluid dilution rate is increased. Molar percentages of isobutyrate, isovalerate, and valerate were similar for both diets.

Rumen ammonia was slightly lower when cows consumed the whey diet. Previous studies (72, 107, 132, 133) also observed lowered rumen ammonia concentrations when whey was fed. The lower ammonia concentration may indicate that more ammonia was being utilized for microbial protein synthesis, since most microbes use ammonia as their principal nitrogen source (4, 7). Since the soluble-N content of the whey concentrate was almost three times higher than that of the

control concentrate (Table 2), one might expect rumen ammonia to be higher with the whey diet. However, this was not the case, which lends more evidence to the possibility that more ammonia was being used for microbial protein production.

Rumen pH was higher when cows were fed the whey ration ( $P < .01$ ). This may be related to the high mineral content of the whey ration. Minerals in whey tend to have a buffering effect in the rumen (56, 134). Increased ruminal pH observed with the whey diet may also be partly attributed to decreased VFA production caused by an increased rumen fluid dilution rate (117, 119).

It should also be pointed out that cow #3906 had a rumen pH that was consistently low ( $\sim 5.40$ ) three out of the four periods, regardless of which diet she was fed. This may contribute somewhat to the differences in rumen pH observed between the two diets. No explanation can be given for the consistently low pH observed with this cow.

#### Feed Intake and Body Weights

Feed intake, body weights, and body weight changes are listed in Table 10. Dry matter intake of both rations was less than 3% of body weight, but was more than adequate to meet these cow's nutritional needs since the diets were high in net energy and the cows were in late lactation. There was a tendency for cows to consume less dried whey ration during the last two periods of the trial. Hot weather during these two periods could have contributed to the

decreased feed intake. Possibly, the dried whey may lose some of its palatability during hot weather. Flies are also more of a problem when feeding whey during hotter weather (128). Cows tended to gain weight when fed the whey diet, whereas a slight loss in body weight was observed when cows consumed the control diet.

TABLE 10. Body weights (BW) and dry matter (DM) intakes of cows fed control and dried whey diets.

	Diet	
	Control	Whey
DM intake, kg/day	16.4	15.3
DM intake, % BW	2.91	2.67
Avg BW, kg	566	574
Weight change, kg/day	-.03	+.41

## SUMMARY AND CONCLUSIONS

Results of this study indicated that rumen microbial protein synthesis was stimulated when cows were fed a ration containing about 38% dried whole whey. Bacterial production was increased and protozoal production was slightly decreased when the whey ration was fed. The high level of soluble carbohydrate (lactose) in dried whole whey provided an excellent fermentable energy source to the microbes. Lowered rumen ammonia concentrations with the whey diet suggested that more ammonia was being used for microbial protein production.

Rumen fluid dilution rate was increased with the whey diet. This was probably due to the higher mineral (especially Na) content of this diet. An increased fluid dilution rate is beneficial to the host animal because fermentation products, including microbial cells, will be carried to the lower gut at a faster rate. Thus, growth rates of rumen microbes are increased in order to compensate for the faster dilution rate.

The fermentation of whey in the rumen resulted in increased proportions of butyrate and decreased proportions of propionate. Other volatile fatty acids were not affected. Rumen pH was higher with the whey diet, possibly as a result of the buffering effect exhibited by the whey minerals.

Dry matter intake of the whey diet was similar to that of the control diet. Cows tended to gain weight with the whey diet, whereas a slight loss in body weight was observed with the control diet.



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