DL-methionine as a winter supplement for gestating cows grazing native range
by David William Lodman

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University

Abstract:
Two grazing studies (Trial 1; 1986 and Trial 2; 1987) were conducted during consecutive winters to determine the replacement value of DL-methionine for soybean meal as a protein supplement fed gestating beef cows. Approximately sixty crossbred cows grazed native foothill range for 75 days each winter. Cows were assigned to one of three supplement groups consisting of, no supplement (CON); methionine in beet pulp carrier (BPM) .5 kg/day; or soybean meal (SBM) .4 kg/day. The BPM supplement was formulated to supply an average of 8 g/day of methionine. The supplements were fed individually on alternate days. The CON treatment experienced ($P < .1$) the greatest body weight loss in both trials and lost more ($P < .05$) body condition during Trial 1. Cows receiving BPM showed less weight and body condition loss than CON which was probably due to changes in ruminal function, however the magnitude of these changes were less than those experienced by cows receiving SBM. Blood samples were obtained on consecutive days twice during each trial (45 days and 25 days prepartum) and analyzed for blood urea nitrogen, total bilirubin, creatinine, albumin, total protein, and cholesterol. Blood urea nitrogen (BUN) showed a treat x period interaction in which BPM had the lowest value which did not differ between periods. Neutral detergent fiber (NDF) disappearance rate was faster ($P < .05$) for SBM than BPM. During Trial 2 whole ruminal purine concentrations had a treatment x hour interaction indicating SBM maintained higher purine concentration than BPM. Ruminal measurements used to ascertain the mechanisms by which supplementation effects ruminal function using a principal component analysis were liquid and particle bound carboxymethylcellulase (CMCase) activity, whole, liquid, and particle bound purine concentrations, in situ NDF disappearance, pH, fluid and particle dilution rates and ammonia concentrations. The first factor accounting for the greatest variation was composed of NDF disappearance, particle dilution rate and CMCase activity in liquid fraction. Second factor consisted of particle bound purine concentration and bound CMCase activity and the third factor consisted of whole rumen and liquid purine concentration, and ammonia. Supplementation has the greatest effect on NDF disappearance and particle dilution rates, which were influenced by increased enzymatic activity. Supplementation increases overall microbial density in the rumen. This effect has little influence on cellulose digestion. The effect of supplementation appears to occur via increases of cellulolytic activity. These findings would indicate alternate day supplementation of methionine may not be sufficient for full substitution of methionine for soybean meal.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Two grazing studies (Trial 1; 1986 and Trial 2; 1987) were conducted during consecutive winters to determine the replacement value of DL-methionine for soybean meal as a protein supplement fed gestating beef cows. Approximately sixty crossbred cows grazed native foothill range for 75 days each winter. Cows were assigned to one of three supplement groups consisting of, no supplement (CON); methionine in beet pulp carrier (BPM) .5 kg/day; or soybean meal (SBM) .4 kg/day. The BPM supplement was formulated to supply an average of 8 g/day of methionine. The supplements were fed individually on alternate days. The CON treatment experienced ($P < .1$) the greatest body weight loss in both trials and lost more ($P < .05$) body condition during Trial 1. Cows receiving BPM showed less weight and body condition loss than CON which was probably due to changes in ruminal function, however the magnitude of these changes were less than those experienced by cows receiving SBM. Blood samples were obtained on consecutive days twice during each trial (45 days and 25 days prepartum) and analyzed for blood urea nitrogen, total bilirubin, creatinine, albumin, total protein, and cholesterol. Blood urea nitrogen (BUN) showed a treat x period interaction in which BPM had the lowest value which did not differ between periods. Neutral detergent fiber (NDF) disappearance rate was faster ($P < .05$) for SBM than BPM. During Trial 2 whole ruminal purine concentrations had a treatment x hour interaction indicating SBM maintained higher purine concentration than BPM. Ruminal measurements used to ascertain the mechanisms by which supplementation effects ruminal function using a principal component analysis were liquid and particle bound carboxymethylcellulase (CMCase) activity, whole, liquid, and particle bound purine concentrations, in situ NDF disappearance, pH, fluid and particle dilution rates and ammonia concentrations. The first factor accounting for the greatest variation was composed of NDF disappearance, particle dilution rate and CMCase activity in liquid fraction. Second factor consisted of particle bound purine concentration and bound CMCase activity and the third factor consisted of whole rumen and liquid purine concentration, and ammonia. Supplementation has the greatest effect on NDF disappearance and particle dilution rates, which were influenced by increased enzymatic activity. Supplementation increases overall microbial density in the rumen. This effect has little influence on cellulose digestion. The effect of supplementation appears to occur via increases of cellulolytic activity. These findings would indicate alternate day supplementation of methionine may not be sufficient for full substitution of methionine for soybean meal.

CHAPTER 1

INTRODUCTION

Protein supplementation of cows grazing native winter range is a common practice in areas where standing forage is accessible. However, cows in these environments usually do not consume sufficient quantities of protein and energy to meet NRC (1984) requirements (Allison, 1985). The goal of supplementation has been to add nutrients deficient in the gestating beef cow's diet. In vitro digestibility of range forage is typically low with reported values of between 40 and 50 % digestible dry matter (Rittenhouse et al., 1970; Kartchner, 1981), however Miner and Petersen (1989) reported DM digestibilities of 62 to 65 % for winter forage. This variation suggests there are many factors which influence the digestibility of mature forage. These factors may include environmental effects of the previous or current year such as drought, time of forage intake, supplementation and others. Protein supplementation has been shown to increase digestibility of mature forages (Rittenhouse et al.,1970), which indicates a ruminal effect via microflora. The mechanisms explaining changes in forage digestion are not fully understood.

Ruminal microbial population may have specific nutrient requirements for optimal growth. Low quality forages may not satisfy these needs and subsequently restrict microbial growth. Cellulolytic bacteria have been shown to use ammonia for most of their N

requirements (Al-Rabbat et. al., 1971). However, Kropp et al. (1976) demonstrated decreased nitrogen retention and forage digestibility as soybean meal was replaced by urea in steers fed prairie hay. Since microbial yield is not increased with increasing ammonia concentrations above 5 mg NH_3-N/dl (Satter and Slyter, 1974), this indicates soybean meal must be supplying growth factors other then NH_3 to stimulate microbial growth. Maeng and Baldwin (1976) showed additions of amino acids improved ruminal microbial growth rate. Gil et al. (1973) using urea as a N source found the addition of DL-methionine accelerated bacterial nitrogen incorporation and concurrent cellulose digestion rate. DL-methionine has also been shown to be the limiting amino acid for ruminal bacterial growth on diets low in protein (Salter et. al., 1979). Arambel et. al. (1987) found efficiency of bacterial nitrogen synthesis was improved 40 % with dairy cattle fed methionine. Methionine was also shown to increase dry matter, organic matter and crude protein degradation when given orally to dairy cattle in a liquid or protected form (Huisman et. al., 1988). DL-methionine and urea in a beet pulp carrier increased ruminal NDF fermentation rate 30% over either a soybean meal or an inorganic sulfur, urea and beet pulp supplement with similar weight gains to soybean meal supplemented heifers. The objectives of this study were to determine if DL-methionine could replace soybean meal as a supplement for cows grazing winter range and to interpret the effects of protein supplementation on ruminal function using principal components.

CHAPTER 2

LITERATURE REVIEW

Cellulose is one of the most abundant organic material in the world. However, it cannot be directly utilized by mankind as a food source. The ruminant animal is able to utilize this resource and convert cellulose into products usable by man, through a synergistic relationship with microorganisms residing in the rumen. The ruminant supplies a suitable environment for microbial growth and reproduction by regulating temperature, pH and supplying needed substrate and nutrients. The microbes in return digest the fibrous plant material which the animal would not be able to utilize otherwise. This digestion of plant material releases additional nutrients to the host for its own use.

Mechanisms of Fiber Digestion

The organic makeup of the plant cell wall consists of cellulose, hemicellulose, pectin, lignin and small amounts of bound protein (Akin and Barton, 1983). Cellulose and hemicellulose in the cell wall are two of the major carbon sources which are transformed into metabolizable forms for the animal in the fermentation process. Microbes produce the enzymes cellulase and hemicellulase which breakdown this fibrous material into volatile fatty acids, and these acids are utilized by the host animal (Smith et al., 1972; Leatherwood, 1973).

Fiber digestion is a complicated process which has been partitioned into distinct actions. Mertens (1977) describes these components as: rate of digestion, digestion lag, extent of digestion, and passage rate of the fibrous feed. These four components have proven useful in describing the integral relationships between the forage, animal and rumen microbial population.

Digestion rate is related to extent of digestion. Factors that effect rate are those inherent to the cell wall and those that affect the microbial population. Digestion rate has a significant effect on dry matter digestibility especially in plants having high cell wall contents (Mertens,1977). Smith et al. (1972) correlated the rate of digestion to cell wall components and found the highest relationship between rate and soluble dry matter. Lignin, while affecting the proportion of undigestible fiber, doesn't affect the digestion rate (Waldo et al.,1972; Mertens,1977). Since the enzymes responsible for forage degradation are produced by the microorganisms within the rumen, it would be possible to assume that factors which affect the microbial population would also alter the rate of digestion (Mertens and Ely, 1982). Investigators (Akin,1979; Akin and Barton, 1983; Goetsch and Owens,1987) have shown microorganisms attack different structures of the cell walls at different rates. Thus the composition, structural characteristics and the relative amounts of cell wall components will affect digestion rate. Mesophyll is the most rapidly attacked and degraded tissue while bundle sheaths and epidermis are slower to be degraded. The sclerenchyma is a ligninified tissue which is usually undegraded (Akin and Barton, 1983). Also, compounds found in the cell

wall, such as phenylpropanoids and tannins depress microbial activity (Hoover, 1986; Jung, 1985).

Lag time is a complicated process which can be caused by the physical characteristic of digestion as well as microbial factors. Some of the physical characteristics which determine lag time are ingestion of air, temperature of feed and water and changes in osmotic pressure (Varga, 1986). Hydration of feed may also play a significant role in lag time (Mertens and Ely, 1982). Forages differ in their rate of hydration which alters the time it takes for enzymatic degradation to begin. It is possible hydration causes a swelling of tissues which allows microbial attachment and enzymatic degradation (Mertens and Ely, 1982). Another important cause of lag time is microbial attachment to fiber particles. Akins and Barton (1983) stated that electron microscopic observations indicated rumen bacteria must directly adhere, or be close to plant cell walls before digestion occurs. Adhesion is the first step in attachment of microbes to forage particles and is effected by pH, surface charge and ionic composition of the media and substrate. Particle size , temperature, substrate, and the growth phase of the microorganisms can also affect adhesion (Varga, 1986).

The amount of mastication of forages will also affect lag time. Microorganisms primarily attack forage particles when cuts or shredded edges occur (Cheng et al., 1977). Pond et al.(1984) showed mastication decreased particle size which exposed more surface area and digestible tissue to microbial degradation. Mastication also disrupted the protective nature of intact plant tissues, which when left intact

increased lag time by resisting microbial attachment and enzyme accessibility to the plant's digestible fractions .

Another factor which might affect lag time is the status of the microbial population in the rumen. If the microbial population is in the rapid growth phase then other factors such as those mentioned above will have greater affect. However, if the bacterial population is in a maintenance state the ratio of cellulolytic bacteria to substrate is low, thus requiring a period of time for growth in microbial number to acheive maximal fiber digestion (Mertens, 1987). Availability of other substrates such as starch (El-Shazly et al.,1961; Mertens and Lofton, 1980) can increase lag time due to a competition among microbes for essential nutrients.

Extent of digestion characterizes the proportion of the plant cell wall that is digestible. Bull et al. (1979) stated the effect of rumen turnover rate on total digestion depends on feed composition and physical form. When turnover time is increased total fiber digestion is usually reduced. There are many components of plant material that can affect the extent of digestibility. Lignin content is one of the major contributors to the undigestible fraction of forage (Van Soest, 1982; Hoover, 1986; Mertens, 1987). There are also fractions of cellulose which are undigestible. Lippke et al. (1986) used indigestible NDF as an internal marker. This indicates there is a consistent portion of NDF that is unavailable for digestion.

Other compounds found in association with fibrous feed can also limit or affect the digestibility of the forage as reviewed by Van Soest (1982). These include the phenylpropanoid substances such as

flavones, coumarins, tannins and isoflavones. These substances inhibit digestion by either interfering with animal metabolism or by inhibiting rumen microorganisms. Varel and Jung (1986) found that phenolics inhibit digestibility of cellulose and xylan by affecting attachment of fibrolytic bacteria to fiber particles. Terpenes such as essential oils, saponins, steroids, latex and rubber can be toxic to animals or rumen bacteria. Cutin and other lipid compounds in plants form protective barriers and are indigestible and also prevent microbes from attacking other digestible parts of the forage. Silica has also been shown to decrease digestion of forage and is usually associated with the lignin fraction (Van Soest, 1982).

Mertens and Ely (1982) using a computer model suggested that the fraction of fiberous feeds that is undigestible accounts for differences in digestibility and intake between forages. Since intake is partly a function of rumen volume, particle size and passage rate, it would be logical to assume a more digestible forage would increase intake of that forage.

Passage rate is the final component affecting digestibility of forages. The two major factors influencing passage rate are intake and particle size (Mertens, 1977). Particle size is a function of mastication, rumination, and digestion. In ruminants fed forage diets particle size can range from 200 to 1200 um. Particles size must be <1200 um for them to escape the rumen (Martz and Belyea, 1986). Smith et al. (1983) found a mean size of 5.3 u escaped the rumen from wethers fed orchard grass pellets and dosed with carbon-14 labeled cell walls of different particle sizes. Although particle size is important in

movement out of the rumen, it doesn't seem to be the rate limiting step (Galyean, 1987). Martz and Belyea (1986) proposed passage is a function of flow associated with contractions of rumen compartments and the consistency of the digesta.

Increased level of intake and addition of coarse fiber to rations have been shown to increase ruminal liquid turnover (Bull et al., 1979; Firkins et al., 1986). This increase in liquid turnover rate caused by increased intake effects the efficiency of microbial yield. Owens and Isaacson (1977) found accelerating fluid dilution rate will increase the efficiency of bacterial protein snythesis.

Rumen Microorganisms

Ruminal microflora consists of a large and diverse population of microorganisms. The major role of these organisms is fermentation of cellulose and hemicellulose into products usable by the host animal. Bacteria seem to be most important in cellulose digestion, but protozoa and fungi also play roles.

Although there are numerous species of bacteria in the rumen, it has been recognized that certain species are primary fiber degraders (Table 1). These are Bacteroides succinogenes, Ruminococcus albus, Ruminococcus flavefaciens (Akin, 1986; Bryant, 1973). These three bacteria, which are usually physically associated with fiber particles, are present in high concentrations and have enzymes that attack structural carbohydrates (Hungate 1966). Bacterial enzymes consist of several different cellulase proteins which act in combination to degrade cellulose (Groleau and Forsberg, 1981). Although some extracellular digestion does occur (Akin and Barton, 1983), most

cellulolytic enzymes are cell bound which requires physical association of bacteria to the cell wall. Other bacteria species such as Butyrivibrio fibrisolvens and Lachnospira multiparus do not have cellulytic ability but do contain enzymes that degrade hemicellulose and pectins (Table 1). These bacteria also have been shown to facilitate degradation by cellulytic bacteria and be significant in the degradation of more easily digested fiber (Akin, 1986).

Another classification (Hungate, 1966) of bacteria in the rumen are mainly starch digestors. Although a few strains of cellulytic bacteria can digest starch, the major amylolytic bacteria are non-cellulolytic (Table 1). Strains of Bacteroides ruminicola, Bacteroides amylophilus, Selenomonas ruminantium and Streptococcus bovis are a few of the species involved in this group (Russell, 1983).

Proteolytic bacteria are also found in the rumen. Many species of bacteria have proteolytic action but Cotta and Hespell (1986) stated the most important protein degrading species are Bacteroides amylophilus, Bacteroides ruminicola and Butyrivibrio fibrisolvens (Table 1).

There have been a large number of ruminal bacteria identified (over 200), however only a few species have been shown to play a major role in rumen fermentation. Many of these bacteria have complex nutrient requirements and substrate preferences (Russell, 1983). A summary of these major bacteria and substrates is included in Table 1. However, even though only a few species have been singled out, one should be careful when assessing the significance of other rumen species. Some species even if a minor part of the total microbial

population can be important, especially if they have some ecological impact on other species (Hungate, 1966).

Protozoa are also found in large numbers in the rumen (Hungate, 1966). However their role in fiber fermentation is not clear. Ciliated protozoa have been shown to attack and consume plant cell walls but they are not essential for cell wall degradation (Akin and Amos, 1975). Protozoa numbers usually increase rapidly after a meal when legume hay is fed. This increase is due to rapid availability of nutrients supplied by the legume that allows the protozoa to attach to fiber particles (Murphy et al., 1985). Protozoa actively ingest bacteria and use them as a protein source. The presence of protozoa in the rumen is responsible for bacterial turnover amounting to 20-50% of bacterial N (Tamminga, 1979). In defaunated sheep digestibility of straw was decreased and particle passage rate was nearly doubled. This decreased fiber digestion observed after defaunation was attributed to both a decrease in degrading activity of rumen microorganisms and a decrease in rumen retention time (Kayouli et al., 1983/84). Demeyer and Van Nevel (1986) summarized protozoal action is determined by the presence of spaces provided by roughage diets. These protozoa depress microbial N yield but allow more complete fiber digestion.

Fungi are the most recently discovered ruminal microorganisms and were originally thought to be flagellated protozoa (Akin, 1987). Several studies have established fungi are definitely able to digest fiber in the rumen (Bauchop, 1979). Fungi seem to colonize areas of lignified vascular tissues more readily than soft leafy tissue low in fiber. This would indicate fungi might play a substantial role in

TABLE 1. DEGRADATION AND UTILAZATION OF SUBSTRATE BY RUMEN BACTERIA

	Substrate		
Bacteria	Hydrolyzes cellulose	Ferments cellulose	Ferments cellobiose
Ruminococcus albus	+(1)(16)	+(1)(16)	+(2)(16)
Ruminococcus flavefaciens	+(1)(2)(4)	+(1)	+(2)
Bacteroides succinogenes	+(1)(2)(4)	+(6)	+(2)
Butyrivibrio fibrisolvens	-	+(1)	+(2)
Bacteroides ruminicola	-	-	+(2)
Bacteroides amylophilus	-	-	-
Selenomonas ruminantium	-	+(6)	+(2)
Streptococcus bovis	-	-	+(2)
Megasphaera elsdenii	-	-	-
Lachnospira multiparus	-	-	+(2)
Succinomonas amylophilus	-	-	-
Eubacterium ruminantium	-	-	+(2)

TABLE 1. Continued

Bacteria	Substrate		
	Hydrolyzes hemicellulose	Ferments xylan	Ferments pentose
Ruminococcus albus	+(3)(16)	+(5)(16)	-
Ruminococcus flavefaciens	+(3)	+(5)	-
Bacteroides succinogenes	+(3)	-	-
Butyrivibrio fibrisolvens	+(3)	+(7)	+(3)
Bacteroides ruminicola	+(3)	+(7)	+(3)
Bacteroides amylophilus	-	-	-
Selenomonas ruminantium	-	+(5)	+(3)
Streptococcus bovis	-	-	-
Megasphaera elsdenii	-	-	-
Lachnospira multiparus	-	-	-
Succinomonas amylophilus	-	-	-
Eubacterium ruminantium	-	+(7)	+(3)

TABLE 1. Continued

Bacteria	Substrate		
	Hydrolyzes starch	Ferments dextrin	Ferments maltose
Ruminococcus albus	-	-	-
Ruminococcus flavefaciens	-	-	-
Bacteroides succinogenes	-	+(8)(9)	+(8)
Butyrivibrio fibrisolvens	+(8)	+(8)(9)	+(8)
Bacteroides ruminicola	+(8)	+(8)(9)	+(8)
Bacteroides amylophilus	+(8)	+(8)(9)	+(8)
Selenomonas ruminantium	+(8)(10)	+(8)(9)	+(8)
Streptococcus bovis	+(8)	+(8)(9)	+(8)
Megasphaera elsdenii	-	-	+(8)
Lachnospira multiparus	-	-	-
Succinomonas amylophilus	+(8)	+(8)(9)	+(8)
Eubacterium ruminantium	-	+(8)(9)	+(8)

TABLE 1. Continued

Bacteria	Substrate		
	Hydrolyzes pectin	Ferments pectin	Ferments glycerol
Ruminococcus albus	+(11)	-	-
Ruminococcus flavefaciens	+(11)	-	-
Bacteroides succinogenes	+(11)	+(11)	-
Butyrivibrio fibrisolvens	+(11)	+(11)	-
Bacteroides ruminicola	+(11)	+(11)	-
Bacteroides amylophilus	-	-	-
Selenomonas ruminantium		+(14)	
Streptococcus bovis	+(12)	-	-
Megasphaera elsdenii	-	-	+(10)
Lachnospira multiparus	+(11)(13)	+(11)(13)	-
Succinomonas amylophilus		-	
Eubacterium ruminantium		-	

TABLE 1. Continued

Bacteria	Substrate		
	Ferments (15) sucrose	Ferments (15) fructose	Ferments (15) glucose
Ruminococcus albus	-	-	-
Ruminococcus flavefaciens	-	-	-
Bacteroides succinogenes	-	-	+
Butyrivibrio fibrisolvens	+	+	+
Bacteroides ruminicola	+	+	+
Bacteroides amylophilus	-	-	-
Selenomonas ruminantium	+	+	+
Streptococcus bovis	+	+	+
Megasphaera elsdenii	+	+	+
Lachnospira multiparus	+	+	+
Succinomonas amylophilus	+	+	+
Eubacterium ruminantium	+		+

TABLE 1. Continued

Bacteria	Substrate: Ferments Amino acids	Substrate: Incorporates Amino acids
Ruminococcus albus		+(17)
Ruminococcus flavefaciens		+(17)
Bacteroides succinogenes		+(17)
Butyrivibrio fibrisolvens		+(17)
Bacteroides ruminicola	-	+(17)
Bacteroides amylophilus	-	+(17)
Selenomonas ruminantium	+(18)	+(17)
Streptococcus bovis	+(18)	+(17)
Megasphaera elsdenii	+(18)	+(17)
Lachnospira multiparus	+(18)	+(17)
Succinomonas amylophilus		
Eubacterium ruminantium		+(17)

TABLE 1. Continued

Bacteria	Substrate: Hydrolizes protein	Substrate: Ferments peptides
Ruminococcus albus	-	-
Ruminococcus flavefaciens	-	-
Bacteroides succinogenes	-	-
Butyrivibrio fibrisolvens	+(9)(21)	+(18)(21)
Bacteroides ruminicola	+(9)(20)	+(18)
Bacteroides amylophilus	+(19)	+(18)
Selenomonas ruminantium	+(9)	+(18)
Streptococcus bovis	+(9)	+(18)
Megasphaera elsdenii	-	+(18)
Lachnospira multiparus	+(9)	-
Succinomonas amylophilus		-
Eubacterium ruminantium	+(9)	+(18)

Table 1 Continued (footnotes)

(1) Bryant M.P., 1973
(2) Russell J.B., 1983
(3) Dehority B.A. 1973
(4) Pettipher G.L. and Latham M.J., 1979
(5) Coen J.A. and Dehority B.A., 1970
(6) Scheifinger C.C. and Wolin M.J., 1973
(7) Dehority B.A., 1965
(8) Hungate R.E., 1966
(9) Hamlin L.H. and Hungate R.E., 1956
(10) Bryant M.P.,1965
(11) Dehority B.A., 1969
(12) Gradel C. and Dehority B.A., 1972
(13) Bryant M.P., et al., 1960
(14) Harfoot C.G., 1978
(15) Russell J.B. and Baldwin R.L., 1978
(16) Halliwell G. and Bryant M.P., 1963
(17) Satter L.D., 1975
(18) Bryant M.P. and Robinson I.M., 1963
(19) Blackburn T.H., 1968
(20) Hazelwood G.P., et al., 1981
(21) Cotta M.A. and Hespell R.B., 1986

fiber digestion especially with diets high in fiber and cell wall tissue (Akin and Rigsby, 1987). Wood et. al. (1986) isolated a highly active extracellular cellulase from the anaerobic rumen fungus Neocallimastix frontalis, which was several times more active than other cellulases. However, the interactions involved in fungal degradation are complex and not fully understood (Akin, 1987).

Nutritional Interactions of Rumen Microorganisms

The nutritional requirements of rumen microorganisms are complex. Bryant (1973) reviewed the requirements of cellulolytic bacteria. The main cellulolytic bacteria Bacteroides succinogenes and Ruminococcus albus require ammonia as their major source of nitrogen (Table 1). Ammonia is the most abundant nitrogen compound in the rumen which is available for microbial growth and between 50 and 78% of bacterial

nitrogen is derived from it (Hespell and Bryant, 1979). Some bacteria cannot utilize ammonia and must receive their nitrogen from other sources such as amino acids or peptides.

Amino acid degradation by rumen bacteria was investigated by Chalupa (1976). He found specific amino acids are degraded at different rates and there are interactions between specific amino acids. However free amino acids with the exception of methionine cannot escape rumen degradation. Scheifinger et al. (1976) found methionine was unique in that it was produced by members of the Megosphaera, Eubacterium and Streptococcus families while being degraded by others. Therefore the type and amount of certain bacteria present in the rumen may influence the utilization rate of methionine and available pool size. Nader and Walker (1970) using radio-labeled methionine found at most only 11% of methionine in the free pool was incorporated unchanged into microbial protein. Gil et al. (1973) reported the addition of methionine or its analog increased rumen fermentation the first 18 h with starch and 24 h with cellulose when compared to the addition of inorganic sulfate. Other investigators, when studying supplemention of mixed amino acids and urea, found the optimum ratio to be 75% urea to 25% amino acids (Maeng et al.,1976). The microbial growth rate was doubled and the mean doubling time was halved by the addition of 18 amino acids substituted for 25% of the urea (Maeng and Baldwin, 1976).

Branched chain C4 and C5 fatty acids (isobutyrate, valerate, isovalerate, and 2-methyl buturate) are required by the predominate cellulolytic bacteria (Bryant, 1973). These acids are required for

biosynthesis of branch chain amino acids or higher fatty acids (Russell, 1983). Gorosito et al. (1985) found an increase in bacterial growth and cell wall digestion with the addition of C4 and C5 fatty acids in an artificial medium. The addition of branched chained fatty acids or amino acids has shown an increase in digestion of barley straw and to a lesser extent alfalfa (Mir et al., 1986). Mir determined that alfalfa diets have the potential to provide branch chained fatty acids and supplementation of these products would not satisfy a limitation to optimal microbial yield. It has been shown there is a nutritional interdependence among rumen bacteria for branch chained fatty acids and amino acids. Miura et al.(1980) showed when grown on starch and ammonia, Bacteroides amylophilus synthesizes branched-chain amino acids and incorporates them into protein and upon death it lysis releasing these branched-chain amino acids which can be deaminated to branch chained-chain fatty acids by Megasphaera elsdenii (Table 1). The branched-chain acids produced will then be used by Ruminococcus albus and other bacteria for their growth (Table 1).

Protein Supplementation

Protein supplementation of cows on grazing low quality winter forage has been a common practice for many years (Clanton 1982). Nitrogen supplementation is practiced to improve profit of livestock production by enhancing the utilization of grazed forage (Petersen, 1987). Supplementation with appropriate nitrogen-containing compounds can produce positive associative effects. These effects seem to be greatest when forage quality is poor and protein content of forage is below 8 to 10% crude protein (Clanton, 1981; Allison, 1985; Branine

and Gaylean, 1985).

Supplementation may satisfy minimum requirements for nitrogen, specific amino acids or carbon chains (Petersen, 1987). Clark (1985) found the amino acids methionine, arginine, and cysteine increased both DM and NDF fermentation rate. Maeng and Baldwin (1976) showed the addition of amino acids improved ruminal microbial growth rates. Methionine has also been shown to increase ruminal bacterial growth (Salter et. al., 1979; Arambel et. al., 1987) and ruminal fermentation rates (Gil et. al., 1973; Wiley et. al., 1988; Clark and Petersen, 1988; Huisman et. al., 1988).

Allison (1985) indicated the effect of supplements on intake and digestibility probably involves increased ruminal microbial activity. Protein supplementation has been shown to increase forage digestibility and intake (Rittenhouse et. al., 1970: Seibert and Hunter, 1981). For a more complete review of supplementation effects on intake and digestibility see Miner (1986).

Protein supplementation may also affect rumen dynamics and the flow of nitrogen and non-nitrogen containing compounds from the rumen (Petersen, 1987). Numerous studies (McCollum and Galyean, 1985; Clark and Petersen, 1988; Caton et. al. 1988: Miner et. al, 1989) have shown increased fluid and particle dilution rates with protein supplementation.

Protein supplementation may also satisfy animal protein requirements for quantity and quality of protein reaching the small intestine (Petersen, 1987). This could be accomplished through increased microbial production or from feed protein. As stated above,

protein supplementation has been shown (as stated above) to enhance microbial growth and increase dilution rates from the rumen. Therefore it would seem plausible that a combination of these two factors would culminate in increasing microbial protein to the small intestine. Ruminal escape protein plus soybean meal has been shown to reduce pregnant-cow weight loss and increase fermentation more than did a soybean meal supplement (Miner and Petersen, 1989). This would suggest that gestating cows may need more or higher quality protein in the small intestine than microbial protein can supply.

CHAPTER 3

MATERIALS AND METHODS

Animals

Two winter trials were conducted at the Red Bluff Research Ranch, Norris, Montana. Trial 1 began December 23, 1986 and ended March 1 1987. Trial 2 began December 26, 1987 and ended March 3, 1988. Cows for Trial 1 consisted of pregnant 3- to 8-yr-old crossbred cows (n = 48). Cows were randomly allocated within age and expected calving date for the supplemented groups except for 5-yr-olds which were assigned to the non-supplemented treatment. Miner et. al. (1988) found no effect due to age (between 4 to 9 yr) for the measurements reported in this study. This selection method had to be applied due to constraints placed upon the study by pasture size and compatibility with other research. Trial 2 cows were pregnant 4- to 8-yr-old crossbred cows (n = 42). Animals were randomly selected within age group and expected calving date from the ranch herd of 150 cows. Cows selected for the studies were restricted to those expected to calve within the first half of the calving season between March 6 and March 30 of each year. Twelve rumen cannulated cows were also selected from the cannulated cows present on the ranch and included in each trial. Genotypes for all cows consisted of varying percentages of Hereford, Angus and/or Tarentaise. Preceding the trials cows grazed native range and did not receive any protein supplements.

Pasture

Both trials were conducted on the same 324 ha native range pasture with north-facing, slight to moderate slopes, and areas of steep ravines and rock outcrops. Elevation of the pasture ranged from 1400 to 1900 m with annual precipitation between 350 to 406 mm. This pasture was classified by the Soil Conservation Service in 1980 as a silty range site in good condition with vegetation composed of 65% grasses and 35% forbs and woody species. Dominant grasses include Agropyron spicatum, Stipa comata, Festuca idahoensis and Elymus cinereus. Estimated carrying capacity was 1.2 ha per animal unit month (USDA-SCS, 1976).

Samples of grazed forage were collected using total rumen evacuation technique (Lesperance et. al., 1960) from two cows grazing with the experimental herd. After the initial rumen evacuation these cows were allowed to graze for 1 h and forage consumed was evacuated and freezed dried. Composited samples of the forage were 5.0% crude protein and 63% neutral detergent fiber (NDF) for Trial 1 and 4.3% crude protein and 68.8% NDF for Trial 2.

Climate

The average median daily temperature and total precipitation for Trial 1 were -2.2½ C and 9.4 mm during January, and 1.3½ C and 6.1 mm during February (National Oceanic and Atmospheric Administration, 1987). The average daily temperature and total precipitation for Trial 2 were -3.9½ C and 2.5 mm during January, and 1.1½ C and 5.1 mm during February (National Oceanic and Atmospheric Administration, 1988).

Treatments

Cows were randomly allocated to one of three treatment groups. Four rumen cannulated cows were also randomly assigned to each treatment. However one cannulated cow during Trial 2 was determined to be ill and removed from the trial. Both trials utilized the same treatment groups which included: control (CON) which received no supplement, methionine in beet pulp carrier (BPM), and soybean meal (SBM). Supplements (Table 2) were formulated to be isocaloric but not

TABLE 2. WINTER SUPPLEMENT FORMULATION PERCENTAGES ON AN AS FED BASIS

	Supplements[a]			
Ingredient	BPM		SBM	
	Trial 1	Trial 2	Trial 1	Trial 2
	----------%		----------	
Beet pulp	79.2	78.9	-	-
Soybean Meal	-	-	82.1	81.8
DL-methionine	3	3.3	-	-
Dicalcium phosphate	9.65	9.65	9.2	9.2
Calcium sulfate	-	-	3	3.3
Potassium chloride	3	3	.55	.55
Vitamin A[b]	.1	.1	.1	.1
Molasses	5	5	5	5
Molasses booster[c]	.05	.05	.05	.05
Supplement fed Kg/hd/d	.5	.5	.4	.4
Protein	8.9	8.8	36.8	36.8
TDN	85	81	85	81

[a] BPM = methionine, and SBM = Soybean meal.
[b] vitamin A 15000 IU.
[c] Feed Flavours, inc.

isonitrogenous (BPM = 8.9%; 8.8% protein, SBM = 36.5%; 36.5% protein for Trial 1 and Trial 2, respectively). Supplements were similar between trials except BPM was formulated to supply 7g/h/each day of DL-methionine in Trial 1 and 9g/h/each day in Trial 2. SBM supplied 1.5g/h/each day of L-methionine in each trial. These supplements were

designed to provide an extreme in protein nutrition in order to minimize experimental confounding of N intake with DL-methionine intake. At the initiation of both trials cows were injected I.M. with 2,000,000 IU of vitamin A. Animals also had free access to loose iodized salt mixture. Supplements contained an additional source of vitamin A, dicalcium phosphate, and potassium chloride. In addition, the SBM supplement contained calcium sulfate to provide an equal amount of sulfur in the supplements. Supplements were fed individually on alternating d at approximately 1200 h. Cows received supplement to provide BPM at.5 kg/h/each day and SBM at .4 kg/h/each day. Cows in the CON group were treated similarly to the supplemented cows.

Measurements

Cow Weights, Condition Scores and Calf Birthweights

Palpable condition scores (1 to 10; Bellows et. al., 1971) by two technicians and two consecutive days body weights were recorded at the start and finish of each trial. For Trial 1 cows were limit fed hay and had access to water for 24 h before each weight. Trial 2 cows were immediately weighed upon entering scale pen from pasture and weighed again 12 h later. They did not receive any feed between weights. The difference in weighing procedures was an effort to minimize weighing error between days. Initial mean body weight and condition score were 539 kg and 4.2 for Trial 1 and 548 kg and 5.1 for Trial 2, respectively.

In situ Disappearance

To adequately determine the effects of supplementation, parameters

other than body weight and condition score are needed. An in situ digestion trial was initiated the last 4 d of each study to measure ruminal NDF disappearance rate. Nylon bags were incubated as described by Orskov (1982). Each bag contained a 3 g sample, ground in Wiley mill through a 2 mm screen, representative of forage obtained by total rumen evacuation. Bags were constructed from nylon mesh fabric of 44 Ìm pore size and were double zig-zag stitched with polyester thread. Finished bags had 533 cm^2 of surface area. Bags containing no sample (blanks) were also incubated to adjust for flow of ruminal contents into the bags. All bags were suspended in the rumen on a 75 cm circular stringer composed of tygon tubing, bath plug chain, and fishing swivels. The stringer was then placed in a 35 x 50 cm fishnet bag tied with 150 cm nylon cord attached to the cannula. This large bag was used to facilitate removal of the entire stringer from the rumen. Trial 1 bags were suspended in the rumen 4 h prior to supplementation at 0800. Trial 2 bags were suspended just prior to supplementation at approximately 1200. At 0, 4, 8, 12, 24, 36, 48, 72, and 96 h post supplementation for Trial 1 and at 3, 6, 9, 20, 24, 30, 48, 72, and 96 h during Trial 2, two nylon bags were removed from each cow and one blank bag from one cow on each treatment. Upon removal, all bags and contents were frozen for later analysis. Bags were washed in cold water until rinse water was clear and dried in forced-air oven at 60½ C for 48 h and weighed back to determine DM residue. Neutral detergent fiber was also determined (Van Soest and Robertson, 1980) on the bag and residue. Dry matter and NDF values were subjected to nonlinear regression procedure to estimate rate of disappearance and

were fitted to the equation:

$$Y = ae^{-kt} + u$$

Where Y = residue remaining, a = potential digestibility, -k = rate constant, t = time-lag period, and u = undigestible fraction (Robinson et al., 1986).

Ruminal ammonia, pH, purine concentrations and CMCase activity

In both trials, whole rumen digesta samples were obtained near the reticulo-omasal orifice and mixed with 1 ml of 5% $HgCl_2$ to stop microbial activity. Ruminal digesta samples were taken to represent a 48 h supplementation cycle. Samples in Trial 1 were obtained at -4, 0, 4, 8, 20, 32 and 44 h post supplementation. In Trial 2, samples were obtained at 0, 3, 6, 9, 20, 24, 30 and 48 h post supplementation. Acidity (pH) of ruminal fluid was determined immediately using a portable electrode (Anonymous, 1984). Ruminal fluid samples (50 ml) were acidified with 3 ml of 6 N HCL for later ammonia analysis by magnesium oxide distillation procedure (AOAC, 1980). Whole ruminal digesta samples were dried at 100½ C for 48 h, ground through a 1 mm screen and analyzed for purine concentration (Zinn and Owens, 1986). Ruminal purine concentrations at the same time intervals during the 48 h supplemental cycle were measured to estimate microbial density of ruminal contents.

In addition during Trial 2, liquid ruminal samples (¼ 350 ml) were obtained near the reticulo-omasal orifice and mixed with 1 ml of 5% $HgCl_2$ to stop microbial activity. Samples were obtained at 0, 3, 6, 9, 20, 24, 30 and 48 h post supplementation to represent the 48 hr supplementation cycle. Nylon bags (15.2cm x 35.6 cm), filled with 6 g

of forage collected and prepared the same as forage for the in situ nylon bag trials, were placed in the rumen at 0 h and removed at the same times as ruminal samples. Liquid ruminal samples were strained through eight layers of cheese cloth and ¼ 75 ml were dried at 100½ C for 48 h to obtain approximately 1 g of DM for purine analysis (Zinn and Owens, 1986). The nylon bags were thawed and rinsed in cold water to remove non-adherent microbes and ruminal debris. Remaining contents were divided, with half being dried at 100½ C for 48 h and analyzed for purine concentration (Zinn and Owens, 1986), and the remainder utilized for carboxymethycellulase (CMCase) analysis.

Carboxymethylcellulase extraction was conducted on the contents of the nylon bags as described by Silva et. al. (1987) using a carbon tetrachloride procedure. Ten ml of each ruminal liquid sample was also analyzed, however due to discoloration of the solution by the ruminal fluid, an individual blank was run for each sample. Hydrolysis of sodium carboxymethylcellulose was assayed by measuring the formation of reducing sugar using 3,5-dinitrosalicylic acid (DNS) reagent. D-glucose (NBS) was used as the standard. CMCase activity was expressed as Ìmol glucose released/g per hour of forage sample or Ìmol glucose released/ml per hour of liquid sample.

Ruminal flow kinetics

During the in situ trial, ruminal grab samples used to determine particulate and liquid dilution rates were also taken from the reticulo-omasal orifice, and mixed with 1 ml of 5% $HgCl_2$ to cease microbial activity. Sampling times were the same as those for the in situ trial.

Chromium ethylenediaminetetraacetic acid (CrEDTA) and ytterbium labeled forage were used as markers for dilution rates of fluid and particulate matter, respectively. Trial 1 CrEDTA was prepared as described by Uden et al. (1980) and Trial 2 CrEDTA was prepared as described by Galyean (1986). The forage used for the ytterbium marker was a low quality chopped grass hay similar in digestibility and nutrient content (5.5% CP, 65% TDN) to the range forage being consumed by the experimental herd. The forage was prepared as described by Galyean (1986).

Markers were administered via rumen cannula at the start of each in situ trial (0800 Trial 1, 1200 Trial 2). Doses were 100 g of ytterbium marked forage in both trials and 5 g of CrEDTA in Trial 1 and 350 ml CrEDTA in Trial 2. Cows received .77 g of ytterbium from marker forage in each trial and .5 g of chromium from the CrEDTA in each trial.

Digesta samples were frozen for later analysis. Upon thawing, ruminal fluid samples were filtered and centrifuged at 1000 x g for 15 min. Analysis for Cr was done by atomic absorption spectrophotometry (New Mexico State University). Particle samples were dried at 60½ C and ground in a Wiley mill through a 1 mm screen. Ytterbium concentration was determined by neutron activation (Washington State University - neutron activation laboratory).

Ruminal dilution rates were calculated (Clark and Petersen, 1988). Marker concentration in the early post-dosing samples of both trials were below the concentration of later sampling times. Thus, inadequate mixing was assumed. Marker concentrations did not stabilize until the 8 h sample in Trial 1 and the 9 h sample in Trial 2, thus samples prior

to these were excluded from analyses.

Blood Metabolites

Blood samples from all cows were drawn into untreated vacuum tubes from the artery or vein near the base of the tail, on consecutive days twice during the studies. Samples were taken approximately 45 and 25 d prepartum at 1300 h in both trials and analyzed for blood urea nitrogen, total bilirubin, creatinine, albumin, total protein, and cholesterol. (Techincon Instruments Corp., Tarrytown, NY).

Statistical Analysis

All estimates of both trials were subject to analysis of variance using General Linear Models Procedures (SAS,1987). Weight and condition score data was analyzed using treatment, initial weight, birthweight and sex of calf, and breed as main effects. Treatment least squares means were separated with single degree of freedom orthogonal contrasts. The blood metabolites used treatment, period, treatment x period interaction in the model. The F statistic for each was calculated using cow(treatment) mean square as the error term. Ruminal pH, ammonia, and purine concentration models used treatment, hour and treatment x hour interaction. Blood metabolites, ruminal dynamics models all used single degree of freedom orthogonal contrasts with cow(treatment) mean square as the error term. Orthogonal contrasts utilized were, CON vs Supplement, and BPM vs SBM. When an interaction was found Least Significant Differences was used to compare means.

Principal component analysis (Brown et. al., 1973) used the Factor Procedure (SAS, 1987). A principal component analysis is concerned with

explaining the variance-covariance structure through a few linear combinations of the orginal variables. This analysis can often reveal relationships that were not previously suspected (Johnson and Wichern, 1982). The principal components procedure provides that successive components describe the maximum variation among a set of observations that is orthogonal to all previous components in the set and the first component represents the major axis of variation with successive components representing minor axis of variation (Baker et al., 1988).

CHAPTER 4

RESULTS AND DISCUSSION

Cow Weights, Condition Scores and Calf Birthweights

Trial 1

Cow body weight change tended to be affected (P = .18) by supplementation (Table 3). Control cows lost 17.7 kg during the study while supplemented lost an average of 11.6 kg. Weight loss between the supplemented groups tended to be different (P = .13; BPM = -15.3 kg vs SBM = -7.9 kg).

Supplement affected (P = .08) cow body condition score change (Table 3). Controls lost .75 units of condition, while supplemented cows lost less condition. Cows receiving BPM lost more (P < .05) condition (.66 units) compared to SBM which lost only .38 units. Supplementation had no affect on calf birth weight (Table 3).

Trial 2

Cow body weight change was affected (P < .01) by supplementation (Table 3). Control cows lost 9.3 kg while supplemented cows gained an average of 7.8 kg. Cows receiving BPM gained less weight (P = .11) 3.4 kg than SBM which gained 12.3 kg. Neither condition score nor calf birth weight were affected by supplementation (Table 3).

TABLE 3. LEAST-SQUARES MEANS OF GESTATING RANGE COWS FOR COW WEIGHT AND CONDITION SCORE CHANGE, AND CALF BIRTHWEIGHT (TRIAL 1 AND TRIAL 2)

Measurements	Supplements[a]			
	CON	BPM	SBM	SE[h]
Trial 1				
Cow weight (kg)				
Initial wt.	541.5	541.7	534.4	11.2
Final wt.	523.6	526.5	526.9	11.6
Change[b]	-17.7	-15.3	-7.9	3.3
Condition Score (1 to 10)				
Beginning	4.1	4.4	4.3	.13
Ending	3.4	3.8	3.9	.12
Change[c]	-.75	-.66	-.38	.095
Calf birthweight (kg)[f]	37.2	37.5	36.5	1.09
Trial 2				
Cow weight (kg)				
Initial wt.	560.7	543.7	545.5	11.3
Final wt.	551.1	547.2	558	10.1
Change[d]	-9.3	3.4	12.3	4.07
Condition Score (1 to 10)				
Beginning	5.1	5.1	5.1	.18
Ending	3.9	4.2	4.2	.16
Change[e]	-1.4	-1.2	-1.3	.18
Calf birthweight (kg)[g]	35.9	36.4	36.3	1.3

[a]CON = control, BPM = methionine, SBM = soybean meal.
[b]Trial 1: CON vs Supplement, P = .18; BPM vs SBM, P = .13.
[c]Trial 1: CON vs Supplement, P = .08; BPM vs SBM, P = .04.
[d]Trial 2: CON vs Supplement, P = .002; BPM vs SBM, P = .11.
[e]Trial 2: CON vs Supplement, P = .44; BPM vs SBM, P = .79.
[f]Trial 1: CON vs Supplement; P = .85; BPM vs SBM, P = .5.
[g]Trial 2: CON vs Supplement; P = .77; BPM vs SBM, P = .98.
[h]Pooled standard error.

Ruminal disappearance rate

Trial 1

Ruminal DM and NDF disappearance rates were slower (P < .01; Table

4) for CON (2.65%/h, DM; 1.34 %/h, NDF) than forsupplemented groups. Disappearance rate for BPM (3.06 %/h, DM; 1.54 %/h, NDF) were slower ($P < .05$) than SBM (3.57 %/h, DM; 1.78 %/h NDF; Table 4).

Trial 2

Ruminal DM and NDF disappearance rates were slower ($P < .05$; Table 4) for CON (3.02 %/h, DM; 1.86 %/h, NDF) than for supplemented groups. Disappearance rate for BPM (3.25%/h, DM; 2.02 %/h, NDF) were slower ($P < .05$) than SBM (3.89 %/h, DM; 2.38 %/h NDF; Table 4).

Ruminal ammonia, pH and purine concentration

Trial 1

Ruminal ammonia concentration (Table 4) was not different between CON (1.2 mg/dl) and supplemented groups. However, SBM (1.25 mg/dl) tended to be higher ($P = .18$) than BPM (0.97 mg/dl).

Ruminal pH (Table 4) tended to be higher ($P = .09$) for CON (6.90) than supplemented groups. Cows receiving BPM (6.87) tended to be higher ($P = .09$) than SBM (6.81).

Whole ruminal purine concentration (Table 4) tended to be lower ($P = .15$) for CON (7.26 mg purine/g DM ruminal contents) than supplemented groups (7.43 and 7.96 mg purine/g DM ruminal contents, respectively for BPM and SBM). Cows receiving BPM (7.43 mg/g) tended to be lower ($P = .13$) than SBM (7.96).

Trial 2

Ruminal ammonia concentration (Table 4) were not different among any of the treatment groups. Ruminal ammonia (mg/dl)

were 1.68 for CON, 1.47 for BPM and 2.17 for SBM. Treatments ranked similarly as in Trial 1.

Ruminal pH (Table 4) was not different among any of the treatment groups. Ruminal pH were 6.8 for CON, 6.88 for BPM and 6.83 for SBM.

Whole ruminal purine concentration (Figure 1) had a significant ($P < .05$) treatment x period interaction. The reason for interaction was due to a peak in purine production in the 6 h post supplementation sample. CON (9.7 mg/g) was lower ($P < .05$) than BPM (13.8 mg/g) or SBM (15.9 mg/g) which were also different ($P < .05$) from each other.

Blood metabolites

Trial 1

Cholesterol (Table 5) showed a significant treatment affect with CON (104.14 mg/dl) being lower ($P < .05$) than supplemented groups (BPM, 115.75 mg/dl; SBM, 111.80 mg/dl). Total protein also showed a treatment effect (Table 5) with CON (6.37 mg/dl) being higher ($P = .05$) than supplemented groups (BPM, 6.19 mg/dl; SBM, 6.26 mg/dl). There were no effects due to treatment for creatinine, total bilirubin, and serum albumin.

Total bilirubin (Table 6) had a significant period effect with Period 1 (.68 mg/dl) being higher ($P < .01$) than period 2 (.57 mg/dl). There were no effects due to period for cholesterol, creatinine, total protein, and serum albumin.

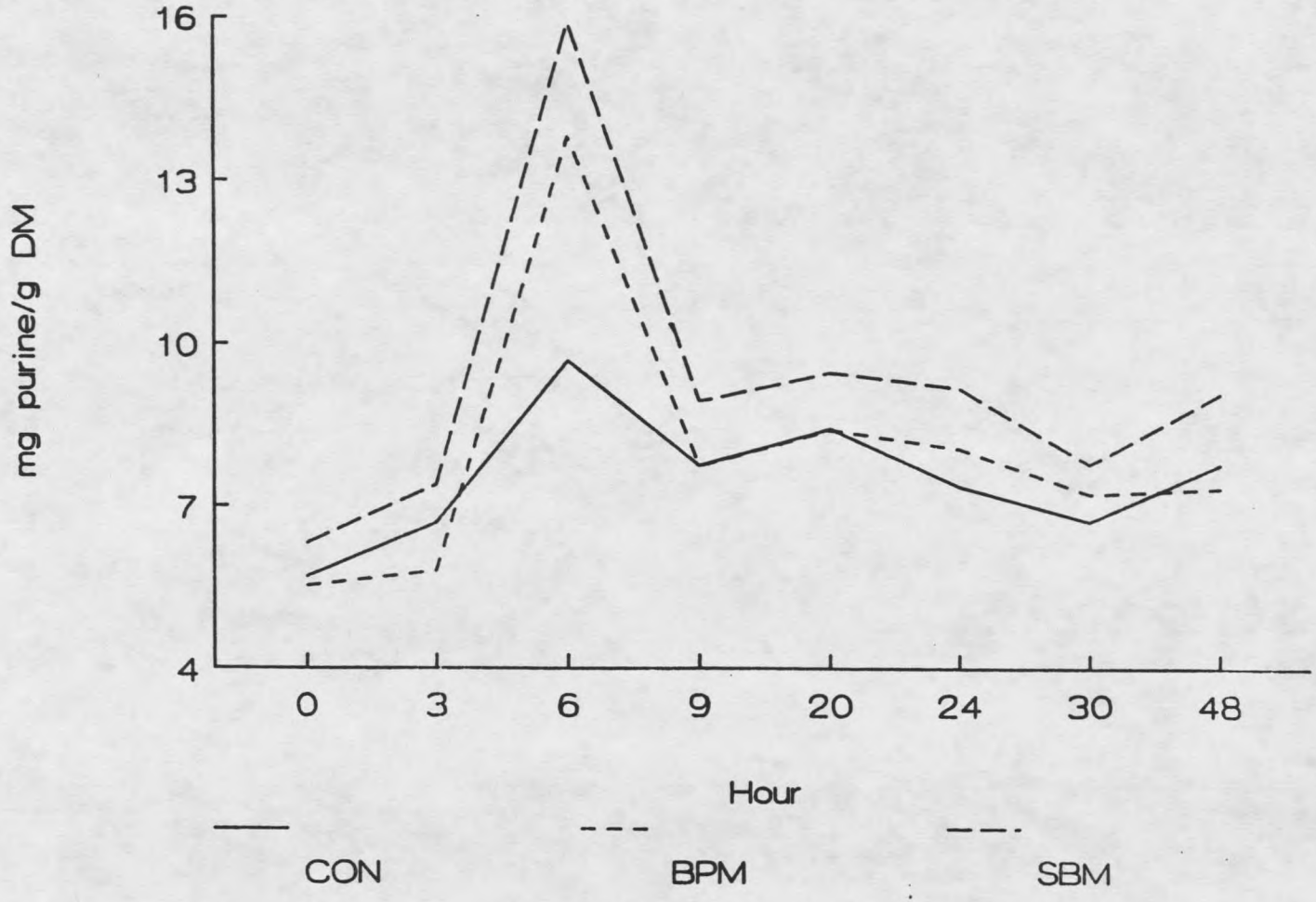
Blood urea nitrogen (BUN; Table 7) had a significant ($P < .05$) treatment x period interaction. All treatment groups had higher ($P < .05$) BUN in period 1 than period 2, also BUN was lower in period 1 and period 2 ($P < .01$) for BPM (3.95 and 3.40 mg/dl) than CON (6.37 and

TABLE 4. LEAST-SQUARES MEANS FOR RUMINAL AMMONIA, pH, PURINE CONCENTRATION, IN SITU FERMENTATION RATE AND FLUID AND PARTICULATE DILUTION RATE (TRIAL 1 AND TRIAL 2)

Measurements	Supplements[a]			
	CON	BPM	SBM	SE[n]
Trial 1				
Rumen ammonia (mg/dl)[b]	1.2	0.97	1.25	.13
pH[c]	6.9	6.87	6.81	.023
Purine concentration (mg/g)[d]	7.26	7.43	7.96	.22
DM disappearance rate (%/h)[e]	2.65	3.06	3.57	.1
NDF disappearance rate (%/h)[f]	1.34	1.54	1.78	.07
Fluid dilution rate (%/h)[g]	8.5	9.1	9.2	.48
Particulate dilution rate (%/h)[h]	2.66	2.89	3.28	.19
Trial 2				
Rumen ammonia (mg/dl)[i]	1.69	1.47	2.17	.42
pH[j]	6.80	6.88	6.83	.04
DM disappearance rate (%/h)[k]	3.02	3.25	3.89	.17
NDF disappearance rate (%/h)[l]	1.86	2.02	2.38	.09
Fluid dilution rate (%/h)[m]	7.95	8.79	8.73	.28

[a]CON = control, BPM = methionine, SBM = soybean meal.
[b]Trial 1: CON vs Supplement, P = .59; BPM vs SBM, P = .18.
[c]Trial 1: CON vs Supplement, P = .09; BPM vs SBM, P = .09.
[d]Trial 1: CON vs Supplement, P = .15; BPM vs SBM, P = .13.
[e]Trial 1: CON vs Supplement, P = .0009; BPM vs SBM, P = .01.
[f]Trial 1: CON vs Supplement, P = .006; BPM vs SBM, P = .04.
[g]Trial 1: CON vs Supplement, P = .29; BPM vs SBM, P = .89.
[h]Trial 1: CON vs Supplement, P = .1; BPM vs SBM, P = .19.
[i]Trial 2: CON vs Supplement, P = .73; BPM vs SBM, P = .28.
[j]Trial 2: CON vs Supplement, P = .32; BPM vs SBM, P = .41.
[k]Trial 2: CON vs Supplement, P = .03; BPM vs SBM, P = .03.
[l]Trial 2: CON vs Supplement, P = .02; BPM vs SBM, P = .03.
[m]Trial 2: CON vs Supplement, P = .04; BPM vs SBM, P = .88.
[n]Pooled standard error.

Figure 1. Whole rumen purine concentration (Trial 2).



4.07 mg/dl) or SBM (6.59 and 4.28 mg/dl). However, the treatment * period interaction was cause by a difference in magnitude of the decline in BUN between periods. Between period 1 and 2, BPM had a 16 % decrease in BUN while CON and SBM decreased approximately 56 %.

TABLE 5. LEAST-SQUARES MEANS FOR CHOLESTEROL, CREATININE, SERUM ALBUMIN, TOTAL BILIRUBIN AND TOTAL PROTEIN CONCENTRATIONS BY TREATMENT (mg/dl; TRIAL 1 AND TRIAL 2)

	Supplements[a]			
Metabolite	CON	BPM	SBM	SE[k]
Trial 1				
Cholesterol[b]	104.14	115.75	111.80	3.33
Creatinine[c]	2.13	2.25	2.17	.057
Total bilirubin[d]	.65	.60	.61	.027
Total protein[e]	6.37	6.19	6.26	.055
Serum albumin[f]	3.93	3.90	3.98	.061
Trial 2				
Cholesterol[g]	108.02	111.93	102.58	3.79
Creatinine[h]	1.98	1.95	1.83	.046
Total bilirubin[i]	.58	.55	.52	.026
Total protein[j]	6.30	6.33	6.48	.079

[a]CON = control, BPM = methionine, SBM = soybean meal.
[b]Trial 1: CON vs Supplement, P = .02; BPM vs SBM, P = .4.
[c]Trial 1: CON vs Supplement, P = .27; BPM vs SBM, P = .36.
[d]Trial 1: CON vs Supplement, P = .17; BPM vs SBM, P = .84.
[e]Trial 1: CON vs Supplement, P = .05; BPM vs SBM, P = .35.
[f]Trial 1: CON vs Supplement, P = .9; BPM vs SBM, P = .36.
[g]Trial 2: CON vs Supplement, P = .87; BPM vs SBM, P = .09.
[h]Trial 2: CON vs Supplement, P = .14; BPM vs SBM, P = .07.
[i]Trial 2: CON vs Supplement, P = .17; BPM vs SBM, P = .4.
[j]Trial 2: CON vs Supplement, P = .28; BPM vs SBM, P = .2.
[k]Pooled standard error.

Trial 2

There were no significant treatment effects in Trial 2 (Table 5) for cholesterol, creatinine, total bilirubin and total protein, or period effects (Table 6) for cholesterol and total protein. Creatinine was higher ($P < .01$) in period 1 (2.00 mg/dl) than in period

TABLE 6. LEAST-SQUARES MEANS FOR CHOLESTEROL, CREATININE, SERUM ALBUMIN, TOTAL BILIRUBIN AND TOTAL PROTEIN CONCENTRATIONS BY PERIOD (mg/dl; TRIAL 1 AND TRIAL 2)

	Periods[a]		
Metabolite	1	2	SE[b]
Trial 1			
Cholesterol[c]	110.77	110.36	.91
Creatinine[d]	2.20	2.17	.016
Total bilirubin[e]	.68	.57	.016
Total protein[f]	6.23	6.32	.022
Serum albumin[g]	3.90	3.97	.016
Trial 2			
Cholesterol[h]	107.11	107.92	3.08
Creatinine[i]	2.00	1.84	.039
Total bilirubin[j]	.33	.76	.021
Total protein[k]	6.39	6.35	.064

[a]Periods represent sampling interval in relation to d prior to calving (Period 1 = 45d and Period 2 = 45 d)
[b]Pooled standard error.
[c]Trial 1: Period effect, P = .91.
[d]Trial 1: Period effect, P = .66.
[e]Trial 1: Period effect, P = .0009.
[f]Trial 1: Period effect, P = .16.
[g]Trial 1: Period effect, P = .33.
[h]Trial 2: Period effect, P = .85.
[i]Trial 2: Period effect, P = .003.
[j]Trial 2: Period effect, P = .0001.
[k]Trial 2: Period effect, P = .65.

2 (1.84 mg/dl). Total bilirubin was lower (P < .01) in period 1 (.33 mg/dl) than in period 2 (.76 mg/dl).

Blood urea nitrogen had a significant (P = .02) treatment x period interaction (Table 7). Blood urea nitrogen was higher (P < .01) for CON (7.95 mg/dl) in period 1 than BPM (5.39 mg/dl) and SBM (5.71mg/dl). However in period 2, CON had lower (P < .01) BUN than in period 1 but was not different than the supplemented groups.

Serum albumin showed (P < .01) a treatment x period interaction similar to the BUN. Serum albumin was higher (P < .01) for CON (4.32

TABLE 7. LEAST-SQUARES MEANS BLOOD UREA NITROGEN AND SERUM ALBUMIN WITH TREATMENT * PERIOD INTERACTION (mg/dl) (TRIAL 1 AND TRIAL 2)

Metabolite	Period[f]	Supplements[a] CON	BPM	SBM	SE[e]
Trial 1					
Blood urea nitrogen[b]	1	6.39	3.95	6.59	.188
	2	4.07	3.40	4.28	.188
Trial 2					
Blood urea nitrogen[c]	1	7.95	5.39	5.71	.445
	2	6.02	5.50	6.00	.441
Serum albumin[d]	1	4.32	3.92	4.09	.072
	2	3.95	4.04	3.97	.072

[a]CON = control, BPM = methionine, SBM = soybean meal.
[b]Trial 1: Treatment * period interaction, P = .02.
[c]Trial 2: Treatment * period interaction, P = .023.
[d]Trial 2: Treatment * period interaction, P = .005.
[e]Pooled standard error.
[f]Period represents sampling intervals in relation to d prior to calving (Period 1 = 45 d and Period 2 = 25 d)

mg/dl) in period 1 than BPM (3.92 mg/dl) or SBM (4.09 mg/dl). However in period 2, CON had lower ($P < .01$) ALB than in period 1 but was not different than supplemented groups. These results would indicate that DL-methionine did not substitute for soybean meal as a winter protein supplement. However, environmental conditions of the two trials may not have allowed for effects to be manifested, since both winters were mild. Mean average temperatures ranged 2 to 3½ warmer than normal and snowfall was 50% of normal (National Oceanic and Atmospheric Administration, 1987-1988). During these years supplementation may not have been needed. This would be supported by the small differences in cow weight change, condition score change and blood metabolite data. Differences in cow weight change between SBM

and CON were only 10 kg in Trial 1 and 22 kg in Trial 2. Miner et. al.(1989) showed differences of 34 kg and 26 kg between CON and SBM on the same site in 1985 and 1986, respectively. The small differences in condition score and blood metabolite data would also support the conclusion that all cows were in relatively similar nutritional status.

Measurements of ruminal dynamics should give an idea of effects the supplements have on digestion. Soybean meal had increased DM and NDF disappearances rates and greater whole rumen purine concentrations than BPM. Cows receiving supplements had similar particulate and liquid dilution rates which were faster than CON. This would agree with Miner et. al.(1989) who found increased NDF disappearance rate and dilution rates with SBM compared to CON.

Cows fed BPM had faster DM and NDF disappearance rates and greater whole rumen purine concentrations than CON. Purine concentration can be used as an indirect measurement of microbial density. The interpretation of our ruminal purine concentration data agrees with Arambel et. al. (1987). He found increased microbial counts when supplementing with a protected methionine product which was 28 % ruminally degradable.

The increase in purine concentration we observed might be attributed to the beet pulp carrier. Silva and Orskov (1988) showed increased NDF disappearance rates of barley straw with beet pulp supplement. However, Clark and Petersen (1988) demonstrated beet pulp with methionine increased NDF disappearance compared to beet pulp with ammonium sulfate. Wiley et. al.(1988) reported increased NDF disappearance rates when methionine was supplemented without carrier

via rumen fistula. Therefore, we attribute the majority of observed ruminal effects to the methionine and not the carrier.

Clark and Petersen (1988) indicated DL-methionine may replace soybean meal as suggested by ruminal measurements. They achieved a 30% greater NDF disappearance rate with methionine containing supplement compared to soybean meal. The present study did not elicit the same response. One explanation might be due to the absence of urea fed with DL-methionine. Clark and Petersen (1988) showed the beet pulp-methionine-urea treatment had tenfold higher ruminal ammonia concentrations in comparison to the values reported here. In this study, the BPM supplement shows a trend for lower ruminal ammonia concentration than CON. This trend for lower ruminal ammonia concentration with BPM may demonstrate a stimulation of ruminal microbial utilization of available ammonia. The increase in NDF disappearance and whole purine concentration compared to the CON certainly suggests increased microbial activity. The low ruminal ammonia concentration may be reflected by BUN in which BPM had lower concentrations than CON in Periods 1 and 2 of both trials. Since the ruminal ammonia concentrations were below the minimum level of 5 mg/dl ammonia reported by Satter and Slyter (1974), ammonia may have been the limiting factor for BPM.

Another possible reason for the difference between this study and that of Clark and Petersen (1988) is the supplementation scheme. They fed methionine everyday in a completely mixed diet while in the present study methionine was supplemented on alternate days. Methionine could possibly have a short term effect on the microbial population in the

rumen. This is evident by the treatment x hour interaction of whole ruminal purine concentration (Figure 1). An interaction was not observed in Trial 1 due to a sampling protocol which missed the short term peak in purines concentration due to supplementation, which occurred near 6 h after supplementation. These data agree with Wiley et. al. (1988) who found a similar peak due to supplementation using purines as a microbial marker. Leedle et. al. (1982) reported a similarly shaped peak while establishing microbial growth curves using actual microbial counts in relation to substrate availability. After the supplementation peak in Trial 2, purine production in the BPM was reduced to a baseline value equal in magnitude to the CON while SBM maintained higher values throughout the 48 h supplementation cycle. This response may demonstrate the need for more frequent supplementation of methionine than every-other-day, thereby stimulating the peak response on a daily basis and potentially elevating the base value.

Principal components of rumen function

Trial 2

Ten ruminal measurements were subjected to principal component analysis and results are presented in Table 8. The analysis grouped the ruminal measurements into four factors.

The first factor, which represents 46.4% of the variation caused by supplementation on ruminal function, is composed of three major components. These components are NDF disappearance rate, particle dilution rate and CMCase activity in the ruminal liquid fraction, all of which had values greater than .88. The fourth component of a lesser

TABLE 8. COEFFICIENTS OF PRINCIPAL COMPONENTS OBTAINED FROM RUMINAL MEASUREMENTS OF GESTATING COWS GRAZING WINTER RANGE

	FACTORS			
Ruminal Measurement	1	2	3	4
Purine Concentrations				
Incubated forage	-0.52	0.75	0.17	-0.04
Liquid fraction	-0.33	-0.35	0.51	-0.38
Whole rumen	0.18	0.46	0.71	-0.24
CMCase Activity				
Incubated forage	-0.05	0.77	-.10	0.20
Liquid fraction	0.88	0.08	0.02	0.16
Ruminal ammonia	0.15	-0.46	0.59	0.34
pH	-0.31	-0.03	0.21	0.79
NDF disappearance	0.90	-0.10	-0.19	-0.07
Liquid dilution	0.77	0.20	0.20	0.01
Particulate dilution	0.91	0.18	0.09	0.02
% total variance	46.4	23.5	16.8	13.3

prediction value was fluid dilution rate with a value of .77. The NDF disappearance rate and particle passage rate would be expected to be highly affected by supplementation considering the fiber content ofdiets the grazing animal consumes. This would agree with Clark and Petersen (1988) who found increased fermentation rate and McCollum and Galyean (1985), Caton et. al.(1988) and Miner and Petersen (1989) who found increased dilution rates with supplementation of animals fed low quality forage. The importance of CMCase activity in the liquid fraction would indicate the microbial population in the rumen can produce extracellular enzymes in response to supplementation or a feeding bout, and probably contributes to the initial digestion of cellulose. Extracellular cellulase could be produced by sources other than bacteria. Wood et. al.(1986) isolated ruminal fungi which secretes a very active extracellular cellulase.

The second factor, which accounts for 23.5% of the variation, has

two major components: purine concentration and CMCase activity isolated from incubated forage in situ. These components are of approximately equal importance with values of .75 and .71. These two account for only half as much variation as explained by factor 1. This relationship, between the factors, may be due to the time lag that occurs for cellulolytic bacteria to adhere and initiate degradation of fibrous particles (Akin and Barton, 1983).

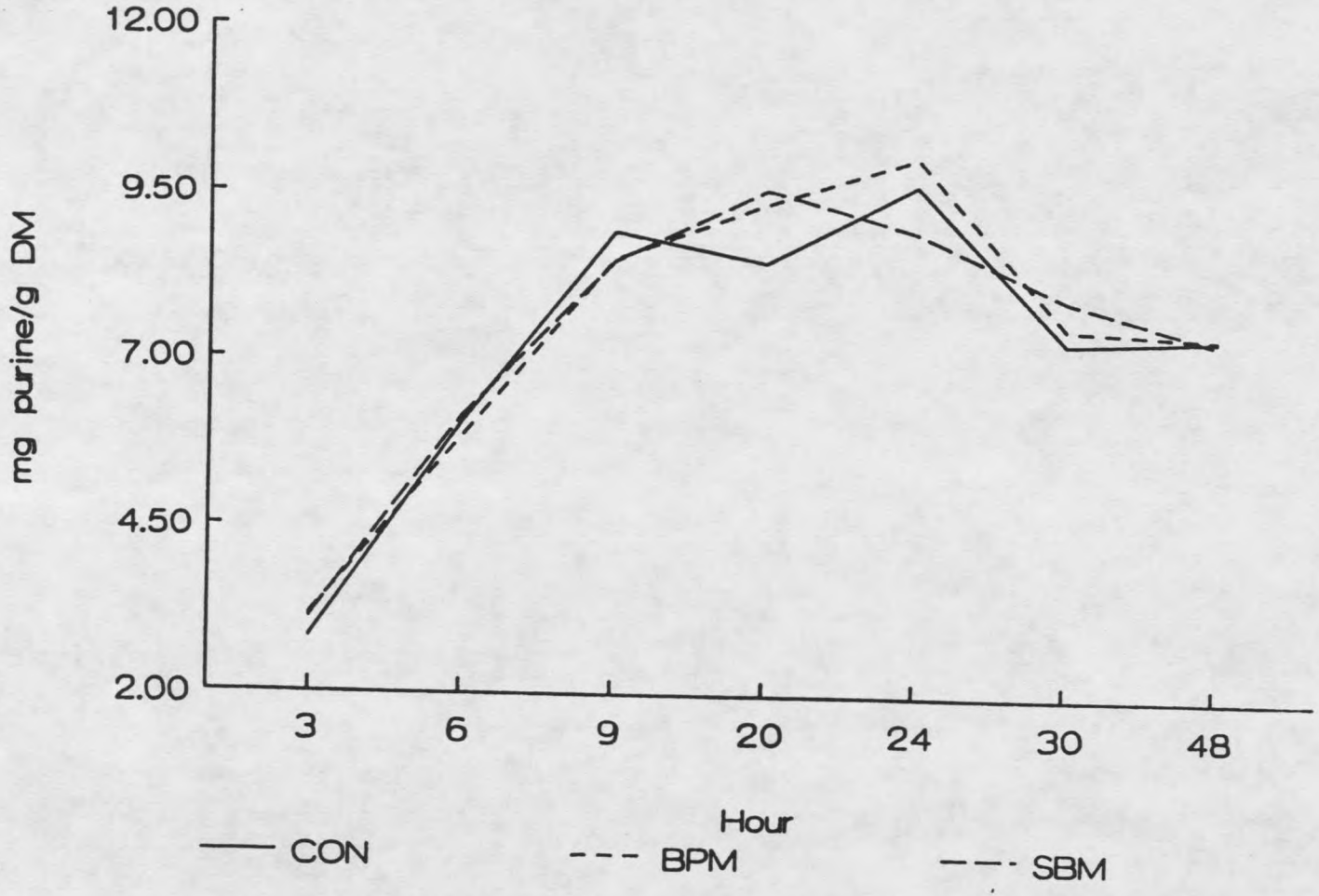
The third factor, which accounts for 16.8% of the variation, is composed of three components. These are ruminal liquid and whole ruminal purine concentrations and ruminal ammonia concentration, with values of .51 .71 and .59 respectively. The whole ruminal purine concentration is probably closely related to changes seen in liquid purine concentration since purine concentration on the incubated forage remained constant after an initial increase through 9 h (Figure 2).

Ruminal ammonia, a component in this factor was measured between .7 and 4.0 mg/dl throughout the study. These levels may have caused ammonia to become a significant component because the highest value reported was below the 5 mg/dl reported by Satter and Slyter (1974) needed for maximal microbial yield, indicating ammonia might have been a limiting factor.

The fourth and final factor, which represents 13.3% of the variation, has only one important component pH. Due to the narrow range of pH in this study it is probably of minor biological importance.

This analysis demonstrates a possible mechanisms describing thr effect of supplementation, having the greatest influence on NDF disappearance and ruminal dilution rates. These parameters are most

Figure 2. Ruminal purine concentration on incubated forage.



strongly influenced by enzymatic activity first from the liquid fraction (component of factor 1) and later by the cellulolytic microbes attached to the forage (factor 2). Finally supplementation also changed the overall microbial density. Supplementation effects therumen, as shown by the increase in liquid purine concentration (factor 3) but also increased CMCase activity (in ruminal liquid-factor 1 and on incubated forage-factor 2).

Supplementation effects

Among the important components in the first factor, NDF disappearance rate and particle dilution rate reported above showed a difference ($P < .05$ and $P < .1$, respectively) between CON (1.34 %/h; 2.66 %/h) and SUP (1.66 %/h; 3.09 5/h) respectively for NDF disappearance and particle dilution rate. Supplement source did have an effect ($P < .05$) on NDF disappearance (2.02 %/h vs 2.38 %/h for BPM and SBM, respectively) but did not affect ($P > .05$) particle dilution rate. The CMCase activity in the liquid fraction (Figure 3) showed no treatment differences, however activity in SUP groups was higher ($P < .01$) at 20 h than CON where activity dropped after 9 h. This period of increased activity in the SUP group corresponded to a period in which the slope of the NDF disappearance curve is the greatest. This could indicate the increase in NDF disappearance due to supplementation might possibly be influenced by a short term stimulation of extracellular cellulase secretion by a portion of the ruminal microbial population. It is also interesting to note that CMCase activity is twice as high in the liquid fraction (Figure 3) as compared to particle bound activity (Figure 4), which indicates extracellular enzymes may be

Figure 3. Ruminal CMCase activity in the liquid fraction.

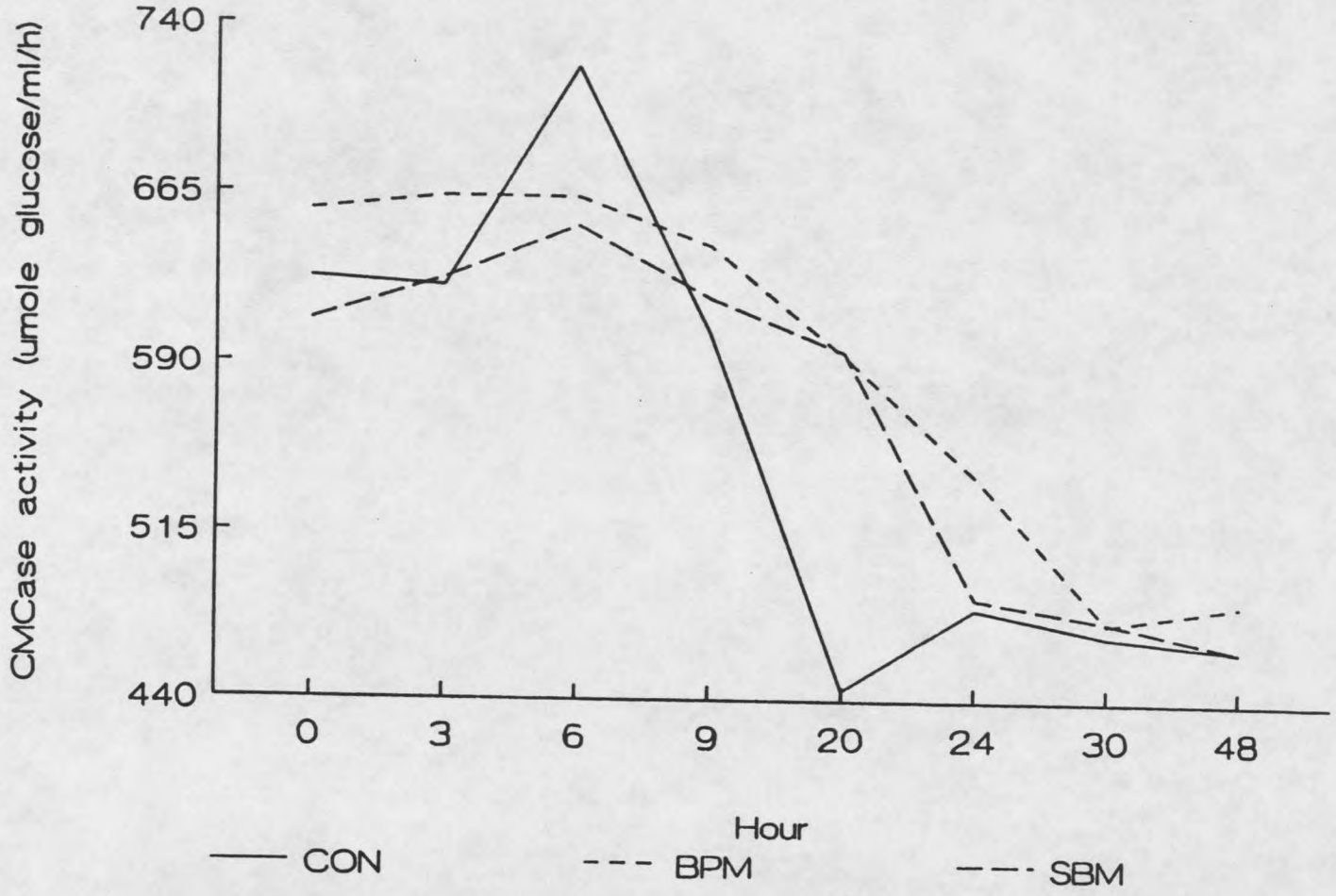
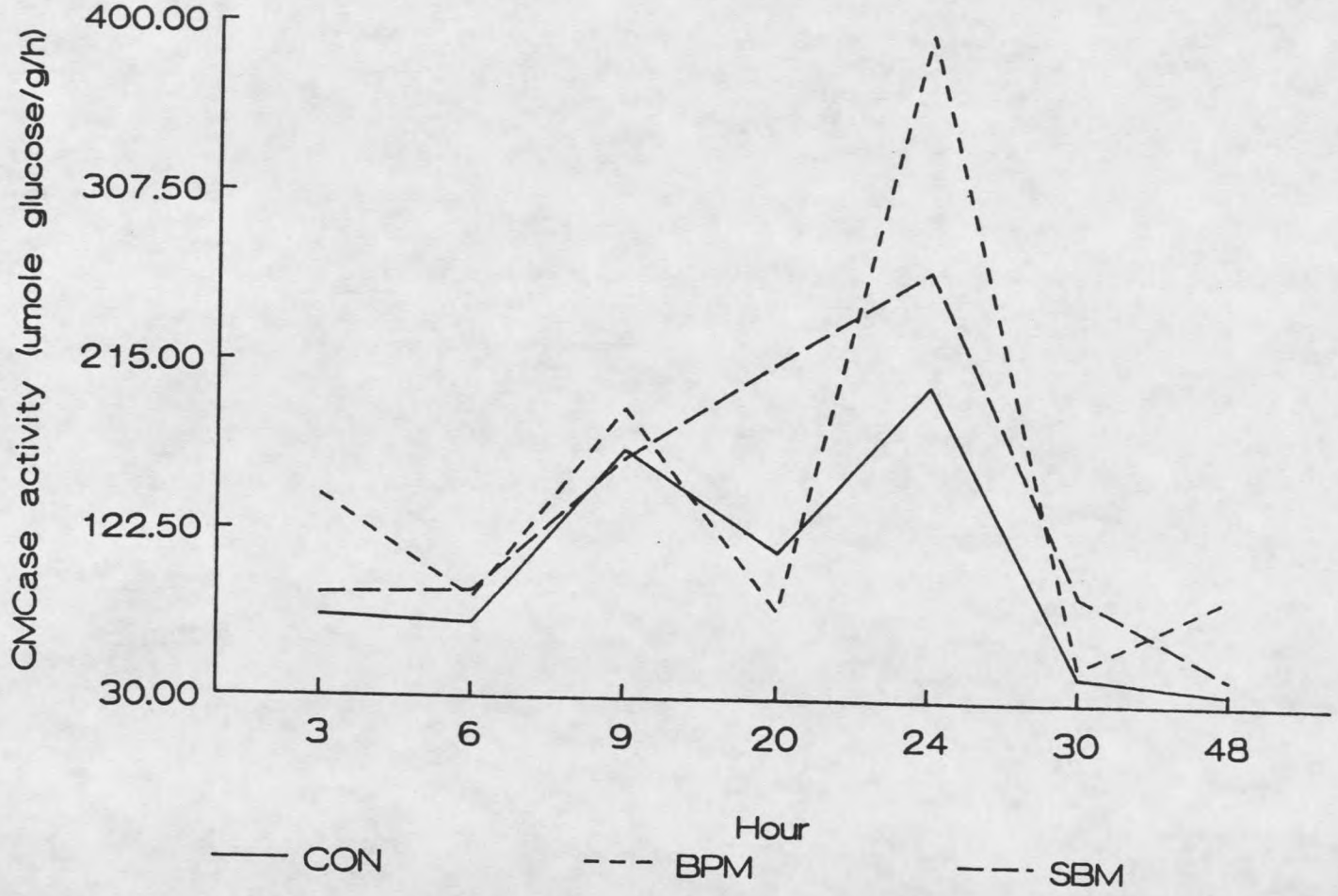


Figure 4. Ruminal CMCase activity on incubated forage.



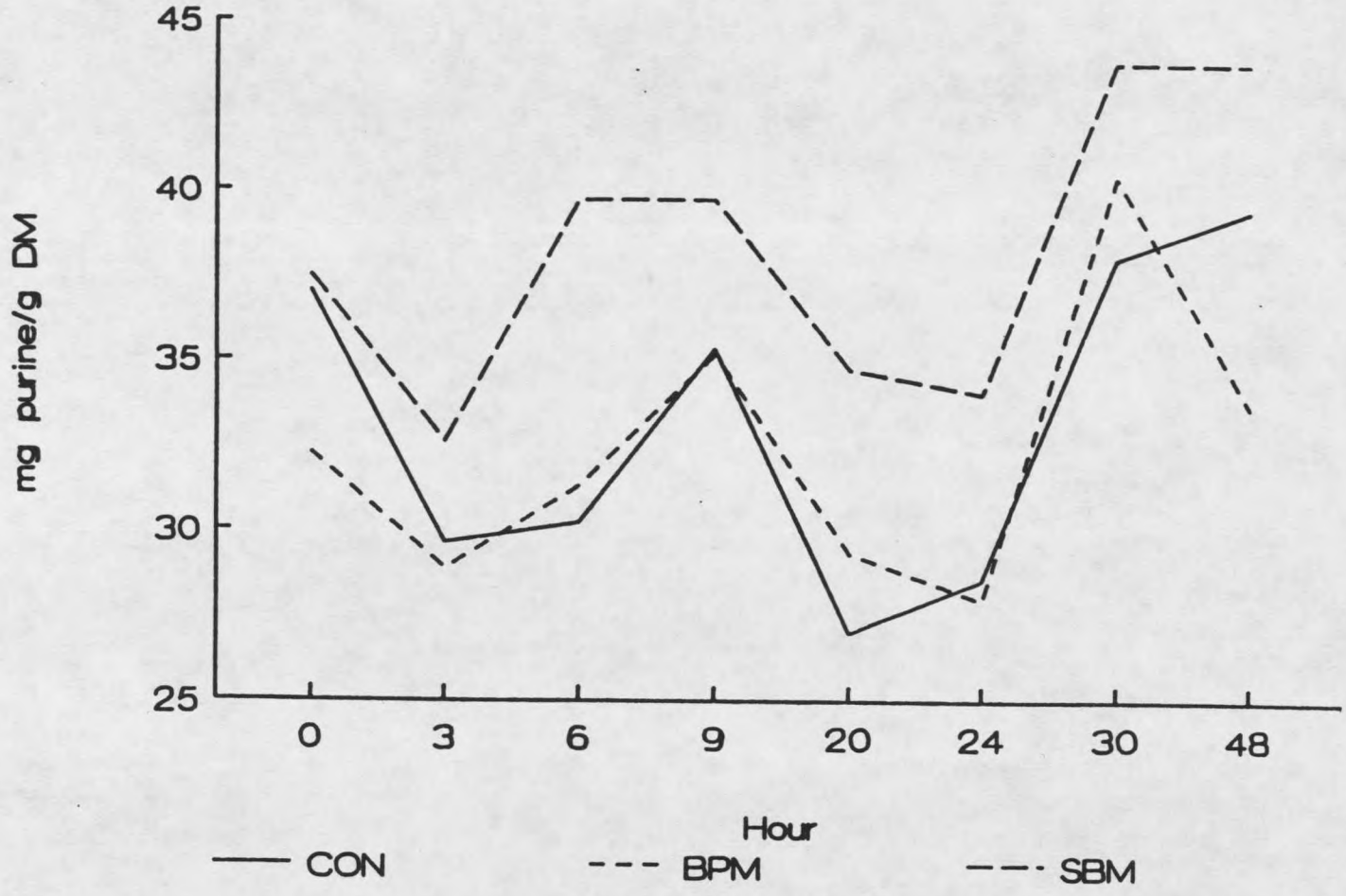
of major importance in fiber degradation.

No difference ($P > .05$) due to treatment was shown by the important components of the second factor. The increase in purine concentration (Figure 2) indicates that approximately 9 h are required for microorganisms to become adhered to the forage. After that point purine levels remained fairly constant. This would indicate there are only a limited number of attachment sites on the forage particles and any increase in forage degradation from this microbial population would come in the form of increased activity. The CMCase activity of adhered microbes was not significantly different between treatments. However there was a trend for the SUP groups to have higher activity (Figure 4) at specific times. Soybean meal tended to have higher activity ($P = .06$) at 20 h and BPM had higher activity ($P < .05$) at 24 h. This would indicate that we stimulated activity without increasing the microbial population. Correlations between CMCase activity on incubated forage and NDF disappearance had a r value of -0.098 and the correlation between purine concentration on incubated forage and NDF disappearance was -0.55. These data agree closely with Silva et. al. (1987). He showed a similar amount of activity on ammonia treated straw and either beet pulp, barley or barley and fishmeal. Using NAD-linked glutamate dehydrogenase as a microbial marker, he also saw a poor relationship between microbial numbers attached to the straw and their ability to digest it.

The important components in the third factor were whole rumen and liquid purine concentrations and ruminal ammonia. Whole rumen purine reported above showed a treatment x time interaction, which was caused

by a sharp increase in purine concentration 6 h post-supplementation. Soybean meal had a higher peak, however, this peak did not translate into increased CMCase activity as shown in Figure 3 and 4. Soybean meal did have higher liquid purine concentration ($P < .05$) than BPM (Figure 5). Once again there was no corresponding increase in CMCase activity in the liquid fraction (Figure 3). These data would indicate that SBM must be stimulating a portion of the microbial population associated with the ruminal liquor that do not produce CMCase. Ruminal NH_3 concentration was the other important component of the third factor. There were no differences between treatment groups for ruminal ammonia concentration. Ammonia levels at all sampling times were below the 5 mg/dl indicated by Satter and Slyter (1974) for maximal microbial synthesis. Soybean meal supplemented cows did receive approximately 3.5 times the quantity (44 g/h/each day vs 146 g/h/each day, BPM and SBM respectively) of protein as BPM and they still had similar NH_3 concentrations. This would indicate that the non-CMCase producing microbes were utilizing the available ruminal nitrogen derived from SBM to increase their concentration as suggested by liquid and whole ruminal purine concentrations.

Figure 5. Ruminal purine concentration in the liquid fraction.



CHAPTER 5

CONCLUSION

From the production data reported in this thesis, it is apparent that BPM is not a complete substitute for SBM for gestating cows grazing winter range. Soybean meal cows gained weight during the study while BPM fed cows maintained their weight. However, ruminal kinetics indicate BPMand SBM had similar effects on the ruminal parameters, except for total liquid and whole ruminal purine concentrations. It would seem that SBM, due to a greater supply of ruminal ammonia, was utilized to multiply by non-cellulolytic microorganisms. This increase in concentration may have resulted in an increase in microbial protein delivered to the small intestine. Miner et. al. (1989) demonstrated the use of escape protein with SBM decreased weight loss of beef cows grazing winter range, thus showing a need for additional protein delivered to the small intestine. Therefore, this study strongly suggests the effect of protein supplementation that influences cow weight change is the quantity of microbial protein delivered to the small intestine as enhanced by the supplement. Cows receiving BPM did not supply additional nitrogen for ruminal ammonia production which may have limited microbial protein delivered to the small intestine. Therefore the differences noted in the production data could be due to an increase in microbial protein production from the liquid portion of the rumen and subsequently reaching the small intestine of cows on the

SBM treatment.

Methionine supplementation did show a positive ruminal response, however, if methionine is to substitute for SBM on winter range certain questions must be answered. First, was ruminal ammonia a limiting factor by restricting a total microbial response to methionine, and would the addition of urea to the supplement have an additive effect? Secondly, would daily feeding of methionine improve animal response? Continued research is needed to answer these questions.

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APPENDIX

Table 9. Least-Squares Analysis of Variance for Cow Weight Change, Condition Score Change and Calf Birth Weight (Trial 1).

Item	Cow Weight Change			Condition Score Change			Calf Birth Weight		
	df	Mean square	P	df	Mean square	P	df	Mean square	P
Treatment(TRT)[a]	2	2356.94	.08	2	.6025	.03	2	26.32	.79
CON vs SUP	1	1652.72	.18	1	.5011	.08	1	3.883	.85
BPM vs SBM	1	2125.47	.13	1	.7163	.04	1	49.67	.50
INITIAL WT.	1	838.29	.34	-	-	-	1	1223.8	<.01
SEX (calf)	1	92.994	.75	1	.0173	.74	1	420.35	.06
BIRTH WT. (calf)	1	1.169	.97	1	.2936	.18	-	-	-
BREED	1	2.1683	.96	-	-	-	1	.1184	.39
ERROR	49	891.11		50	.1560		51	109.05	

[a]Least-squares means separated by orthogonal contrasts.

Table 10. Least-Squares Analysis of Variance for Cow Weight Change, Condition Score Change and Calf Birth Weight (Trial 2).

Item	Cow Weight Change df	Cow Weight Change Mean square	Cow Weight Change P	Condition Score Change df	Condition Score Change Mean square	Condition Score Change P	Calf Birth Weight df	Calf Birth Weight Mean square	Calf Birth Weight P
Treatment(TRT)[a]	2	9352.21	<.01	2	.3056	.56	2	50.61	.60
CON vs SUP	1	16202.39	<.01	1	.5525	.31	1	20.48	.65
BPM vs SBM	1	2762.35	.15	1	.0612	.73	1	78.58	.38
BIRTH WT. (calf)	1	653.01	.48	1	.0020	.95	-	-	-
SEX (calf)	1	1.9125	.97	1	.0242	.83	1	642.17	.01
BREED	1	2868.68	.14	1	.1504	.60	1	447.14	.04
ERROR	44	1278.43		45	.5260		44	99.35	

[a]Least-squares means separated by orthogonal contrasts.

Table 11. Least-Squares Analysis of Variance for Concentrations of Blood Urea Nitrogen (BUN), Cholesterol (CHO), Creatinine (CRE), Total Bilirubin (BIL), Total Protein (PRO), and Albumin (ALB; Trial 1).

		BUN		CHO		CRE		BIL		PRO		ALB	
Item	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Treatment (TRT)[a]	2	71.7	<.01	2672	.05	.26	.36	.06	.37	.61	.09	.13	.65
CON vs SUP	1	23.6	.03	4762	.02	.31	.27	.11	.17	1	.05	.01	.91
BPM vs SBM	1	120	<.01	608	.40	.22	.36	.01	.84	.21	.35	.25	.36
Period(PER)	1	175	<.01	9.9	.75	.05	.22	.69	<.01	.50	<.01	.28	<.01
TRT X PER[b]	2	20.3	.02	66	.93	.27	.35	.05	.45	.61	.09	.19	.53
COW(TRT)	57	4.8	<.01	857	<.01	.25	<.01	.06	<.01	.24	<.01	.29	<.01
ERROR	174	1.37		96		.03		.03		.06		.03	

[a]Mean square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.
[b]Mean square for COW(TRT) used as error term.

Table 12. Least-Squares Analysis of Variance for Concentrations of Blood Urea Nitrogen (BUN), Cholesterol (CHO), Creatinine (CRE), Total Bilirubin (BIL), Total Protein (PRO), and Albumin (ALB; Trial 2).

		BUN		CHO		CRE		BIL		PRO		ALB	
Item	df	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P	Mean square	P
Treatment (TRT)[a]	2	40.6	<.01	1444	.23	.43	.06	.06	.29	.59	.25	.39	.11
CON vs SUP	1	76.7	<.01	25.2	.87	.34	.14	.08	.18	.48	.29	.73	.04
BPM vs SBM	1	5.4	.37	2849	.09	.51	.07	.03	.40	.67	.21	.07	.52
Period PER)	1	13.2	.01	32.6	.40	1.4	<.01	9.2	<.01	.09	.20	.70	<.01
TRT X PER[b]	2	25.8	.02	75.3	.92	.17	.33	.01	.89	.77	.16	.99	<.01
COW(TRT)	49	6.5	<.01	939	<.01	.15	<.01	.04	.04	.41	<.01	.17	<.01
ERROR	145	2.04		47.1		.03		.03		.05		.03	

[a]Mean square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.
[b]Mean square for COW(TRT) used as error term.

Table 13. Least-Squares Analysis of Variance for Whole Ruminal Purine Concentration, Ruminal Ammonia, and Ruminal pH (Trial 1).

Item	Ruminal Ammonia df	Ruminal Ammonia Mean square	Ruminal Ammonia P	Ruminal pH df	Ruminal pH Mean square	Ruminal pH P	Whole Ruminal Purine Concentration df	Whole Ruminal Purine Concentration Mean square	Whole Ruminal Purine Concentration P
Treatment(TRT)[a]	2	.4342	.34	2	.0546	.07	2	3.737	.12
CON vs SUP	1	.1130	.59	1	.0565	.09	1	3.537	.15
BPM vs SBM	1	.7554	.18	1	.0528	.10	1	3.937	.13
COW(TRT)	9	.3597	.04	9	.0153	.16	9	1.398	.29
HOUR	4	.8109	<.01	6	.0667	<.01	6	5.951	<.01
TRT X HOUR	8	.2122	.26	12	.0091	.53	12	.9221	.63
ERROR	36	.1606		54	.0099		54	1.131	

[a]Mean square for COW(TRT) used as error term for treatment and least-square means separated by orthogonal contrasts.

Table 14. Least-Squares Analysis of Variance for Neutral Detergent Fiber (NDF) Disappearance Rate, Ruminal Fluid Dilution and Ruminal Particle Dilution Rate (Trial 1 and Trial 2).

	NDF Disappearance Rate			Fluid Dilution Rate			Particle Dilution Rate		
Item	df	Mean square	P	df	Mean square	P	df	Mean square	P
Trial 1									
Treatment(TRT)[a]	2	.00002	<.01	2	.00006	.55	2	.00004	.12
CON vs SUP	1	.00003	<.01	1	.00012	.29	1	.00005	.11
BPM vs SBM	1	.00001	.04	1	.000002	.89	1	.00003	.19
ERROR	9	.000002		9	.00009		9	.00001	
Trial 2									
Treatment(TRT)[a]	2	.00003	.01	2	.00008	.1			
CON vs SUP	1	.00002	.02	1	.00017	.04			
BPM vs SBM	1	.00002	.03	1	.00000	.88			
ERROR	8	.000003		8	.00003				

[a]Mean square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.

Table 15. Least-Squares Analysis of Variance for Liquid CMCase Activity and Incubated Forage CMCase Activity (Trial 2).

Item	CMCase Activity Liquid df	Mean square	P	CMCase Activity Forage df	Mean square	P
Treatment(TRT)[a]	2	8877.29	.46	2	14106.1	.18
CON vs SUP	1	11290.9	.32	1	27867.5	.07
BPM vs SBM	1	7975.93	.40	1	1356.68	.66
COW(TRT)	8	10229.1	.06	8	6535.15	.27
HOUR	7	73135.5	<.01	6	72149.7	<.01
TRT X HOUR	14	4891.39	.49	12	7760.36	.15
ERROR	56	5001.25		46	5066.19	

[a]Means square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.

Table 16. Least-Squares Analysis of Variance for Ruminal Ammonia and Ruminal pH (Trial 2).

Item	Ruminal Ammonia df	Mean square	P	Ruminal pH df	Mean square	P
Treatment(TRT)[a]	2	3.709	.51	2	.0405	.46
CON vs SUP	1	.6537	.73	1	.0357	.32
BPM vs SBM	1	6.742	.28	1	.0357	.41
COW(TRT) 8	5.039		<.01	8	.0473	<.01
HOUR 7	9.146		<.01	7	.0731	<.01
TRT X HOUR	14	.3460	.64	14	.0194	.09
ERROR 56	.4216			56	.0118	

[a]Means square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.

Table 17. Least-Squares Analysis of Variance for Whole Ruminal Purine Concentration, Liquid Purine Concentration, and Incubated Forage Purine Concentration (Trial 2).

Item	Whole Ruminal Purine Concentration			Liquid Purine Concentration			Forage Purine Concentration		
	df	Mean square	P	df	Mean square	P	df	Mean square	P
Treatment(TRT)[a]	2	25.91	<.01	2	303.29	.04	2	.2264	.93
CON vs SUP	1	25.13	<.01	1	92.86	.25	1	.4419	.71
BPM vs SBM	1	22.21	<.01	1	472.83	.02	1	.0262	.92
Cow(TRT)	8	1.216	.51	8	59.89	.28	8	3.042	.1
HOUR	7	51.87	<.01	7	184.22	<.01	6	54.82	<.01
TRT X HOUR	14	3.918	<.01	14	13.62	.99	12	.5277	.98
ERROR	56	1.327		56	47.51		48	1.682	

[a]Mean square for COW(TRT) used as error term for treatment and least-squares means separated by orthogonal contrasts.

Table 18. Correlations between Ruminal Parameters: Purine Concentration on Incubated Forage (RNAF), Purine Concentration in Liquid (RNAL), Whole Rumen Purine Concentation (RNAW), CMCase Activity on Incubated Forage (CMCF), CMCase Activity in Liquid (CMCL), Ammonia (NH_3), pH, NDF Disappearance Rate (NDF), Liquid Dilution Rate (LIQ), and Particle Dilution Rate (PAR) measured for Principal Component Analysis (Trial 2).

	Correlations									
	RNAF	RNAL	RNAW	CMCF	CMCL	NH_3	pH	NDF	LIQ	PAR
RNAF	1.0	0.02	0.31	0.49	-0.39	-0.34	0.16	-0.55	-0.15	-0.31
RNAL	0.02	1.0	0.09	-0.22	-0.29	0.14	0.07	-0.20	-0.25	-0.24
RNAW	0.31	0.09	1.0	0.12	0.18	0.08	-0.6	0.01	0.23	0.31
CMCF	0.49	-0.22	0.12	1.0	0.07	-0.20	0.02	-0.08	0.07	0.03
CMCL	-0.39	-0.29	0.18	0.07	1.0	0.14	-0.13	0.78	0.57	0.80
NH_3	-0.34	0.14	0.08	-0.20	0.14	1.0	0.10	-0.03	0.22	0.01
pH	0.16	0.07	-0.06	0.02	-0.13	0.10	1.0	-0.27	-0.25	-0.15
NDF	-0.55	-0.20	0.01	-0.08	0.78	-0.03	-0.27	1.0	0.56	0.79
LIQ	-0.15	-0.25	0.23	0.07	0.57	0.22	-0.25	0.56	1.0	0.71
PAR	-0.31	-0.24	0.31	0.03	0.80	0.01	-0.15	0.79	0.71	1.0