Influence of amino acids and branch chain organic acids on ruminant fiber fermentation
by Connie Kay Clark

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

Abstract:
Amino acids and branched chain organic acids were examined for possible positive effects on fiber fermentation in the rumen. Nine L-isomer amino acids and three branch chain organic acids were added as single amendments to in vitro buffer to evaluate influence of these additions on fermentation kinetics of native winter range forage collected via esophageal fistula. Amino acids provided 50 mg nitrogen per 100 ml buffer and branch chain organic acids were added at a .01% level. Dry matter (DM) and neutral detergent fiber (NDF) disappearance at 48 h (41.2% and 53.1%) were similar (P>.05) for buffer amendments and the control, although treatments differed significantly (P<.05). In comparison to the control, methionine, arginine, and cysteine increased (PC.05) both DM and NDF fermentation rate, and histidine and leucine influenced only DM fermentation rate. The correlation between DM fermentation rate and extent was positive and significant (P<.01). Lag time calculated for DM (29.1 h) and NDF (23.3 h) was similar for all treatments.

Methionine was further evaluated in an in situ trial. Three isonitrogenous isocaloric (.32 kg crude protein and 1.23 kg TDN) supplements, DL-methionine-urea, soybean meal (SBM), and urea, were formulated which contained: 4, 0, 63, 33; 0, 81, 0, 19; and 0, 0, 67, 33% nitrogen from DL-methionine, SBM, urea and beet pulp. Cannulated cows receiving the supplements were maintained on a mixture of 75% mature grass hay and 25% barley straw. DL-methionine-urea supplementation increased (P<.05) DM fermentation rate in comparison to the other supplements. Estimated rumen digestibility (48%) was improved (PC.01) and particulate passage rate (4.2 %/h) was slower (PC.05) with DL-methionine-urea and urea supplementation as compared to SBM. Neutral detergent fiber fermentation followed similar trends; however, no significant differences could be detected. Lag times (1.5 h) were calculated for NDF only.

A heifer growth trial was conducted utilizing the three supplements evaluated in the in situ trial and a SBM-urea supplement. Heifers were fed the same low quality roughage as described for the in situ trial. No differences were detected for average daily gain, intake, or feed efficiency.

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Connie Kay Clark

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ABSTRACT

Amino acids and branched chain organic acids were examined for possible positive effects on fiber fermentation in the rumen. Nine L-isomer amino acids and three branch chain organic acids were added as single amendments to *in vitro* buffer to evaluate influence of these additions on fermentation kinetics of native winter range forage collected via esophageal fistula. Amino acids provided 50 mg nitrogen per 100 ml buffer and branch chain organic acids were added at a .01% level. Dry matter (DM) and neutral detergent fiber (NDF) disappearance at 48 h (41.2% and 53.1%) were similar ($P>.05$) for buffer amendments and the control, although treatments differed significantly ($P<.05$). In comparison to the control, methionine, arginine, and cysteine increased ($P<.05$) both DM and NDF fermentation rate, and histidine and leucine influenced only DM fermentation rate. The correlation between DM fermentation rate and extent was positive and significant ($P<.01$). Lag time calculated for DM (29.1 h) and NDF (23.3 h) was similar for all treatments.

Methionine was further evaluated in an in situ trial. Three isonitrogenous isocaloric (.32 kg crude protein and 1.23 kg TDN) supplements, DL-methionine-urea, soybean meal (SBM), and urea, were formulated which contained: 4, 0, 63, 33; 0, 81, 0, 19; and 0, 0, 67, 33% nitrogen from DL-methionine, SBM, urea and beet pulp. Cannulated cows receiving the supplements were maintained on a mixture of 75% mature grass hay and 25% barley straw. DL-methionine-urea supplementation increased ($P<.05$) DM fermentation rate in comparison to the other supplements. Estimated rumen digestibility (48%) was improved ($P<.01$) and particulate passage rate (4.2 %/h) was slower ($P<.05$) with DL-methionine-urea and urea supplementation as compared to SBM. Neutral detergent fiber fermentation followed similar trends; however, no significant differences could be detected. Lag times (1.5 h) were calculated for NDF only.

A heifer growth trial was conducted utilizing the three supplements evaluated in the in situ trial and a SBM-urea supplement. Heifers were fed the same low quality roughage as described for the in situ trial. No differences were detected for average daily gain, intake, or feed efficiency.

Chapter 1

INTRODUCTION

Agriculture is challenged to feed an ever-increasing population with declining available resources. The ruminant provides man with a vital link to an abundant store of organic energy, forage, which supplies a source of nutrients for the ruminant that cannot be used by man. Fiber is a resource found in many areas unsuitable for cultivation or human residence. Equipped with a unique fermentation vat, ruminants are capable of utilizing fiber and complement man's need for energy and high quality protein without direct competition with man for food or land.

Forage harvesting by the ruminant animal may be the sole means of utilizing the rangelands in Montana. Although range plants can provide an adequate diet for the ruminant, the nutritional status of range plants vary substantially depending on the season of the year. Fiber digestibility may range from sixty-seven percent in June down to forty-nine percent or lower in December. Winter range supplementation with a protein source has been a common management practice to help maintain animals on winter range. A supplement program capable of increasing fiber digestibility and improving diet utilization by the animal could benefit the producer. In viewing the price-cost squeeze experienced by animal industries, a decrease in supplemental feed cost as well as better diet utilization by animals, may improve profits for producers.

In order to approach optimum rumen fermentation of winter range forage, minimum ruminal conditions are required. The rumen provides a suitable environment for anaerobic microorganisms to ferment fiber the animal consumed, and subsequently produce volatile fatty acids which are used by the animal as energy. The microbial population of the rumen may have specific nutritional requirements to obtain an optimum level of growth and reproduction which could enhance fiber fermentation. The importance of meeting nutritional needs of microbes may be magnified for ruminants on winter range as the nutrient supply for rumen microorganisms fermenting low quality forage could put limiting restraints on microbial growth and reproduction.

Cellulolytic bacteria are necessary for fiber digestion, but other bacteria must not be ignored because many of the microorganisms work in a synergistic fashion to accomplish fermentation. Providing rumen microorganisms with readily accessible amino acids or branch chain organic acids may enhance turnover of the microbial population. Although cellulolytic bacteria utilize ammonia as a major source of nitrogen, small amounts of amino acids or branch chain organic acids added to rumen bacteria are known to stimulate growth. It has been demonstrated that amino acids and branch chain organic acids improved microbial growth rate, but it is not known how this may influence fermentation.

This study had three objectives:

1) evaluate influence of amino acids and branch chain organic acids on fermentation of winter range forage,

2) incorporate an amino acid or branch chain organic acid into a supplement for cows consuming a low quality

roughage and evaluate influence of the supplement on fermentation parameters, and

3) evaluate influence of the supplement onperformance of growing heifers.

The study consisted of three phases. The first phase investigated the influence of single amino acid or branch chain organic acid addition to in vitro buffer solution on fermentation of native winter range forage, and served as a screening procedure for the following phases. An in situ trial was completed for phase two, in which a DL-methionine-urea supplement was compared to soybean meal and urea supplements. The final phase evaluated feedlot performance of heifers receiving a low quality forage supplemented with one of four supplements; DL-methionine-urea, urea, soybean meal, or soybean meal-urea.

Chapter 2

LITERATURE REVIEW

The ruminant is unique in its ability to harvest highly fibrous plants and produce nutritious food and products for man. To accomplish fiber utilization, the ruminant animal and rumen microbe populations live in a symbiotic relationship. With ingestion of feed, the ruminant provides a nutrient source for the microorganisms to grow and reproduce. The ruminant also contributes a suitable microbial environment with optimum temperature, pH, and mixing of rumen contents.

The rumen microorganisms provide energy substrate, protein and vitamins for the ruminant animal. An important function of the microbes is the production of cellulase and hemicellulase (Smith et al., 1973; Dehority, 1968), enzymes which enzymatically degrade fibrous digesta. Cellulose and hemicellulose supply an abundant source of carbon and energy in the ruminant's diet and without the fermentation action of the rumen bacteria these nutrients would be unavailable to the animal. The microbe population utilizes the carbon and energy of cellulose to produce volatile fatty acids such as acetic, propionic and butyric acid which the animal absorbs into the blood and uses for energy.

The microbial biomass of the rumen is composed of a multitude of differing microbe species capable of many biochemical functions (Hungate, 1966). Rumen bacteria appear to be more essential than

protozoa because rumen fermentation can proceed normally in defaunated animals (Eadie, 1962). Bacteria can be roughly divided into three groups according to their activity: cellulolytic bacteria, amylolytic bacteria and proteolytic bacteria, however many of the activities of individual species overlap with other species and it is difficult to designate bacteria into specific groups. Important cellulolytic species include Ruminococcus albus, Ruminococcus flavefaciens, Bacteroides succinogens, and Butyrivibrio fibrisolvens (Wolin, 1979).

To understand the process of cellulose fermentation, all species of rumen microbes need to be considered as fermentation occurs through the interaction of many species. Only a few of the predominant species produce cellulase for degradation. The hydrolysis products released from cellulose degradation are utilized as fermentation substrates by noncellulolytic as well as cellulolytic microorganisms. This reduces end product inhibition which improves the efficiency of enzymatic substrate degradation.

Bacterial Nitrogen and Branch Chain Organic Acid Requirements

Bryant (1973) reviewed nutrient requirements of cellulolytic bacteria. Three of the major cellulolytic species, Bacteroides succinogens, Ruminococcus albus, and Ruminococcus flavefaciens, required ammonia as the essential nitrogen source during growth regardless of the amount of amino acid and peptide nitrogen in the medium (Bryant and Robinson, 1962). Some strains are incapable of utilizing organic nitrogen source. These bacteria possibly lack the mechanisms to transport most amino acids or peptides into the cell.

Studies with Butyrivibrio fibrisolvens, a cellulolytic species, have indicated amino acids are essential for growth in some strains (Bryant and Robinson, 1962; Gill and King, 1958).

Branch chain organic acids, isovaleric, isobutyric and 2-methylbutyric, are required by major cellulolytic species (Bryant and Robinson, 1962). Specific requirements for branched chain organic acids of cellulolytic bacteria has been studied (Dehority et al., 1967; Allison et al., 1962a). Bacteroides succinogens require a combination of either isobutyric acid or 2-methylbutyric acid with valeric acid (straight chain fatty acid). Ruminococcus albus exhibited a requirement for isobutyrate or 2-methylbutyerate to achieve maximum growth. The Ruminococcus albus bacteria incorporated the branched chain organic acids into branched chain 14- and 16-carbon fatty acids or 14- and 16-carbon aldehydes (Allison et al., 1962b). Ruminococcus flavefaciens can utilize isobutyric, isovaleric, or 2-methylbutyric acid to maximize growth. Isovaleric acid was found to serve as a carbon skeleton for leucine synthesis by R. flavefaciens and isobutyric acid served the same function for valine synthesis (Allison et al., 1962a). Ruminococcus flavefaciens have limited ability to incorporate exogenous branched chain amino acids. Allison (1969) later reported that in addition to synthesis of valine, leucine, long chain branched fatty acids and aldehydes, the bacteria utilize shorter branched chain organic acids to synthesize isoleucine.

There is evidence of nutritional interdependence among rumen bacteria. Miura, et al. (1980) reported growth stimulation of Bacteroides amylophilus by starch resulted in successive growth of

amino acid-dependent Megasphaera elsdenii and branch chain fatty acid-dependent Ruminococcus albus in a media lacking both amino acids and branch chain fatty acids, indicating interdependence among the three bacteria species. Allison (1978) has also reported branch chain organic acid production by Megasphaera elsdenii. Further investigation by Muira, et al. (1983) demonstrated mixed rumen bacteria interact nutritionally in the same manner as revealed in pure culture mixture. The microbial population changes in relation to alterations of amino acid and branch chain fatty acid concentrations. Preformed amino acids or branch chain fatty acids are not essential for growth of branch chain fatty acid-dependent bacteria (Slyter and Weaver, 1971); however lag in the appearance of cellulose digestion may reflect time required for producing branch chain fatty acids via nutritional interdependence.

Bacterial Growth Rate

Currently in the literature, several publications report responses in rumen bacterial growth rate with the addition of amino acids, however; not much is available on the influence of amino acid additions on fermentation rate in the rumen. There are several factors in the complex ecosystem of the rumen that can have an effect on growth rate of microorganisms. Rates of hydrolysis of polymeric carbon and nitrogen sources, concentrations of permeable macronutrients used by microorganisms, concentrations of potentially growth-limiting micronutrients, inhibitory agents, and pH may be influential factors on growth rate (Wolin, 1979). The effect of

supplying readily available amino acids to rumen microorganisms may have an impact on microbial growth rate. An estimation that 60 to 80% of microbial nitrogen was derived from ammonia and 40 to 60% from amino acid- and peptide-nitrogen has been reported (Al-Rabbat et al., 1971; Nolan and Leng, 1972). An optimum ratio, 75% nonprotein nitrogen to 25% amino acid nitrogen, for rumen microbial growth has been indicated (Maeng et al., 1976).

A study conducted by Maeng and Baldwin (1976a) revealed that the addition of small amounts of amino acids to a diet containing urea as the nitrogen source improved rumen microbial yield. The greatest impact upon microbial yields with amino acid additions occured immediately after feeding and became less evident as time passed. The microbial nitrogen, protein and cell yield per mol ATP and per mol VFA was increased ($P<.01$) by amino acid containing treatments. The projected microbial nitrogen, protein, and cell yields per day and per kg of diet dry matter also was significantly improved with the addition of amino acids. A second study completed by Maeng and Baldwin (1976b) indicated a stimulatory microbial response with partial substitution of amino acids for urea. Amino acids replaced 25% of urea-nitrogen in a growth media. Growth rate of rumen bacteria was twofold and mean doubling time was reduced by half.

The relative degree to which ATP or other energy-rich compounds produced from catabolic activities are utilized by anabolic activities of the cell is referred to as energetic uncoupling. To obtain maximum microbial cell yields per unit of nutrient input, the rate of ATP production from fermentation reactions must equal the usage rate by

biosynthetic reactions at all times or in other words, the energetic uncoupling must be minimized. Hespell and Bryant (1979) suggest the stimulated bacterial growth response observed with amino acid additions is due to a decrease in the degree of energetic uncoupling with the rumen microbial population as a whole.

Studies have indicated the addition of branched chain organic acids have a positive influence on bacterial growth in the rumen. This is especially true of cellulolytics which have been shown to have nutrional requirements for branched chain organic acids. Russell and Sniffen (1984) reported the addition of low concentrations of isovaleric and 2-methylbutyric acid improved synthesis efficiency of rumen bacterial protein from carbohydrates in vitro. Bacterial growth measured by cell protein synthesis was increased 11.2% and 16.4% with the addition of isovaleric and 2-methylbutyric acid. A combination of isovaleric, isobutyric, 2-methylbutyric, and valeric acids produced a 18.7% increase in protein synthesis when added to in vitro inoculum. Branched chain organic acids have also increased bacterial growth measured in the rumen of sheep consuming a low quality forage supplemented with urea, isovalerate, isobutyerate, and 2-methylbutyerate (Hemsley and Moir, 1963).

Branched chain organic acids have been shown to effect digestion of forage. Isovaleric and isobutyric acid were added to an in vitro system fermenting purified cellulose with urea serving as the nitrogen source. Digestibility values at 30 hours revealed a substantial increase for the branched chain fatty acid treatment in comparison to the control (Bentley et al., 1955). Soofi et al. (1982) more recently

reported similar results. The addition of branched chain volatile acids increased ($P<.05$) in vitro dry matter digestibility at 48 hours, 48.1% digested verses 45.3% digested in the control. Gorosito et al. (1985) also studied in vitro digestion of forage as influenced by additions of isovaleric, isobutyric, 2-methylbutyric and valeric acid. All acids except valeric had positive effects on fermentation as indicated by increased cell wall digestibility at 24 hours and improved ammonia utilization. The greatest improvement for cell wall digestion was noted for the grass hay although alfalfa hay cell wall digestibility was also increased.

Hemsley and Moir (1963) reported an increase in intake and passage rate when isobutyric, isovaleric and 2-methylbutyric acid were added to a urea supplement provided to sheep consuming a low quality forage. Felix et al. (1980) fed isoacids with urea to lactating cows and indicated no influence on nitrogen or dry matter digestibility; however loss of urinary nitrogen was decreased and percent of absorbed nitrogen retained was higher indicating an improvement in utilization of nitrogen.

Fiber Fermentation

Forage digestion in the rumen involves complex relationships and interactions among the animal, microbial populations in the rumen, and plant components. Understanding ruminal digestion is simplified when the process is divided into four components: digestion rate, digestion lag, extent of digestibility, and passage rate (Mertens, 1977).

The first component to be addressed is digestion rate. Rate of digestion has been suggested as being important in assessing forage quality and voluntary intake (Crampton, 1957; Thorton and Minson, 1972). The theory is that enhancing rate of digestion would increase disappearance of fiber from the rumen; therefore, freeing space for additional intake. Mertens and Ely (1979) reported simulation results in which values for coastal bermuda grass suggested that a 1.0% increase in digestion rate resulted in a .67% increase in maximum digestible dry matter intake. Digestion rate can have significant effects on dry matter digestibility (Mertens, 1977). The effect of rate is magnified in grasses with high levels of cell wall content.

Several factors have been suggested which may have an affect on digestion rate. Limited growth and reproduction of microorganisms in the rumen may influence rate because microorganisms produce enzymes necessary for fiber degradation; however, few factors that affect microbial population have been studied for their impact on rate of digestion. Plant composition has an impact on rate of digestion. The morphological types of leaf tissues vary in rate of digestion with the mesophyll and phloem tissue being rapidly digested, while bundle sheaths and epidermis tissue are slowly degraded and the vascular bundles and sclerynchema are almost entirely indigestible (Akin et al., 1974; Akin and Amos, 1975). In addition, individual animals may affect digestion rate through mastication and rumination. It has been reported (Dehoirity and Johnson, 1961) that reducing particle size can increase cell wall digestibility in vitro. The animal also influences rumen pH through buffering capacity. Rumen pH affects the ratio of

species in the rumen microbe population (Russell et al., 1979), and inhibits cellulase activity when pH falls below 6.0 (Stewart, 1977).

Fiber digestion lag time is a period at the initial onset of digestion in which very little digestion occurs. The effect of lag time increases exponentially with increasing levels of fiber in the diet, indicating its importance as a variable in digestion (Mertens, 1977). There have been several explanations to account for the phenomenon of lag time. Brazle and Harbers (1977) completed work that indicated penetration of the epidermal layer may provide an initial barrier to digestion. It may also be possible that physical or chemical inhibitors must be removed or that swelling of fibers is required before enzymes can contact and react with fiber particles. Attachment of bacteria to fiber is necessary for some fiber digestion (Akin, 1979). These suggestions all imply that plant and bacteria interactions are responsible for lag time.

Mertens and Ely (1982) reviewed other explanations for lag time. Period of lag may be due to delay in digestion until bacterial numbers and concentration of fiber-digesting enzymes reach levels that are no longer limiting to digestion. A preference for starch or other readily fermentable carbohydrates may also be a factor involved in lag time. The addition of starch to forage has shown to increase fiber digestion lag time (Mertens and Loften, 1980).

The third component of fiber digestion, extent of digestibility, has been shown to have a great impact on digestibility of forage. In a simulation (Mertens and Ely, 1979), an increase in the indigestible fraction resulted in a decrease in digestibility and an increase in

neutral detergent fiber content of the rumen and intestines. The dry matter intake was also affected and showed a decrease. Values for coastal bermuda grass indicated that a 1.0% decrease in indigestibility resulted in a 1.0% increase in maximum digestible dry matter intake.

Lignin is generally accepted as an anti-quality factor which inhibits utilization of fibrous carbohydrates in forage. Smith et al. (1972) noted correlations of .78 or higher between lignin and the 72 hour cell wall indigestibility used to predict potential digestibility. This relates to work reported by Akin and Amos (1975) in which the indigestible portion of plant material contains high levels of lignin. Waldo and Smith (1979) suggest cellulose is composed of two fractions; potentially digestible and indigestible. The potentially digestible fraction disappears from the rumen by both passage and digestion whereas the indigestible disappears only with passage. Lignin concentration influences extent of digestibility but increasing lignin does not slow rate of digestion.

Passage rate is the fourth component of fiber digestion. When long forage is consumed by an animal, particle size must be reduced before the forage can escape the rumen (Pearce, 1967). Particle reduction in the rumen may be considered as a rate limiting step in the process of forage escape from the rumen (Smith et al., 1983). Prehension, rumination (Welch, 1982), and microbial weakening of fiber (Akin and Amos, 1975) are factors involved in particle reduction. Contribution of microbial action to particle reduction has been questioned. Murphy and Nicoletti (1984) evaluated the particle

size of coarse ground hay after incubation in vitro for 48 hours and indicated minor contribution of microbial action to particle reduction. Conflicting results were reported by Pearce and Moir (1964) in which sheep fitted with devices to inhibit rumination were able to consume a diet of known high rumination potential, and it was concluded that microbial breakdown was sufficient in the rumen for passage of digesta. In a model presented by Mertens (1977) ruminal digestion of grass decreased exponentially as passage rate increased. With a passage rate of 1.0% per hour grass dry matter digestibility was 67.2%, and when rate was increased to 5.0% disappearance per hour the dry matter digestibility value dropped to 58.4%. Studies have shown that grinding a diet to decrease particle size decreases retention time in the rumen (Moore, 1964, Alwash and Thomas, 1971). Retention time has also been effected by feed intake (Grovum and Williams, 1979). As intake increases retention time in the rumen decreases. It is evident that passage rate can be influenced by physical form of the diet, rumination, and to a lesser degree, microbial action, and passage of feed through the rumen can have an effect on digestion.

The relationship of liquid passage and fermentation is receiving more attention. Fermentation responses to change in fluid dilution rate are complex. Bull et al. (1979) suggest that increased liquid turnover may benefit the animal in terms of microbial growth efficiency, improvement in utilization of nonprotein nitrogen and reduction in fermentation losses.

Adams and Kartchner (1984) reported that increased intake in steers consuming a hay diet increased the fluid dilution rate. Fluid dilution rate was 4.3 %/h for steers consuming 1.40% body weight, and 7.2 %/h for those consuming 2.4% body weight. Estell and Galyean (1985) indicate similar results with compiled data collected from seven beef steer trials.

Diet composition is another factor that may influence liquid dilution rate. Owens and Isaacson (1977) related elevation of percent roughage in the diet to increased intake resulting in higher rate of liquid passage. Other studies revealed a decrease in liquid dilution rate when the level of concentrates were elevated in the diet (Cole et al., 1976; Goetsch and Galyean, 1982).

Liquid dilution rate has also been related to ammonia levels and osmolarity in the rumen (Prigge et al., 1984). Estell and Galyean (1985) showed a positive relationship ($P<.01$) existed between ammonia concentration and fluid dilution rate in a trial where beef steers were grazing blue grama rangeland. Effecting fluid dilution rate with hypertonic solutions infused in the rumen was studied by Rogers et al. (1979). Holstein steers fed a high roughage diet were infused with sodium chloride. Fluid dilution rates increased ($P<.05$) from 5.85 %/h with no infusion to 7.11 %/h with infusion of sodium chloride. The increased dilution rate was not accompanied by changes in rumen volume. Increased transruminal water flux accounted for the higher level of liquid required for increased flow rate.

Chapter 3

EXPERIMENTAL PROCEDURE

In Vitro Trials

Nine amino acids, arginine, cysteine, histidine, leucine, lysine, methionine, tryptophan, phenylalanine, and isoleucine, and three branched chain organic acids, isovaleric, isobutyric, and 2-methylbutyric, were evaluated to determine their influence on in vitro fermentation of winter range forage esophageal extrusa. Fermentation rate, digestibility and lag times were calculated for both dry matter (DM) and neutral detergent fiber (NDF) content of fermented samples. Two replications of triplicate samples incubated with either single amino acid or organic acid additions were conducted.

The winter range forage was a composite of esophageal collections from cows grazing winter pastures on the Red Bluff Range Research Ranch located 56 km west of Bozeman, Montana on the northwest slope of the Madison Range. Grasses composed 65 to 75% of the vegetation, with forbs and woody species making up the remaining 25 to 35%. The forage samples were freeze dried, ground through a 1 mm screen and weighed (.25 gram) into 50 ml in vitro fermentation tubes. McDougall's buffer solution (McDougall, 1948) was modified to contain 50 mg nitrogen per 100 ml buffer. Amino acid was added to the buffer to provide 25% of buffer nitrogen with 75% supplied by urea. All amino acids were L-isomers. Branch chain organic acids were added to buffer at 0.1%, and 100% of buffer nitrogen was contributed by urea. Urea supplied all the

buffer nitrogen in the buffer solution for the control. Following pH adjustment to 6.9, 20 ml of buffer were added to the forage sample. Rumen innoculum was obtained from two fistulated crossbred beef cows fed mature grass hay and barley straw ad libitum. Fluid from donor cows was composited and strained through sixteen layers of cheesecloth to remove large digesta particles. The fluid was continuously agitated and maintained at 39° C while 5 ml were innoculated into each in vitro tube. Fermentation tubes were flushed with CO_2, capped, and incubated at 39° C. At 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 hours postinoculation, fermentation was ceased with the addition of .5 ml 5% mercuric chloride in two blank, triplicate treatment and control tubes. After incubation, tubes were centrifuged at 2000 rpm for 15 min, decanted, dried for 48 hours at 60° C, and weighed to determine DM disappearance. The concentration of NDF was determined for each residue (Van Soest and Robertson, 1980).

Fermentation rates for DM and NDF were determined by nonlinear regression techniques (SAS, 1978). Variable b in the equation, dig. = ae^{bt}, estimated the rate of change of DM or NDF for the entire incubation period. Lag times for DM and NDF were calculated using the first order kinetic equation (Mertens, 1977):

$$\log \frac{A_o}{A} = \frac{K(t-t_L)}{2.303},$$

where A_o = concentration at zero hour, A = concentration at time t, K= rate of disappearance, and t_L = lag time.

Data were subjected to analysis of variance for randomized block design, whereas the replicated in vitro runs were designated as

blocks. When a significant f-value was indicated, means were tested by Least Significant Difference multiple comparison (Snededcor and Cochran, 1980).

In Situ Trial

Methionine additions to in vitro buffers increased ($P<.05$) DM and NDF fermentation rate in comparison to the control and exhibited higher values for DM and NDF digestibility although not significant. Based on the in vitro results methionine was selected for further evaluation. Feed grade methionine is an amino acid commercially available, which could be incorporated into supplements at competitive costs.

Three supplements designated as, DL-methionine-urea, SBM and urea, (table 1) were formulated to provide 1.23 kg total digestible nutrients and .32 kg crude protein equivalence daily, and were evaluated for their influence on forage DM and NDF rumen fermentation. The nitrogen sources for the supplements were DL-methionine, SBM, urea, and beet pulp. DL-methionine provided 4%, urea 63%, and beet pulp 33% of the crude protein in the DL-methionine-urea supplement. Soybean meal contributed 81% of the protein content in the soybean meal supplement and beet pulp supplied 19%. Fifty-three percent of the protein in the urea supplement was derived from urea, 12% from ammonium sulfate, and 31% from beet pulp. Ammonium sulfate was added to the urea supplement to provide 7.9 grams sulfur, an equivalent amount of sulfur as contributed by methionine in the DL-methionine-urea supplement.

Six mature crossbred beef cows fitted with rumen cannulas were utilized in this trial. Each treatment replication (two cows) were confined in one pen and maintained on a forage mix containing 75% mature grass hay and 25% barley straw, chopped by a tub grinder thru a 5 cm screen, and fed ad libitum. The forage was 7.0% crude protein and 62.96% NDF on an as fed basis. Cows consumed approximately 13.33 kg DM of the chopped forage mix daily. The supplements were fed daily at 0800 hour when cows were haltered and tethered to assure each cow consumed all of the daily supplement treatment. The amount of supplement fed per head per day was as follows: 1.95 kg DL-methionine-urea supplements, 1.97 kg urea supplement, and 1.85 kg SBM supplement. Each supplement supplied .32 kg protein and 1.23 kg total digestible nutrients. The experimental periods allowed for 10 days of adaptation to supplements followed by a five day sample collection period.

Bags were made of nylon monofilament material with a pore size of 44 microns. Each nylon bag was double stitched around the bag perimeter and the outer dimensions were 15.2x17.8 cm. The sample fermented in the nylon bags was identical to the chopped forage fed to the cows. The forage was ground by a Wiley mill through a 2 mm screen and three gram samples were placed in each bag. This allowed for a ratio of 7 mg of sample per cm^2 of nylon material (Uden and Van Soest, 1984). Bags were attached to snap swivels on a hoop of tygon tubing filled with a chrome ball chain for suspension Rumen digesta collected from cannulated cows fed the diet previously described was prepared as a mordant according to Uden et al. (1980) and contained

3.27% chromium on the fiber. The mordanted fiber served as a particulate passage marker in the rumen. Cobalt EDTA was prepared as a liquid passage marker in the rumen (Uden et al., 1980).

At zero hour on day one of the collection periods, each cannulated cow was dosed with 100 g of chromium mordanted fiber and 15 g cobalt EDTA. The bags were suspended in the rumen and 15 minutes later rumen contents were sampled. All rumen samples were grab samples (500 ml) from approximately the same location in the cranial sac of the rumen. Collection times were 4, 8, 12, 18, 24, 36, 72, and 96 hours. For each time period, two sample filled bags and rumen extrusa were collected from every cow, and one blank bag was collected from one cow on each treatment.

Rumen samples were immediately treated with 1 ml 5% mercuric chloride to cease any further microbial activity, centrifuged at 2000 rpm for 15 minutes, the fluid was decanted, and both fluid and fiber samples were frozen at -4° C for later analysis. The nylon bags were washed with cold water until the rinse water was clear when squeezed out of the bag. The bags were dried in a forced air oven at 60° C for 48 hours.

Dry matter and NDF (Van Soest and Robertson, 1980) content was determined for each bag. Rumen digesta samples were prepared (Williams, David and Iismaa, 1962) and the chromium concentration was determined with atomic absorption spectrophotometry. Rumen fluid samples were analyzed for cobalt and rumen ammonia concentration. A portion of each fluid sample was centrifuged at 10,000 rpm for 10 minutes and the supernatant was evaluated for cobalt concentration

with an atomic absorption spectrophotometer (Gaylean and McCollum, 1985). Rumen ammonia levels (mg/100 ml rumen fluid) were determined by a macro Kjeldahl procedure (AOAC, 1980).

Dry matter and NDF disappearance values were subjected to nonlinear regression (SAS, 1985) to estimate rate of fermentation for each parameter. The equation used for the nonlinear regression analysis was; $Y = ae^{[-k\ (t-tlag)]} + u$, where as Y = digestibility, a = potential digestibility, -k = rate of fermentation, t = time, tlag = lag period, and u = undigestible fraction. Dry matter data did not indicate a period of fermentation lag, therefore the tlag variable was not included in the DM equation for fermentation parameters estimates. Cobalt and chromium concentrations were transformed into natural logarithms and linear regression techniques (Snedecor and Cochran, 1982) were applied to determine the rate of passage from the rumen of the liquid and particulate fractions.

Dry matter and NDF rumen digestibilities of the forage sample were calculated with the following equation:

$$\text{Rumen Digestibility} = 100 - [a\ (k_1/(k_1+k_2)) + b\ ],$$

a = potential digestibility, k_1 = particulate rate of escape from the rumen, k_2 = fermentation rate, and b = undegradable portion. The potential digestibility value used for calculation was the percent digestible at 72 hours.

The liquid dilution rate constant was utilized to predict three liquid parameters of the rumen, fluid volume, fluid outflow, and fluid turnover time as influenced by the supplements. Fluid volume was estimated using the equation, volume = (ml/a)(b), whereas a =

concentration of cobalt at zero hour (intercept of the linear regression line) and b = amount of cobalt EDTA given at time zero hour. The equation, outflow = liquid volume/ escape rate, provided a measure of fluid outflow from the rumen. The third parameter, fluid turnover time, equals the inverse of the rate constant.

The experimental design of this trial was a 3x3 replicated Latin-square; whereas, over the course of the three periods all cows received each treatment. All estimates were subjected to analysis of variance for replicated Latin-squares (Snedecor and Cochran, 1980). When a significant f-value was indicated then mean differences were determined by the Newman-Keuls means test.

Heifer Growth Trial

Four isonitrogenous isocaloric supplements (table 1) incorporated into roughage diets fed to weanling heifer calves were evaluated by performance measurements. Three supplements, DL-methionine-urea, SBM and urea, were formulated similar to those described in the in situ trial. In the fourth supplement, soybean meal-urea, urea contributed 47%, soybean meal 28%, and beet pulp 25% to the protein content. The supplements and a chopped roughage mixture consisting of 75% mature grass hay and 25% barley straw, were used to formulate rations to meet Nutritional Research Council requirements (NRC, 1976) for heifers gaining .3 kg per day.

The feeding trial was conducted at the Montana Agriculture Experiment Station Feedlot, Bozeman. Fifty-six weanling heifer calves with an average initial weight of 231.5 kg were used in the feeding

trial. Breeds of the heifers included Angus, Hereford, Angus-Hereford crossbred, and Tarantaise crossbred. Initial weights were an average of two consecutive day weights. The heifers were randomly assigned to groups within breed and body weight stratification. Four pens were allotted to each treatment group; two of the pens held four heifers while the other two pens contained three. The pens had fence line bunks protected by partial roof cover, and were bedded with straw. Automatic waterers provided water and trace mineral salt blocks were available in the bunks for each pen.

The heifers were fed once daily in the morning. Diets were mixed individually by treatment in a feed wagon equipped with a scale to weigh the forage. For those diets containing urea, the urea was dissolved in 11.748 liters of water and sprayed over the ration as it was mixing in the feed wagon. This procedure allowed for a more even distribution of the urea and reduction in palatability problems. Water without urea was sprayed on the SBM diet in the same fashion. Feed remaining in the bunks was removed and weighed every other day to estimate DM intake. When heifers were consuming all of their diets the forage level in the ration was increased, however the amount of supplement remained constant throughout the entire feeding period. The supplements were fed to provide the following levels per head per day: 1.95 kg for the DL-methionine-urea treatment, 1.97 kg for the urea treatment, 1.68 kg for the SBM treatment, and 1.85 kg for the SBM-urea treatment.

The duration of the feeding trial was ninety days. During the trial the heifers were weighed every fourteen days before the morning

feeding to monitor animal performance. Water was withheld for twelve hours prior to weighing time. A final weight was an average of weights collected on the last two days of the trial. Intake, daily gain, and feed efficiency were calculated as indications of performance. Individual animals were used as the experimental unit for average daily gain while pen was the experimental unit for intake and feed efficiency. Data was analyzed by analysis of variance for complex randomized block designs utilizing y = treatment, pen(treatment) as the model (SAS, 1985).

Chapter 4

RESULTS

In Vitro Trial

Extent of in vitro fermentation at 48 hours (table 2) for all amino acid, arginine, cysteine, histidine, leucine, lysine, methionine, tryptophan, phenylalanine, and isoleucine, and branch chain organic acid, isobutyric, isovaleric, and 2-methylbutyric, treatments in comparison to forage alone was similar ($P>.05$). The control, containing no amendments, had 41.2% disappearance of DM at 48 hours. Other dry matter disappearance values ranged 36.9% (isobutyric acid) to 47.4% (methionine). The addition of arginine (47.1%) or methionine (47.4%) improved ($P<.05$) disappearance when compared to isobutyric acid, lysine or phenylalanine (36.9, 37.1, and 39.9%, respectively). It is interesting to note that the control had the third lowest disappearance extent (41.5%) which was nearly six percentage units lower than either arginine or methionine addition.

Dry matter fermentation rate (table 2) was increased ($P<.05$) by over 30% with addition of arginine, methionine, histidine, leucine, or cysteine (3.6, 3.6, 3.5, 3.5, and 3.4 %/h, respectively) in comparison to forage alone (2.7 %/h) or lysine (2.6 %/h) addition. Tryptophan, isoleucine, phenylalanine, isobutyric, leucine and 2-methylbutyric (3.1, 3.3, 2.8, 2.9, 3.0, and 3.1 %/h, respectively) were similar ($P>.05$) to forage alone. Linear regression analysis indicated a

positive ($P<.01$) correlation ($R^2 = .78$) between extent and rate of dry matter forage.

Neutral detergent fiber 48 hours disappearance (table 3) was similar ($P>.05$) for all amendments in comparison to the control with no amendments. The addition of histidine, arginine, leucine, methionine, isoleucine, isovaleric and 2-methylbutyric acid (57.8, 56.9, 54.5, 56.2, and 56.6%, respectively) improved NDF disappearance in comparison to phenylalanine (47.9%). Tryptophan, phenylalanine, lysine, isobutyric acid, and forage alone consistantly ranked numerically among those treatments with the least DM and NDF fermentation extent. Arginine, histidine, methionine, and isoleucine appeared in the four treatments which ranked highest for both DM and NDF digestion extent. NDF analysis of the winter range forage indicated the sample contained 67.9% NDF which may account for similar trends noted between DM and NDF data.

The rate of NDF fermentation (table 3) for the control was numerically the slowest (2.5 %/h) of all treatments tested, which was slower ($P<.05$) than cysteine, methionine, and leucine (3.8, 3.8, and 3.4 %/h). These amendments improved NDF fermentation rate by 52, 52, and 36%. Linear regression analysis showed no significant correlation between NDF fermentation extent and rate.

Lag times calculated for DM and NDF fermentation appear in table 4. Statistical analysis revealed no significant differences between treatments for DM or NDF lag times. The estimated lag time for in vitro digestion of the DM ranged from 28.0 hours (control) to 32.1