

Influence of different forms of methionine on gain, intake, and ruminal parameters of heifers grazing improved pastures
by Sharon Joyce Sorensen

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Animal Science
Montana State University
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Abstract:
Four experiments were conducted to determine the effect of DL-methionine (MET) and sustained release methionine (SRM) in salt mineral mixtures on heifer growth, DM intake and ruminal parameters. Two performance experiments using DL-methionine and two experiments using SRM were conducted in four consecutive years on the same pastures using a total of 326 bred yearling English crossbred heifers to determine the influence of DL-methionine and SRM on weight gain and intake. Intake did not differ ($P>.05$) from year to year or by treatment. Gains were significantly greater ($P<.05$) for the MET and SRM heifers than control (CON) in 3 out of 4 years. The year there was no benefit to feeding SRM the forage protein content and quantity were lower than the previous years suggesting that possibly SRM is best utilized in situations of low protein and adequate energy intake. Cannulated cows (2/paddock) were used in the third performance study with SRM to investigate ruminal kinetics. All measured ruminal parameters were similar except liquid purines which tended to be higher in SRM animals ($P<.12$) than CON. A latin square (4X4) with 4 mature ruminally cannulated beef cows was used to measure the following: pH, ammonia, DM and NDF disappearance rates, passage rates, carboxymethylcellulase (CMC) activity and purine concentrations for animals receiving control (CON-no treatment), MET, SRM or sodium sulfate (SUL). Cows receiving sustained release methionine had higher ($P<.05$) liquid purine concentrations than CON. This difference was consistent in all ruminal kinetics trials. An increase in ruminal purine concentration would deliver more microbial protein to the small intestine thus improving the protein status of the animal possibly providing the mechanism for improved animal performance.

INFLUENCE OF DIFFERENT FORMS OF METHIONINE ON GAIN, INTAKE, AND RUMINAL PARAMETERS OF HEIFERS GRAZING IMPROVED PASTURES

by

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TABLE OF CONTENTS

	Page
LIST OF TABLES	vi
ABSTRACT	vii
1. INTRODUCTION	1
2. LITERATURE REVIEW	4
3. MATERIALS AND METHODS	14
Experiment 1	14
Experiment 2	18
Trial 1	17
Trial 2	18
Trial 3	18
Experiment 3	21
Experiment 4	23
4. RESULTS AND DISCUSSION	25
Experiment 1	25
Experiment 2	27
Experiment 3	28
Experiment 4	29
5. CONCLUSION AND SUMMARY	33
LITERATURE CITED	36
APPENDIX	43

LIST OF TABLES

Table Page

1. Supplement composition Exp.2 and 3.....44

2. Supplement composition Exp.4.....44

3. Ruminal kinetics and rates as influenced by no supplememt (CON), N-acetyl DL-methionine (MET), MET+Urea (U), and MET+U four times a day (MET+U4X) Exp.1.....45

4. In situ carboxymethylcellulase activity by hour as influenced by no supplement (CON), N-acetyl DL-methionine MET), MET+Urea (U), and MET+U four times a day (MET+U4X) Exp.1.....45

5. Liquid ruminal purine concentrations over time as influenced by no supplement (CON), N-acetyl DL-methionine (MET), MET+Urea (U) and MET+U four times a day (MET+U4X) Exp.1.....46

6. Ruminal kinetics and rates as influenced by sustained release methionine Exp.2.....46

7. Intake and average daily gain as influenced by sustained release methionine (SRM) Exp.2.....47

8. Intake, salt intake, blood urea nitrogen and average daily gain as influenced by sustained release methionine (SRM) Exp.3.....47

9. Forage clip sample composition and total grams dry matter available forage Exp.2 and 3.....47

10. Ruminal kinetics and rates as influenced by sustained release methionine (SRM) Exp.4.....48

11. Blood urea nitrogen levels as influenced by period Exp.3.....48

12. Least squares analysis of variance Exp.1.....49

13. Least squares analysis of variance Exp.2.....50

14. Least squares analysis of variance Exp.3.....51

15. Least squares analysis of variance Exp.4.....52

ABSTRACT

Four experiments were conducted to determine the effect of DL-methionine (MET) and sustained release methionine (SRM) in salt mineral mixtures on heifer growth, DM intake and ruminal parameters. Two performance experiments using DL-methionine and two experiments using SRM were conducted in four consecutive years on the same pastures using a total of 326 bred yearling English crossbred heifers to determine the influence of DL-methionine and SRM on weight gain and intake. Intake did not differ (P>.05) from year to year or by treatment. Gains were significantly greater (P<.05) for the MET and SRM heifers than control (CON) in 3 out of 4 years. The year there was no benefit to feeding SRM the forage protein content and quantity were lower than the previous years suggesting that possibly SRM is best utilized in situations of low protein and adequate energy intake. Cannulated cows (2/paddock) were used in the third performance study with SRM to investigate ruminal kinetics. All measured ruminal parameters were similar except liquid purines which tended to be higher in SRM animals (P<.12) than CON. A latin square (4X4) with 4 mature ruminally cannulated beef cows was used to measure the following: pH, ammonia, DM and NDF disappearance rates, passage rates, carboxymethylcellulase (CMC) activity and purine concentrations for animals receiving control (CON-no treatment), MET, SRM or sodium sulfate (SUL). Cows receiving sustained release methionine had higher (P<.05) liquid purine concentrations than CON. This difference was consistent in all ruminal kinetics trials. An increase in ruminal purine concentration would deliver more microbial protein to the small intestine thus improving the protein status of the animal possibly providing the mechanism for improved animal performance.

CHAPTER 1

INTRODUCTION

Cattle producers would benefit if less costly protein supplement sources were found for use with low protein forages. Summer and fall pastures may not supply the pregnant growing heifer or growing stocker cattle with adequate quantities of protein. Optimal weight gain of cattle grazing summer pastures is a goal for efficient forage use. Optimization may be improved either by increasing total forage DM intake or by improving the balance of nutrients available to the animal. One method of improving animal nutrient balance is to develop strategies for enhancing the quantity or ratio of products derived from microbial activity which limit animal production. Natural protein supplements which are ruminally degradable, such as soybean meal (SBM), have proven to be effective in improving animal performance when protein content of forage declines in late summer). If the components of soybean meal which cause an increase in animal performance could be identified then they might be fed alone without the unnecessary bulk and cost (10 to 19 cents per .45 kg for soybean meal) associated with the natural protein sources.

L-methionine has been found to be a limiting amino acid for growth of ruminal bacteria (Salter et al., 1979). Lodman et al. (1990) ascertained that the half life of DL-methionine influenced the duration of response to ruminal supplementation of DL-methionine while Mangan (1972) determined that the half

life of free amino acids in the rumen is less than one hour. Storm and Ørskov (1984) also established that methionine is the most limiting amino acid for ruminal microbial growth. A "ruminally protected" methionine source that was 28% degraded in the rumen has been shown to increase total ruminal bacterial counts (Arambel et al., 1987). If ruminal methionine concentration can be sustained then we would expect a sustained increase in the quantity and activity of the ruminal microorganisms in methionine restricted conditions. Ruminally resistant N-acetyl DL-methionine was found to increase (Sorensen et al., 1989) purine concentration (a microbial marker) in the ruminal fluid of cows fed forage containing 4.5% crude protein. Wiley et al. (1991) demonstrated that supplementation of DL-methionine in relation to time of feeding influenced ruminal activity. The best responses were found when DL-methionine was supplied 3 to 7 h after feeding. Cattle grazing pastures have feeding cycles (Beverlin et al., 1989, Dunn, 1986), so it is hypothesized that a continuous source of sustained release DL-methionine may improve ruminal microbial response. A practical method of supplying methionine to cattle grazing pastures must be found in order to realize the potential improvement in weight gains associated with the feeding of DL-methionine. Cattle producers require a method of delivery that is relatively simple, economical and utilizes little or no extra labor.

Salt has been found to be an excellent carrier of other

nutrients and feed additives (Sword et al., 1984) and is readily consumed by grazing cattle. Weight gains and intake levels of monensin were the same whether cattle were self-fed or hand-fed a mixture of salt and monensin (Muller et al., 1986). Sorensen et al., (1990) ascertained that grazing beef heifers would consume a salt mixture containing 20% sustained release methionine in sufficient quantities to improve cattle weight gain performance.

The objectives of these studies were to 1) determine the efficacy of three forms of methionine (DL-methionine, N-acetyl DL-methionine and sustained release methionine (SRM)) on ruminal kinetics, and 2) determine the effects of N-acetyl DL-methionine and SRM on heifer growth, intake and ruminal kinetics while grazing summer pasture.

CHAPTER TWO

LITERATURE REVIEW

The constraints on complete and rapid fiber digestion by ruminal microbes are important factors limiting the productivity of ruminants. Fresh forages are normally high in crude protein (CP), but may not meet the metabolizable protein requirements of the animal (Anderson et al., 1988). The concentration of CP decreases and the fiber content increases as the grazing season progresses. Protein supplementation has been recommended by Adams et al. (1987) for animals grazing late-summer and early fall pastures to sustain or increase animal production. Ruminal escape protein supplementation has been found to increase daily performance of steers grazing spring and fall smooth brome pastures (Anderson et al., 1988). Protein supplements in current use are aimed at increasing the flow of protein to the small intestine to meet the requirement of the animal. In ruminants, protein consumed by the animal is fermented and degraded by the microbial population of the rumen and is resynthesized into microbial protein which is delivered to the small intestine. At the small intestine the microbial protein is digested and absorbed as an amino acid source for the host animal. Microbial protein is synthesized from nitrogen containing compounds in poor quality forages in limited quantities. The cost of satisfying the animal's protein requirement with supplemental escape protein may be reduced if maximal microbial protein

synthesis occurs.

Allen et al. (1988) determined that increased intake is associated with an increase in rate of particulate passage out of the rumen with an accompanying decrease in extent of fiber digestion. This agrees with the findings of Blake et al. (1988) who suggested that fiber digestion may be negatively affected by lower fiber intake. Jones et al. (1988) discovered that the production of microbial protein may be limited by the supply of readily fermentable substrate in some warm season grasses, and suggested feeding a small quantity of supplemental grain to growing animals to increase ruminal microbial protein synthesis and consequently improve performance. Fermentation of feed carbohydrates in the rumen is closely linked to the synthesis of the microbial mass which passes to the small intestine and thus provides the ruminant with a major supply of amino acids (Armstrong, 1982). It has been suggested (Lodman et al., 1990; Miner et al., 1990) that the principle effect of protein supplementation influencing cow weight change on native winter range is the quantity of protein delivered to the small intestine.

Microbial nitrogen is composed of 80% amino acids and 15% nucleic acids (Storm et al., 1983). Storm and Ørskov (1984) and others have reported that the most limiting amino acid for ruminal microbial production is methionine. Methionine was released in the smallest quantity compared to 12 amino acids (1-4 mol/100 mol protein) from feed protein sources (casein,

meat and bone meal, cottonseed meal, and autoclaved cottonseed meal). Craig et al. (1984) suggested that this may indicate that methionine is limiting ruminal microbial growth. Methionine was 94.7% to 100% degraded (corn gluten meal, whole soy extruded, meat and bone meal, soybean meal) in a continuous culture fermentation system. This extensive utilization of methionine may be indicative of a nutrient limitation (Blake and Stern, 1988). Some investigators have suggested that sulfur is limiting microbial growth and that methionine is satisfying that sulfur requirement. Patterson and Kung (1988) found that sulfur source (sodium sulfate, methionine or methionine hydroxy analog (MHA)) had no effect on neutral detergent fiber digestion at 12 h in vitro and NDF digestion was related to quantity of sulfur in the medium and not the sulfur source. They also suggested that methionine was the preferred substrate for incorporation into microbial protein. The primary function of sulfur for ruminal microorganisms is to support the synthesis of sulfur containing amino acids (methionine and cysteine) needed for growth of microbial protein. Forty percent of dietary sulfur and most of the supplementary methionine sulfur in an alfalfa diet is unavailable to the ruminal microbes due to rapid absorption through the rumen wall and passage out of the rumen (Durand and Komisarczuk, 1988). Stimulation of microbial protein synthesis by sulfur addition has been observed in vivo with natural diets. When in vitro studies are evaluated it is

important to be aware that a lack of sulfur may have compounded the effects seen from methionine additions.

Maeng and Baldwin (1976) found that bacterial generation interval halved with the addition of amino acids and urea as opposed to urea alone. More microbial protein available for passage to the small intestine should be seen by halving the generation time. Urea is well utilized for microbial synthesis in animals fed low protein and low digestibility forages (Kropp et al., 1977) with ruminal microbial turnover rates increasing with the addition of urea. A slight increase in efficiency of microbial protein production when urea was fed as measured by grams microbial protein per 100 grams DM ingested (Kropp et al., 1977), may be explained by increasing ruminal turnover rate. Gil et al. (1973), using urea as a nitrogen source and adding methionine accelerated bacterial nitrogen incorporation and concurrent substrate digestion rate in vitro using a mixed ruminal microbial population with glucose or cellulose as the substrate. Gil et al. (1973) also noted that inorganic sulfur sources were as effective as MHA or DL-methionine in stimulating microbial protein production only when fermentation was prolonged beyond 24 h.

Storm and Ørskov (1984) indicated that methionine was the most limiting amino acid for ruminal microbial growth. Lodman et al. (1990) suggested for cows grazing native winter range that the ruminal ammonia level ranks as the second most important nutrient after methionine for limitation of ruminal

microbial growth and that the half life of methionine influences the duration of the response to methionine. Amino acids have a short half life in the rumen. Mangan (1972) found that the half life of amino acids in the rumen was less than one hour. In vitro DM and NDF disappearance can be influenced by amino acid concentration in the buffer with methionine additions having the greatest effect on increasing DM and NDF disappearance rate (methionine treated animals NDF disappearance 52% greater than control) of a low quality forage substrate (Clark and Petersen, 1985). Methionine (10 g per day) and urea increased the DM fermentation rate 30% above that of soybean meal or urea (Clark and Petersen, 1988) for heifers at 90% NRC recommended energy levels, but not at 112% NRC energy levels.

Beever et al. (1986) determined that mean ruminal ammonia concentration was less than 100 mg NH_3-N/l on an all grass diet and 200-300 mg NH_3-N/l for a clover diet. These ruminal ammonia concentrations reflected the forage nitrogen levels with early season grass (100 mg/l NH_3-N) being higher than mid-season (20-40 mg/l NH_3-N) and increasing again in late season (130-200 mg/l NH_3-N). Adams and Kartchner (1984) observed a linear relationship between rumen pH and ammonia N concentrations. They found no effect of forage intake on ruminal NH_3-N if the CP is constant. The concentration of ruminal NH_3-N was affected by forage intake and time post-feeding. Ruminal NH_3-N concentration for maximum microbial

protein synthesis have been reported to be from 0.35 to 29 mg/100ml (Owens and Bergen, 1983), while Satter and Slyter (1974) reported no more than 5 mg /dl of ammonia is required for maximal microbial growth. Song and Kennnelly (1990) infused NH_4HCO_3 into the rumen of cows fed an oat silage diet and found an increase in viable counts of total mixed bacteria in ruminal fluid 1.5 and 10 h post feeding, clearly indicating a positive effect of additional N in the rumen. Infusion of NH_4HCO_3 proportionally increased mixed bacterial counts and total volatile fatty acid concentration, but had little or no impact on ruminal degradation of oat silage and concentrate rations. Wiley et al. (1989) reported that DL-methionine plus urea could be used as a suitable replacement for soybean meal as a protein supplement for ruminants on poor quality forage. By supplying urea with methionine, methionine can be used for other purposes than as a nitrogen source for ruminal microbes. Methionine plus urea was similar to SBM for DM disappearance but faster for NDF disappearance (Wiley et al., 1989).

Polan (1988) indicated that the microbial protein flow from the rumen is dependent on ruminal flow or turnover of ruminal contents. The microbial flow to the duodenum was less for high protein than low protein diets when the additional protein source was dried brewer's grains.. This supports the data of Huisman et al. (1988) who found greater degradation of hay incubated in nylon bags was a result of an increased degradation capacity caused by an increase in number of

microorganisms. Their results suggested that the activity of ruminal microorganisms was increased by oral administration of methionine. In another study top dressing up to 60 g per day of DL-methionine did not affect feed intake of lactating dairy cattle (Higginbotham et al., 1987).

The objectives of the studies cited above as designed were to investigate the effects of ruminal administration of methionine. Other workers have used various forms of ruminally protected methionine. Oke et al. (1986) used ruminally protected methionine and lysine that were available post-ruminally and improved lamb N balance and steer performance. Methionine hydroxy analog has been found to be well protected from ruminal degradation and to be effectively absorbed in the lower gastrointestinal tract (Wright and Loerch, 1988) but had no effect on steer growth rate. Titgemeyer and Merchen (1990) found similar results with growing steers not responding in gain or feed efficiency when supplemented with 20 g/d of rumen protected methionine. Post ruminal methionine did not alter the N retention of the steers, possibly explaining why the ruminally protected methionine did not alter steer performance.

A large percentage (74%) of the N reaching the duodenum was of bacterial origin in animals supplemented with a ruminally protected methionine (RP) while in the animals not receiving RP methionine bacterial N supplied only 53% of the nitrogen delivered to the duodenum (Arambel et al, 1987). A

portion of the RP methionine was degraded in the rumen and probably utilized by microflora thus increasing ruminal microbial protein synthesis. Lodman et al. (1990) fed 7 g DL-methionine on alternate days to cows grazing native winter range and concluded that a minimum methionine concentration in the rumen was not maintained for effective increases in microbial growth rate. They did find increased ruminal fluid purine concentrations in cows fed methionine compared to non-supplemented cows but less than cows receiving SBM based supplement. The ruminal fluid purine concentration was highly correlated to cow weight change. They indicated that ruminal ammonia concentrations may have been co-limiting for cows grazing winter range forage, not allowing full utilization of the methionine by ruminal microbes. A summer study with DL-methionine delivered in a self-fed salt mixture, found that cattle with access to DL- methionine had an increase in gain over the controls of 0.18 kg/d (Sorensen et al., 1990). The methionine supplemented animals had decreased ruminal ammonia levels possibly due to increased microbial growth thus causing greater utilization of ruminal ammonia.

Low quality roughages may lack sufficient available nutrients for optimal level of growth by ruminal microorganisms. Favorable conditions must be created in the rumen for maximal growth of plant cell-wall degrading microorganisms (Silva and Ørskov, 1988). Gijzen et al. (1988) discovered that ciliate protozoa account for 19 to 28% of

total cellulase activity of microbial cultures in vitro, and remaining cellulase activity was attributed to the ruminal bacterial population. Carboxymethylcellulase (CMC) activity can be used as an indication of total population of fiber degrading rumen microorganisms (Silva et al., 1986). A key factor governing DM degradation is not microbial density but the establishment of a specific CMC containing population. Thus CMC activity is a good indicator of DM degradation (Silva et al., 1986).

While investigating the influence of methionine derivatives on effluent flow of methionine from a continuous culture of ruminal bacteria, Windschitl and Stern (1988) observed that N-acetyl DL-methionine had an escape value of 67% suggesting only a 33% degradation in ruminal fluid. This could be a possible source of methionine that could be ruminally available over a longer period of time than DL-methionine, thus stimulating microbial growth.

Cattle grazing pastures have feeding cycles. Wiley et al. (1991) noted an improvement in DM and NDF disappearance rates when cattle were supplemented with DL- methionine 4 and 7 h post feeding. This suggests that the timing of DL-methionine supplementation can be used to enhance utilization of poor quality forages. Salt has long been used as a carrier for nutrients to grazing cattle requiring little added labor. Salt can also be used to regulate the intake of added nutrients (Muller et al. 1986). Taking these factors into

consideration, Clark et al. (1990) used sustained release methionine plus urea to replace SBM as a supplementary protein source for cattle grazing winter range. Trials conducted in the Shirley Basin of Wyoming (Petersen, unpublished data) with heifers on summer range using sustained release methionine mixed with salt showed a 0.1 kg/d increase in gain over heifers not receiving sustained release methionine.

CHAPTER 3

MATERIALS AND METHODS

Experiment 1

Eleven ruminally fistulated mature crossbred beef cows (685 kg ± 9.3) were fed ad libitum a mixture of 75% barley straw and 25% low quality grass hay that was chopped through a 5 cm screen in a tub grinder. Roughage mixture contained 74.6% NDF and 4.5% CP. They were fed once a day at 0730, with the orts weighed back daily. Cows were randomly assigned to one of four treatments. Two cows were assigned to the control group while the three treatment groups had three cows each. The four supplemental treatments were: no supplement (CON), 15 g N-acetyl DL-methionine (MET) once a day; 15 g MET plus 71 g urea (MET+U) once a day; 3.75 g MET plus 17.6 g U four times a day (MET+U4X). After a 14 d adaptation to diets and experimental supplements a 96 h in situ nylon bag study was conducted. All vegetable fat was chosen as a carrier for N-acetyl DL-methionine and urea as it was found to slowly disintegrate in ruminal fluid (Sorensen et al., unpublished data). Fat was thoroughly mixed with N-acetyl DL-methionine and urea in a Hobart mixer. The vegetable fat was mixed by weight with the appropriate quantity of MET and/or U with each animal receiving 9.2 g vegetable fat per day. Half of the daily dose for MET was placed in a #10 gelatin capsule, the remainder of the dose was placed in a smaller #12 gelatin capsule and was inserted into the larger #10 capsule. For

treatment MET+U, N-acetyl DL-methionine was mixed with U and vegetable fat then placed in the capsules in the same manner, with each cow receiving 4 capsules simultaneously once a day. The MET+U4X was prepared the same as MET+U except the cows received one capsule at 6 h intervals throughout the day. Capsules used for MET+U4X were designed for a release of N-acetyl DL-methionine over a period of six hours (Sorensen et al. unpublished data, 1989). The quantity of U added was approximately equivalent to the amount of N in 0.45 kg of soybean meal. Supplements MET and MET+U were given at 1030 h (3 h post feeding) while MET+U4X was given 3, 9, 15, and 21 h post feeding. All treatments were administered via the rumen cannulae.

Nylon bags (44 μm pore size) 15 x 18 cm (used for DM and NDF disappearance) were weighed and filled with 3 g of roughage (ground through a 2 mm screen) representative of what the cows were consuming for DM and NDF disappearance rate determination. Sample bags used for determination of purine concentration and carboxymethylcellulase (CMC) activity were filled with approximately 6 g for the purine bags, ground through a 2 mm screen. Sample bags were attached with snap swivels to a loop of tygon tubing filled with chrome ball chain, placed in a nylon net bag and inserted into the rumen of each cow at 0730 with the draw strings from the bags protruding through the rumen cannulae for easy retrieval. Three bags (two for DM and NDF analysis and one for purine

assay) were removed from each cow after 3, 6, 9, 12, 15, 18, 21, 24, 30 , 36, 48, 72, and 96 h of incubation. One cow receiving each treatment had a single blank bag removed at each collection period. The blank bag value was used to correct for any contamination from ruminal debris. The sample bags used for DM and NDF determination were hand rinsed in running cold water until the rinse water was clear to remove non-adhering microbes and ruminal debris. They were dried at 60° C for 48 h in a forced air oven and weighed to determine DM residue. Intact sample bags and contents were then analyzed for NDF (Van Soest and Robertson, 1980). To estimate disappearance rates, DM and NDF values were fitted to a nonlinear regression equation:

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

where Y = disappearance, a = potential digestibility, -k = disappearance rate, t = time-lag period, and u = undigestible fraction (Robinson et al., 1986). The sample bag used for determination of purine concentration and CMC activity were rinsed in running cold water until outside of bag was clean. The bag was gently squeezed to remove excess water, inverted and contents divided. Half was dried in a 100° C convection oven for 48 h, and analyzed for purine concentration (Zinn and Owens, 1986). The remainder was frozen for later CMC analysis (Silva et al., 1986). Using the carbon tetrachloride extraction procedure as described by Silva et al. (1986), CMC activity on the sample content of the nylon bags was

performed.

At 0, 3, 6, 9, 12, 15, 18, 21, 24, 30 36, and 48 h post feeding ruminal grab samples (approximately 500 ml) were collected from the cranial sac of the rumen. The whole sample was filtered through eight layers of grade 50 cheesecloth. Immediately after filtration, pH was determined using a pH meter and glass electrode. A 50 ml subsample was placed in a preweighed weigh boat and dried at 60° C for 48 h, reweighed, manually ground in a mortar and pestle and later purine analyzed for purine concentration. Three ml of 6 N HCl was added to a 50 ml subsample for ammonia analysis using the magnesium oxide distillation method (AOAC, 1980). A 30 ml subsample was frozen for later CMC determination. Ten ml of the thawed ruminal liquid was used for CMC analysis. Due to discoloration of the reagents by the ruminal fluid, each sample served as its own blank. The formation of reducing sugar using 3,5-dinitrosalicylic acid (DNS) was used to measure the hydrolysis of sodium carboxymethylcellulose. D-glucose was used as the standard.

All analysis were subjected to analysis of variance using General Linear Models Procedure (SAS, 1988), with hour and treatment included in the model. Least significance difference was utilized to compare treatment means.

Experiment 2

This experiment consisted of three trials all conducted

at Ft. Ellis, Montana in three consecutive years (1987-89). Trial 1. Sixty-two yearling bred english crossbred heifers (initial weight 397 kg ± 3.39) were randomly allotted to one of two supplement treatments: 1. self-fed salt:monosodium phosphate 50:50 mix or 2. self-fed salt:monosodium phosphate:DL-methionine 43:43:14 mix. The salt mineral mix was contained in wind vane type covered mineral feeders. Each supplement group was randomly assigned to one of four paddocks. The paddocks contained a mix of Medicago sativa, Bromus inermis, Poa pratensis and Agropyron cristatum. Each replication grazed a 48 ha paddock containing one mineral feeder. Cattle were moved between each paddock biweekly in conjunction with biweekly weight records and mineral feeders were rotated at that time. The duration of trial 1 was 56 d, beginning July 7 and terminating September 5, 1987. Total gain and average daily gain were measured.

Trial 2. This trial utilized 84 bred yearling english crossbred heifers similar to trial 1 (initial weight 374 kg ± 2.54). This study was designed to be similar to trial 1. However, due to a drought, the replications of each treatment were combined after 42 d into two adjacent paddocks. This study was initiated June 25 and terminated September 17, 1988. Total gain and average daily gain were measurements taken.

Trial 3. Sixty-four bred english crossbred heifers (initial weight 375 kg ± 3.2) were blocked by weight and randomly allotted within weight blocks to one of two salt mixture

treatments: control (CON) salt:monosodium phosphate mixture or sustained release methionine (SRM)[1], salt:monosodium phosphate:sustained release methionine. The form of DL-methionine used was treated to slowly release (Clark, 1989 unpublished data) in the rumen. The salt mineral mixture was contained in wind-vane type covered feeders. Each supplement group was randomly divided into two replications. Each replication was randomly assigned to one of four paddocks. The paddocks were the same as used in trials 1 and 2. Animals were weighed biweekly during the 42 d trial and rotated between the four paddocks to coincide with days weights were recorded. Each paddock contained one wind-vane mineral feeder that was rotated with the cattle. Mineral was added as needed to maintain continuous availability, but individual animal consumption was not monitored.

On d 70 five heifers from each paddock were bolused with a sustained release chromium bolus[2]. Fecal grab samples were taken 14, 15, and 16 d after bolusing. The samples were then frozen for later chromium analysis using the digestion procedure of Williams, et al. (1962) with the chromium concentration determined by inductively coupled plasma (ICP). Relative forage intake was calculated using the formulae of Harris (1970) and an 18 h in situ disappearance value of

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[1] Microsupplements, Inc. Caldwell, Idaho.

[2] Captec (NZ) LTD, 81 Carlton Gore Road, Newmarket, P.O. Box 759, Auckland, New Zealand.

clipped forage was used to estimate indigestibility.

$$\text{Fecal dry matter output (g)} = \frac{\text{external marker X 100}}{\text{\% internal indicator in feces (DM)}}$$

$$\text{Dry matter consumption (g)} = \frac{\text{G fecal output X 100}}{\text{\% indigestible NDF}}$$

Two mature ruminally cannulated beef cows (685 kg ± 9.3) were placed in each paddock on the last biweekly weigh day. Polyester bags with a pore size of 53 ± 10 microns measuring 10 x 20 cm were weighed, and filled with 3 g of dried, ground (2 mm screen) roughage obtained from composited clipped samples (10 cm in height, 64.2 %NDF, 5.34% CP) from the paddocks. The residues from these sample bags were utilized to determine DM and NDF disappearance. Approximately 6 g of dried forage was weighed into sample bags used for analysis of purine concentration and CMC. The bags were attached to loops and inserted into dunk bags as previously described in Exp. 1. Six nylon bags (2 for determination of DM and NDF disappearance, 2 for determination of purine concentration and CMC activity and 2 blank bags) were placed into the rumen of each cow after 14 d adaptation to the paddock and mineral mixtures. The bags were removed after 18 h (Clark et al., 1989) of incubation. The blank bag value was used to correct for influx of contamination from ruminal debris into the bags. All bags were removed from the rumen and immediately placed in

ice water at the collection site. Upon arrival at the analytical facilities the sample and blank bags were rinsed in running cold water until the water was clear, then dried in a forced air oven at 60° C for 48 h, weighed, and DM determined. Dry matter sample bags were then analyzed for NDF using a modification of the Van Soest and Robertson (1980) procedure. The sample bags used for purine and CMC analysis were hand rinsed and analyzed as in Exp. 1. At 0 and 18 h collections, approximately 500 ml of ruminal digesta samples were collected from the cranial sac of the rumen of each cow as in Exp. 1 for analysis of pH, ammonia, purine and CMC.

All analyses were subjected to analysis of variance using General Linear Models Procedure (SAS, 1988) with treatment in the model and cow*treatment in the model for intake. Least significant difference was utilized to compare treatment means. Analysis of average daily gain and total gain, utilized treatment, replication (treatment) and initial weight in the model. Replication (treatment) was used as the error term.

Experiment 3

Ninety-six bred heifers (inial weight 369 kg ± 2.4) were blocked by weight and randomly allotted to one of the two salt mixture treatments: control (CON) receiving no methionine and methionine (SRM) containing sustained release methionine (Table 1). Each treatment group was divided into 4

replications of 12 animals and placed in 8 paddocks. Animals were weighed and bled (for blood urea nitrogen (BUN)) triweekly during the 72 d trial. Clipped forage samples were taken from each paddock (5 cm in height) at three random locations each weigh day and analyzed for NDF, DM, CP, S and forage composition (legume vs grass). Clipped forage samples were divided into grass and legume fractions by visual determination and percentages obtained. Sulfur was digested using perchloric-nitric acids and then analyzed by inductively coupled plasma. Each paddock contained a wind-vane mineral feeder, with salt mixture checked weekly and added as needed to maintain continuous availability.

On d 40 three heifers randomly chosen from each paddock were bolused with a sustained release chromium bolus. Fecal grab samples were taken 5, 6, 7, 12, 13, and 14 d after bolusing. The samples were frozen for later chromium analysis as in Exp. 2. Relative intake was calculated as in Exp. 2, trial 3.

Intake analysis was subjected to analysis of variance using General Linear Models Procedure (SAS, 1988), with treatment, paddock and cow (treatment) as factors in the model. Analysis of average daily gain and total gain utilized treatment, replication (treatment) and initial weight as factors in the model. Least significant differences were utilized to compare treatment means.

Experiment 4

Four mature ruminally cannulated beef cows (685 kg ± 9.3) were used in a 4 X 4 latin square design trial for measurement of ruminal kinetics. Ruminal measurements included: pH, ammonia, carboxymethylcellulase, purine, DM, NDF, and ruminal dilution rates using cobalt-EDTA for a fluid marker and chromium mordanted fiber for a particulate marker. Cows were weighed and fed a medium quality grass hay at 0700 h (9.3% CP) at 2% of body weight. Cows were randomly provided one of four dietary treatments which were: 1) Control (CON): salt, dicalcium phosphate and monoammonium phosphate, 2) DL-methionine plus CON (MET), 3) sustained release methionine plus CON (SRM) and 4) sodium sulfate plus CON (SUL). See Table 2 for formulation. Dietary supplements (56.75 g) were placed directly in the rumen via the cannulae 3 h post feeding at 1000 h. Polyester bags (same as used in Exp. 2) were weighed, filled with 3 g (for DM and NDF) of ground forage (2 mm screen) representative of what the cows were eating. Approximately 6 g of ground forage was placed into the bags to be used for determination of purine and CMC analysis. The sample bags were inserted after a 10 d dietary adaptation period while collections and analysis were conducted as in Exp. 2, trial 3.

Cobalt EDTA was used as a digesta fluid marker and chromium mordanted fiber (Uden et al., 1984) representative of the forage the animals were fed was used as a particulate

digesta marker for the determination of ruminal dilution rate. Markers were dosed when sample bags were introduced into the rumen and collected at the same time the sample bags were removed from the rumen.

The analysis of variance for the latin square utilized period, treatment, cow in the model, with cow (treatment) as the error term.

CHAPTER 4

RESULTS AND DISCUSSION

Experiment 1

In situ extent and rate of DM disappearance were similar (P>.1) for all treatments. All treatment rate constants were numerically at least 15% faster than the control (Table 1). Neutral detergent fiber disappearance rate and ruminal digestibility showed non-significant trends similar to the DM disappearance (P>.1, Table 3).

Ruminal ammonia concentrations differed (P<.03) between supplemented and control, with supplemented cows having the greatest concentrations. Ruminal fluid ruminal ammonia concentrations were highest for cows fed MET+U4X (P<.01) and lowest for cows fed CON (Table 3). This was anticipated as urea was added to the continuous release formulation of MET+U4X. Ruminal pH means were similar (P>.5) for cows receiving CON, MET and MET+U4X while cows fed MET+U was lower than the other treatments (P<.01, Table 3).

Carboxymethylcellulase (CMC) activity eluted from incubated forage was similar (P>.1) for cows consuming any treatment except at 21 and 24 h of incubation (Table 4). The CMC activity increased (P<.01) from 488 μmoles (3 h) to a peak of 1338 μmoles at 18 h then returned to 431 μmoles at 24 h (Table 4). This would suggest that CMC activity is associated with microbial attachment to the forage, but does not appear to be influenced by either MET or U supplementation.

Overall mean purine concentration on incubated feed samples had similar values (P>.1) at each incubation period for cows receiving each treatment (Table 5). The concentration of purine increased (P<.01) from 3 h post feeding to 18 h post feeding, then reached a plateau and remained constant (P>.1) at 21 and 24 h post feeding. The CMC activity shows the same tendency to increase to 18 h post feeding as the purine concentration on the incubated forages. This is in agreement with Wiley et al. (1991) who found that initial colonization of microbes (purine accumulation) was rapid at 0-3 h and that between 15 to 18 h the highest concentrations were found. At 15 to 18 h the purine concentration remains stable suggesting no further growth of microbial mass. Mean ruminal fluid purine concentrations (mg/g of dried rumen fluid) differed (P<.01) with cows fed MET+U4X being the greatest (Table 5).

Ruminal fluid purine concentrations (mg/g dried rumen fluid) were similar (P>.1) for cows receiving treatment CON and MET, while MET+U fed cows had greater liquid purine density (P<.1) at 9 and 12 h compared to CON and MET. Cows given treatment MET+U4X had denser purine concentrations (P<.05) than CON, MET and MET+U fed cows at 6, 9, 12, 15 and 18 h post feeding (Table 5). As fluid purine concentrations rose, ruminal ammonia levels dropped, suggesting usage of the ruminal ammonia by the microflora for growth. The availability of ruminal ammonia when methionine is

supplemented may influence microbial yield associated with the liquid fraction of the rumen (Lodman, et al., 1990).

When N-acetyl DL-methionine and urea were delivered to the rumen at a constant rate (MET+U4X) increases in the amount of ruminally fluid purine concentration were detected which may influence the quantity of microbial protein delivered to the small intestine, thus enhancing the protein status of the animal.

Experiment 2

Intake of the salt mineral mix by ruminally cannulated cows in trial three was not designed to be measured, but cows were observed consuming the mineral mix.

Dry matter 18 h extent of disappearance was similar ($P>.24$) for cows receiving both treatments (Table 6). Neutral detergent fiber 18 h extent of disappearance in cows fed SRM exhibited a trend ($P<.16$) to be numerically greater than the cows receiving CON (53.7 and 51.9% respectively, Table 6).

Ruminal particulate and fluid fraction CMC activity was similar ($P>.23$) for cows receiving either treatment (Table 6). Ruminal pH and ammonia concentration (mg/dl) were similar ($P>.50$) for both treatments (Table 6). Particulate purine concentration was similar for cows with access to CON or SRM while the fluid fraction purine concentrations was different ($P<.04$) at 18 h with the SRM being greater. Experiment 1 found that the fluid ruminal purine density was higher ($P<.01$) in animals fed a slow release form of N-acetyl DL-methionine.

These two studies indicate that a sustained concentration of DL-methionine increases purine (microbial) density which may have implications on the growth of microflora associated with the fluid fraction. Lodman (1990) suggested that alternate day supplementation of DL-methionine was not sufficient to maintain cow weight in winter grazing situations because of the short term effect of DL-methionine on the microbial population of the rumen, even though overall, DL-methionine improved cow performance over unsupplemented animals.

Average daily gain was numerically greater ($P<.18$) for heifers receiving the salt plus mineral mix containing either DL-methionine or SRM, possibly reflecting the increased concentrations of ruminal liquid purines shown to be present when SRM was used (Table 7).

Forage intake was similar for animals receiving either CON or SRM groups ($P>.83$, Table 7).

Experiment 3

Heifers consuming the CON treatment had a significantly higher ($P<.01$) average daily gain than heifers receiving SRM. Forage intake was similar in both groups of cows (Table 8) and similar to values reported in Exp. 2. Salt consumption was similar for CON and SRM (87.9 and 82.3 g/day, respectively).

Forage quality and availability deteriorated throughout the trial. Crude protein initially was 9.9% (July 3, 1990) and decreased to 5.4 % (September 13, 1990) while NDF increased from 53.5% to 71.1% from the first weigh period to

the last weigh period (Table 9).

Experiment 4

Ruminal fluid dilution rates were similar (P>.19) for cows receiving CON compared to cows fed other dietary treatments. While cows fed SRM had slower fluid dilution rates (P>.16) than cows consuming SUL (Table 10). Cows fed SUL were significantly (P<.04) slower than MET fed cows but similar (P>.30) to both the CON and SRM treatment groups. These results agree with those reported in Exp. 1. Particulate passage rates were similar for all treatments (P>.22).

Ruminal pH values were similar (P>.22) for cows receiving any treatment. Ruminal ammonia levels were numerically higher in the ruminally cannulated cows receiving the SUL treatment than in the animals fed CON and MET (P<.15, Table 10) but similar to SRM treated cows. The ruminal fluid purine concentrations tended to be greater (P<.12) for SRM fed cows than those receiving CON. Cows fed MET and SUL had intermediate concentrations which were similar to MET and SRM treated cows (P>.21).

Methionine and SUL dietary supplements were isosulfurous. All basal diets were deficient in sulfur (.08% S) and the additional 1.22 g/d of sulfur intake did not increase microbial protein. This finding does not agree with Morrison et al. (1990) who found that the amount of fluid borne ruminal bacteria increased significantly with sulfur supplementation

when fed a sulfur deficient diet. Both MET and SUL fed cows had similar fluid purine concentration which was numerically less than that found in cows receiving SRM. This suggests that even though sulfur was deficient, the availability and possibly form of sulfur was important in the higher purine concentration found in the SRM fed cows compared to CON. Particulate purine concentrations were similar (P>.51) for all treatments, with SRM being numerically higher (P>.21) than CON (Table 10).

Dry matter and NDF disappearance rates were similar (P>.31) for all treatments (Table 10).

Ruminal fluid CMC activity was lower (P<.05) in CON fed cows compared to cows consuming MET, but all sulfur containing treatments had similar (P>.45) activity. The CMC activity eluted from incubated forage from cows receiving all treatments had similar (P>.56) particulate CMC activity levels (Table 8).

In all experiments where ruminal kinetics were measured an increase in the liquid ruminal purine concentrations was noted in animals receiving methionine. This has been observed in sheep consuming a poor quality grass hay (Morrison et al., 1990) and cows grazing native Montana winter range (Lodman, et al., 1990). Lodman also noted that ruminal NH_3 is second limiting after methionine for microbial growth. In Exp. 2 ruminal NH_3 levels (2.48, 2.73 mg/dl; CON and SRM respectively) were in the lower end of the 2-5 mg/dl

range suggested by Satter and Slyter (1974) even though approximately 2.93 g N/d was added to salt mineral mix. Thus ruminal ammonia levels may not have been adequate for optimal microbial growth to occur.

Forage sampling in Exp. 3 indicated a marked decrease in forage quality as well as quantity from mid to late summer. Crude protein concentrations rose from 4.6% to 5.4% (the level comparable to the CP level in the forage in Exp. 2) during the last three week weigh period due to forage regrowth but forage mass (availability) decreased by over 50% (Table 7). This would suggest that even though an adequate CP may have been present in the forage, the total CP intake was not sufficient for maintenance or growth. Blood urea nitrogen levels at the beginning of Exp. 3 were 13.75 mg/dl, three weeks later dropped slightly to 12.14 mg/dl, in the middle of August dropped dramatically to 7.44 mg/dl and then rose to 14.26 mg/dl by the end of the Exp. 3 (Table 8). The drop to 7.44 mg/dl would indicate a drop in the ruminal ammonia levels and the rise at the end could possibly signify catabolism of body protein stores and low ruminal ammonia levels. The heifers were actively losing weight (-.39 CON, -.55 SRM kg/d) during this time period.

In Exp. 3 the biomass at the end of the trial was 15.6% of the amount at the beginning suggesting that energy intake was not adequate to maintain body condition. The same paddocks were used in Exp. 2 and 3 with Exp. 3 having twice

the stocking rate thus the animals in Exp. 3 grazed the available forage much sooner.

CHAPTER 5

CONCLUSIONS AND SUMMARY

One parameter stands out in every trial where ruminal parameters were measured: the increase in liquid purine concentrations with the addition of methionine. Even though the passage rates did not differ, this increase would deliver more microbial protein to the small intestine of the host animal receiving the SRM. To an animal on a low protein diet with an adequate energy intake this would allow improvement in animal performance through increased microbial protein for utilization by the host animal. In situations where protein is adequate and energy is limiting, animals have the ability to mobilize body fat reserves to utilize the protein and maintain lean mass growth at the expense of energy reserves (Ørskov, 1991) thus maintaining body weight.

Lodman (1990) demonstrated that DL-methionine can be detrimental to microbial growth when given at the improper time post feeding (Wiley et al., 1990). Sustained release methionine was designed to provide a constant source of methionine to the ruminal population to minimize the negative effects by synchronizing DL-methionine in grazing situations. The increases shown in these studies in fluid purine concentrations demonstrate potential effectiveness of this system when self-fed in a salt mineral mixture. Sustained release methionine did not influence salt consumption at the levels used.

In 3 out of 4 performance trials using DL-methionine or SRM an increase in average daily gain was demonstrated compared to animals receiving no form of DL-methionine. These trials had lower stocking rates than in Exp. 3 where SRM showed no advantage. One possible explanation for the failure of SRM to increase gain in Exp. 3 is that the animal stocking rate was 150% of the previous year and forage availability and quality decreased at a faster rate. This caused a decrease in protein intake as well as energy. Weight loss found in the SRM supplemented cows in the last period of Exp. 3 was greater than the CON treated cow weight loss. One possible explanation is that the SRM was successful in maintaining an elevated methionine level in the rumen but ruminal ammonia levels (as estimated by BUN) were not sufficient during this period to allow full utilization of the SRM by the microbial population. This agrees with Lodman et al. (1990) who demonstrated that ruminal ammonia levels were second limiting to methionine for ruminal microbial growth. The supplemental methionine may initially stimulate a microbial growth increase until the available ruminal ammonia supply is exhausted. Substrate utilization is then depressed causing a greater weight loss.

These findings are in agreement with those of Jones et al. (1988) who found that the microbial protein production is limited by the amount of readily fermentable substrate in grasses. They suggested feeding a small amount of grain to

provide fermentable substrate. The addition of some form of readily fermentable energy source to the salt mineral mix containing SRM may possibly result in a higher level of animal performance when in a crude protein and energy deficient state as may be found in late summer grazing. Further studies in this area would be beneficial to our understanding of the mechanism involved.

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APPENDIX

Table 1. Supplement Composition, Experiments 2 and 3.

Ingredient	CON	SRM
	%	
Wheat mill run	4.1	4.1
Dicalcium phosphate	20.0	0
Salt	50.0	50.0
Monoammonium phosphate	22.0	22.0
Trace mineral	0.1	0.1
Vegetable oil	2.8	2.8
Dried Molasses	1.0	1.0
Sustained release methionine	0	20.0
Total	100.0	100.0

Table 2. Supplement Composition, Experiment 4

Ingredient	CON	MET	SRM	SUL
	%			
Wheat mill run	4.1	4.1	4.1	4.1
Dicalcium phosphate	20.0	10.0	0	10.4
Salt	50.0	50.0	50.0	50.0
Monoammonium phosphate	22.0	22.0	22.0	22.0
Trace mineral	0.1	0.1	0.1	0.1
Vegetable oil	2.8	2.8	2.8	2.8
Dried molasses	1.0	1.0	1.0	1.0
Sustained release methionine	0	0	20.0	0
DL-methionine	0	10.0	0	0
Sodium sulfate	0	0	0	9.6
TOTAL	100.0	100.0	100.0	100.0

Table 3. Ruminal Kinetics and Rates as Influenced by No Supplement (CON), N-Acetyl DL-methionine (MET), MET + UREA (U) and MET + U four times a day (MET + U4X) (Experiment 1)[a]

Measurements	Treatments				SE[b]	P value
	CON	MET	MET+U	MET+U4X		
Undigested DM %	38.3	40.2	41.9	40.7	1.56	.17
Undigested NDF %	31.0[c]	34.1[cd]	36.0[d]	34.4[cd]	1.42	.05
DM disappearance, %/h	4.27	5.06	5.26	4.93	.52	.25
NDF disappearance, %/h	4.70	5.58	6.12	5.39	.53	.12
Ruminal NH_3, mg/dl	.79[c]	1.54[c]	7.07[d]	10.99[e]	1.25	.03
pH	6.96[c]	6.64[d]	6.29[c]	6.96[c]	.04	.01
Fluid purine, mg/ml	1.70[c]	1.61[c]	1.71[c]	2.12[d]	.07	.01
Particulate purine, mg/g	.40[c]	.39[c]	.44[d]	.41[c]	.01	.10

a Least square means.
b Pooled standard error of the mean.
c,d,e Means within the same row with different superscripts differ.

Table 4. In Situ Carboxymethylcellulase Activity by Hour as Influenced by No Supplement (CON), N-Acetyl DL-methionine (MET), MET + UREA (U) and MET + U four times a day (MET+U4X) (Experiment 1)[a]

Hour	Treatments				SE[b]	P value
	CON	MET	MET+U	MET+U4X		
3	407	471	528	508	99	.51
6	606	563	516	537	104	.57
9	634	756	754	543	113	.20
12	640	446	745	692	154	.19
15	704	736	864	868	104	.32
18	803	1512	1613	1236	298	.11
21	956[cd]	1338[c]	694[de]	343[e]	123	.10
24	419[cd]	492[d]	400[c]	400[c]	28	.04

a Least square means.
b Pooled standard error of the mean.
c,d,e Means within the same row with different superscripts differ.

Table 5. Liquid Ruminal Purine Concentrations Over Time as Influenced by No Supplement (CON), N-acetyl DL-methionine (MET), MET + Urea (U) and MET+U four times a day (MET+U4X) (Experiment 1)[a]

Hour	Treatments				SE[b]	P value
	CON	MET	MET+U	MET+U4X		
	---------- mg/g dried ruminal fluid ----------					
0	.44	.40	.40	.51	.08	.25
3	.72	.60	.61	.81	.13	.21
6	.51[d]	.62[cd]	.63[cd]	.72[c]	.08	.08
9	.48[c]	.67[d]	.51[c]	.72[d]	.07	.08
12	.46[c]	.54[c]	.59[c]	.89[d]	.07	.01
15	.57[c]	.55[c]	.49[c]	.88[d]	.06	.01
18	.45[c]	.39[ce]	.53[cd]	.79[def]	.04	.02
21	.59	.55	.59	.67	.06	.13
24	.46	.55	.53	.51	.09	.59

a Least square means.
b Pooled standard error of the mean.
c,d,e,f Means within the same row with different superscripts differ.

Table 6. Ruminal Kinetics and Rates as Influended by Sustained Release Methionine (SRM) (Experiment 2)[a]

Measurements	Treatments		SE[b]	P value
	CON	SRM		
DM undigested %, 18 h	53.6	52.2	.75	.24
NDF undigested %, 18 h	48.1	46.3	.79	.16
pH	6.64	6.72	.09	.57
Ruminal NH_3, mg/dl	2.48	2.73	.44	.70
Fluid CMC, μmoles/ml	303.3	299.4	2.2	.23
Particulate CMC, μmoles/g	312.4	315.9	4.0	.56
Fluid purine, mg/g[c]	2.27[d]	2.34[e]	.02	.04
Particulate purine, mg/g	.25	.26	.01	.57

a Least square mean.
b Pooled standard error of the mean.
c Dried rumen fluid
d,e Means within the same row with different superscripts differ.

Table 7. Intake and Average Daily Gain (ADG) as Influenced by Sustained Release Methionine (SRM) (Experiment 2)[a]

Measurements	Treatments CON	SRM	SE[b]	P value
Intake, g DM/d	8959	9370	1216	.83
ADG, kg/d	.7	.8	.03	.18

a Least square means.
b Pooled standard error of the mean.

Table 8. Intake, Salt Intake, Blood Urea Nitrogen (BUN) and Average Daily Gain (ADG) as influended by Sustained Release Methionine (SRM) (Experiment 3)[a]

Measurements	Treatments CON	SRM	SE[b]	P value
Intake, g DM/d	9500	9304	710	.86
ADG, kg/d	.8[c]	.7[d]	.05	.01
Salt consumed, g/d/hd	87.9	82.3		
BUN, mg/dl	11.88	11.92	.29	.91

a Least square means.
b Pooled standard error of the mean.
c,d Means within the same row with different superscripts differ.

Table 9. Forage Clip Sample Composition and Total Grams DM Available Forage (Experiments 2 and 3)

Exp.	Date	Component % CP	% NDF	% Sulfur	Total grams DM Available Forage
2	8/25/89	5.3	64.2	---	---
3	7/06/90	9.9	53.5	.10	2120
3	7/27/90	6.2	59.2	.07	1314
3	8/16/90	4.6	68.3	.06	747
3	9/06/90	5.4	71.1	.07	331

Table 10. Ruminal Kinetics and Rates as Influenced by Sustained Release Methionine (SRM) (Experiment 4)[a]

Measurements	Treatments				SE[b]	P value
	CON	MET	SRM	SUL		
DM disappearance, %/h	8.86	8.71	7.95	8.35	.49	.24
NDF disappearance, %/h	4.15	4.01	3.73	3.75	.24	.28
Fluid passage rate, %/h	11.1[cd]	10.1[c]	11.0[cd]	12.3[d]	.59	.04
Particulate passage rate, %/h	1.50	1.61	1.65	1.97	.24	.22
pH	6.86	6.80	6.80	6.83	.035	.22
Ruminal NH_3, mg/dl	7.41[c]	7.41[c]	7.86[c]	8.80[d]	.67	.14
Fluid CMC, μmoles/ml	70.3[c]	81.1[d]	76.8[cd]	75.2[cd]	3.72	.05
Particulate CMC, μmoles/g	472.6	461.4	479.8	467.2	22.5	.56
Fluid purine, mg/g[e]	2.96[c]	3.09[cd]	3.16[d]	3.06[cd]	.09	.12
Particulate purine, mg/g	.31	.30	.28	.29	.02	.21

a Lease square means
b Pooled standard error of the means.
c,d Means within the same row with different superscripts differ.
e Dried rumen fluid.

Table 11. Blood Urea Nitrogen (BUN) Levels as Influenced by Period (Experiment 3)[a]

Measurement	Periods[b]				SE[c]	P value
	1	2	3	4		
BUN mg/dl	13.75[d]	12.14[d]	7.44[d]	14.26[e]	.30	.01

a Least square mean.
b Period 1 = July 3 - 24, 1990
Period 2 = July 24 - August 14, 1990
Period 3 = August 17 - September 14, 1990
Period 4 = September 4 - 14, 1990
c Pooled standard error of the means.
d,e Means within the same row with different superscripts differ.

Table 12. Least Squares Analysis of Variance (Experiment 1)

	Source	df	Mean Square	P
pH	treatment	3	.82219	.0001
	hour	11	.08777	.0824
	error	117	.05174	
ammonia	treatment	3	568.08	.0001
	hour	8	79.241	.0497
	error	87	38.676	
DM disappearance rate	treatment	3	22.343	.0858
	error	7	6.7018	
NDF disappearance rate	treatment	3	37.228	.0201
	error	7	5.7749	
Liquid purine	treatment	3	549.53	.0001
	hour	8	335.94	.0001
	error	87	46.745	
Particulate purine	treatment	3	6.2325	.0432
	hour	10	131.27	.0001
	error	106	2.2207	
Liquid CMC	treatment	3	871.05	.1095
	hour	11	21273.1	.0001
	error	116	423.06	
Particulate CMC	treatment	3	25523.3	.9164
	hour	10	2964011.1	.0001
	error	120	150057.9	
Liquid passage rate	treatment	3	2.5636	.0178
	error	7	.37944	
Particulate passage rate	treatment	3	1.3177	.0224
	error	7	.21382	

Table 13. Least Squares Analysis of Variance (Experiment 2)

Trial 1, 2, and 3	Source	df	Mean Square	P
Total gain	Treatment	1	6016.2	.0007
	Year	2	11.925	.0001
	Rep (trt)	2	1575.7	.0469
	Heifer	1	1298.9	.1111
	Initial wt	1	155.17	.5808
	Error	198	507.06	
Average Daily Gain	Treatment	1	1.7392	.0007
	Year	2	48.473	.0001
	Rep (trt)	2	.41965	.0603
	Heifer	1	.38982	.1054
	Initial wt	1	.04143	.5965
	Error	198	.14732	
Trial 3				
Intake	Treatment	1	36612204	.0008
	Day	2	11386540	.0254
	Cow (trt)	2	33055674	.0001
	Day * trt	2	3435647	.3135
	Error	52	2887829	
pH	Treatment	1	.02403	.5717
	Error	14	.07164	
Ammonia	Treatment	1	.24726	.6966
	Error	14	1.5603	
DM 18h extent of disappearance	Treatment	1	4.4639	.21
	Error	6	2.2324	
NDF 18h extent of disappearance	Treatment	1	6.4815	.16
	Error	6	2.4835	
Liquid purine	Treatment	1	.04119	.39
	Hour	1	.15575	.10
	Error	13	.05153	
Particulate purine	Treatment	1	.00017	.57
	Error	1	.00052	
Liquid CMC	Treatment	1	61.330	.23
	Hour	1	239.45	.03
	Error	13	39.377	
Particulate CMC	Treatment	1	24.124	.56
	Error	6	64.984	

Table 14. Least Squares Analysis of Variance (Experiment 3)

	Source	df	Mean square	P
Average daily gain	Treatment	1	.7674	.0119
	Cow (trt)	2	9758625	.1022
	Paddock	3	.43332	.0143
	Begin avg	1	.69516	.0165
	Paddock * trt	3	.72763	.0007
	Error	87	.11638	
Intake	Period	1	18594387	.0381
	Treatment	1	7841579	.1707
	Per * trt	1	7848695	.1705
	Error	36	4012425	
BUN[a]	Period	3	925.15	.0001
	Treatment	1	.00946	.9145
	Error	1	8.2033	

a Blood Urea Nitrogen (BUN)

Table 15. Least Squares Analysis of Variance (Experiment 4)

	Source	df	Mean square	P
pH	Cow	3	.22582	.0011
	Period	3	.06804	.2161
	Treatment	3	.02846	.5971
	Error	134	.04520	
Ammonia	Cow	3	32.260	.1150
	Period	3	1.1893	.9737
	Treatment	3	15.569	.4081
	Error	134	16.020	
	CON vs trt	1	10.236	.4255
	MET vs SRM	1	3.6288	.6349
	SRM vs SUL	1	16.086	.3181
Liquid purine	Cow	3	.41162	.2426
	Period	3	4.1252	.0001
	Treatment	3	.24114	.4817
	Error	134	.29188	
	CON vs trt	1	.55909	.1687
	MET vs SRM	1	.08329	.5941
	SRM vs SUL	1	.15275	.4707
Particulate purine	Cow	3	.00669	.4756
	Period	3	.56382	.0001
	Treatment	3	.00459	.6328
	Error	116	.00799	
	CON vs trt	1	.00639	.3728
	MET vs SRM	1	.00685	.3566
	SRM vs SUL	1	.00354	.5070
Liquid CMC	Cow	3	139.44	.8642
	Period	3	321.89	.4526
	Treatment	3	707.34	.2954
	Error	134	567.34	
	CON vs trt	1	1544.6	.1024
	MET vs SRM	1	321.89	.4526
	SRM vs SUL	1	26.425	.8295

Table 15. Least Squares Analysis of Variance (Experiment 4) CONT.

	Source	df	Mean square	P
Particulate CMC	Cow	3	10654	.5757
	Period	3	76660	.0035
	Treatment	3	1965.8	.9466
	Error	117	16039	
	CON vs trt	1	234.98	.9039
	MET vs SRM	1	5405.4	.5627
	SRM vs SUL	1	2569.3	.6897
Liquid passage rate	Cow	3	.000659	.0489
	Period	3	.000238	.2591
	Treatment	3	.000318	.1750
	Error	6	.000137	
	CON vs trt	1	.000002	.9153
	MET vs SRM	1	.000141	.3492
	SRM vs SUL	1	.000351	.1609
Pariculate passage rate	Cow	3	.000016	.5882
	Period	3	.000126	.0401
	Treatment	3	.000034	.2734
	Error	6	.000024	
	CON vs trt	1	.000017	.4198
	MET vs SRM	1	.000000	.9009
	SRM vs SUL	1	.000020	.3857

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