# SUBSTITUTION OF DL-METHIONINE FOR SOYBEAN MEAL AS A WINTER SUPPLEMENT FOR GESTATING COWS GRAZING NATIVE RANGE[1,2,3]

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## ABSTRACT

A winter grazing study was conducted to determine whether DL-methionine could replace soybean meal as a N supplement for gestating beef cows. During two winters (Trial 1, n = 51; Trial 2, n = 60), crossbred beef cows grazed native foothill range. Three treatment groups were supplemented with either none (CON), DL-methionine (7.5 g Trial 1 and 9 g Trial 2) in .5 kg beet pulp carrier (BPM) or .4 kg soybean meal (SBM). Cows were supplemented individually every other day. Small differences were noted in cow BW, condition score and blood metabolites. Unsupplemented cows lost the greatest amount of BW ($P < .01$) in both trials and lost more ($P < .05$) condition during Trial 1 than cows fed BPM or SBM supplements. Blood samples were obtained on two consecutive days during each trial (45 d and 25 d prepartum) and analyzed for blood urea N, total bilirubin, creatinine, albumin, total protein and cholesterol. A treatment × day prepartum interaction ($P < .05$) was noted for blood urea. Blood urea nitrogen declined as gestation length increased for CON and SBM cows, but blood urea of BPM-supplemented cows remained low and unchanged. In situ forage digestion was measured in 12 ruminally cannulated cows (four/treatment). In both trials, in situ rate of NDF disappearance was greater ($P < .05$) for SBM than for BPM. In Trial 2, a treatment × sampling hour interaction was detected for purine concentration of whole ruminal contents; SBM maintained greater purine concentrations throughout the 48-h supplementation cycle than BPM did. Principal component analysis suggested that ruminal ammonia limited the microbial growth response to DL-methionine. Therefore, alternate-day supplementation of DL-methionine plus beet pulp did not effectively substitute for soybean meal in these trials.

(Key Words: Beef Cattle, Methionine, Protein Supplements, Range Pastures.)

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## Introduction

Protein supplementation of cows grazing native range during the winter is a routine practice in areas where standing forage is accessible through most of the winter. The goal of supplementation is to supply nutrients that are deficient in the gestating beef cow's diet. Protein supplementation has increased digestibility of mature forages (Rittenhouse et al., 1970), which suggests that it affects the ruminal microflora; however, mechanisms of changes in forage digestion and other responses to protein supplementation are not fully understood.

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The microbial population in the rumen has specific nutrient requirements for optimal growth. Low-quality forages may not satisfy these needs, thereby restricting microbial growth. Cellulolytic bacteria can use ammonia for most of their N requirements (Al-Rabbat et al., 1971), but Maeng and Baldwin (1976) showed that absence of amino acids decreased ruminal microbial growth rate in vitro. Gil et al. (1973), using urea as a N source, found that the addition of DL-methionine accelerated bacterial N incorporation and rate of cellulose digestion. DL-methionine also has been shown to be the limiting amino acid for ruminal bacterial growth with low-protein diets (Salter et al., 1979). DL-methionine increased the ruminal NDF fermentation rate 30% over a soybean meal or inorganic sulfur, urea and beet pulp supplement (Clark and Petersen, 1988). Moreover, weight gains of methionine-supplemented heifers were similar to those of heifers supplemented with soybean meal. The objective of the present study was to determine whether DL-methionine in a beet pulp carrier could replace soybean meal as a supplement for cows grazing winter range and to determine the principle components of various ruminal kinetic measures.

## Materials and Methods

*Animals.* Two winter trials were conducted at the Red Bluff Research Ranch, Norris, MT. Trial 1 began December 23, 1986 and ended March 1, 1987; Trial 2 began December 26, 1987 and ended March 3, 1988. Cows for Trial 1 (48 pregnant 3- to 8-yr-old crossbred cows, n = 16/supplement treatment) were allocated randomly within age and expected calving date to treatment groups. Forty-two cows used in Trial 2 were pregnant 4- to 8-yr-old crossbred cows (n = 14/supplement treatment) that were selected randomly within each age group and expected calving date from the experimental ranch herd of 150 cows. Cows selected for the two studies were restricted to those expected to calve within the first half of the calving season (between March 6 and March 30) of each year. Twelve ruminally cannulated cows (Dougherty, 1981) also were selected from the cannulated cows present on the experimental ranch and were included in each trial. Genotypes for all cows consisted of varying degrees of Hereford, Angus and(or) Tarentaise. Preceding the trials, cows had grazed native range without supplementation since the previous spring.

*Pasture.* Both trials were conducted on the same 324-ha native range pasture with north-facing, slight to moderate slopes and areas of steep ravines and rock outcrops. Elevation ranged from 1,400 to 1,900 m; annual precipitation was between 350 and 406 mm. The Soil Conservation Service classified the pasture in 1980 as a silty range site in good condition with vegetation composed of 65% grasses and 35% forbs and woody species. Dominant grasses included *Agropyron spicatum, Stipa comata, Festuca idahoensis* and *Elymus cinereus.* Estimated carrying capacity was 1.2 ha per animal unit month (USDA-SCS, 1976). The pasture had been grazed since 1980 below the estimated carrying capacity. The pasture was grazed in each year only during the experimental period.

Samples of grazed forage were collected using the total rumen evacuation technique (Lesperance et al., 1960) from two ruminally cannulated cows grazing with the experimental herd. After the initial rumen evacuation, these cows were allowed to graze for 1 h; forage consumed was evacuated and freeze-dried. Composited samples of the forage were 5.0% CP and 63% NDF for Trial 1 and 4.3% CP and 68.8% NDF for Trial 2 on a dry matter basis.

*Climatic.* Average median daily temperature and total precipitation for Trial 1 were –2.2°C and 9.4 mm during January and –1.3°C and 6.1 mm during February (National Oceanic and Atmospheric Administration, 1987). Average median daily temperature and total precipitation for Trial 2 were –3.9°C and 2.5 mm during January and 1.1°C and 5.1 mm during February (National Oceanic and Atmospheric Administration, 1988).

*Treatments.* Cows were allotted randomly to one of three treatment groups. Four ruminally cannulated cows also were assigned randomly to each treatment. However, during Trial 2 one cannulated cow (receiving the supplement containing DL-methionine) became ill and was removed from the trial. Both trials used the same three treatments, including an unsupplemented control (CON), DL-methionine in beet pulp carrier (BPM) and soybean meal (SBM). Supplements (Table 1) were formulated to be isoenergetic but not isonitrogenous (BPM = 8.9% and 8.8% crude protein, SBM = 36.8% and 36.8% crude protein for Trial 1 and Trial 2, respectively). Supplements

TABLE 1. FORMULATION OF SUPPLEMENTS, AS-FED BASIS

| Item | Supplements[a] | | | |
|---|---|---|---|---|
| | BPM | | SBM | |
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Ingredient | | | | |
| Beet pulp, % | 79.2 | 78.9 | | |
| Soybean meal, % | | | 82.1 | 81.8 |
| DL-methionine, % | 3 | 3.3 | | |
| Dicalcium phosphate, % | 9.65 | 9.65 | 9.2 | 9.2 |
| Calcium sulfate, % | | | 3.0 | 3.3 |
| Potassium chloride, % | 3 | 3 | .55 | .55 |
| Vitamin A[b], % | .1 | .1 | .1 | .1 |
| Molasses, % | .5 | .5 | .5 | .5 |
| Molasses booster[c], % | .05 | .05 | .05 | .05 |
| Supplement fed, kg·hd$^{-1}$·d$^{-1}$ | .5 | .5 | .4 | .4 |
| CP % | 8.9 | 8.9 | 36.8 | 36.8 |
| TDN, % | 69 | 69 | 81 | 81 |

[a]BPM = methionine; SBM = soybean meal.

[b]Vitamin A, 15,000 IU/head daily.

[c]Feed Flavours, Inc., Wheeling, IL.

were identical between trials, except that BPM was formulated to supply 7.5 g·head$^{-1}$·d$^{-1}$ of DL-methionine in Trial 1 vs 9 g·head$^{-1}$·d$^{-1}$ in Trial 2. The SBM supplied 1.5 g·head$^{-1}$·d$^{-1}$ of L-methionine in each Trial[7]. At the initiation of both trials, cows were injected i.m. with 2 × $10^6$ IU of vitamin A; cows had free access to a loose iodized salt mixture. Supplements contained an additional source of vitamin A, dicalcium phosphate and potassium chloride. In addition, the SBM supplement contained calcium sulfate to provide an amount of sulfur equal to that supplied by BPM. Supplements were fed individually on alternating days at approximately 1200. Cows received .5 kg·head$^{-1}$·d$^{-1}$ of BPM and .4 kg·head$^{-1}$·d$^{-1}$ of SBM. Cows in the CON group were gathered and restricted from grazing while supplemented cows received their supplements.

## *Measurements*

*Cow Weights, Condition Scores and Calf Birth Weights.* Palpable condition scores (1 to 10; Bellows et al., 1971) by two technicians and two consecutive days BW were recorded at the start and end of each trial. Cows in Trial 1 were limit-fed hay and had access to water for 24 h before each weighing. Cows in Trial 2 were weighed immediately upon entering the scale pen from the pasture and weighed again 12 h later. Cows in Trial 2 were not fed between weighings. The difference in weighing procedures between Trial 1 and 2 was an effort to minimize weighing error between days (comparing Trial 2 to Trial 1). Initial mean BW and condition scores were 539 kg and 4.2 for Trial 1 and 548 kg and 5.1 for Trial 2, respectively (Table 2). Calves were weighed within 24 h of birth in both trials.

*In Situ Disappearance.* An in situ digestion trial began during the last 4 d of each trial to measure ruminal NDF disappearance rate. Nylon bags[8] were incubated as described by Ørskov (1982). Each bag contained a 3-g sample, ground in a Wiley mill to pass a 2-mm screen. The sample was representative of forage obtained by total rumen evacuation. Bags were constructed from a nylon mesh fabric of 44-μm pore size and were double zigzag stitched with polyester thread. Finished bags had a surface area of 533 cm$^2$. Bags containing no sample (blanks) also were incubated to adjust for flow of ruminal contents into the bags. All bags were suspended in the rumen on a 75-cm circular stringer composed of tygon tubing, a bath plug chain and fishing swivels. The stringer then was placed in a 35-cm × 5-cm fishnet bag tied with 150-cm nylon cord attached to the ruminal cannula. This large bag was used to facilitate the removal of the entire stringer from the rumen. Bags in Trial 1 were

[7]AAA Laboratory, Mercer Island, WA.

[8]Nitex 44 Fabric (#HD3-44), H. R. Williams Mill Supply, Kansas City, MO.

TABLE 2. LEAST SQUARES MEANS OF GESTATING RANGE COWS FOR COW WEIGHT AND CONDITION SCORE CHANGE AND CALF BIRTH WEIGHT (TRIALS 1 AND 2)

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

[a]CON = control; BPM = methionine; SBM = soybean meal.

[b]Trial 1: CON vs supplement, $P = .05$; BPM vs SBM, $P = .13$.

[c]1 = emaciated and 10 = excessively fat.

[d]Trial 1: CON vs supplement, $P = .08$; BPM vs SBM, $P = .04$.

[e]Trial 2: CON vs supplement, $P < .01$; BPM vs SBM, $P = .11$.

suspended in the rumen 4 h before supplementation (0800); bags in Trial 2 were suspended just before supplementation at approximately 1200. At 0, 4, 8, 12, 24, 36, 48, 72 and 96 h after supplementation during Trial 1 and at 3, 6, 9, 20, 24, 30, 48, 72 and 96 h after supplementation during Trial 2, two sample bags were removed from each cow and one blank bag from one cow on each treatment. Upon removal, all bags were frozen for later analysis. Bags were washed in cold water until the rinse water was clear, dried in a forced-air oven at 60°C for 48 h and weighed to determine DM residue. Neutral detergent fiber also was determined (Van Soest and Robertson, 1980) on the bag and residue. Dry matter and NDF values at various incubation times were subjected to a nonlinear regression procedure to estimate the rate of disappearance (Robinson et al., 1986).

*Ruminal Ammonia, pH, Purine Concentration and Carboxymethylcellulase Activity.* Ruminal samples in Trial 1 were obtained at −4, 0, 4, 8, 12, 24, 36 and 48 h after supplementation. In Trial 2, samples were obtained at 0, 3, 6, 9, 20, 24, 30 and 48 h after supplementation. In both trials, while sample bags were removed, whole digesta samples were obtained from near the reticulo-omasal orifice and mixed with 1 ml of 5% $HgCl_2$ to stop microbial activity. The pH of ruminal fluid was determined immediately upon sampling using a portable electrode (Anonymous, 1984). Ruminal fluid subsamples (50 ml) were acidified with 3 ml of 6 *N* HCl for later ammonia analysis by a magnesium oxide distillation procedure (AOAC, 1980). Whole ruminal digesta subsamples were dried at 100°C for 48 h, ground to pass a 1-mm screen and analyzed for purine concentration (Zinn and Owens, 1986). In Trial 2 additional ruminal fluid subsamples were strained through eight layers of cheesecloth; 75 ml were collected and dried at 100°C for 48 h to obtain approximately 1 g of DM for purine analysis. Ruminal purine concentrations were determined at the same time intervals that nylon bags were removed during the 48-h supplementation cycle to estimate the microbial density of ruminal contents. A fourth nylon bag used in Trial 2,

filled with 6 g of forage, was prepared and incubated similarly to all the sample bags used for rate and extent determination (1,080 $cm^2$ of surface area). The contents of this bag was divided; half was dried at 100°C for 48 h and analyzed for purine concentration, and the remainder was used for carboxymethylcellulase (CMCase) analysis (Silva et al., 1987).

*Ruminal Flow Kinetics.* During the recovery of sample bags, ruminal subsamples were obtained (in the sampling procedure previously described) to determine particulate and fluid dilution rates. Sampling times were the same as those for the in situ trial.

Chromium ethylenediaminetetraacetic acid (Cr-EDTA) and ytterbium (Yb)-labeled forage were used as markers for dilution rates of fluid and particulate matter, respectively. In Trial 1 Cr-EDTA was prepared as described by Uden et al. (1980), and in Trial 2 Cr-EDTA was prepared as described by Binnerts et al. (1968). The forage used for the Yb marker was a low-quality chopped native grass hay similar in digestibility and nutrient content (5.5% CP, 65% NDF) to the range forage being consumed by the experimental herd. The forage was prepared as described by the immersion labeling technique of Teeter et al. (1984).

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

Digesta samples were frozen for later analysis. Upon thawing, ruminal fluid samples were filtered (Whatman No. 2 filter paper) and centrifuged at 1,000 × *g* for 15 min. Analysis for Cr was determined by atomic absorption spectrophotometry with a nitrous oxide/acetylene flame[9]. Particle samples from filtered digesta samples were dried at 60°C and ground in a Wiley mill to pass a 1-mm screen. Ytterbium concentration was determined by neutron activation[10].

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

*Blood Metabolites.* Blood samples from all cows were drawn into untreated vacuum tubes from the artery or vein near the base of the tail on two consecutive days twice during the studies. Samples were taken approximately 45 and 25 d prepartum at 1300 in both trials and analyzed for blood urea nitrogen (BUN), total bilirubin, creatinine, albumin, total protein and cholesterol[11].

*Statistical Analysis.* Data from within each trial were analyzed by analysis of variance using the General Linear Models procedure (SAS, 1987). Weight and condition score data were analyzed using treatment, sex of calf and breed of cow as main effects. Initial weight and birth weight were included as linear covariates. Initial weight was an important ($P < .01$) source of variation. Treatment least squares means were separated by orthogonal contrasts (CON vs supplement and BPM vs SBM). Blood metabolites and ruminal measurements were analyzed by a split-plot analysis of variance with repeated measures (Gill and Hafs, 1971). The statistical model for blood metabolites included treatment, days prepartum, and the treatment × day prepartum interaction. The *F* statistic for treatment was calculated using cow (treatment) mean square as the error term. Ruminal pH, ammonia, purine concentration and CMCase models used treatment, hour and the treatment × hour interaction. Models for blood metabolites and ruminal dynamics models utilized orthogonal contrasts with cow (treatment) mean square as the error term to compare treatments. The orthogonal contrasts utilized were CON vs supplement and BPM vs SBM. Least significant difference techniques were used to compare means. In Trial 2, all ruminal variables measured (in situ disappearance, dilution rates, purine concentration, CMCase activity, ammonia and pH) were selected for a principal component analysis. In this analysis, variables are grouped together to account for multiple correlations among observed variables. With similar variables grouped, only the uncor-

[9]Analysis conducted by Ruminant Nutrition Laboratory, New Mexico State University, Las Cruces.

[10]Analysis conducted by Neutron Activation Laboratory, Washington State University, Pullman.

[11]Analyses conducted by Marsh Laboratory, Montana State University, Bozeman.

related factors remain (which make up the principal components). Therefore, this analysis was used to determine the combination of components that account for variation of ruminal function due to supplementation. Principal component analysis used the Factor Procedure (SAS, 1987).

## Results

### *Cow Weights, Condition Scores and Calf Birth Weights*

*Trial 1.* Cow BW change was affected ($P = .05$) by SBM supplementation compared to the control (Table 2). Weight loss between the supplemented groups also was different ($P = .13$), with the SBM group losing less weight.

Cows fed supplement lost less condition score ($P = .08$) than did control cows (Table 2). Controls lost .75 units of condition, whereas supplemented cows lost an average of .52 units. However, cows fed BPM lost more ($P = .04$) condition than those fed SBM (.68 vs .38 units). Supplementation had no effect ($P > .20$) on calf birth weight (Table 2).

*Trial 2.* Cow BW change was affected ($P < .01$) by supplementation (Table 2). Control cows lost 9.3 kg (Table 2), whereas supplemented cows gained an average of 7.8 kg ($P < .01$). Cows fed BPM gained less weight ($P = .11$) than those fed SBM. In contrast to Trial 1, neither condition score nor calf birth weight was affected ($P > .05$) by supplementation (Table 2).

### *Blood Metabolites*

*Trial 1.* Control cows had lower ($P = .02$) serum cholesterol than the supplemented group (Table 3). Further, serum total protein was greater ($P = .05$) in the control cows than in the supplemented group. There were no effects ($P > .20$) due to treatment for creatinine, total bilirubin and serum albumin.

Serum total bilirubin (Table 4) was greater ($P < .01$) at 45 vs 25 d prepartum. There were no effects, however ($P > .20$), due to days prepartum on serum cholesterol, creatinine, total protein or serum albumin.

A treatment × days prepartum interaction ($P = .02$) resulted from a difference in magnitude of the decline in BUN between 45 and 25 d prepartum (Table 5). Between the two dates, the cows fed BPM had a 16% decrease in BUN, whereas BUN of cows fed CON and SBM decreased approximately 56%. All treatment groups had greater BUN at 45 d vs 25 d prepartum. The BUN was less at 45 d and 25 d ($P < .01$) for the cows fed BPM than for the CON cows or those fed SBM.

*Trial 2.* There were no significant treatment effects in Trial 2 (Table 3) for serum cholesterol, creatinine, total bilirubin and total protein. Likewise, day prepartum did not affect (Table 4) serum cholesterol and total protein. Creatinine was greater ($P < .01$) at 45 d prepartum than at 25 d prepartum. Conversely, bilirubin was lower ($P < .01$) at 45 d prepartum than at 25 d prepartum.

Effects of treatment and days prepartum interacted with respect to BUN ($P = .02$; Table 5); control cows had greater ($P < .1$) BUN at 45 d prepartum than did cows supplemented with BPM or SBM. At 25 d prepartum, CON cows had less ($P < .01$) BUN than at 45 d prepartum, but BUN of CON cows was not different from that of supplemented cows.

As with BUN, a treatment × days prepartum interaction ($P < .01$) was detected for serum albumin. Serum albumin was greater ($P < .01$) in CON cows at 45 d prepartum than in cows fed BPM or SBM. At 25 d prepartum, the CON cows had less ($P < .01$) albumin than at 45 d prepartum, but they were not different from the supplemented cows (Table 5).

### *Ruminal Disappearance, Fluid, and Particulate Dilution Rate*

*Trial 1.* Ruminal DM and NDF disappearance rates were slower ($P < .01$; Table 6) for the CON cows than for supplemented cows. Further, disappearance rates for cows fed BPM were slower ($P = .01$ and $P = .04$ for DM and NDF, respectively) than for those fed SBM (Table 6).

Ruminal fluid dilution rates (Table 6) were similar ($P > .20$) among treatments. Particulate dilution rates (Table 6) were faster ($P = .1$) for the supplemented than for the control cows.

*Trial 2.* As in Trial 1, ruminal DM ($P = .03$) and NDF ($P = .02$) disappearance rates were slower (Table 6) for CON cows than for supplemented cows. Likewise, disappearance rates for cows fed BPM were slower ($P = .03$) than for the cows fed SBM (Table 6).

Both ruminal fluid and particulate dilution rates (Table 6) were faster ($P = .04$ and $P = .08$, respectively) for supplemented than for CON cows.

TABLE 3. LEAST SQUARES MEANS CHOLESTEROL, CREATININE, SERUM ALBUMIN, TOTAL BILIRUBIN AND TOTAL PROTEIN CONCENTRATIONS BY TREATMENT (mg/dl) (TRIALS 1 AND 2)

| Metabolite | Supplements[a] | | | SE |
|---|---|---|---|---|
| | CON | BPM | SBM | |
| Trial 1 | | | | |
| Cholesterol[b] | 104.1 | 115.8 | 111.8 | 3.3 |
| Creatinine | 2.1 | 2.3 | 2.2 | .06 |
| Total bilirubin | .7 | .6 | .6 | .03 |
| Total protein[c] | 6.4 | 6.2 | 6.3 | .06 |
| Serum albumin | 3.9 | 3.9 | 4.0 | .06 |
| Trial 2 | | | | |
| Cholesterol | 108.0 | 111.9 | 102.6 | 3.8 |
| Creatinine | 2.0 | 2.0 | 1.8 | .05 |
| Total bilirubin | .6 | .6 | .5 | .03 |
| Total protein | 6.3 | 6.3 | 6.5 | .08 |

[a]CON = control; BPM = methionine; SBM = soybean meal.

[b]CON vs supplement, $P = .02$.

[c]CON vs supplement, $P = .05$.

### *Ruminal Ammonia, pH, Purine Concentration and Carboxymethylcellulase Activity*

*Trial 1.* Mean ruminal ammonia concentrations within the 48-h sampling interval (Table 6) were not different between CON and the supplemented groups; however, cows fed SBM (1.3 mg/dl) tended to have greater ($P = .18$) ammonia concentrations than those fed BPM (1.0 mg/dl).

Ruminal pH (Table 6) was greater ($P = .09$) for CON cows than for the two supplemented groups of cows, and pH was greater ($P = .09$) in cows fed BPM than in those fed SBM.

Purine concentration of whole ruminal contents (Table 6) tended to be less ($P = .15$) for the CON cows than for cows receiving supplement. Further, cows fed BPM tended to have lower purine concentrations ($P = .13$) than those fed SBM.

*Trial 2.* Ruminal ammonia concentrations and pH (Table 6) were not different among the treatment groups.

A significant sampling time × treatment ($P < .05$) interaction was detected for purine concentration in whole ruminal contents (Figure 1). The interaction resulted from a peak in purine concentration in a sample taken 6 h after supplementation. The purine concentration of CON cows was less ($P < .01$) than values observed for the mean of cows fed BPM or SBM, which also were different ($P < .01$) from each other.

TABLE 4. LEAST SQUARES MEANS CHOLESTEROL, CREATININE, SERUM ALBUMIN, TOTAL BILIRUBIN AND TOTAL PROTEIN CONCENTRATIONS BY PERIOD (mg/dl) (TRIALS 1 AND 2)

| Metabolite | Days before calving | | SE |
|---|---|---|---|
| | 45 | 25 | |
| Trial 1 | | | |
| Cholesterol | 110.8 | 110.4 | .9 |
| Creatinine | 2.2 | 2.2 | .02 |
| Total bilirubin[a] | .7 | .6 | .02 |
| Total protein | 6.2 | 6.3 | .02 |
| Serum albumin | 3.90 | 4.0 | .02 |
| Trial 2 | | | |
| Cholesterol | 107.1 | 107.9 | 3.1 |
| Creatinine[b] | 2.0 | 1.8 | .04 |
| Total bilirubin[c] | .3 | .8 | .02 |
| Total protein | 6.4 | 6.6 | .06 |

[a]Period effect, $P < .01$.

[b]Period effect, $P < .01$.

[c]Period effect, $P < .01$.

TABLE 5. LEAST SQUARES MEANS BLOOD UREA NITROGEN AND SERUM ALBUMIN WITH TREATMENT × PERIOD INTERACTION (mg/dl) (TRIALS 1 AND 2)

| Metabolite | Days[b] | Supplements[a] CON | BPM | SBM | SE |
|---|---|---|---|---|---|
| Trial 1 | | | | | |
| Blood urea N[c] | 45 | 6.4 | 4.0 | 6.6 | .29 |
| | 25 | 4.1 | 3.4 | 4.3 | .29 |
| Trial 2 | | | | | |
| Blood urea N[d] | 45 | 8.0 | 5.4 | 5.7 | .4 |
| | 25 | 6.0 | 5.5 | 6.0 | .4 |
| Serum albumin[e] | 45 | 4.3 | 3.9 | 4.1 | .1 |
| | 25 | 4.0 | 4.0 | 4.0 | .1 |

[a]CON = control; BPM = methionine; SBM = soybean meal.

[b]Days prior to calving.

[c]Trial 1: Treatment × period interaction, $P = .02$.

[d]Trial 2: Treatment × period interaction, $P = .02$.

[e]Trial 2: Treatment × period interaction, $P < .01$.

Purine concentration in strained ruminal fluid was greater ($P = .05$) in cows fed SBM (38.3 mg/g) than in cows fed BPM (32.4 mg/g) or CON cows (33.1 mg/g) (Figure 2). Mean purine concentration found on the samples within the nylon bags were similar ($P > .05$) among the treatments (Figure 3) (7.2, 7.4 and 7.4 mg/g for CON, BPM and SBM, respectively). There was an effect of sampling time that showed that a maximum concentration (9.0 mg/g) was achieved within 9 h of incubation.

The mean (for all sampling times) CMCase activity in the fluid fraction (Figure 4) was similar ($P > .20$) among treatments (554, 590 and 565 μmol glucose·$g^{-1}$·$h^{-1}$ for CON, BPM and SBM, respectively). Activity in cows fed BPM and SBM was greater ($P < .01$) at 20 h than for CON cows. Mean CMCase activity associated with the samples incubated in the nylon bags was greater ($P < .08$) for cows fed BPM (147 μmol glucose·$g^{-1}$·$h^{-1}$) and SBM (136 μmol glucose·$g^{-1}$·$h^{-1}$) compared to CON (101 μmol glucose·$g^{-1}$·$h^{-1}$) (Figure 5).

### *Principal Components of Ruminal Function as Influenced by Protein Supplementation*

*Correlations Between Ruminal Measures.* In Trial 2, factors that were positively related ($P < .01$, Table 7) to the purine concentration of the in situ incubated forage were purine concentration of the whole ruminal contents and CMCase activity of the in situ incubated forage. Factors related negatively ($P < .01$) were CMCase activity in strained ruminal fluid, ammonia concentration, NDF disappearance and particle dilution. Four factors related negatively ($P < .05$) to purine concentration of strained ruminal fluid were CMCase activity of the incubated forage and strained ruminal fluid along with particulate and fluid dilution. Both particulate and fluid dilution were related positively ($P < .05$) to purine concentration of whole ruminal contents. CMCase activity of strained ruminal fluid was related positively ($P < .01$) to NDF disappearance rate and ruminal dilution. Fluid dilution was negatively related ($P < .05$) to pH and related positively ($P < .01$) to ammonia concentration and particulate dilution. Disappearance rate of NDF was related positively ($P < .01$) to particulate and fluid dilution rates.

*Principal Components of Ruminal Function.* Ten ruminal measurements were subjected to principal component analysis, and results are presented in Table 8. The analysis grouped the ruminal measurements into four components.

The first component, with an Eigenvalue of 3.5 accounted for 46.4% of the variation caused by supplementation in ruminal measurements taken and had three major terms. These were NDF disappearance, particle dilution and CMCase activity in the ruminal fluid fraction, all of which had coefficients with magnitudes greater than .88. The fourth term of a lesser predictive value was fluid dilution, with a coefficient of .77.

The second component, with an Eigenvalue of 1.8, accounted for 23.5% of the variation and has two major terms dominated by

TABLE 6. LEAST SQUARES MEANS FOR RUMINAL AMMONIA, pH, PURINE CONCENTRATION, IN SITU FERMENTATION RATE AND FLUID AND PARTICULATE DILUTION RATE (TRIALS 1 AND 2)

| Measurements | Supplements[a] | | | SE |
|---|---|---|---|---|
| | CON | BPM | SBM | |
| Trial 1 | | | | |
| DM disappearance, %/h[b] | 2.65 | 3.06 | 3.57 | .1 |
| NDF disappearance rate, %/h[c] | 1.34 | 1.54 | 1.78 | .07 |
| Fluid dilution rate, %/h | 8.5 | 9.1 | 9.2 | .48 |
| Particulate dilution rate, %/h[d] | 2.66 | 2.89 | 3.28 | .19 |
| Rumen ammonia, mg/dl[e] | 1.2 | 1.0 | 1.3 | .1 |
| pH[f] | 6.9 | 6.9 | 6.8 | .1 |
| Purine concentration mg/g[g] | 7.3 | 7.4 | 8.0 | .2 |
| Trial 2 | | | | |
| DM disappearance rate, %/h[h] | 3.02 | 3.25 | 3.89 | .17 |
| NDF disappearance rate, %/h[i] | 1.86 | 2.02 | 2.38 | .09 |
| Fluid dilution rate, %/h[j] | 7.95 | 8.79 | 8.73 | .28 |
| Particulate dilution rate, %/h[k] | 2.58 | 3.06 | 3.20 | .23 |
| Rumen ammonia, mg/dl | 1.7 | 1.5 | 2.2 | .42 |
| pH | 6.8 | 6.9 | 6.8 | .04 |

[a]CON = control; BPM = methionine; SBM = soybean meal.

[b]CON vs supplement, $P > .01$; BPM vs SBM, $P = .01$.

[c]CON vs supplement, $P > .01$; BPM vs SBM, $P = .04$.

[d]CON vs supplement, $P = .1$.

[e]BPM vs SBM, $P = .18$.

[f]CON vs supplement, $P = .09$; BPM vs SBM, $P = .09$.

[g]CON vs supplement, $P = .15$; BPM vs SBM, $P = .13$.

[h]CON vs supplement, $P = .03$; BPM vs SBM, $P = .03$.

[i]CON vs supplement, $P = .02$; BPM vs SBM, $P = .03$.

[j]CON vs supplement, $P = .04$.

[k]CON vs supplement, $P = .08$.

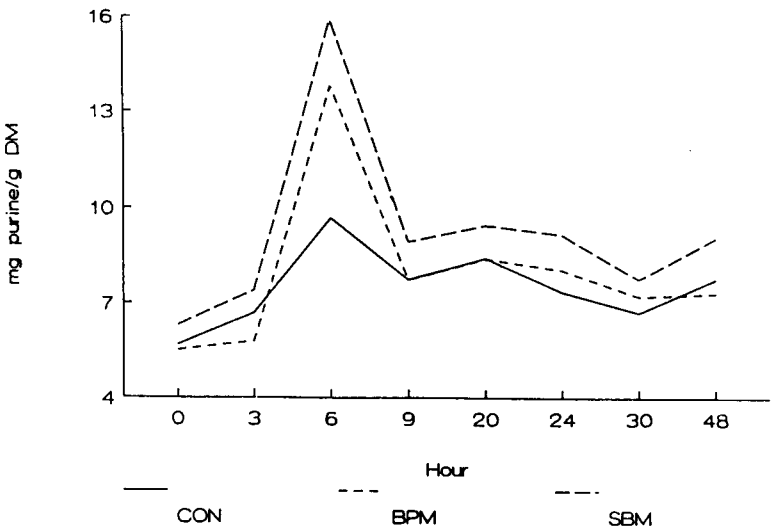


Figure 1. Mean whole ruminal purine concentrations of whole ruminal contents (mg purine/g DM) at times after supplementation (0 h = time of supplementation) of prepartum beef cows grazing native winter range (Trial 2). Treatments are CON = no supplement, BPM = methionine in beet pulp carrier and SBM = soybean meal. Linear contrast of CON vs supplement, $P < .01$ and for BPM vs SBM, $P < .01$. Pooled standard error ± .62.

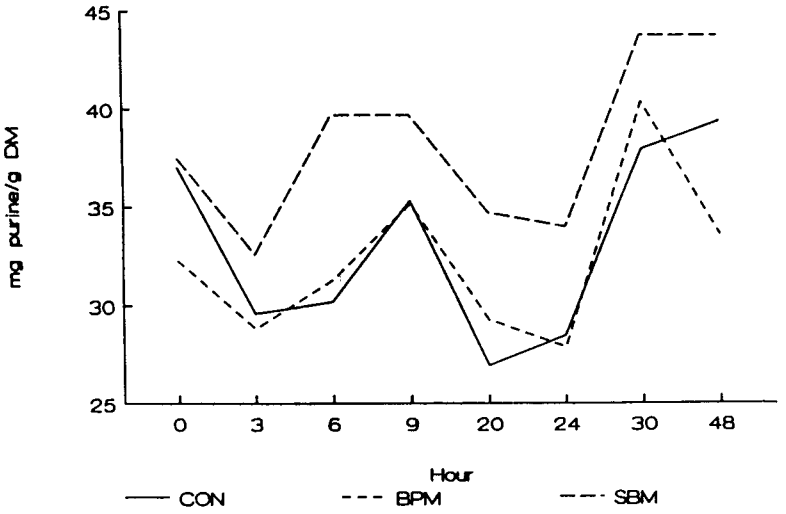


Figure 2. Mean ruminal purine concentrations in strained ruminal fluid (mg purine/g DM) at times after supplementation (0 h = time of supplementation) of prepartum beef cows grazing native winter range. Treatments are CON = no supplement, BPM = methionine in beet pulp carrier and SBM = soybean meal. Linear contrast of CON vs supplement, $P = .25$ and for BPM vs SBM, $P < .05$. Pooled standard error ± 3.7.

microbially related measures of the sample bags, purine concentration and CMCase activity. These terms were of approximately equal importance, with coefficients of .75.

The third component, with an Eigenvalue of 1.3, accounted for less than 17% of the variation and was composed of three terms, ruminal fluid and whole ruminal purine concentrations and ruminal ammonia concentration, with coefficients of .51, .71 and .59, respectively.

The fourth and final component, with an Eigenvalue of 1.0, accounted for 13.3% of the variation and had pH as its only important component. Due to the narrow range of pH in this study it probably had only minor biological importance.

## Discussion

DL-methionine in a beet pulp carrier did not substitute for soybean meal as a winter supplement compared with no supplement. However, the environmental conditions of the two trials may not have allowed supplementation effects to be manifested because both winters were mild. Mean average temperatures ranged from 2 to 3°C warmer than normal, and snowfall was 50% of normal (National Oceanic and Atmospheric Administration, 1987, 1988). Snow cover was minimal or nonexistent, thus giving cows unlimited access to available forage, in contrast to years with accumulating snow when access is limited to aerial forage. Warmer temperatures also decreased maintenance requirements. During these years, supplementation may not have been needed. This observation is supported by the small differences in cow weight change and condition score change, and by the similarity of blood metabolite data. Differences in cow weight change between the SBM and the CON were only 10 kg in Trial 1 and 22 kg in Trial 2. Miner et al. (1990) found differences of 34 kg and 26 kg between CON and SBM on the same site in 1985 and 1986, respectively, both years with normal snowfall.

Measurements of ruminal dynamics should detect effects that supplements have on digestion. Neutral detergent fiber disappearance rate and particle dilution rate were different between CON and supplemented cows. Mean CMCase activity in the strained ruminal fluid (Figure 2) showed no treatment differences; however, activity in both supplemented groups was greater at 20 h than in CON cows, in which activity dropped after 9 h. This period of increased activity in supplemented cows corresponded to a period in which the slope of the NDF disappearance curve was the greatest. These results could indicate that the increase in NDF disappearance caused by supplementation might be influenced by a short-term stimulation of fluid cellulase. Furthermore, CMCase

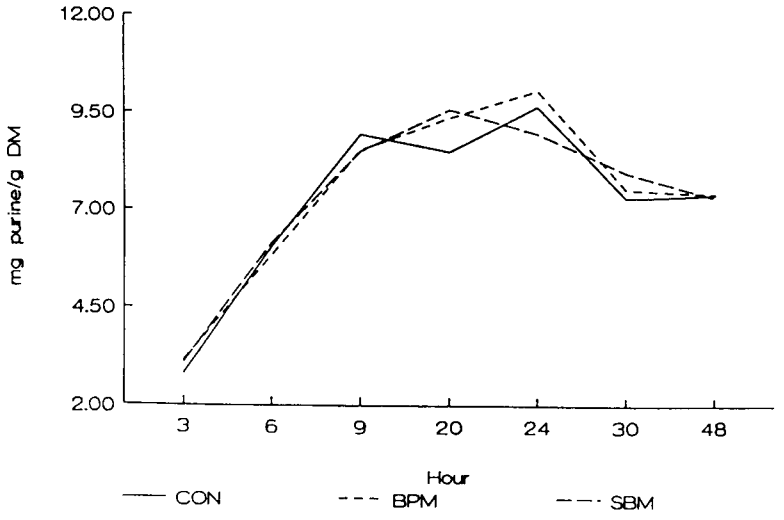


Figure 3. Mean ruminal purine concentrations on incubated forage (mg purine/g DM) at times after supplementation (0 h = time of supplementation) of prepartum beef cows grazing native winter range. Treatments are CON = no supplement, BPM = methionine in beet pulp carrier and SBM = soybean meal. Linear contrast of CON vs supplement, $P$ = .71 and for BPM vs SBM, $P$ = .93. Pooled standard error ± .70.

activity was twice as high in the fluid fraction (Figure 4) compared with particle-bound activity (Figure 5), which indicates that fluid enzymes may be of major importance in fiber degradation. However, these values reflect dried ruminal fluid samples, which obviously concentrate CMCase activity.

Purine concentration (Figure 3) on the incubated forage increased for approximately 9 h, after which purine concentration remained fairly constant. This pattern indicates that an increase in forage degradation rate would arise from increased enzymatic activity. Mean CMCase activity eluted from sample residue was different among supplementation treatments. There was a trend for the supplemented cows to have greater activity (Figure 5) at specific times (between 20 and 30 h of

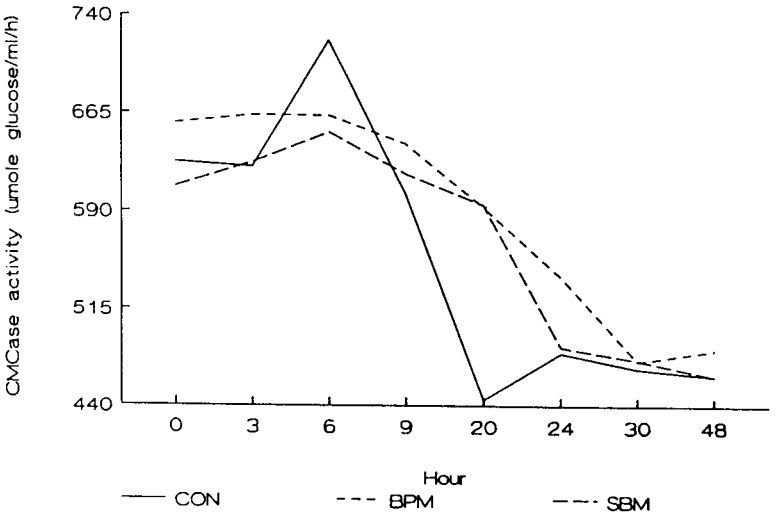


Figure 4. Mean carboxymethylcellulase (CMCase) activity (μmole glucose released·$g^{-1}$·$h^{-1}$) in liquid fraction at times after supplementation (0 h - time of supplementation) of prepartum beef cows grazing native winter range. Treatment are CON = no supplement, BPM = methionine in beet pulp carrier and SBM = soybean meal. Linear contrast of CON vs supplement, $P$ = .32 and for BPM vs SBM, $P$ = .40. Pooled standard error ± 38.

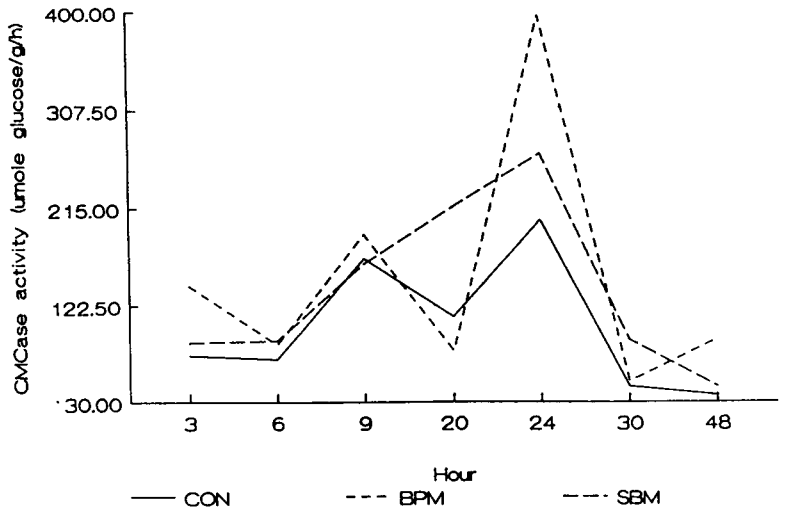


Figure 5. Mean carboxymethylcellulase (CMCase) activity (μmole glucose released·$g^{-1}$·$h^{-1}$) on incubated forage at times after supplementation (0 h = time of supplementation) of prepartum beef cows grazing native winter range. Treatments are CON = no supplement, BPM = methionine in beet pulp carrier and SBM = soybean meal. Linear contrast of CON vs supplement, $P$ = .07 and for BPM vs SBM, $P$ = .66. Pooled standard error ± 38.2.

incubation). Cows fed SBM had greater activity at 20 h, and those fed BPM had greater activity at 24 h than did CON cows (data not shown). This would indicate that CMCase activity was stimulated without increasing the microbial concentration, although it may be indicative of a shift in the activity of the population. However, correlations between CMCase activity on incubated forage and NDF disappearance had an r value of only –.08 and the correlation between purine concentration on incubated forage and NDF disappearance was –.55 ($P$ < .01). These data agree with those of Silva et al. (1987), who showed a similar amount of CMCase activity on ammonia-treated straw fed with either beet pulp, barley or barley plus fishmeal. Using NAD-linked glutamate dehydrogenase as a microbial marker, these authors also saw a moderate negative relationship between microbial concentration attached to the straw and the residue remaining.

Cows fed BPM had greater purine concentrations in whole ruminal contents than did CON cows, which agrees with results of Arambel et al. (1987), who found increased ruminal microbial counts when supplementing dairy cows with a protected methionine product that was 28% ruminally degraded.

The increased whole ruminal purine concentration observed with BPM might be attributed to the beet pulp carrier. Silva and Ørskov (1988) reported that NDF disappearance rates of barley straw increased when supplemental beet pulp was fed. However, Clark and Petersen (1988) demonstrated that beet pulp plus methionine increased NDF disappearance compared to beet pulp with ammonium sulfate. Wiley et al. (1988) reported that NDF disappearance rates increased when methionine was supplemented without a carrier via ruminal fistula in cows fed a roughage diet (6.8% crude protein, 68% NDF). Therefore, we attribute the observed ruminal effects (increase in disappearance rates, dilution rates, and CMCase activity compared to control) to the methionine and not to the carrier.

Clark and Petersen (1988) indicated that DL-methionine may replace soybean meal, as suggested by ruminal measurements and heifer performance. They found a 30% greater NDF disappearance rate in situ with a methionine-containing supplement compared with soybean meal. In the present study, methionine did not elicit the same response. One explanation might be the absence of urea fed with DL-methionine. Clark and Petersen (1988) showed that the beet pulp-methionine-urea treatment had 10-fold greater ruminal ammonia concentrations compared with values reported in the present study, in which the BPM supplement resulted in lower ruminal ammonia concentrations than did the SBM supplement. This

TABLE 7. PEARSON CORRELATION COEFFICIENTS BETWEEN RUMINAL PARAMETERS: PURINE CONCENTRATION ON INCUBATED FORAGE (RNAF), PURINE CONCENTRATION IN RUMINAL FLUID (RNAL), PURINE CONCENTRATION OF WHOLE RUMINAL SAMPLES (RNAW), CARBOXYMETHYLCELLULASE (CMCASE) ACTIVITY ON INCUBATED FORAGE (CMCF), CMCASE ACTIVITY IN LIQUID (CMCL), AMMONIA ($NH_3$), pH, NDF DISAPPEARANCE (NDF), LIQUID DILUTION (LIQ) AND PARTICLE DILUTION (PAR) MEASURED FOR PRINCIPAL COMPONENT ANALYSIS (TRIAL 2, N = 88)

| | Correlations | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | RNAF | RNAL | RNAW | CMCF | CMCL | $NH_3$ | pH | NDF | LIQ | PAR |
| PAR | −.31[a] | −.24[b] | .31[a] | .03 | .80[a] | .01 | −.15 | .79[a] | .71[a] | |
| LIQ | −.15 | −.25[b] | .23[b] | .07 | .57[a] | .22[b] | −.25[b] | .56[a] | | |
| NDF | −.55[a] | −.20 | .01 | −.08 | .78[a] | −.03 | −.27[b] | | | |
| pH | .16 | .07 | −.06 | .02 | −.13 | .10 | | | | |
| $NH_3$ | −.34[a] | .14 | .08 | −.20 | .14 | | | | | |
| CMCL | −.39[a] | −.29[a] | .18 | .07 | | | | | | |
| CMCF | .49[a] | −.22[b] | .12 | | | | | | | |
| RNAW | .31[a] | .09 | | | | | | | | |
| RNAL | .02 | | | | | | | | | |
| RNAF | | | | | | | | | | |

[a] $P < .01$.

[b] $P < .05$.

finding is supported by the lower BUN in the BPM cows in both trials. This trend toward lower ruminal ammonia concentration with BPM may reflect increased utilization of available ammonia by ruminal microbes. In fact, the relatively low ruminal ammonia with SBM compared to CON also may be indicative of utilization. Increased NDF disappearance rate and purine concentration of whole contents compared with values in CON cows suggests that microbial activity was increased for both BPM and SBM. Because ruminal ammonia concentrations were well below the suggested level of 5 mg/dl ammonia reported by Satter and Slyter (1974), ammonia may have been the factor limiting weight gain of cows fed BPM and, to a lesser extent, of cows fed SBM.

This interpretation that ammonia concentration may have limited ruminal function is supported by the principal component analysis of ruminal measures. The first component, which accounted for nearly 50% of the variation due to supplementation, was composed of four terms. In three of the four terms, the two supplement treatments elicited similar responses, which were different from the control. The fourth term of the first component was NDF disappearance rate, in which all three treatments were different, with SBM being the fastest, followed by BPM and the control. Factors of component 1 such as ruminal

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

| Terms | Factors | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Purine concentrations | | | | |
| Incubated forage | −.52 | .75 | .17 | −.04 |
| Ruminal liquid | −.33 | −.35 | .51 | −.38 |
| Whole ruminal sample | .18 | .46 | .71 | −.24 |
| Carboxymethylcellulase activity | | | | |
| Incubated forage | −.05 | .77 | −.10 | .20 |
| Ruminal liquid | .88 | .08 | .02 | .16 |
| Ruminal ammonia | .15 | −.46 | .59 | .34 |
| pH | −.31 | −.03 | .21 | .79 |
| NDF disappearance | .90 | −.10 | −.19 | −.07 |
| Fluid dilution | .77 | .20 | .20 | .01 |
| Particulate dilution | .91 | .18 | .09 | .02 |
| Total variance, % | 46.4 | 23.5 | 16.8 | 13.3 |

digestion and passage rates make up the classical responses reported to be influenced by the feeding of ruminally degraded protein sources compared with none. Therefore, the first component is correlated to the differences that occurred in weight change between unsupplemented and supplemented cows, but not between different types of supplement. Component 2, which is composed of terms relating to the sample bags (purine concentration and CMCase activity), did not separate effects caused by type of supplement, but by whether or not cows received supplement. Component 3 may identify the mechanism that controlled the BW change difference between cows fed BPM vs SBM. Terms making up component 3 included fluid purine concentration, in which SBM elicited a greater concentration throughout the 48-h supplementation cycle than did BPM. Another important term of component 3 was ruminal ammonia concentration, in which cows fed BPM had lower concentrations than those fed SBM. Horn and McCollum (1987) reported that ruminal ammonia decreased in response to microbial stimulation via the supplementation of rapidly degraded energy sources. Therefore, the differences in animal performance reported here vs those of Clark and Petersen (1988) may be due to a stimulation of the ruminal microflora by BPM, which ultimately reduced ruminal ammonia (and BUN), and which in turn may be related to lower purine concentration within the ruminal fluid. If protein reaching the small intestine of winter-grazing beef cows limits cow performance (as proposed by Miner et al., 1990), then cows supplied with BPM would not be expected to maintain body weight as well as SBM-supplemented cows because ruminal ammonia may have limited the concentration of purines found in the fluid portion of the rumen and, thus, potentially reduced microbial protein reaching the small intestine.

Another possible explanation for the lack of agreement between this study and that of Clark and Petersen (1988) is the supplementation scheme. They fed DL-methionine daily in a completely mixed diet; in the present study, DL-methionine was supplemented as a single pulse dose on alternating days. DL-methionine probably has a short-term effect on the microbial population in the rumen, as evidenced in Trial 2 by the treatment × sampling time interaction with whole ruminal purine concentrations (Figure 1). An interaction was not observed in Trial 1, but this probably was the result of the sampling protocol, which missed the short-term peak in purine concentration that occurred 6 h after supplementation. These data agree with those of Wiley et al. (1988), who found a similar peak after supplementation using purines as a microbial marker. Leedle et al. (1982) reported a similarly shaped peak while establishing microbial growth curves using microbial counts in relation to substrate availability. After the supplementation peak in Trial 2, purine production in BPM-supplemented cows was reduced to a baseline value equal in magnitude to that of the CON cows, whereas the SBM-fed cows maintained greater values throughout the 48-h supplementation cycle. This response may demonstrate the need for supplementation of methionine more frequently than every other day, thereby stimulating the peak response on a daily basis and potentially elevating the base value and microbial protein reaching the small intestine.

## Implications

Based on overall means, DL-methionine supplementation showed improvement in ruminal function compared with unsupplemented cows (for in situ disappearance rate, particulate dilution rate and carboxymethylcellulase activity). However, if methionine is to substitute for soybean meal on winter range, certain questions must be answered. First, Did ruminally degraded protein supplementation alter weight change of winter grazing beef cows via concentration of purines found in ruminal contents? Continued research is needed to determine whether microbial protein production is the mechanism by which protein supplements improve animal weight and body condition of winter-grazing range cows.

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