

**INFLUENCE OF UNDEGRADABLE INTAKE PROTEIN MIXED WITH MINERALIZED SALT ON INTAKE AND FERMENTATION PROFILES OF COWS GRAZING DORMANT WINTER RANGE.**

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**ABSTRACT:** Six crossbred, mature beef cows (609 kg) fitted with ruminal cannulas were used in a crossover design to evaluate the effects of small quantities of supplemental blood meal and feather meal combined with mineralized salt (40% NaCl, 41% CaHPO<sub>4</sub>, 7% KCl, 7% MgO, 5% trace minerals) on serum urea nitrogen, ruminal fermentation profiles, in situ organic matter disappearance and fecal output. Cows grazed dormant winter range in a 481 ha pasture at the New Mexico State University Chihuahuan Desert Rangeland Research Center. Mineralized salt, blood meal, and feather meal (SBF) were combined in a 2:1:1 ratio and dosed directly through the cannula of three cows daily for 14 d at a rate of 128 g/d, while mineralized salt (MS) was dosed into another three cows at a rate of 64 g/d. To determine the appropriate amount to dose, SBF was offered free choice in open tubs to 53 cows in three pastures for 60 d. In each period cows were bolused with a slow release Cr device. Fecal samples were collected and supplement and forage samples were incubated in situ for 0, 6, 12, 24, 36, 48, 72, and 96 h on d 7 to 12. Blood and ruminal fluid samples were collected at 0, 3, 6, 9, 12, 15, 18, and 24 h after supplementation on d 12. To assess forage quality, rumen extrusa was collected from two cows allowed to graze for 1 h following complete rumen evacuation. Extrusa was 7.4% CP, 53% NDF, and 14% ash. Serum urea nitrogen concentration was greater (8.4 vs. 7.7 mg/dL,  $P < 0.05$ ) for SBF-treated cows. Ruminal ammonia nitrogen and total VFA concentrations, in situ DM disappearance, and fecal output, were 3.85 vs. 3.49 mg/dL ( $P = 0.21$ ), 56.0 vs 55.5 mM ( $P = 0.86$ ), 2.2 vs. 2.8 %/h ( $P = 0.41$ ), and 4.61 vs. 4.48 kg/d ( $P = 0.21$ ) for SBF and MS treated cows, respectively. Results suggest that the physical nature of the forage rendered supplemental protein inadequate to alter intake and fermentation profiles. However, supplemental UIP may have increased metabolizable protein supply as evidenced by increased blood urea nitrogen.

Key words: Cattle, Protein supplementation, Mineral supplementation

### Introduction

Optimum beef cattle production in western rangelands often necessitates supplementation of protein and minerals. Efficiently utilized supplements supplied in cost effective delivery systems contribute to economic efficiency (Kunkle et al., 2000). Efficiency of supplemental protein utilization can be improved by capitalizing on ability of ruminants to improve nitrogen (N) conservation through increased recycling during periods of N deprivation (Bunting et al.,

1989). Excessive ruminal N has been shown to impair N recycling (Siddons et al., 1985). Because of the innate resistance of undegradable intake proteins (UIP) to microbial degradation, supplements containing UIP can result in reduced ruminal nitrogen loss without compromising ruminal function (Coomer et al., 1993). Therefore, small quantities of supplemental UIP fed to cows maintained on low quality diets may be more efficiently utilized than larger amounts of degradable intake protein. Sawyer (2000) found that small quantities of supplemental blood meal and feather meal resulted in greater N flow to the duodenum and similar ruminal function to soybean meal fed on an equivalent CP basis to cows maintained on a low quality diet.

Ruminants have a strong appetite for salt (NRC, 1996), making it an excellent delivery mechanism for other nutrients. Supplemental minerals lacking in the diet are frequently provided in combination with salt (Salt Institute, 2001). We theorized that mineralized salt would make an effective vehicle for delivery of small quantities of supplemental blood meal and feather meal. One objective of this study was to quantify the ad libitum intake of blood meal and feather meal combined with mineralized salt by cows on native range. A second objective was to test the effects of small quantities of blood meal and feather meal supplied in combination with supplemental minerals on intake and ruminal function of cows grazing dormant winter range.

### Materials and Methods

*Experiment 1.* Mineralized salt (MS; 40% NaCl, 41% CaHPO<sub>4</sub>, 7% KCl, 7% MgO, 5% trace minerals) was offered free choice in open rubber tubs to 53 cows grazing 3 pastures at the New Mexico State University Chihuahuan Desert Rangeland Research Center (NMSU CDRRC), for 28 d. A single tub was placed in each pasture equidistant (50 m) from water. Amount of MS not consumed was weighed weekly. Sub samples were collected at each weighing and dried at 100° C for 24 h. To correct intake measures for environmental losses, tubs filled with MS were placed in adjacent enclosures. Amount of MS consumed was calculated as the difference between amount offered and amount refused, on a DM basis, after correcting for environmental loss. Following the MS intake period mineralized salt was combined with blood meal and feather meal (SBF) in a 2:1:1 ratio and offered for 60 d. Intake of SBF was calculated similarly to MS.

*Experiment 2.* Six ruminally cannulated mature beef cows (609 kg) were used in a cross over design to measure the influence of small amounts of blood meal and feather meal combined with mineralized salt on forage intake and

fermentation profiles of cows grazing poor quality forage. Cows grazed dormant winter range during the months of December and January in a 481 ha pasture at the NMSU CDRRC. Average amount of SBF consumed in Exp 1 (128 g/(animal · d)) was dosed directly through the cannula of three cows daily for 14 d, while MS only was dosed into the other three cows at a rate of 64 g/(animal · d). Cows were administered slow release-chromium sesquioxide boluses (Cattle Chrome, Captec NZ Ltd., Auckland, New Zealand) in each period. At the end of each period boluses were recovered and mean Cr release rate calculated.

**Sampling.** Diet quality was assessed from ruminal extrusa obtained from two cows allowed to graze for 1 h following complete ruminal evacuation. Cows grazed the pasture for 24 d prior to extrusa collection and were removed from the pasture without access to feed and water 8 h before collection. Extrusa was analyzed for DM, CP, ash (AOAC, 1990) and NDF by procedures outlined by ANKOM Technology Corp. (Fairport, NY). Chemical composition of extrusa was 7.4% CP, 53 % NDF, and 14% ash.

Each period of the experiment consisted of a 7-d adaptation period followed by a 7-d sample collection period. Fecal grab samples were collected at 24-h intervals and extrusa and supplement samples were incubated *in situ* for 0, 6, 12, 24, 48, 72, and 96 h on d 7 to 12. Fecal samples were dried at 50° C in a forced air dryer for 72 h, ashed, and analyzed for Cr concentration by atomic absorption spectroscopy (3110 Atomic Absorption Spectrophotometer, Perkin-Elmer, Norwalk, CT) using an air-plus-acetylene flame. Fecal output was calculated by dividing the mean Cr release rate from the boluses by the concentration of Cr in the feces. Ruminal extrusa samples used for *in situ* analysis were ground to pass a 2 mm screen, and Dacron bags (10 X 20-cm, 53 µm pore size; Bar Diamond Inc., Parma, ID) were filled with 5 g extrusa or SBF and sealed with an impulse heat sealer. Triplicate Dacron bags of extrusa and SBF for each time period were placed into 36 x 50-cm mesh bags and inserted into the ventral rumen. All bags were removed simultaneously and immediately frozen. Zero-h bags were submerged in ruminal fluid before freezing. Prior to analysis, bags were rinsed 5 times in 47 L cold water (1 min agitation, 2 min spin per rinse) in a top-loading washing machine. After rinsing, all bags were dried to a constant weight at 50 C in a forced air dryer and allowed to equilibrate with the atmosphere for 12 h prior to weighing (Vanzant et al., 1996). Beginning on d 12 blood and ruminal fluid samples were collected at 0 (before dosing), 3, 6, 9, 12, 15, 18, 21, and 24 h after dosing. Blood samples were collected in evacuated serum separator tubes, allowed to coagulate at room temperature for 30 min then centrifuged at 1,500 X g for 15 min. Following centrifugation, serum was decanted and frozen (-20° C) until analysis for serum urea nitrogen (SUN; Sigma procedure 640, Sigma diagnostics, St. Louis, MO). Ruminal fluid samples were strained through four layers of cheesecloth and divided into two aliquots for analysis of ruminal NH<sub>3</sub> N and VFA. For ruminal NH<sub>3</sub> N analysis, 10 mL ruminal fluid were added to 1 mL 6 N HCl and frozen (-20° C) until analysis by the phenol hypochlorite method (Broderick and Kang, 1980). For VFA analysis, aliquot was immediately frozen then combined with 25% (wt/vol) metaphosphoric acid before analysis. A 10-d recovery period

followed the first treatment period and cows were reassigned to the opposite treatment.

**Statistics.** Statistical analyses were performed to determine differences in mean response in supplemented versus unsupplemented treatments. Repeated measures data collected after supplementation (SUN, VFA, extent *in situ* OM disappearance (ISOMD), and NH<sub>3</sub> N) were analyzed using the MIXED procedure of SAS (SAS Inst. Inc.) with an ARH(1) (first order autoregressive, heterogeneous variance) covariance structure among times within animal. The model included fixed effects of treatment, time, and treatment x time and random effects of period and animal. Rate of forage *in situ* OM disappearance was analyzed using the segmented model of Gunter and Galyean (2000). Fecal output, and percent OM disappearance were analyzed using a mixed model that included treatment as a fixed effect and period and animal as random effect. Each cow received supplement on an individual basis and thus served as the experimental unit.

## Results and Discussion

**Experiment 1.** Mean environmental loss of MS and SBF was 0.04% and 0.06% of the amount offered, respectively. These data support the findings of Dean et al. (1999) who reported negligible environmental loss of loose mineral in open tubs. Intake of MS and SBF was variable among pastures (CV = 58.0%) and across weeks (CV = 39.7) with mean intakes of 44.5 g/(hd · d) and 128 g/(hd · d), respectively (Figure 1). The variability of intake is well within the range of variation for supplement consumption reported by Bowman and Sowell (1997). Actual intake of SBF was close to the target intake of 113 g/(hd · d).

**Experiment 2.** Ruminal NH<sub>3</sub> N concentrations averaged 3.49 and 3.85 mg/dL ( $P = 0.21$ ) for MS and SBF treated cows, respectively, across all sampling times (Table 1). Ruminal NH<sub>3</sub> N levels represent the balance between NH<sub>3</sub> production and utilization by ruminal microorganisms. Energy generated from fermentation is required for the incorporation of N from NH<sub>3</sub> into microbial CP. Elevated NH<sub>3</sub> N concentrations may indicate that protein is in excess relative to energy availability. *In situ* degradation of SBF was 32% after 24 h, yet mean NH<sub>3</sub> N concentrations of cows receiving SBF did not differ from cows receiving MS. Since NH<sub>3</sub> N did not accumulate, SBF degradation in the rumen may have resulted in NH<sub>3</sub> N supply in synchrony with energy availability.

Extent *in situ* OM disappearance in cows treated with SBF was similar to those treated with MS ( $P > 0.10$ ) at all incubation times except after 48 h ( $P = 0.03$ ; Table 2). Overall rate of OM disappearance was similar between MS and SBF treated cows (1.0 vs 0.9 %, respectively,  $P = 0.54$ ), however, rate of ISOMD was slower than would be expected in either group considering the CP content of diet extrusa samples (7.4%). Forage species composition of diet extrusa samples may be responsible for this finding. Daniel et al (1993) reported forbs and shrubs comprise a significant portion of the diet for cattle grazing Chihuahuan Desert rangelands. Forbs contain more lignin and CP than grasses (Cook, 1983). The physical nature of the forage may have prevented an increase in ISOMD in response to supplemental N similar to the finding of Hunter and Siebert (1985). Sawyer

(2000) noted improved DM disappearance of sudangrass after 24 h when supplemental blood meal and feather meal were provided in small quantities. Indicating that SBF supplementation may be best suited to situations where more fermentable grasses comprise the majority of the diet.

Similar concentrations ( $P > 0.10$ ) of total VFA, molar proportions of acetate, propionate, and butyrate, and acetate:propionate ratios were observed between cows fed MS and SBF supplements (Table 1). No difference in total fecal OM output was detected ( $P = 0.21$ ) between supplement groups. Fecal OM output is related to intake and would not be expected to differ when no difference in fermentation characteristics were noted.

Serum urea nitrogen concentrations are indicative of N released from deamination of dietary or endogenous protein and N lost from the rumen (Roseler et al., 1993). A treatment by time interaction was observed for SUN. Cows receiving SBF had greater SUN concentrations ( $P < 0.05$ ) at all sampling times except h 6 than did cows receiving MS (Figure 2). Elevated SUN concentrations in SBF supplemented cows suggests metabolizable protein supply was increased by UIP supplementation. This conclusion is supported by the findings of Sawyer (2000) who reported increased SUN and CP flow to the duodenum in steers supplemented with blood meal and feather meal at levels similar to the present study. Coomer et al. (1993) also observed increased total and apparent dietary CP flow to the abomasum of steers fed supplemental UIP. Serum urea nitrogen concentrations also reflect the protein to energy ratio of the diet (Hammond et al. 1994). The generally low SUN values (Kaneko, 1989) observed, in conjunction with ruminal  $\text{NH}_3$  N levels similar to MS-treated cows, following SBF supplementation, indicate that dietary protein supply was not in excess relative to digestible energy intake. Furthermore, these results suggest that the pattern of ruminal  $\text{NH}_3$  N release from blood meal and feather meal was balanced with energy release from forage fermentation.

### Implications

Mineralized salt is an effective vehicle for delivery of small quantities of blood and feather meal. Small quantities of supplemental undegradable intake protein can provide  $\text{NH}_3$  N in synchrony with energy availability leading to increased metabolizable protein supply to the abomasum and elevated serum urea nitrogen concentration. However, this supplement may be best suited for situations where grass is a large component of the diet.

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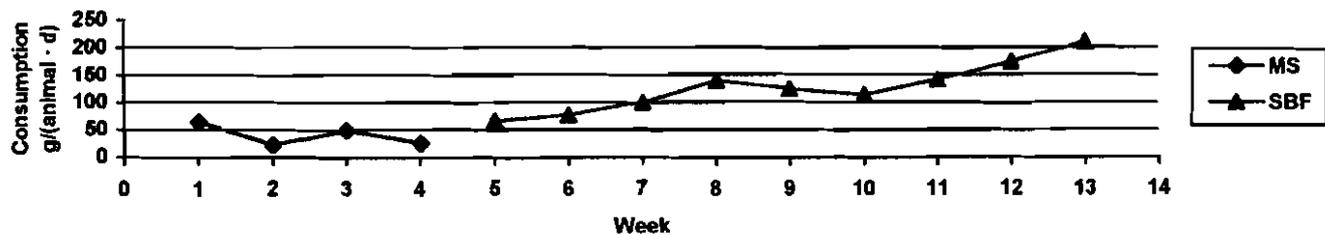


Figure 1. Intake of mineralized salt (MS) and mineralized salt combined with blood meal and feather meal (SBF), combined in a 2:1:1 ratio, offered in open tubs to cattle grazing native rangeland (Exp. 1).

Table 1. Effects of 64 g/(animal · d) of mineralized salt (MS) or 128 g/(animal · d) mineralized salt combined with blood meal and feather meal (SBF) on serum urea nitrogen (SUN), ammonia nitrogen (NH<sub>3</sub>-N) and VFA concentrations, fecal output and in situ OM disappearance (ISOMD)(Exp. 2)

Item	Treatment		SE	P-value
	MS	SBF		
SUN, mg/dL	7.76	8.53	0.2	0.05
NH <sub>3</sub> -N, mg/dL	3.49	3.85	0.1	0.21
Total VFA, mM	54.7	54.0	1.9	0.85
Acetate (% of total)	76.0	78.9	1.5	0.81
Propionate (% of total)	14.6	14.4	0.3	0.74
Butyrate (% of total)	6.6	6.7	0.2	0.92
Acetate:Propionate	5.3	5.5	0.1	0.24
Fecal output, kg/d	4.48	4.61	0.1	0.21
ISOMD, %/h	1.0	0.9	0.1	0.54

Table 2. Extent in situ OM disappearance (ISOMD) from diet extrusa samples of cows grazing winter range and ISOMD of mineralized salt combined with blood meal and feather meal (SBF) in a 2:1:1 ratio (Exp. 2)

Incubation time, h	Extrusa				SBF	
	MS%	SBF%	SE	P-value	%	SE
0	12.8	12.7	0.42	0.79	16.8	0.6
6	13.8	14.0	0.43	0.76	23.2	1.5
12	18.2	19.0	0.38	0.28	29.5	1.5
24	30.1	27.8	0.74	0.12	32.0	1.6
48	45.2	41.6	0.80	0.03	41.8	1.8
72	50.9	52.1	0.91	0.50	50.1	1.1
96	54.2	54.7	0.56	0.64	51.6	0.6

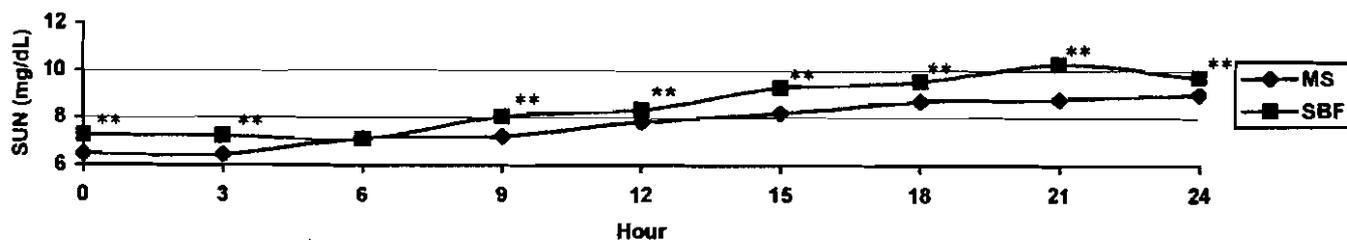


Figure 2. Serum urea nitrogen (SUN) concentrations of cows receiving 64 g/(animal · d) mineralized salt (MS) or 128 g/(animal · d) mineralized salt combined with blood meal and feather meal (SBF) over a 24 h sampling period (Exp. 2). \*\**P* < 0.05.