Published January 20, 2015

Effects of supplemental molybdenum on animal performance, liver copper concentrations, ruminal hydrogen sulfide concentrations, and the appearance of sulfur and molybdenum toxicity in steers receiving fiber-based diets[1]

K. L. Kessler,* K. C. Olson,† C. L. Wright,† K. J. Austin,* P. S. Johnson,‡ and K. M. Cammack*[2]

*Department of Animal Science, University of Wyoming, Laramie 82071; †Department of Animal Science, South Dakota State University, Brookings 57007; and ‡Department of Natural Resources, South Dakota State University, Brookings 57007

ABSTRACT: Poor performance and S-induced polioencephalomalacia (sPEM) have been observed in ruminant livestock in high-S drinking water regions. No gainful method of removing S from drinking water is available and therefore a feed supplement that negates the effects of high-S water is needed. Our objective was to determine if supplementing Mo improves health and performance of steers administered a high-fiber diet and high-S drinking water. We hypothesized that if the supplemental Mo adequately bound excess S in the rumen, it would not be available at toxic concentrations. Yearling steers (n = 96; 260.0 ± 1.3 kg BW) were stratified by pretrial BW into 12 feedlot pens (n = 8 steers per pen). One of 3 treatments, low-S water (LS; 375 mg SO_4/L), high-S water (HS; 2,218 mg SO_4/L), or high-S water plus Mo (HSMO; 2,218 mg SO_4/L; 187.5 mg Mo/kg DM), were randomly assigned to pens within 4 blocks for a 56-d trial. Body weights were recorded on d –2, –1, 29, 56, and 57, ruminal H_2S concentrations were measured by rumenocentesis on d –1, 29, and 57, and liver biopsies were performed on d –1 and 57. Performance data were analyzed over the 56-d trial period (overall) as well as over 2 periods: Period 1 (d 0 to d 28) and Period 2 (d 29 to d 56). One case of sPEM was confirmed by the presence of cortical lesions in the HS treatment group. Daily DMI and ADG were affected by treatment and period ($P < 0.001$) main effects. The LS steers had the greatest ($P < 0.05$) DMI followed by HS and HSMO steers, respectively. Similar results were observed for ADG. Daily water intake was affected ($P < 0.001$) by period only, with greater daily water intake in Period 2 than Period 1. Change in hepatic concentrations of Cu, Fe, and Mo over the course of the trial were all affected ($P < 0.001$) by treatment. Hepatic Cu increased from d 1 to 57 in LS and HS steers but was depleted in HSMO steers. Hepatic Fe and Mo increased in HSMO steers only. Ruminal H_2S concentrations were affected by treatment ($P < 0.021$), with greater H_2S concentrations in HSMO compared with LS and HS steers. Signs of Mo toxicity such as severe diarrhea, loss of body condition, anorexia, changes in hair color, and stiffness in joints were observed in the Mo supplemented steers. These results indicate that added dietary Mo does not adequately bind excess S in the rumen, causing aggravated toxic effects from potentially both the high dietary S and Mo.

Key words: beef cattle, molybdenum, polioencephalomalacia, sulfur, toxicity

 J. Anim. Sci. 2012.90:5005–5012
doi:10.2527/jas2011-4453

INTRODUCTION

Increased S is common in livestock water sources. Excess intake of S in ruminants causes overproduction of free sulfide (S_2) in the rumen, interfering with cellular processes and binding with hydrogen to form toxic hydrogen sulfide (H_2S) gas; it is associated with poor performance and S-induced polioencephalomalacia (**sPEM**).

There is interest in supplementing minerals to bind free S_2 in the rumen. Molybdenum (as molybdate) interacts with S_2 in the rumen to form various isoforms of thiomolybdate (MoO_nS_{4-n}, in which n is 0 to 3), which are either absorbed or passed through the gastrointestinal tract (Price et al., 1987). It is proposed that Mo inhibits sulfate (SO_4)-reducing bacteria without compromising ruminal fermentation (Bryden and

[1]The authors acknowledge the support of the University of Wyoming Agriculture Experiment Station, the South Dakota Agriculture Experiment Station, and NCR-SARE Project No. LNC05-260.
[2]Corresponding author: kcammack@uwyo.edu
Received July 7, 2012.
Accepted July 20, 2012.

Bray, 1972) and that it blocks the SO_4 activation step catalyzed by ATP sulfurylase. Also, Mo has the ability to inhibit S_2 production in ruminal fermentations and cultures. Reports by Huber et al. (1971), Loneragan et al. (1998b), and Majak et al. (2004) suggest that up to 100 mg Mo/kg DM does not markedly reduce animal performance. We conducted a preliminary in vitro experiment that showed inhibition of H_2S gas production when ≥100 mg/kg Na_2MoO_4 was added to fermentations with 2,500 mg/kg SO_4; this was in agreement with in vitro data reported by Kung et al. (2000).

Excess Mo can lead to toxicity, causing poor growth, anemia, diarrhea, joint and bone deformities, and hair discoloration (Ward, 1978). Production of thiomolybdates in the rumen can decrease Mo absorption from the diet and reduce Cu availability due to the formation of nonabsorbable complexes between Cu and MoS_4 (Suttle and Field, 1983).

We aimed to determine if supplementing cattle with 100 mg Mo/kg DM prevented 1) the poor performance associated with increased dietary S and 2) the accumulation of H_2S in the rumen. We hypothesized that if the supplemental Mo adequately bound excess S in steers administered high-SO_4 water, it would not be available at toxic concentrations.

MATERIALS AND METHODS

All experimental procedures were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Treatments

This study was conducted June 18 through August 12, 2009 at the South Dakota State University Cottonwood Range and Livestock Research Station (Cottonwood, SD).

Yearling steers (n = 96; 260.0 ± 1.3 kg BW) were stratified by pretrial BW into 12 feedlot pens (n = 8 steers per pen). Three treatments were allocated in a randomized complete block design, providing 4 replicate pens for each treatment, for a 56-d trial. Treatments consisted of low-S water (**LS**; 375 mg SO_4/L), high-S water (**HS**; 2,218 mg SO_4/L), or high-S water plus Mo (**HSMO**; 2,218 mg SO_4/L). Although it was intended to supplement Mo at 100 mg/kg DM, the final concentration was actually 187.5 mg Mo/kg DM. All treatments received 10 mg supplemental Cu/kg DM to meet NRC (2000) requirements. Treatment diets consisted of 50:50 ground hay and pelleted supplement on an as-fed basis. Hay was ground to an approximate length of 10 cm. Pellets were produced at a commercial feed mill. Pellets for the LS and HS treatments consisted of 94% wheat middlings, 6% limestone, and Cu (as tribasic $CuCl_3$). Pellets for the HSMO treatment were the same, except Mo (as Na_2MoO) was substituted for a portion of the limestone. The pellets did not contain any other ingredients such as supplemental minerals or vitamins. White salt was offered ad libitum. Grass hay was used to reflect nutritive value of summer pasture. Samples of each pellet type and hay were composited daily throughout the trial, and a random sampling of each was analyzed after the trial at the South Dakota State University Analytical Services Laboratory (Brookings, SD; Table 1). The concentration of SO_4 used for this study was based on previous data that demonstrated that water with >3,000 mg SO_4/L can be lethal to crossbred steers (Patterson et al., 2003). In the current study, 2,500 mg SO_4/L was used to provide a chronic exposure at a concentration of SO_4 that would elicit a biological response but not result in a marked increase in incidence of sPEM.

Rural water was used for the LS treatment and averaged 375 mg SO_4/L throughout the duration of the trial. High-S water was mixed for the trial using a Dosatron injection pump (Dosatron – North America, Clearwater, FL) with Na_2SO_4 as the source of additional S added to rural water to achieve a target concentration of 2,500 mg SO_4/L (actual average dose achieved was 2,218 mg SO_4/L). Samples of HS were collected from each batch mixed by the Dosatron and analyzed by Energy Laboratories (Rapid City, SD) using ion chromatography to measure SO_4 concentrations.

Table 1. Nutrient composition of feedstuffs[1]

Item	Hay	No Mo pellet[2]	Mo pellet[3]
DM, %	91.6	92.2 (0.2)	92.2 (0.3)
		% of DM	
CP	7.5	17.6 (0.2)	17.2 (0.4)
NDF	72.9	38.4 (1.3)	37.5 (0.5)
ADF	29.9	12.3 (0.4)	12.1 (0.4)
Ca	0.4	2.6 (0.2)	2.4 (0.2)
K	1.86	1.2 (0.0)	1.3 (0.0)
Mg	0.1	0.5 (0.1)	0.5 (0.0)
Na	0.004	0.02 (0.01)	0.03 (0.01)
P	0.16	1.1 (0.1)	1.2 (0.0)
S	0.12	0.2 (0.0)	0.2 (0.0)
Cu, mg/kg	7.5	44.8 (5.1)	48.4 (16.9)
Fe, mg/kg	111	212.7 (43.2)	188.5 (5.0)
Mn, mg/kg	101	173.3 (55.4)	161.0 (7.1)
Mo, mg/kg	2.5	6.2 (7.6)	375.0 (21.2)
Zn, mg/kg	31.9	106.7 (30.6)	102.5 (2.1)

[1]Treatment diets consisted of 50:50 ground hay and pelleted supplement on an as-fed basis. No Mo pellets consisted of 94% wheat middlings and 6% limestone. They also contained 10 mg Cu/kg DM as tribasic $CuCl_3$. For Mo-supplemented pellets, Mo (as Na_2MoO_4) was substituted for limestone.

[2]Three batches of pellets without supplemental Mo were used for the trial. Mean (SD) is shown for each nutrient. No Mo Pellets 1 and 2 were produced in consecutive years, respectively, and fed on alternating days. Pellets from an aborted attempt to initiate this experiment in the previous year provided the first batch of pellets. No Mo Pellet 3 was produced and fed in the last 2 wk of the trial when supplies of No Mo Pellets 1 and 2 were depleted.

[3]Two batches of pellets with supplemental Mo were used for the trial. Mean (SD) is shown for each nutrient. Mo pellets 1 and 2 were produced in consecutive years, respectively, and fed on alternating days. Pellets from an aborted attempt to initiate this experiment in the previous year provided the first batch of pellets.

Data Collection

Steers were weighed before feeding and watering on d –2, –1, 29, 56, and 57, with 2 d means calculated for initial (d –1 and –2) and final (d 56 and 57) BW. Ruminal H_2S gas cap concentrations were measured on d –1, 29, and 57 to determine the changes in rumen H_2S gas production in the presence of high-S water and supplemental Mo.

Ruminal gas cap concentrations were collected by rumenocentesis from all animals for determination of H_2S concentrations using a modification of the method described by Loneragan et al. (1998a). Collections were performed before daily feed and water administration to minimize effects of feed and water intake on sampling. Briefly, rumen H_2S was collected by inserting a needle into an incision approximately 5 cm behind the 13th rib, in the paralumbar fossa on the left side of the animal and approximately 15 cm down from the spinal column. The needle was connected to a syringe into which 50 mL of rumen gas was collected twice; the first collection was taken to purge the syringe of the rumen gas of the previous animal and the second collection was expelled through a graduated H_2S detection tube (Draeger Safety Inc., Pittsburgh, PA). Color change in the tube indicated the presence of H_2S gas, and the distance of color change traveled through the tube was used to estimate H_2S concentrations according to manufacturer's instructions.

Liver biopsies were conducted on d –1 and 57 using the true-cut technique (Pearson and Craig, 1980) as adapted by Engle and Spears (2000). Samples were immediately rinsed with 0.01 M phosphate buffered saline, placed into 1.5 mL polypropylene tubes, snap-frozen in dry ice, and stored at –20°C until shipped for a trace nutrient element panel.

Feed and Water Administration

Steers were housed in outdoor dry-lot pens with wind breaks in each pen. Water and feed were provided on an ad libitum basis, and intake of both was estimated daily (starting on d 1). Feed was provided in concrete fence line bunks. Each steer was allowed 1.1 m of linear bunk space. Feed refusals were collected and weighed each morning, and representative samples of the refusals of each pen were used to calculate DM of refusals. Feed was offered ad libitum at 110% of mean intake for the previous 3 d on an as-fed basis. After refusal collection, hay was weighed into feed bunks using a mixer wagon. Pellets were weighed in separate containers for each pen and top dressed on the hay. This was done to prevent uneven mixing of low-density hay and high-density pellets to ensure that hay:pellets was 50:50 for each pen. It was observed that there was a mild aversion to the Mo pellets with refusals for those pens having a greater amount of pellets than hay left in the bunk. Because facilities to implement pair feeding were not available, it was attempted to minimize this sorting by the HSMO steers by providing approximately 100 to 105% of mean intake for the previous 3 d on an as-fed basis (as compared with 110% for the LS and HS treatment groups). Therefore, steers in the HSMO consumed almost all of their diet on a daily basis as intended, and the potential to sort out pellets was lessened. Water was administered in tanks to each pen according to treatment, up to 3 times daily. One oval water tank was placed at the fence line in each pen. Each steer was allowed 22 cm of linear tank space. These tanks were the only source of water in the pens. Pen floors were flat and sloped adequately so that water did not accumulate in puddles during precipitation events. Water tanks were emptied at least twice weekly to avoid residual Na_2SO_4 accumulation and fecal contamination inside the tanks. Water consumption was determined by the daily change in water volume of each tank, adjusted for precipitation and evaporation each day. Climate measurements, including precipitation, temperature, and evaporation, were collected at the onsite weather station. Evaporation was estimated daily from the difference in water depth using a round nonelectric evaporation tank from the measurement of the previous day. Precipitation was collected in a rain gauge and measured daily to aid in determining water depth change in the evaporation tank. High-S water was mixed into large (3,785 L) water storage tanks and distributed to each pen receiving HS, and a separate large water storage tank was filled with rural water to distribute to each pen receiving LS.

Animal Health

Animals were monitored at least 3 times daily for general health and signs of sPEM. Steers displaying early signs of sPEM, including staggers, separation from herd, anorexia, diarrhea, or muscle tremors, and those exhibiting acute signs of sPEM, including blindness, head pressing, repetitive chewing, or star gazing, were removed from the trial and treated with thiamin at 2.2 g/100 kg BW intramuscularly (**IM**) twice on d 1 and then twice daily for 6 d at 889 mg/100 kg BW IM, dexamethasone at 311 mg/100 kg BW IM once on d 1 and then at 4 mg/100 kg BW IM once per day for the next 6 d, B-complex at 4.4 mg/100 kg BW IM once on d 1, 15 g per animal over 180 kg of Probios Bovine Oral Gel (Bomac Vets Plus Inc., Knapp, WI) on alternate days starting on d 3, and drenching with 19 L of water once daily for 7 d. Removed steers were provided LS, hay, and low Mo pellets. Body weights, liver biopsies, and ruminal H_2S concentrations were collected from steers immediately upon removal from the trial because

of sPEM symptoms. Brain tissues were collected from steers that expired due to treatment; tissues were shipped overnight to the South Dakota State University Animal Disease Research and Diagnostic Laboratory (Brookings, SD) where they were analyzed for the presence of the necrotic lesions characteristic of sPEM.

Liver Mineral Analysis

Initial (d –1) and final (d 57) collections of liver tissues from all steers were sent to the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing, MI) for determination of mineral concentrations of Cu, Fe, and Mo using inductively coupled plasma-mass spectroscopy.

Statistical Analysis

The data were analyzed over the 56 d trial period (overall) and in 2 periods: **Period 1** (d 0 to 28) and **Period 2** (d 29 to 56). This provided opportunity to identify early changes in performance that might be compensated for after adjustment to the HS or supplemental Mo. Daily DMI and water consumption were analyzed as repeated measures (autoregressive variance–covariance matrix) with pen(treatment) as the subject in the MIXED procedure (SAS Inst. Inc., Cary, NC) to test for the fixed effects of treatment, period, and period × treatment interaction. Individual mineral concentrations from liver biopsies were averaged by pen, and the change in hepatic concentration of each mineral was estimated as the difference between initial and final pen means (d 57 minus d –1). These changes were analyzed for the fixed effect of treatment using the MIXED procedure of SAS, with d –1 concentration included as a covariate for each mineral. Average daily gain was averaged by pen and analyzed for fixed effects of treatment, period, and treatment × period interaction using the MIXED procedure of SAS. Total S from feed, total S from water, and total S from feed and water were also analyzed using repeated measures (heterogeneous autoregressive variance–covariance matrix) in the MIXED procedure of SAS. Concentrations of H_2S collected on d 29 (end of Period 1) and d 57 (end of Period 2) were each averaged by pen and analyzed for treatment, period, and treatment × period interaction using the MIXED procedure of SAS, with the respective initial H_2S concentrations (d –1) used as a covariate.

RESULTS AND DISCUSSION

Average maximum ambient temperatures were 30.9 and 31.2°C for Period 1 and Period 2, respectively. Around mid trial, on d 25 and d 29, 2 steers from the same HS treatment pen were removed from the experiment due to signs of sPEM and were treated as described above. One of the removed steers exhibited blindness but responded to the LS and medicinal treatments. The second steer died due to the HS exposure; brain tissue from this steer was collected within 2 h of death by a veterinarian. Samples from the cortical region showed lesions indicative of sPEM. No steers from any other treatment were removed from the trial due to signs of sPEM.

Daily DMI was not affected (Table 2) by a period × treatment interaction but was affected ($P < 0.001$) by main effects of both period and treatment. Daily DMI was greater ($P < 0.001$) in Period 2 than in Period 1. Daily DMI was less ($P < 0.001$) in HSMO steers compared with LS and HS steers, but no differences were observed between LS and HS steers. Patterson et al. (2003) reported a 37% decline in DMI between treatments that contained 441 mg SO_4/L and 4,654 mg SO_4/L. Tjardes et al. (2004), however, observed no differences ($P > 0.40$) in DMI for steers consuming HS at 400, 3,090, or 3,950 mg SO_4/L over a 126 d trial. Given that there was no difference in DMI between LS and HS steers in the current experiment, it is possible that the reduced intake in HSMO steers was due to the excessive Mo intake. In comparison with the LS treatment, the HSMO treatment caused an additional 23% decline in DMI beyond that caused by the HS treatment (9%), indicating that that the negative impact of the added dietary Mo was 2.5 greater than that of the high S alone. The formulation for the HSMO steers was for 100 mg Mo/kg DM, but a mixing error resulted in the cattle being supplemented with 187.5 mg Mo/kg DM. Both concentrations exceed the maximum tolerable concentrations suggested by NRC (2005). Data reported by Huber et al. (1971) suggest that cattle can be fed up to 100 mg Mo/kg with no signs of Mo toxicity. Dietary Mo concentrations in excess of 100 mg/kg resulted in diarrhea, emaciation, depressed appetite, and reduced milk yield (Huber et al., 1971). However, our hypothesis was that if S and Mo were bound together as a thiomolybdate isoform, then neither would be available to the animal and neither S nor Mo toxicity should occur.

Daily water intake was also different ($P < 0.001$) between periods, with greater water intake in Period 2 compared with Period 1; however, there was no treatment main effect or period × treatment interaction observed for daily water intake. Previous reports published on the effects of HS on water intake (using Na_2SO_4 as the additional source of S) are variable. Weeth and Capps (1972) found no differences in water intake in heifers administered low-S tap water (110 mg SO_4/L), moderate-S water (1,462 mg SO_4/L), or high-S water (2,814 mg SO_4/L). In a similar experiment, Digesti and Weeth (1976) reported no differences in water intake between heifers administered low-S tap water (110 mg SO_4/L), moderate-S water (1,250 mg SO_4/L), or high-S

Table 2. Least square means for daily DMI, daily water intake, total S from DMI, total S from water intake, and total S intake, ADG, and ruminal H_2S

	Treatment[1]			
Item	LS	HS	HSMO	SEM
DMI, [2] kg/d				
Period 1[3]	9.3	8.2	5.9	0.3
Period 2[3]	10.4	9.7	7.8	0.3
Overall[3]	9.8[a]	9.3[b]	6.9[c]	0.2
Daily water intake, [4] L/(animal/d)				
Period 1	43.4	41.0	41.4	1.3
Period 2	55.0	54.0	55.5	1.3
Overall	49.2	47.5	48.4	1.1
S intake from diet, [5] g/d				
Period 1	16.0	14.3	9.9	0.8
Period 2	19.7	18.2	14.1	0.8
Overall	17.8[a]	17.0[a]	12.0[b]	0.5
S intake from water, [6] g/d				
Period 1	5.4[a]	30.4[b]	30.7[b]	0.8
Period 2	6.9[a]	40.0[b]	41.1[b]	0.8
Overall	6.2[a]	35.2[b]	35.9[b]	0.6
Total S intake, [6] g/d				
Period 1	21.4[a]	44.7[b]	40.5[b]	1.3
Period 2	26.6[a]	58.2[b]	55.1[b]	1.3
Overall	24.0[a]	51.5[b]	47.8[c]	0.9
ADG, kg/d[5]				
Period 1	0.82	0.59	0.03	0.13
Period 2	1.40	1.46	0.77	0.13
Overall	1.11[a]	1.02[a]	0.40[b]	0.09
Ruminal H_2S, [7] mg/L				
Period 1	81.4	286.0	527.6	156.6
Period 2	71.7	115.1	610.6	156.6
Overall	76.6[a]	200.5[ab]	569.1[b]	112.4

[a–c]Overall treatment means within a row lacking a common superscript differ ($P < 0.05$) based on Tukey's protected least significant difference. Mean separation was performed on treatment means within period only when the treatment × period interaction was significant ($P < 0.05$); treatment × period means within a row lacking a common superscript differ ($P < 0.05$) based on Tukey's protected least significant difference

[1]Treatments: LS = low-S water (375 mg SO_4/L; n = 32), HS = high-S water (2,218 mg SO_4/L; n = 32), and HSMO = high-S water plus Mo (2,218 mg SO_4/L + 187.5 mg Mo/kg DM; n = 32).

[2]$P < 0.001$ for treatment main effect; $P < 0.001$ for period main effect; $P = 0.32$ for treatment × period interaction.

[3]Period 1 = d 0 to d 28; Period 2 = d 29 to d 56; Overall = d 0 to d 56.

[4]$P < 0.22$ for treatment main effect; $P < 0.001$ for period main effect; $P = 0.47$ for treatment × period interaction.

[5]$P < 0.001$ for treatment main effect; $P < 0.001$ for period main effect; $P \geq 0.54$ for treatment × period interaction.

[6]$P < 0.001$ for treatment main effect; $P < 0.001$ for period main effect; $P < 0.001$ for treatment × period interaction.

[7]$P = 0.021$ for treatment main effect; $P = 0.80$ for period main effect; $P = 0.71$ for treatment × period interaction.

water (2,500 mg SO_4/L). In contrast, Cammack et al. (2010) observed greater water intake in steers receiving low-S water (566 mg of SO_4/L) than in those receiving high-S water (3,651 mg SO_4/L). This is similar to results reported by Patterson et al. (2003) who mixed water from 3 natural water sources to achieve the desired SO_4 concentrations and observed a linear decrease in water intake with increasing concentrations of SO_4 (mg/L) by steers administered 1 of 4 water treatments: 1) 441 SO_4, 2) 1,725 SO_4, 3) 2,919 SO_4, or 4) 4,654 SO_4.

Intake of S from feed was affected by treatment and period main effects ($P < 0.001$; Table 2), but there was no period × treatment interaction ($P = 0.61$). Total S from DM was less ($P < 0.001$) in HSMO steers than LS and HS steers; however, no differences ($P \geq 0.77$) were observed between LS and HS steers. Differences in S intake from DM can be explained by differences in DMI between treatment groups (Table 2), where DMI was not different between LS and HS steers but was less ($P < 0.001$) in the HSMO steers.

Intake in S from water was affected by a period × treatment interaction ($P < 0.001$) as well as period and treatment main effects ($P < 0.001$; Table 2). By design, the LS steers had less ($P < 0.001$) S intake from water than HS and HSMO steers. The change in S intake from water from Period 1 to Period 2 was affected ($P < 0.001$) by treatment, with greater ($P < 0.001$) changes in HS and HSMO treatments than the LS treatment, accounting for the period × treatment interaction effect. No differences ($P = 0.67$) in S intake from water were observed between HS and HSMO treatment groups.

Total S intake (from DMI and water) was similarly affected by a period × treatment interaction ($P < 0.001$; Table 2) as well as period and treatment main effects ($P < 0.001$). Total S intake was greater ($P = 0.002$) in HS than HSMO steers due to differences in DMI (i.e., S intake from the diet), because there were no differences in the concentrations of SO_4 both treatments were receiving or in daily water intake. Additionally, total S intake was lesser ($P < 0.001$) in LS steers than HS and HSMO steers, as would be expected.

Average daily gain was not affected by a treatment × period interaction ($P = 0.54$) but was affected by both treatment and period ($P < 0.001$; Table 2) main effects. Average daily gain was greater ($P < 0.001$) in Period 2 than Period 1. Overall, HSMO steers had less ($P \leq 0.001$) ADG than LS and HS steer, with no overall differences in ADG observed between LS and HS steers. Loneragan et al. (2001) observed less ADG ($P < 0.001$) with increasing concentrations of water SO_4 concentrations in steers administered 1 of 5 concentrations of SO_4 water (136.1, 291.3, 582.6, 1,219.2, or 2,360.4 mg SO_4/L). Our results indicate consumption of HS alone was not enough to significantly reduce ADG; however, the addition of Mo did decrease ADG. This response is clearly explained by the dramatic reduction in DMI in the HSMO steers. Although a LS treatment with supplemental Mo was not included in this study, previous research has shown that Mo supplementation at up to 100 mg/kg (our original targeted concentration of supple-

mentation) does not cause signs of toxicity (Huber et al., 1971). If the Mo was binding S, toxic concentrations of Mo should not have been available to depress DMI and ADG. The negative influence of the HSMO treatment suggests our hypothesis that the Mo would bind with the S was not supported.

Ruminal H_2S concentrations were affected (P = 0.021; Table 2) by treatment, with HSMO steers having greater ($P \leq 0.072$) H_2S concentrations than LS and HS steers. No period (P = 0.80) or treatment × period (P = 0.71) effects were observed. There was considerable variation in the H_2S measurements. This is comparable with variation in H_2S measurements observed by Drewnoski et al. (2012), who cited potential contributing factors of ruminal pH, S intake, or ruminant bacterial population differences. Molybdate has been shown to inhibit SO_4–reducing bacteria in the rumen that ultimately produce H_2S gas (Taylor and Oremland, 1979). Kung et al. (2000) showed a reduction in H_2S concentrations when 10 to 25 mg Mo/kg was added to in vitro cultures. A study by Price et al. (1987) estimated that 30% of Mo in the rumen fluid used in their study consisted of di-, tri-, and tetrathiomolybdates bound to the solid phase and were not detectable in the liquid phase of these samples. In the rumen they showed that 41 and 34% of the thiomolybdates are trithiomolybdates ($MoOS_3^{2-}$) and tetrathiomolybdates (MoS_4^{2-}), respectively. Tetrathiomolybdates are thought to bind to the solid phase of digesta and evade absorption in the rumen, thus taking captive the S_2 that would be used otherwise to form H_2S. Although the increased ruminal H_2S concentrations in the Mo-supplemented steers was not expected in this study, conflicting reports from literature suggest that the effects of Mo supplementation on the rumen environment are not completely understood. Jones et al. (1982) reported that in sediment, methanogenesis was inhibited by MoO_4 when H_2 was in excess, regardless of SO_4^{2-}. This may suggest that Mo is not exclusive to inhibiting SO_4–reducing bacteria (Kung et al., 1998). Although S was abundant in the current study, it may be possible that that Mo supplementation did not specifically inhibit ruminal SO_4–reducing bacteria but perhaps inhibited methanogenesis, which would cause greater H_2S production in a high S environment. Additionally, it has been proposed that rumen microbes, particularly SO_4–reducing bacteria, may adapt to increased dietary S. The SO_4–reducing bacterial populations may change in numbers in response to a high S environment, or the metabolism of these bacteria may be altered (Drewnoski et al., 2012). It is possible that greater dietary Mo, especially when in the toxic range, may inhibit the ability of SO_4–reducing bacteria to adapt to a high S environment and in turn contribute to greater H_2S production. Further research is needed to determine the mechanism by which Mo supplementation of ruminants administered high dietary S would cause an increase in H_2S production.

It was observed that steers receiving the Mo supplement experienced Mo toxicity, as dietary Mo concentrations between 20 and 100 mg/kg of DMI can be toxic in cattle (Underwood, 1981). The Mo may not have been sufficiently binding the excess S from the water to prevent toxic concentrations of free Mo. Ward (1978) described the signs of Mo toxicity as severe diarrhea, loss of body condition, anorexia, changes in hair color, and stiffness in joints, all of which were observed in the Mo supplemented steers in the present study. Huber et al. (1971) reported signs of Mo toxicity in lactating cows, including diarrhea, emaciation, unthriftiness, decreased appetite, and reduced milk yields in cows receiving 200 mg Mo/kg in their diet. These signs became notably worse when the Mo in the ration was increased to 300 mg/kg with 0.26% of SO_4 added to the diet. Similarly, sheep administered 50 mg of Mo and 10 g of SO_4 per d for 45 d exhibited diarrhea and loss of appetite. However, once the supplemented Mo and SO_4 concentrations were reduced by 50% of the original diet, the sheep regained normal eating habits and had no continued issues with diarrhea (Suttle and Field, 1968). These studies suggest Mo toxicity can be intensified in the presence of excess SO_4. The inclusion of a Mo control treatment group in this study may have helped explain the unexpected results observed in the HSMO steers. However, the intended inclusion concentration of Mo in this study was not expected to elicit toxicity symptoms and, as previously discussed, was not expected to adversely affect animal performance based on work by Huber et al. (1971).

Hepatic mineral concentrations of Cu ($P < 0.001$), Fe ($P = 0.002$), and Mo ($P < 0.001$) were all affected by a treatment × collection day (d –1 or d 57) interaction. Copper ($P < 0.001$), Fe ($P < 0.001$), and Mo ($P < 0.001$) were affected by the main effect of treatment and Cu ($P < 0.001$) and Mo ($P < 0.001$) were also affected by the main effect of collection day (Table 3). Changes in hepatic mineral concentra-

Table 3. Least square means for hepatic mineral concentrations of Cu, Fe, and Mo

	Treatment[1]						
	LS		HS		HSMO		
Mineral	d –1	d 57	d –1	d 57	d –1	d 57	SEM
Cu,[2] mg/kg	101.3[a]	377.8[b]	100.5[a]	187.8[c]	98.0[a]	55.0[d]	9.4
Fe,[3] mg/kg	196.2[a]	167.8[a]	180.3[a]	183.1[a]	181.1[a]	296.5[b]	17.9
Mo,[2] mg/kg	3.5[a]	4.1[a]	3.5[a]	4.0[a]	3.7[a]	12.8[b]	0.2

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$) based on Tukey's protected least significant difference.

[1]Treatments: LS = low-S water (375 mg SO_4/L; n = 32), HS = high-S water (2,218 mg SO_4/L; n = 32), and HSMO = high-S water plus Mo (2,218 mg SO_4/L + 187.5 mg Mo/kg DM; n = 32).

[2]$P < 0.001$ for treatment main effect; $P < 0.001$ for period main effect; $P < 0.001$ for treatment × period interaction.

[3]$P < 0.001$ for treatment main effect; $P = 0.055$ for period main effect; $P = 0.002$ for treatment × period interaction.

Table 4. Least square means for change (d 57 to d –1) in hepatic mineral concentrations of Cu, Fe, and Mo

	Treatment[1]			
Mineral	LS	HS	HSMO	SEM
Cu,[2] mg/kg	277.7[a]	87.8[b]	–44.8[c]	11.7
Fe,[2] mg/kg	–26.7[a]	1.9[a]	114.6[c]	26.9
Mo,[2] mg/kg	0.65[a]	0.66[a]	8.92[b]	0.23

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$) based on Tukey's protected least significant difference.

[1]Treatments: LS = low-S water (375 mg SO_4/L; n = 32), HS = high-S water (2,218 mg SO_4/L; n = 32), and HSMO = high-S water plus Mo (2,218 mg SO_4/L + 187.5 mg Mo/kg DM; n = 32).

[2]$P \leq 0.013$ for treatment main effect.

tions (i.e., concentration difference between initial and final sample collections) of Cu ($P < 0.001$), Fe ($P = 0.013$), and Mo ($P < 0.001$) were all affected by treatment (Table 4). Because of its interaction with S and Mo, Cu concentrations were of particular interest in this study. Both LS and HS treatment groups showed increases in hepatic Cu concentrations from d –1 to d 57 due to the supplemental Cu provided. The increase in Cu status was less ($P < 0.001$) for HS steers than LS steers, suggesting that the added S did negatively influence Cu availability. A decrease in hepatic Cu was observed in the HSMO treatment group despite the supplemental Cu. This response was not unexpected given the well-defined antagonism of S and Mo on Cu absorption and Cu status (Gooneratne et al., 1989; Suttle, 1991). Due to the formation of Cu-thiomolybdates, Cu deposition in organs is reduced and Cu loss through urine is increased. Therefore, suitable concentrations of Cu to meet NRC requirements may be inadequate in the presence of excess Mo and S or when the Cu to Mo ratio is less than 4:1 (Paterson and Engle, 2005). Thornton et al. (1972) observed that dietary Cu concentrations of 7 to 14 mg/kg were insufficient to overcome the antagonism created by Mo concentrations ranging from 3 to 20 mg/kg.

The addition of Mo to the diet itself was evident for the HSMO treatment group, with a greater ($P \leq 0.020$) increase in hepatic Mo concentration observed in the HSMO treatment group compared with the LS and HS groups (Table 3). The HSMO treatment group also had the greatest ($P \leq 0.013$) concentration of hepatic Fe. The HSMO group showed an increase in hepatic Fe concentration over the trial period; in contrast, the LS treatment group showed a decrease in Fe concentration, and the HS group showed no change. These data may suggest that interaction of Mo and S may be preventing Fe from binding with S, increasing Fe concentrations in the HSMO steers compared with the LS and HS steers. A study by Harrison et al. (1992) indicates Fe and SO_4 would bind to form a black precipitate in the rumen if Fe was able to interact with SO_4, explaining the increase in Fe seen in the HSMO steers in the current study.

Conclusion

Although the dietary Mo concentrations fed in this experiment were in the range considered potentially toxic, it was hypothesized that only limited amounts of Mo would actually be available to the animal because of the substantial S administered in the water. However, it appears that supplemental Mo, at least at the concentrations provided in this experiment, did not effectively bind excess dietary S and prevent H_2S accumulation in the rumen, resulting in Mo toxicity signs and even greater H_2S production. Additionally, it can be presumed that this excess Mo is being excreted by the cattle and therefore could become an environmental pollutant in a confined animal feeding operation. This study provides new data that contribute to the current understanding of high dietary S and potential interactions with Mo in feedlot steers in addition to new information on Mo toxicity in cattle. Supplemental Mo has been speculated as a potential means of alleviating the effects of excess dietary S. Preliminary in vitro results suggested the opposite response from that obtained in vivo, at least at the concentrations of Mo used in this study. In particular, a reduction of gaseous H_2S was not observed in vivo. Sulfate-reducing bacteria may have responded differently between in vitro and in vivo cultures. Evaluation of responses of ruminal microbial populations (particularly SO_4–reducing bacteria) to similar mineral supplements may elucidate mechanisms involved and provide opportunities to overcome S and Mo toxicities. Further research regarding possible feed additives or supplements to counteract high dietary S is warranted.

LITERATURE CITED

Bryden, J. M., and A. C. Bray. 1972. The effect of dietary molybdenum on the reduction of inorganic sulphate in the rumen. Proc. Aust. Soc. Anim. Prod. 9:335–340.

Cammack, K. M., C. L. Wright, K. J. Austin, P. S. Johnson, R. R. Cockrum, K. L. Kessler, and K. C. Olson. 2010. Effects of high-sulfur water and clinoptiloite on health and growth performance of steers fed forage-based diets. J. Anim. Sci. 88:1777–1785.

Digesti, R. D., and H. J. Weeth. 1976. A defensible maximum for inorganic sulfate in drinking water of cattle. J. Anim. Sci. 42:1498–1502.

Drewnoski, M. E., E. L. Richter, and S. L. Hansen. 2012. Dietary sulfur concentration affects rumen hydrogen sulfide concentrations in feedlot steers during transition and finishing. J. Anim. Sci. doi:10.2527/jas.2011-5078

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446–2451.

Gooneratne, S. R., W. T. Buckley, and D. A. Christensen. 1989. Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci. 69:819–845.

Harrison, G. A., K. A. Dawson, and R. W. Hemken. 1992. Effects of high iron and sulfate ion concentrations on dry matter digestion and volatile fatty acid production by ruminal microorganisms. J. Anim. Sci. 70:1188–1194.

Huber, J. T., N. O. Price, and R. W. Engel. 1971. Response of lactating dairy cows to high levels of dietary molybdenum. J. Anim. Sci. 32:364–367.

Jones, J. G., B. M. Simon, and S. Gardener. 1982. Factors affecting methanogenesis and associated processes in the sediments of a stratified eutrophic lake. J. Can. Microbiol. 128:1–11.

Kung, Jr., L., J. P. Bracht, A. O. Hession, and J. Y. Tavares. 1998. High sulfate induced polioencephalomalacia (PEM) in cattle – Burping can be dangerous if you are a ruminant. Pacific Northwest Nutr. Conf., Vancouver, BC, Canada.

Kung, Jr., L., J. P. Bracht, and J. Y. Tavares. 2000. Effects of various compounds on in vitro ruminal fermentation and production of sulfide. Anim. Feed Sci. Tech. 84:69–81.

Loneragan, G. H., D. H. Gould, R. J. Callan, C. J. Sigurdson, and D. W. Hamar. 1998a. Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal gas cap of recently weaned beef calves with polioencephalomalacia. J. Am. Vet. Med. Assoc. 213:1599–1604.

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and S. R. Goodall. 1998b. Effect of dietary molybdenum and copper on ruminal gas cap H_2S levels and liver copper stores of feedlot steers. J. Dairy Sci. 81(Suppl. 1):329(Abstr.).

Loneragan, G. H., J. J. Wagner, D. H. Gould, F. B. Garry, and M. A. Thoren. 2001. Effects of water sulfate concentration on performance, water intake, and carcass characteristics of feedlot steers. J. Anim. Sci. 79:2941–2948.

Majak, W., D. Steinke, J. McGillivray, and T. Lysyk. 2004. Clinical signs in cattle grazing high molybdenum forage. J. Range Manage. 57:269–274.

NRC. 2000. Nutrient Requirements of Beef Cattle. 7th ed. Natl. Acad. Press. Washington, DC.

NRC. 2005. Mineral Tolerance of Animals. 2nd ed. Natl. Acad. Press. Washington, DC.

Paterson, J. A., and T. E. Engle. 2005. Trace mineral nutrition in beef cattle. 2005 Nutr. Conf. Knoxville, TN.

Patterson, H. H., P. S. Johnson, and W. B. Epperson. 2003. Effect of total dissolved solids and sulfates in drinking water for growing steers. Proc. West. Sec. Amer. Soc. Anim. Sci. 54:378–380.

Pearson, E. G., and A. M. Craig. 1980. Diagnosis of liver disease in equine and food animals. Mod. Vet. Pract. 61:233–237.

Price, J., A. M. Will, G. Paschaleris, and J. K. Chesters. 1987. Identification of thiomolybdates in digesta and plasma from sheep after administration of ^{99}Mo-labelled compounds into the rumen. Br. J. Nutr. 58:127–138.

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulfur in ruminant nutrition. Ann. Rev. Nutr. 11:121–140.

Suttle, N. F., and A. C. Field. 1968. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep. J. Comp. Path. 78:351–362.

Suttle, N. F., and A. C. Field. 1983. Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. J. Comp. Path. 93:379–389.

Taylor, B. F., and R. S. Oremland. 1979. Depletion of adenosine triphosphate in Desulfovibrio by oxyanions of group VI elements. Curr. Microbiol. 3:101–103.

Thornton, I., G. F. Kershaw, and M. K. Davies. 1972. An investigation into copper deficiency in cattle in the Southern Pennines. II. Response to copper supplementation. J. Agric. Sci. Camb. 78:165–171.

Tjardes, K. E., H. H. Patterson, C. L. Wright, and B. D. Rops. 2004. Effects of supplying water with varying levels of total dissolved solids and sulfates to steers during the growing period on subsequent finishing performance. Proc. West. Sec. Amer. Soc. Anim. Sci. 55:141–150.

Underwood, E. J. 1981. The Mineral Nutrition of Livestock. 2nd ed. Commonwealth Agricultural Bureaux, Franham Royal, UK. pp. 91–108.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: A review. J. Anim. Sci. 46:1078–1085.

Weeth, H. J., and D. L. Capps. 1972. Tolerance of growing cattle for sulfate-water. J. Anim. Sci. 34:256–260.