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High dietary S decreases the retention of copper, manganese, and zinc in steers

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ABSTRACT: To examine the effects of dietary S on diet digestibility and apparent mineral absorption and retention 16 steers [8 ruminally fistulated (368 ± 12 kg BW) and 8 unmodified (388 ± 10 kg BW)] were paired within modification status and BW, and within each of the two consecutive 28 d periods, four pairs of steers were randomly assigned to either a low S (0.24%) or high S (0.68%) pelleted diet. Bromegrass hay was fed at 5 or 7% of the diet, during period 1 and 2, respectively. Sodium sulfate was used to increase the S content of the high S diet. The low S steers were fed the amount of feed their high S counterpart consumed the previous day, while the high S steers received 110 % of previous day's intake. Steers were adapted to individual metabolism stalls for 4 d (d -3 to 0 of period), acclimated to diet for 7 d (d 1 to 7 of period), and after high S steers were consuming ad-libitum intake for 7 d (d 14 of period), total urine and feces were collected for 5 d. Feed intake and orts were recorded daily. Dry matter and OM digestibility were determined. Jugular blood was collected before and after each collection period on d 14 and 20, and liver biopsies were collected on d 0 and 27. Macro (Ca, K, Mg, and Na) and micro (Cu, Mn, and Zn) mineral concentrations were determined for pellets and hay, orts, feces, urine, and plasma and liver samples from each steer via inductively coupled plasma spectrometry. Dry matter intake, DM and OM digestibility, and urine volume were not affected ($P \geq 0.11$) by dietary treatment, but fecal output was greater ($P = 0.02$) in the low S steers than the high S steers. A high S diet decreased plasma Cu ($P = 0.04$) and liver Zn ($P = 0.03$) compared to low S steers. No differences ($P \geq 0.20$) were noted among urinary excretion of Cu, Mn, and Zn. Sodium absorption was greater ($P < 0.01$) and Cu, Mn, and Zn retention was lesser ($P \leq 0.01$) in the high S steers than the low S steers. Apparent absorption of Ca, K, and Mg was not affected ($P \geq 0.18$) by dietary treatment, while absorption of Cu, Mn, and Zn in the high S treatment was lesser ($P \leq 0.06$). In conclusion, consumption of a high S diet for 28 d had limited

effects on Ca, K, Mg, and Na absorption and retention, but decreased Cu, Mn, and Zn retention, which may limit growth and production of cattle consuming a high S diet long-term.

Key words: cattle, copper, manganese, mineral retention, sulfur, zinc

INTRODUCTION

Ethanol co-product inclusion to cattle diets is an economic and nutritional alternative to corn-based finishing diets. However, the variability in the S content (0.3 to greater than 1.0% S; Kim et al., 2012) of co-products poses a risk for performance and health of feedlot cattle. Also, in some areas of the United States high sulfate drinking water can introduce greater quantities of S. Currently, the NRC (2005) recommends 0.15% S in cattle diets. However, maximum tolerable limits currently range from 0.3 to 0.5% S for diets containing less than 15 % forage or at least 40% forage, respectively. This range was set to avoid the deleterious effects of high S diets on cattle health, and feedlot and carcass-based performance (Kandylis, 1984; Spears et al., 2011; Uwituze et al., 2011).

A component to successful cattle performance in the feedyard is adequate mineral nutrition, as minerals play critical roles in nerve conduction, cell signaling, and energy metabolism, often acting as enzyme cofactors among other biological roles (Underwood and Suttle, 1991). In cattle diets, S is necessary because it is a component of the S-containing amino acids, B-vitamins, and sulfhydryl bonds for some enzymes (Suttle, 2010). However, the mineral status of cattle fed a high S diet may be compromised, as increased dietary S may antagonize Cu, Se, Zn, Ca, and Mg, thus limiting their absorption, availability, and use by the animal (Suttle, 1974; Spears et al., 1985; Suttle, 1991). While the negative effects of high S diets on

performance, health, and carcass characteristics have been investigated, research data concerning macro and trace mineral retention of cattle consuming a high S diet are limited. Therefore, the objective of the present study was to determine the effect of dietary S on diet digestibility, mineral status, and apparent digestibility, absorption, and retention of minerals in steers consuming either a low (0.24%) or high (0.68%) S pelleted diet.

MATERIALS and METHODS

Procedures and protocols for cattle experiments were approved by the Iowa State University Institutional Animal Care and Use Committee (8-09-6796-B).

Animals and Experimental Design

This study was conducted in conjunction with a 28-d diagnostic study designed to induce polioencephalomalacia (**PEM**), which is further described by Drewnoski et al. (2012). Sixteen Angus-crossbred steers were to determine the effect of dietary S on diet digestibility and apparent mineral absorption and retention. Eight of the 16 steers were fitted with a ruminal cannula (368 ± 12 kg BW) while the other eight remained unmodified (388 ± 10 kg BW). The steers were paired by their modification status and BW, and steers within each pair were randomly assigned to either a low S (**low S**; 0.24%) or high S (**high S**; 0.68%) pelleted diet (Table 1). The pellet was manufactured by a commercial feed mill in Creston, IA. Two sets of eight steers (four pairs of cannulated and unmodified steers) were used in one of two consecutive 28 d periods, in which steers were adapted to individual metabolism stalls [213.4 (length) × 182.9 (height) × 91.4 (width) cm] for 4 d (d -3 to 0 of period) while consuming the low S diet. Following stall adaptation, steers were stepped up to ad-libitum intake for a 7 d period (d 1 to 7

of period), during which steers received 1% of BW (DM) on d 1 and increased by 0.25% BW over each of the following 6 d. Because high S diets have been reported to decrease DMI (Spears et al., 2011; Uwituze et al., 2011), steers in the present study were pair-fed to decrease difference in DMI between the low and high S treatments. Therefore, starting on d 8 of each period, steers consuming the high S diet were fed 110% of their previous day's intake, while the low S steers were fed the low S diet, but at the quantity of feed that was consumed on the previous day by their high S counterpart. Steers were fed once daily; at 0800, hay was fed and at 0815, pellets were fed. Chopped, smooth bromegrass hay was offered to steers at 5% (period 1) of their previous day's pellet intake to decrease the likelihood of acidosis and this was increased to 7% in period 2 because ruminal pH was observed to be very low during period 1 (Drewnoski et al., 2012). An automatic cup waterer was available in each stall to allow the steers ad-libitum access to water. On d 14 of each period, after 7 d of ad-libitum feed consumption by the high S steers, the 5 d collection period of total urine and feces was initiated. Prior to and at the completion (d 14 and 19 of period) of collection steers were removed from their stalls so the stalls could be thoroughly cleaned.

During each period, a composite of pellets and a composite of hay were made, composed of daily samples of pellets and hay, and on a daily basis, the amount of feed offered (pellet and hay) and feed refused (orts; pellet and hay) for each steer was recorded. Samples of feed offered (pellet and hay) and feed refused (orts; pellet and hay) were collected daily and dried at the end of each period (feed offered) or daily (feed refused) in a convection oven at 70 °C for 48 h.

Prior to the initiation of the collection period, all plastic containers for fecal and urine collection were acid-washed for 4 h in 10% hydrochloric acid, rinsed three times with de-ionized water, and allowed to dry thoroughly before use. Total urine output was collected daily into 20 L

plastic carboys that contained 6 M trace metal grade acetic acid and de-ionized water to prevent crystallization and volatilization of urine N. Daily during the collection period, prior to removing a 1% aliquot of urine, urine pH was determined and additional acetic acid was included to achieve a pH of ≤ 5. The 1% urine aliquot was composited in a 2 L plastic container over the 5 d collection period. Urine samples were frozen at -20 °C until further analysis. Total fecal output was collected on a tared piece of plastic sheeting (122 × 155 cm) and weighed daily. A 3% fecal aliquot was collected daily, composited, and dried in a convection oven at 70 °C for 48 h. All 70 °C dried samples (feed, orts, and feces) were ground through a 2 mm screen (Retsch Zm100 grinder, Glen Mills Inc., Clifton, NJ) and stored in ziplock bags until further analysis.

Dry matter and OM of feed, orts, and fecal samples was determined according to AOAC (1999) procedures. Briefly, 1.0 g of sample was dried for 24 h at 105 °C, the weight was recorded, then the dried sample was ashed in a muffle furnace (630 °C for 6 h), and the weight of the ashed sample was recorded. Organic matter intake was calculated by multiplying the percent OM of the appropriate feed or ort sample for each steer by the 105 °C DM adjusted value for that sample. Fecal OM was determined by multiplying the percent OM of the appropriate fecal sample by the 105 °C DM adjusted total fecal sample collected.

Dry matter intake was calculated by multiplying the 105 °C DM adjusted value of the appropriate feed and ort sample for each steer by the daily as-fed feed offered and the daily ort values collected for each steer and subtracting orts (DM basis) from feed offered (DM basis). Fecal DM was determined by multiplying the 105 °C DM adjusted value of the appropriate fecal sample for each steer by the total fecal sample collected. Digestibility, both DM and OM, was calculated by subtracting the DM or OM adjusted total fecal output from the DM or OM adjusted

total intake (pellets and hay minus pellets and hay orts), dividing the DM or OM adjusted total intake, and subtracting the value from 100.

Jugular blood for plasma mineral analysis was collected in trace mineral grade potassium EDTA vacuum tubes (7 mL/steer; Becton, Dickinson, and Company, Franklin Lakes, NJ) from all steers (n = 16) prior to the start of and after the collection period (on d 14 and 20 of each period). Samples were centrifuged at 1,000 × *g* for 10 min at 4 °C and plasma was removed and stored at -20 °C until further analysis. Liver biopsies were conducted using the method of Engle and Spears (2000) on d 0 and 27 of each period. Biopsy samples were transported on ice to the laboratory and frozen at -20 °C until further analysis. Liver samples were dried in a forced air oven at 70 °C for 48 h prior to acid digestion.

Feed (pellet and hay), ort (pellet and hay), and fecal samples were acid digested prior to mineral analysis according to the method described by Richter et al. (2012), while the liver and plasma samples were digested according to Pogge and Hansen (2013). Urine and fecal samples were diluted 1:60 and 1:9, respectively, in 1 % nitric acid for the analysis of Ca, K, Mg, and Na. Urine samples were diluted 1:2 with 2% nitric acid for the analysis of Cu, Mn, and Zn. No additional dilutions were required for the analysis of feed and orts macro or trace minerals, or fecal trace minerals. Sulfur and Fe concentrations of the pellet and hay samples only were determined, and no additional dilutions were required for analysis. Mineral content was analyzed using inductively coupled plasma optical emission spectrometry (**ICP**; Optima 7000 DV, Perkin Elmer, Waltham, MA). A bovine liver standard from the National Institute of Standards and Technology (Gaithersburg, MA) was included with each run to verify instrument accuracy and Yttrium (Inorganic Ventures, Christiansburg, VA) was added as an internal standard to all samples to account for variation in sample introduction. The concentration of sulfate in the water

was determined by the Water/Wastewater Laboratory (Ames, IA) and Mo concentrations of the pellets and hay were determined by Dairyland Laboratories (Arcadia, WI) according to AOAC (1999) methods.

Total mineral content of individual components (feed offered and refused, feces, and urine) was calculated by multiplying the respective value determined via ICP (mg/kg or mg/L) by the total quantity of the feed offered or refused, total fecal output, or total urine output volume. Daily mineral content of mineral intake (pellets and hay), refused pellets and hay, fecal output, and urine output were determined by dividing total mineral content of the respective components by the number of days of collection. Mineral retention was calculated by subtracting mineral excreted from mineral consumed for each steer. The percent mineral retained was calculated by dividing the daily mineral retained by the mineral consumed, multiplied by 100. The percent of apparent mineral absorption was calculated by subtracting fecal mineral from consumed mineral, divided by consumed mineral, and multiplying by 100. The mineral S is not reported, due to the inability to account for the amount of S exiting the body as hydrogen sulfide during eructation or as a thiomolybdate complex when Cu is excreted (Gooneratne, 1994).

One steer receiving the high S diet in period 2 was removed from the study after being diagnosed with PEM on d 2 of the collection period (d 16 of the period). Final plasma and liver biopsy samples were collected from that steer (on d 16 of the period) prior to his removal from the study. Following the collection period, the low S counterpart to the steer that developed PEM was removed from study.

Statistical Analysis

Data were analyzed by ANOVA using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC). The model for the analysis of DMI, DM and OM digestibility, daily fecal and urine output, plasma and liver minerals, and mineral intake, excretion, and retention data included the fixed effects of treatment, period, and the interaction. Interactions were removed from the model when $P \geq 0.10$. Modification status of the steers was tested as a fixed effect, but was not significant and was removed from the model. Steer was the experimental unit for all analyses ($n = 16$). Day 14 plasma and initial (d 0) liver mineral concentrations were tested as covariates for their respective final mineral concentrations, but were removed from the model when $P \geq 0.20$. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10. The values reported in the tables are least squares means.

RESULTS

Dry Matter Intake, Diet Digestibility, and Fecal and Urine Output

Dry matter intake, diet digestibility, and fecal and urine output data are reported in Table 2. Because steers were pair fed, DMI was not influenced ($P = 0.41$) by S content of the diet; however, a period effect ($P = 0.002$) was noted, in which the steers in period 1 consumed approximately 1.07 kg/d more than period 2 steers. Dry matter intake displayed no interaction between treatment and period ($P = 0.26$). Treatment did not affect the DM ($P = 0.18$) or OM ($P = 0.54$) digestibility or urine output ($P = 0.22$). Fecal excretion showed a treatment by period interaction ($P = 0.004$), which is likely caused by the lesser ($P \leq 0.001$) amount of feces produced by the high S steers in period 2 compared with the low S steers in period 2, as fecal excretion did not differ ($P = 0.79$) due to treatment in period 1.

Final Plasma and Liver Mineral Concentrations

Final plasma and liver mineral data are reported in Table 3. On d 14 plasma Cu was lesser ($P = 0.05$) in the high S steers than the low S steers, but no differences ($P \geq 0.39$) were noted among plasma Mg or Zn. On d 20, the high S steers had lesser ($P = 0.04$) plasma Cu concentrations compared to low S steers, but no differences ($P \geq 0.47$) were noted among plasma Mg or Zn concentrations. Initial (d 0) mineral concentrations Cu, Mn, or Zn were not different ($P \geq 0.47$) among the steers that were assigned to either dietary treatment; however, on d 27 the high S steers showed a lesser ($P = 0.03$) concentration of liver Zn than the low S steers, and there were no differences ($P \geq 0.34$) in liver Cu or Mn concentrations.

Daily Macro Mineral Intake, Excretion, and Retention

Macro mineral intake, excretion, and retention data are reported in Tables 4 (g/d) and 5 (%). As designed, the high S steers consumed a greater ($P < 0.001$) amount of S per day than the low S steers. Because S was introduced to the diet as sodium sulfate the concentration of Na consumed was greater ($P < 0.01$) in the high S treatment than the low S steers; however, steers in period 2 tended ($P = 0.06$) to consume less Na than steers in period 1. Magnesium intake tended to decrease ($P = 0.09$) with the greater S concentration in the diet, while Ca and K were not different ($P \geq 0.15$) due to S treatment. No treatment × period effect ($P \geq 0.25$) was observed for Ca, Mg, or K intake.

The amount of fecal Na (g/d) was greater ($P = 0.04$), while fecal Ca (g/d) and K (g/d) were lesser ($P \leq 0.08$) in the high S steers than the low S steers. Treatment did not affect ($P \geq 0.50$) fecal amounts of Mg (g/d). However, the amount of fecal Ca, K, and Mg (g/d) displayed a period effect ($P \leq 0.07$), in which steers in period 1 excreted more Ca, K, and Mg (g/d) than in

period 2. Urinary excretion of Na (g/d) was greater ($P < 0.01$) in steers consuming the high S diet than the low S, and Na (g/d) excretion was greater ($P = 0.02$) in period 1 compared to period 2. The concentration of dietary S did not influence ($P \geq 0.22$) the urinary excretion (g/d) of Ca, K, or Mg; however, period 1 steers had a greater excretion (g/d; $P \leq 0.08$) of K and Mg compared to period 2 steers. No treatment × day ($P \geq 0.35$) interaction was observed for Ca, K, or Mg urine excretion (g/d).

Fecal excretion of Na (as % of intake) was lesser ($P = 0.002$) in the high S steers than the low S steers. Fecal excretion (as % of intake) of Ca, K, and Mg were greater ($P \leq 0.05$) in period 1 steers compared to period 2. A treatment × period effect ($P = 0.05$) was noted among fecal Ca excretion (%), which is primarily being caused by greater ($P = 0.01$) fecal Ca excretion by the high S steers in period 1 than those in period 2. A treatment × period effect ($P = 0.03$) was observed for fecal Mg excretion (%), which is being driven by a tendency for greater ($P = 0.07$) fecal Mg excretion by the high S period 1 steers than period 2 steers. Urinary excretion of Na (%) was greater ($P = 0.001$) in high S steers compared to low S steers.

Steers consuming the high S diet tended to retain a greater amount ($P = 0.07$) of Na (g/d); however, as a percent of their daily Na intake they actually retained less ($P < 0.01$) Na than the steers fed the low S diet. The high S diet did not affect the amount of mineral retained (g/d; $P \geq 0.11$) or percent ($P \geq 0.16$) retention of Ca, K, or Mg. Steers in period 1, regardless of S concentration of the diet, retained a lesser ($P \leq 0.07$) amount of Ca (g/d, percent retention, and percent apparent absorption) compared to steers in period 2, likely attributed to lesser fecal excretion of Ca in period 2. However, no effects of period were observed between the amount (g/d; $P \geq 0.48$) or percent ($P \geq 0.11$) of K, Mg, or Na retention. A treatment × period effect ($P = 0.03$) was observed in the percent Na retention, an interaction likely due to the greater ($P = 0.01$)

proportion of Na retention by the low S period 1 steers compared with the low S steers in period 2, while no difference ($P = 0.58$) was noted between the high S treatments of period 1 and 2. As a percent of intake, Na was greater ($P < 0.01$) in the high S steers compared to the low S steers, and dietary treatment did not affect ($P = 0.18$) the apparent absorption of Ca, K, or Mg. Steers in period 1, regardless of dietary S concentration, had lesser ($P < 0.01$) apparent absorption of K and greater ($P = 0.05$) absorption of Na. The apparent absorption of Mg displayed a treatment by period effect ($P = 0.03$), which was likely a result of greater absorption ($P = 0.02$) of Mg by the low S steers in period 1 than the period 2 steers, whereas Mg absorption in the high S treatment tended to be lesser ($P = 0.07$) in period 1 steers compared to period 2 steers. A treatment × period effect ($P = 0.05$) was also noted among apparent absorption of Ca, which is due to the lesser absorption of Ca by the high S period 1 steers than the high S period 2 steers.

Daily Trace Mineral Intake, Excretion, and Retention

Trace mineral intake, excretion, and retention data are reported in Tables 6 (mg/d) and 7 (%). The intake of Mn and Zn was lesser ($P \leq 0.05$) in steers consuming the high S diet than the low S diet, as the high S diet contained lesser concentrations of these minerals. Steers in period 1, regardless of dietary S concentration, consumed more (mg/d) of Cu and Mn compared to period 2 steers. A treatment × period effect was observed for the intake of Mn ($P = 0.04$) and Zn ($P = 0.04$). Iron intake was not different due to dietary S treatment ($P = 0.51$) or period ($P = 0.29$).

Fecal Cu (mg/d) and Mn (mg/d) were greater ($P < 0.01$) in steers consuming the high S diet, but fecal Zn (mg/d) was not affected ($P = 0.68$) by dietary treatment. However, steers in period 1 excreted more ($P < 0.01$) fecal Cu, Mn, and Zn (mg/d) compared to period 2 steers.

There was a treatment × period effect for fecal Cu and Zn ($P < 0.01$), which is related to lesser ($P \leq 0.02$) fecal Cu and Zn excretion (mg/d) by the low S period 1 steers compared to the low S period 2 steers. Urinary excretion (mg/d) of Cu, Mn, and Zn were not different as a result of dietary treatment ($P \geq 0.20$) and Cu (mg/d) and Mn (mg/d) were not different due to period ($P \geq 0.62$); however, urinary Zn excretion (mg/d) was greater ($P = 0.04$) amongst steers in period 1 compared to period 2. No treatment × period interaction was observed ($P \geq 0.28$) for the urinary excretion of Cu, Mn, and Zn.

As a percent of total intake, fecal excretion of Cu, Mn, and Zn were greater ($P \leq 0.06$) in the high S steers than the low S steers. Urinary excretion of Mn (%) was greater ($P = 0.05$) in the high S steers compared to the low S steers, and urinary Zn excretion (%) tended to be greater ($P = 0.07$) in period 1 steers than period 2 steers.

The high S diet decreased the amount (mg/d) of retained ($P < 0.01$), the percent retention ($P \leq 0.06$), and the apparent absorption ($P \leq 0.06$) of Cu, Mn, and Zn compared to the low S diet. The amount of Cu, Mn, and Zn retained were not influenced ($P \geq 0.14$) by period. No treatment × period interactions ($P \geq 0.10$) were noted among mineral retention (mg/d or percent) and apparent absorption for any of the trace minerals.

DISCUSSION

Ethanol co-products are attractive in price and nutrient profile as feedstuffs for finishing cattle, but a greater inclusion in diets may increase the S content of the diet and decreases cattle performance and health (Kandylis, 1984; Spears et al., 2011; Uwituze et al., 2011; Richter et al., 2012). In the present study, DMI of steers was not different due to treatment as steers were pair fed in order to maintain similar intakes between the low and high S steers. However, in the

feedyard the impact of high S diets on DMI may vary. It is important to note that the dietary S concentration of 0.68% used in the present study was designed to induce PEM and is less likely to be use in a typical feedlot diet.

Dietary S exceeding 0.20% has decreased DMI of growing goats (Qi et al., 1993a), Holstein steers (Zinn et al., 1997), and feedlot steers (Spears et al., 2011; Uwituze et al., 2011). Because steers in the present study were pair-fed DMI was not different, which may help explain the lack of differences observed among diet digestibility (DM and OM) between the high and low S steers. Similar to the present study, when DMI was similar between treatments, total tract digestibility was not affected by the consumption of either 17.8 or 32.9 g S/d (Robertson et al., 1996) or dietary S concentrations of 0.15 to 0.25% (Zinn et al., 1997). Alternately, Uwituze et al. (2011) reported that steers consuming a 0.65% S diet consumed less DM (approximately 0.7 kg DM/d) and increased DM and OM apparent digestibility than the steers consuming a 0.42% S diet, which may indicate that rate of passage is contributing to diet digestibility.

The formation of Cu-sulfide is a notable and well-studied antagonism of S in the ruminant (Suttle, 1991; Spears, 2003). Furthermore, Mo in the rumen or circulation results in the scavenging of Cu by the S-Mo compound thiomolybdate, which may be found in multiple forms as di-, tri-, and tetra-thiomolybdates, and may further diminish the availability of Cu for utilization in biochemical processes (Suttle, 1991; Gould and Kendall, 2011). Because the antagonism between Cu and Mo results in rapid depletion of Cu status of the ruminant, it is recommended that for every 1 mg Mo/kg diet, the concentration of available Cu should be increased by 8 mg Cu/kg diet (Marston, 1999). Kelleher et al. (1983) showed that radiolabeled tri- and tetra-thiomolybdates were absorbed from the rumen of sheep, gained entry into the blood and could bind to Cu in albumin. In the present study, S treatment did not change Cu intake, but

an increase in fecal Cu (mg/d) resulted in lesser apparent absorption of Cu by the high S steers. The greater amount of Cu lost may be attributed to the inability of Cu-sulfide or the complex formed between Cu, S, and Mo to be absorbed in the gastrointestinal tract, thus contributing to greater concentrations of Cu in the feces (Suttle, 1991). In the present study while Mo retention was not determined, the high S pellets contained a greater quantity of Mo compared to the low S pellets, which may be a contributing factor to the greater Cu excretion by the high S steers. Additionally, Gooneratne et al. (1994; 2011) reported increasing concentrations of both dietary S and Mo increased both biliary and urinary excretion of Cu in Angus and Simmental heifers after 2 mo of diet consumption. Copper is primarily excreted in the bile, which ultimately contributes to fecal losses of Cu.

In addition to a Cu, Mo, and S-complex limiting the availability of Cu, the introduction of large quantities of Fe, such as during grazing or in silage-based diets, may further limit the availability of Cu for utilization by the animal (Suttle, 1991). Iron concentrations greater than 500 mg/kg have been reported to decrease the Cu status of cattle (Phillippo et al., 1987). In the present study, the apparent absorption of Cu was least in the period 1 high S steers, which may be related to a tendency for a greater consumption of S and Fe by this treatment than the period 2 high S steers. In a review, Suttle (1991) suggested that Cu availability may be further hindered by the presence of Fe with Mo and S, as Fe can complex with Mo and S, which can still have a high affinity for Cu (Simpson and Mills, 1982), or during the formation of Fe-sulfide a sulfide is released that can bind Cu as Cu-sulfide (Suttle, 1991).

Consuming a high S diet for 28 d decreased plasma Cu approximately 34% compared to the low S steers. There is a similar decrease in circulating Cu concentrations when cattle consumed a high S diet for 76 d (Suttle, 1974), 149 d (Pogge and Hansen, 2013), or 155 d

(Richter et al., 2012). Alternately, when S is consumed for a similar time period (28 d) as the present study, no differences in serum Cu concentration were noted in steers consuming S-fertilized tall-fescue (0.33 or 0.40% S for control and fertilized, respectively) or orchard-grass (0.29 or 0.37% S for control and fertilized, respectively; Spears et al., 1985) or Angora goats consuming 0.16 and 0.34% S diets (Qi et al., 1993b). Contrary to plasma, liver Cu concentration was not affected by the 28 d consumption of a high S diet in the present study. Arthington et al. (2002) similarly reported no difference in liver Cu concentration of cows consuming S-fertilized grasses over a 117 d period, but others have observed a decrease in liver Cu caused by greater S content of the diet [van Ryssen et al., 1998 (74 d study); Spears et al., 2011 (116 d study); Richter et al., 2012 (155 d study); Pogge and Hansen, 2013 (149 d study)]. However, these contradictory results may be caused by the number of days consuming a high S diet, as the current study (28 d) may not have been of sufficient length for cattle to deplete liver Cu stores.

In contrast to the well-defined S-Cu interaction, little is known about how S may impact Mn and Zn absorption and retention. Often plasma and liver Zn are often used as biomarkers of Zn status, but because pools of Zn in the body undergo constant exchange, no ideal biomarker of Zn status has been identified (Hambidge, 2003; Suttle, 2010). Plasma Zn was not altered because of dietary S concentration in the current study. These data concur with findings reported in steers (Spears et al., 1985), goats (Qi et al., 1993b), and sheep (Smith and White, 1997). Increasing the S content of the diet decreased liver Zn in the present study, while others have reported an increase in liver Zn of lambs consuming 0.35% S compared to those consuming 0.18% S (Felix et al., 2012) or no difference in liver Zn concentration of sheep (Smith and White, 1997), steers (Richter et al., 2012), or cows (Arthington et al., 2002) consuming increased dietary S. Similar to Felix et al. (2012) and Pogge and Hansen (2013), liver Mn concentration was not different as a

result of dietary S content in the present study. The lack of S effect observed on liver Cu and Mn concentrations might be related to the short period of time that cattle received a high S diet. The percent retention and apparent absorption of Mn and Zn were approximately 3-fold less in the high S steers compared to the low S steers, and as far as the authors are aware, this paper is the first to report a decreased retention of Mn and Zn in cattle consuming a high S diet. Because Mn- and Zn-sulfide formation can occur in biological systems (Rickard and Luther, 2006), it was our hypothesis that Mn- and Zn-sulfide complexes may be formed within the rumen that may limit the availability of these trace minerals for absorption. This possible interaction of S and Mn or Zn may help explain the greater percentage of consumed Mn and Zn that were excreted in the feces of the steers consuming a high S diet. However, further research is necessary to elucidate the impact of S on these divalent metals and the subsequent impact on cattle performance.

In contrast to the trace minerals, dietary S had limited impacts on the macro mineral balance of steers. The use of sodium sulfate to increase the concentration of S in the diet is likely a contributing factor to the greater Na intake and urinary excretion as a percent of total intake by the high S steers. Similarly, the consumption of high sulfate coal-pit water by steers increased Na excretion approximately 4-fold (Robertson et al., 1996), even though water consumption and urine volume were not different. Robertson et al. (1996) suggested that greater Na excretion might be related to overall greater solute excretion in the urine to maintain osmotic balance. However, regardless of greater Na excretion by the high S steers, the urine volume was not altered due to dietary treatment.

Circulating Mg concentrations were not altered by S treatment in the present study. Richter et al. (2012) and Pogge and Hansen (2013) also reported no difference in plasma Mg when steers consuming diets containing 0.22 to 0.6% S finishing diets. While Ca and Mg intake

and retention in the present study were not altered by dietary S concentration, Spears et al. (1985) reported the apparent absorption of Ca and Mg of Hereford steers consuming 0.37% forage S from S-fertilized cool season grasses was lesser than the control steers (no S-fertilization). Robertson et al. (1996) reported no difference in Ca retention of steers consuming high sulfate coal-pit water compared to the low sulfate town water.

Because cattle consuming a high S diet can experience a decrease in DMI, thus decreased mineral intake, and reduced mineral absorption due to S antagonisms, the provision of adequate trace minerals to feedlot cattle consuming a high S diet is important, as the availability of these minerals for absorption and utilization by the animal is likely compromised. Minerals play an essential role in immunity and growth, and the recommended trace mineral concentrations of cattle consuming a high S diet should be evaluated. The NRC (2000) recommends increasing the trace minerals supplemented to stressed or newly received cattle, which often consume less DMI, by approximately two times the recommended concentrations for non-stressed cattle. Similarly, an increase in supplemented mineral in a high S diet may aid in maintaining mineral status in the animal. Additionally, an injectable mineral may limit the depletion of trace minerals by high S diets because it bypasses the gastrointestinal tract antagonisms. An injectable mineral may also help bolster the trace mineral status of cattle consuming a high S diet, which may slightly lessen the dependence of cattle on gastrointestinal absorption of trace minerals for incorporation into biological processes. When an injectable trace mineral (containing Cu, Se, Mn, and Zn) is used in concert with NRC (1996) recommended trace mineral supplementation, plasma Cu and Se (Pogge et al., 2012) and liver Cu (Daugherty et al., 2002, Pogge et al., 2012) and Se (Pogge et al., 2012) concentrations were increased in cattle. However it is important to note these cattle were not consuming a high S diet.

Sulfur is a potential contributor toward the development of oxidative stress (Truong et al., 2006; Pogge and Hansen, 2013), which would occur via the depletion of the antioxidant glutathione, involved in the clearance of excess S from the body, and the disruption of cytochrome *c* oxidase (a Cu dependent enzyme), which results in increased production of reactive oxygen species and reactive S species (Truong et al., 2006). Because high S diets can decrease the trace mineral status of an animal the cellular antioxidants superoxide dismutase (Cu, Mn, Zn dependent) and glutathione peroxidase (Se dependent) may be unable to efficiently remove reactive oxygen species and reactive S species, thus further contributing to oxidative stress.

In conclusion, the consumption of high S diets decreased the retention of Cu, Mn, and Zn and had little to no impact on macro minerals, Ca, K, Mg, and Na, or diet digestibility, respectively. Because trace mineral retention was decreased after 20 d of consuming a high S diet, cattle exposed to high S diets for a longer period of time may require additional trace mineral supplementation to ensure the maintenance of adequate mineral nutrition for optimal growth and production.

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Table 1. Ingredient composition of pellet and mineral content of pellet and hay (DM basis, %)[1]

	Period 1		Period 2	
Pellet composition	Low S	High S	Low S	High S
Wheat middlings	40.8	40.1	40.8	40.1
Corn dried distiller's grains plus solubles	25.4	24.7	25.4	24.7
Corn starch	23.1	22.4	23.1	22.4
Molasses	3.9	3.9	3.9	3.9
Cottonseed hulls	3.7	3.7	3.7	3.7
Sodium sulfate	--	2.0	--	2.0
Limestone	1.7	1.7	1.7	1.7
Trace minerals and vitamin A[2]	0.1	0.1	0.1	0.1
Analyzed mineral content				
Ca, %	0.82	0.71	0.90	1.02
K, %	1.08	1.03	1.06	1.03
Na, %	0.14	0.71	0.14	0.69
Mg, %	0.34	0.31	0.34	0.34
S, %	0.25	0.61	0.29	0.68
Cu, mg/kg	14.8	17.1	16.2	17.0
Fe, mg/kg	134.5	141.0	145.6	142.1
Mn, mg/kg	117.7	97.9	131.8	89.2
Mo, mg/kg	1.57	2.50	1.86	2.19
Zn, mg/kg	106.5	107.5	132.2	110.9

Bromegrass hay[3]	Period 1	Period 2
Analyzed mineral content		
Ca, %	1.06	1.26
K, %	1.70	1.77
Na, %	0.003	0.004
Mg, %	0.14	0.17
S, %	0.13	0.17
Cu, mg/kg	7.6	8.2
Fe, mg/kg	199.9	436.3
Mn, mg/kg	47.5	57.3
Mo, mg/kg	0.99	0.91
Zn, mg/kg	14.9	18.4

	Period 1		Period 2	
Calculated diet composition[4]	Low S	High S	Low S	High S
CP, %	16.1	15.7	15.3	15.9
Lipid, %	4.14	4.04	4.18	4.09
NEg, Mcal/kg	1.84	1.83	1.82	1.81

[1]Water sulfate concentration was 101 mg/L (Water/Wastewater Laboratory, Ames, IA)

[2]Trace minerals ($CoCO_3$, $CuSO_4$, $Ca(IO_3)_2(H_2O)$, $MnSO_4$, Na_2SeO_3, and $ZnSO_4$) were

added to provide 100% of the requirement based on NRC (1996) and vitamin A was included at 4,400 IU/kg of diet DM.

[3]Bromegrass hay included at 5 or 7% of the previous day's pellet intake in period 1 and 2, respectively.

[4]Crude protein, lipid, and NEg were calculated based on NRC (1996) values for each ingredient, except the cornstarch value, which was reported by Gunawardena et al. (2010).

Table 2. Influence of dietary S concentration on daily DMI, diet digestibility, and daily fecal and urine output of steers fed a low sulfur (0.24% S) or high sulfur (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P*-Value[1]		
Steers (*n*)	4	4	4	4		Trt	Prd	Trt*prd
DMI, kg/d	6.28	5.34	5.85	4.01	0.479	0.10	0.01	0.37
DM digestibility, %	71.7	71.7	69.0	73.0	1.44	0.18	0.65	0.20
OM digestibility, %	69.5	68.7	66.8	69.7	1.79	0.54	0.63	0.32
Daily output								
Fecal, kg DM/d	8.94	8.70	8.16	5.09	0.619	0.02	0.004	0.04
Urine, L/d	6.7	10.4	6.2	6.5	1.34	0.23	0.13	0.18

[1]Trt: treatment; Prd: period; Trt*Prd: treatment × period.

Table 3. Influence of dietary S concentration on final plasma (d 20) and liver (d 0 and 27) mineral concentrations of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Low S	High S	SEM	*P*-Value
Steers (*n*)	8	8		
Plasma mineral, mg/L, d 20[1]				
Cu[2]	1.06	0.75	0.096	0.04
Zn[2]	1.13	1.25	0.138	0.57
Mg[2]	22.32	24.76	2.285	0.47
Initial liver mineral[3], mg/kg DM				
Cu[4]	132.6	143.8	21.26	0.71
Mn[4]	10.5	10.9	0.43	0.47
Zn[4]	112.0	109.1	9.00	0.82
Liver mineral, d 27[3], mg/kg DM				
Cu[4,5]	181.2	145.8	27.65	0.34
Mn[4]	12.7	12.3	0.52	0.62
Zn[4]	114.5	93.6	5.86	0.03

[1]Plasma mineral collected at the end of 5 d collection period (d 20), one high S steers was removed from study d 2 of collection period after developing polioencephalomalacia symptoms

[2]Period: $P \geq 0.15$; treatment × period: $P \geq 0.15$.

[3]Liver mineral collected on d 0 and 27 of the study.

[4]Period: $P \geq 0.20$; treatment × period: $P \geq 0.14$.

[5]Day 0 liver Cu used as a covariate.

Table 4. Influence of dietary S concentration on the amount (g/d) of daily macro mineral intake, fecal and urine excretion, and mineral retention of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P*-Value[1]		
Steers (*n*)	4	4	4	4		Trt	Prd	Trt*prd
Mineral intake								
Ca	51.4	44.3	47.7	48.8	3.37	0.38	0.91	0.25
K	69.0	65.1	58.5	50.9	3.75	0.15	<0.01	0.63
Mg	21.1	19.1	17.8	15.8	1.09	0.09	0.01	0.98
Na	8.5	42.4	7.0	30.9	2.37	<0.01	0.02	0.06
S	15.5	36.8	15.1	31.1	2.21	<0.01	0.19	0.26
Fecal excretion								
Ca	32.8	30.4	30.1	17.0	3.99	0.08	0.07	0.20
K	9.5	7.5	5.0	3.7	0.71	0.04	<0.01	0.56
Mg	15.9	17.4	15.1	12.3	0.91	0.50	<0.01	0.03
Na	1.3	4.0	1.9	3.6	0.94	0.04	0.89	0.61
Urinary excretion								
Ca	0.2	0.3	0.3	0.4	0.10	0.70	0.39	0.75
K	42.4	45.3	38.1	34.5	3.41	0.91	0.05	0.35
Mg	1.6	1.8	0.9	1.4	0.28	0.22	0.08	0.55
Na	1.2	30.5	2.6	18.8	1.99	<0.01	0.02	<0.01
Mineral retention								
Ca	18.4	13.6	17.3	31.5	4.29	0.30	0.07	0.05
K	17.1	12.4	15.4	12.7	4.28	0.40	0.87	0.81
Mg	3.7	-0.1	1.9	2.1	1.02	0.11	0.85	0.08
Na	5.9	7.9	2.5	8.4	2.02	0.07	0.48	0.34

[1]Trt: treatment; Prd: period; Trt*Prd: treatment × period.

Table 5. Influence of dietary S concentration on macro mineral fecal and urine excretion, mineral retention, and mineral absorption as a percent of intake of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P*-Value[1]		
Steers (*n*)	4	4	4	4		Trt	Prd	Trt*prd
Fecal excretion, %								
Ca	63.6	69.4	62.8	34.6	7.79	0.18	0.04	0.05
K	13.8	11.7	8.6	7.3	1.20	0.18	<0.01	0.71
Mg	75.2	91.4	84.6	79.2	4.34	0.23	0.74	0.03
Na	15.5	9.2	27.5	10.7	3.07	<0.01	0.05	0.11
Urinary excretion, %								
Ca	0.4	0.7	0.7	0.8	0.24	0.49	0.40	0.74
K	61.3	69.1	65.5	71.5	7.07	0.35	0.65	0.90
Mg	7.5	9.2	4.8	10.4	2.52	0.17	0.77	0.46
Na	14.4	71.7	38.5	64.3	10.09	<0.01	0.43	0.14
Mineral retention, %								
Ca	35.9	29.9	36.5	64.6	7.86	0.18	0.05	0.05
K	24.8	19.3	25.9	21.2	6.86	0.46	0.83	0.95
Mg	17.3	-0.6	10.6	10.4	6.02	0.16	0.72	0.17
Na	70.1	18.8	33.9	25.0	8.70	<0.01	0.11	0.03
Apparent absorption, %								
Ca	36.4	30.6	37.2	65.4	7.79	0.18	0.04	0.05
K	86.2	88.3	91.4	92.7	1.20	0.18	<0.01	0.71
Mg	24.8	8.6	15.4	20.8	4.34	0.23	0.74	0.03
Na	84.5	90.8	72.5	89.3	3.07	<0.01	0.05	0.11

[1]Trt: treatment; Prd: period; Trt*Prd: treatment × period.

Table 6. Influence of dietary S concentration on the amount (mg/d) of daily micro mineral intake, fecal and urine excretion, and mineral retention of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2					
	Low S	High S	Low S	High S	SEM	*P*-Value[1]		
Steers (*n*)	4	4	4	4		Trt	Prd	Trt*prd
Mineral intake								
Cu	90.9	105.0	84.7	79.9	5.18	0.39	0.01	0.09
Fe	826.8	863.3	838.2	722.5	58.73	0.51	0.29	0.22
Mn	715.9	594.9	684.1	415.7	32.44	<0.01	<0.01	0.04
Zn	638.9	642.2	676.9	510.8	37.38	0.05	0.23	0.04
Fecal excretion								
Cu	68.1	105.1	53.2	66.0	4.60	<0.01	<0.01	<0.01
Mn	597.2	558.7	553.0	84.0	32.58	<0.01	<0.01	0.07
Zn	550.9	663.6	551.8	464.6	30.34	0.68	<0.01	<0.01
Urinary excretion								
Cu	0.2	0.4	0.3	0.3	0.06	0.20	0.96	0.28
Mn	0.4	0.6	0.4	0.5	0.13	0.24	0.62	0.88
Zn	2.2	2.7	1.6	0.9	0.51	0.85	0.04	0.27
Mineral retention								
Cu	22.0	0.3	21.1	13.7	3.93	<0.01	0.14	0.10
Mn	117.6	37.3	133.2	34.2	28.35	<0.01	0.83	0.75
Zn	86.6	-17.4	126.3	49.8	25.65	<0.01	0.06	0.60

[1]Trt: treatment; Prd: period; Trt*Prd: treatment × period.

Table 7. Influence of dietary S concentration on daily micro mineral fecal and urine excretion, mineral retention, and apparent mineral absorption as a percent of intake of steers fed a low S (0.24% S) or high S (0.68% S) diet

Dietary treatment	Period 1		Period 2			*P*-Value[1]		
	Low S	High S	Low S	High S	SEM			
Steers (*n*)	4	4	4	4		Trt	Trt	Trt
Fecal excretion, %								
Cu	74.9	99.8	74.5	85.0	4.79	<0.01	0.14	0.56
Mn	83.4	94.0	80.7	95.9	6.24	0.06	0.95	0.72
Zn	86.2	103.4	81.3	94.3	5.54	0.02	0.23	0.71
Urinary excretion, %								
Cu	0.3	0.4	0.4	0.4	0.07	0.30	0.29	0.75
Mn	0.06	0.10	0.06	0.14	0.028	0.05	0.55	0.48
Zn	0.3	0.4	0.2	0.2	0.08	0.99	0.07	0.41
Mineral retention, %								
Cu	24.8	-0.2	25.1	14.6	4.81	<0.01	0.14	0.16
Mn	16.5	5.9	19.3	4.0	6.24	0.06	0.85	0.72
Zn	13.4	-3.8	18.5	5.6	5.54	0.02	0.22	0.70
Apparent absorption, %								
Cu	25.1	0.2	25.5	15.1	4.79	<0.01	0.14	0.16
Mn	16.6	6.0	19.3	4.1	6.24	0.06	0.94	0.72
Zn	13.8	-3.4	18.7	5.7	5.54	0.02	0.23	0.71

[1]Trt: treatment; Prd: period; Trt*Prd: treatment × period.