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Ruminant Nutrition

RUMINANT NUTRITION

Trace mineral source impacts rumen trace mineral metabolism and fiber digestion in steers fed a medium-quality grass hay diet

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Abstract

Twelve Angus steers (BW 452.8 ± 6.1 kg) fitted with ruminal cannulae were used to determine the impact of trace mineral (TM) source on digestibility, ruminal volatile fatty acid (VFA) composition, ruminal soluble concentrations of Cu, Zn, and Mn, and relative binding strength of trace minerals located in the rumen insoluble digesta fraction. Steers were fed a medium-quality grass hay diet (DM basis: 10.8% CP, 63.1% neutral detergent fiber [NDF], 6.9 mg Cu/kg, 65.5 mg Mn/kg, and 39.4 mg Zn/kg) supplemented with protein for 21 d. Treatments consisted of either sulfate (STM) or hydroxy (HTM) sources (n = 6 steers/treatment) to provide 20, 40, and 60 mg supplemental Cu, Mn, and Zn/kg DM, respectively. Following a 21-d adaptation period, total fecal output was collected for 5 d. Dry matter ($P < 0.07$) and CP ($P < 0.06$) digestibility tended to be reduced, and NDF ($P < 0.04$) and acid detergent fiber (ADF) ($P < 0.05$) digestibility were reduced in STM- vs. HTM-supplemented steers. On day 6, ruminal fluid was collected at 0, 2, and 4 h post-feeding and analyzed for VFA. There were no treatment x time interactions for VFA. Steers receiving HTM had less ($P < 0.02$) molar proportions of butyric acid and greater ($P < 0.05$) total VFA concentrations than STM-supplemented steers. Steers were then fed the same diet without supplemental Cu, Zn, or Mn for 14 d. On day 15 steers received a pulse dose of 20 mg Cu, 40 mg Mn, and 60 mg Zn/kg DM from either STM or HTM (n = 6 steers/treatment). Ruminal samples were obtained at 2-h intervals starting at –4 and ending at 24 h relative to dosing. There was a treatment x time interaction ($P < 0.03$) for ruminal soluble Cu, Mn, and Zn concentrations. Ruminal soluble mineral concentrations were greater ($P < 0.05$) for Cu at 4, 6, 8, 10, 12, and 14 h; for Mn at 4 and 6 h; and for Zn at 4, 6, and 8 h post-dosing in STM compared with HTM-supplemented steers. Copper concentrations were greater ($P < 0.05$) at 12 and 24 h and Zn concentrations in ruminal solid digesta were greater at 24 h in HTM-supplemented steers. Upon dialysis against Tris-EDTA, the percent Zn released from digesta was greater ($P < 0.05$) at 12 h ($P < 0.03$) and 24 h ($P < 0.05$), and the percent Cu released was greater ($P < 0.02$) at 24 h post-dosing in HTM steers when compared with STM-supplemented steers. Results indicate that Cu and Zn from HTM have low solubility in the rumen and appear to be less tightly bound to ruminal solid digesta than Cu and Zn from STM. The lower ruminal soluble concentrations of Cu and Zn in steers given HTM were associated with greater fiber digestibility.

Key words: binding strength, copper, volatile fatty acids, zinc

Abbreviations

ADF	acid detergent fiber
BW	body weight
CP	crude protein
DM	dry matter
DMI	dry matter intake
EDTA	Ethylenediaminetetraacetate
HTM	hydroxy trace mineral
NDF	neutral detergent fiber
N	nitrogen
OM	organic matter
STM	sulfate trace mineral
TM	trace mineral
VFA	volatile fatty acid

Introduction

Lactating dairy cows supplemented with hydroxy trace minerals (HTM) had greater neutral detergent fiber (NDF) digestibility than those supplemented with sulfate TM (STM) sources (Faulkner and Weiss, 2017). The authors suggested that the impact of trace mineral source on fiber digestion may be due to differences in ruminal solubility of Cu and Zn. More recent studies with lactating dairy cows have also indicated that replacing STM with HTM improves NDF digestibility (Daniel et al., 2020; Miller et al., 2020). In agreement with these findings, NDF digestibility tended to be lower in steers fed STM compared to those receiving HTM (Caldera et al., 2019). Furthermore, ruminal soluble Cu and Zn concentrations were greater (at multiple time points over a 24-h period) in steers following a single bolus dose of Cu, Mn, and Zn from STM compared to steers given HTM (Caldera et al., 2019). Ruminal pH averaged 6.23 in this experiment. These findings agree with earlier studies indicating that HTM forms of Cu and Zn are relatively insoluble under slightly acidic pH conditions and increase in solubility as pH decreases, whereas STM forms of Cu and Zn are almost completely soluble in water and acidic conditions (Cao et al., 2000; Spears et al., 2004).

Collectively, these data suggest that ruminal solubility of Cu and Zn may influence ruminal fermentation. Diets used in previous studies examining NDF digestion in cattle supplemented with STM or HTM were comprised primarily of corn silage and corn and ranged from 28% to 36% NDF (Faulkner and Weiss, 2017; Caldera et al., 2019). In similar fashion, we hypothesized that ruminal solubility of Cu and Zn would be lower and fiber digestibility greater in steers fed a medium-quality grass hay diet when supplemented with HTM than in steers receiving STM. Therefore, the objectives of the current experiment were to examine the influence of TM source on 1) fiber digestion and ruminal fermentation characteristics, 2) ruminal soluble concentrations of Cu, Mn, and Zn, and 3) relative binding strength of trace minerals located in the ruminal insoluble digesta fraction in steers fed a medium-quality grass hay-based diet.

Materials and Methods

Prior to the initiation of this experiment, all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee (IACUC approval #17-7182A).

Experiment 1

Twelve crossbred Angus steers fitted with ruminal *cannulae* (initial BW 452.8 ± 6.1 kg) were utilized in this experiment. Steers were housed at Colorado State University Agriculture, Research, Development, and Education Center (ARDEC) in Fort Collins, CO. Steers were initially stratified by BW and housed in two feedlot pens (6 steers per pen) and fed a high-fiber, medium-quality grass hay (chopped) diet balanced to meet the CP, Na, Cl, Ca, P, Se, I, Co, and vitamin A, D, and E requirements for growing steers (Table 1), with no supplemental Cu, Mn, or Zn for 21 d. After the 21-d adaptation period, steers received one of two treatments. Treatments consisted of either sulfate (STM) or hydroxychloride (HTM; IntelliBond C, M, and Z; Micronutrients USA LLC., Indianapolis, IN) sources (n = 6 steers/treatment) to provide 20, 40, and 60 mg supplemental Cu, Mn, and Zn/kg DM, respectively. Dietary levels per chemical composition of the basal diet were 6.6, 58.4, and 37.4 mg/kg of Cu, Mn, and Zn, respectively. Supplemental levels of Cu, Mn, and Zn were provided at 20, 40, and 60 mg/kg per day, respectively, based on concentration typically supplied in free choice mineral supplement for grazing beef cattle. Appropriate TM treatment supplements were manufactured prior to the initiation of the experiment. Soybean meal was used as the carrier for the TM treatments. The appropriate TM supplement amounts (60% of the ration in the morning and 40% of the ration in the afternoon) were top-dressed onto the basal diet and mixed thoroughly by hand for each feeding within a day. After receiving treatments for 7 d, steers were moved indoors and housed in individual pens (2.5 × 2.5 m pens equipped with automatic waters, individual feeders, and rubber matted floors) for 2 d and allowed to acclimate to their new surroundings. Steers were then relocated into individual metabolism stalls (3.0 × 1.1 m pens equipped with automatic waters, individual plastic feeders, and rubber matted floors) for a 5-d acclimation period. During the acclimation period, DMI for each steer was determined. At the end of the acclimation period, steers were paired across treatments based on their mean DMI over the 5-d period. Once animals were appropriately paired by DMI, each steer within a pair was fed the same amount of feed. Feed delivered to each steer within a pair was calculated to be

Table 1. Ingredient composition of the basal medium-quality grass hay-based diet

Ingredient	%DM
Grass hay	90.0
Soybean meal (46% CP)	4.7
Beet pulp	4.6
Mineral premix[1]	0.70
Total	100.00
Chemical composition[2]	
Dry matter, %	91.8
Crude protein, %	13.0
NEm, Mcal/kg	1.25
NEg, Mcal/kg	1.4
Fat, %	2.8
Acid detergent fiber, %	29
Neutral detergent fiber, %	49.0
Calcium, %	0.65
Phosphorus, %	0.30
Sulfur, %	0.30
Copper, mg/kg	6.6
Manganese, mg/kg	58.4
Zinc, mg/kg	37.4

[1]Mineral premix contained white salt, urea, vitamin A, D and E, iodine (EDDI), cobalt, and selenium.
[2]Chemical composition of the basal diet without supplemental treatments of Cu, Zn, and Mn.

90.0% of the steer within the pair with the lowest average DMI during the acclimation period. This ensured equal amounts of feed and minerals offered for individual steers within a pair (block) during the 5-d total fecal collection period as described by Caldera et al. (2019). Diets were fed twice daily (60% of the ration in the morning and 40% of the ration in the afternoon) and appropriate TM treatments were provided using the method described above.

Sample collection

Total fecal output was measured daily for individual steers during the 5-d collection period as described by Caldera et al. (2019). Feces collected each day (over a 24-h period) were quantified by wet-weight (feces), thoroughly mixed, and sampled (10.0% of wet weight). Duplicate, individual fecal samples were sealed in plastic bags, labeled, and stored at –20 °C. Prior to chemical analysis of feces and feed, samples were proportionally composited across all collection days for each animal. Dry matter analysis was determined by placing a known mass of wet material in a forced-air drying oven for 48 h at 100 °C. After drying, samples were allowed to cool in a desiccator and then weighed. NDF and acid detergent fiber (ADF) were analyzed using an Ankom 200 Fiber analyzer (Ankom Technology Corp.; Van Soest et al., 1991). Four milliliters of alpha amylase and 20 g of sodium sulfite were used per digestion (digestion vessel contained a total of 22 samples including a blank). NDF and ADF analysis were performed sequentially. Samples used for NDF and ADF analysis were previously dried in a forced-air drying oven for 48 h at 100 °C. All TM were quantified via inductively coupled plasma-mass spectrometry (PerkinElmer; NexION 2000 B) and N was quantified using the TruSpec CN Carbon/Nitrogen LECO system (Leco Corp., St. Joseph, MI).

Following the 5-d fecal collection (day 20), ruminal samples were collected at 0, 2, and 4 h post-feeding for the determination of volatile fatty acids (VFA) and pH. Collection was only performed for the morning feeding (60% of total intake). Ruminal contents were centrifuged at 28,000 × *g* at 5 °C for 30 min. A 2.0-ml aliquot of the supernatant was acidified with 25% (vol/vol) meta-phosphoric acid and frozen at –20 °C until analyzed for VFA concentrations via gas chromatography (Agilent 6890N, Santa Clara, CA). Ruminal pH was determined by inserting a portable pH meter (EcoTestr pH 2+; Oaktron 153 Instruments, Vernon Hills, IL) into the geometric center of the rumen at the time of ruminal content collection.

Experiment 2

At the end of experiment 1, steers were moved into outdoor feedlot pens and fed the basal diet without supplemental Cu, Mn, and Zn for 14 days. During this time, steers had ad libitum access to drinking water and the basal diet was fed as described above. On the following day (day 15), steers were moved back into indoor metabolism building. After 1-d adaptation, on day 16, steers received a pulse dose of the TM sources being evaluated. Individual trace mineral treatments were thoroughly mixed with 0.23 kg of ground corn and administered as a single bolus-dose via the rumen fistula to provide two times the NASEM (2016) requirement for Cu (20 mg Cu/kg DM), Mn (40 mg Mn/kg DM), and Zn (60 mg Zn/kg DM). Immediately after bolus dose administration, the rumen contents were thoroughly mixed by hand. Ruminal samples were then obtained at 2-h intervals beginning at –4 and ending at 24 h post-dosing, time zero being the administration of bolus and feeding of the basal diet. Before each sampling time, ruminal contents were thoroughly mixed by hand and a sample was obtained from the geometric center of the rumen (approximately 250 g). After each collection time, ruminal samples were centrifuged 28,000 × *g* in graduated 50-ml centrifuge tubes. Once centrifuged, the volume of supernatant was determined and the supernatant was frozen at –20 °C until TM analysis was performed. The Cu, Mn, and Zn concentrations of the supernatant and pellet fractions were considered the soluble and insoluble fractions of these elements, respectively.

Dialysis of ruminal insoluble digesta

Ruminal solid digesta samples from three different collection times (0, 12, and 24 h) of experiment 2 were exposed to dialysis. Briefly, the insoluble fraction of the ruminal digesta collected was dried at 60 °C for 48 h in a forced air-drying oven, ground using mortar and pestle to analyzed for Cu, Mn, and Zn, and dialyzed against 0.01 M ethylenediaminetetraacetate in 0.05 M Tris buffer (Tris-EDTA). Regenerated cellulose dialysis tubing (31.8 mm diameter, 30 µm wall thickness, molecular weight cut off 6,000 to 8,000; Fisher Scientific, Pittsburgh, PA) was cut into 10-cm segments and treated to remove metal contamination as described previously (Caldera et al., 2019). Dialysis tubing was stored in 50% ethanol, 50% deionized water, 1mM EDTA at 4 °C prior to use. The Tris-EDTA buffer was prepared immediately prior to use and the pH adjusted to 6.8. Samples were placed into 10 ml of buffer, then placed into dialysis tubing pre-wet with deionized water, and the tubing was then sealed with clips. The samples were then dialyzed against 1.0 L of the Tris-EDTA buffer for 16 h at 4 °C with continuous stirring. The buffer was changed and dialysis continued for another 6 h. Samples were removed from dialysis bags, placed into pre-weighed acid-washed crucibles, and dried overnight at 60 °C. After drying, samples were weighed and then ashed at 600 °C in a Thermo-Fisher Thermolyne muffle furnace overnight. After cooling, ashed samples were weighed and re-suspended in 5 ml of boiling 1.2 M HCl and analyzed for Cu, Mn, and Zn as described in experiment 1.

Statistical analysis

Total tract apparent digestibility of DM, ADF, NDF, and CP and initial and post-dialysis Cu, Mn, and Zn concentrations of insoluble digesta at times 0, 12, and 24 h were analyzed using a mixed effects model (PROC MIXED, SAS Inst. Inc., Cary, NC) for a completely randomized block design. A mixed effects model repeated measures analysis (PROC MIXED) for a completely randomized block design was used to analyze ruminal soluble Cu, Mn, and Zn concentrations, pH, and VFA proportions and total concentrations. The fixed effects were treatment, time, and the treatment x time interaction. For all response variables measured, individual animal was considered the experimental unit. Several covariance structures were compared to determine the most appropriate covariance structure for data analysis. The AR(1) covariance structure was the most appropriate for all variables. For all response variables, significance was determined at $P \leq 0.05$ and tendencies were determined at $P > 0.05$ and ≤ 0.10. When a significant treatment × time interaction was detected, treatment means were separated using the PDIFF option of the LSMEANS statement of SAS.

Results and Discussion

Experiment 1

The influence of TM source on nutrient digestibility is shown in Table 2. By design, DM intake was similar across

Table 2. Influence of trace mineral source on dry matter (DM), acid detergent fiber (ADF), neutral detergent fiber (NDF), and crude protein (CP) digestibility in steers fed a high roughage diet

	Treatment			
Item	HTM[1]	STM[2]	SEM	P<
N	6	6	–	–
DM intake, kg DM/animal/d	7.4	7.4	–	–
DM digestibility, %	53.4	51.9	0.52	0.07
ADF digestibility, %	34.1	32.4	0.49	0.05
NDF digestibility, %	42.7	40.4	0.67	0.04
CP digestibility, %	54.3	51.2	0.58	0.06

[1]Hydroxy trace minerals: 20 mg Cu/kg DM from CuOHCl; 40 mg Mn/kg DM from MnOHCl; 60 mg Zn/kg DM from ZnOHCl.
[2]Sulfate trace minerals: 20 mg Cu/kg DM from $CuSO_4$; 40 mg Mn/kg DM from $MnSO_4$; 60 mg Zn/kg DM from $ZnSO_4$.

Table 3. Influence of trace mineral source on ruminal pH and VFA, molar proportion, and total concentration in steers fed a high forage diet

	Treatment			P<		
Item	HTM[1]	STM[2]	SEM	Trt	Time	Trt*time
n	6	6	–	–	–	–
pH	6.68	6.59	0.087	0.47	0.01	0.57
VFA, mM/100mM						
Acetic acid	49.15	48.89	0.539	0.74	0.05	0.92
Propionic acid	21.21	22.38	0.824	0.34	0.44	0.45
Isobutyric acid	5.80	5.57	0.232	0.51	0.001	0.61
Butyric acid	14.93	16.28	0.346	0.02	0.001	0.93
Isovaleric acid	5.09	4.08	0.374	0.09	0.001	0.43
Valeric acid	3.83	3.71	0.220	0.71	0.001	0.91
Total VFA, mM	72.26	59.81	3.93	0.05	0.85	0.86

[1]Hydroxy trace minerals: 20 mg Cu/kg DM from CuOHCl; 40 mg Mn/kg DM from MnOHCl; 60 mg Zn/kg DM from ZnOHCl.
[2]Sulfate trace minerals: 20 mg Cu/kg DM from $CuSO_4$; 40 mg Mn/kg DM from $MnSO_4$; 60 mg Zn/kg DM from $ZnSO_4$.

the treatments. Steers supplemented with HTM had greater NDF ($P < 0.04$) and ADF ($P < 0.05$) digestibility than those supplemented with STM. Lactating dairy cows (Faulkner and Weiss, 2017; Daniel et al., 2020; Miller et al., 2020) and steers (Caldera et al., 2019) supplemented with HTM also had greater NDF digestibility than those receiving STM. However, in situ NDF disappearance did not differ among fistulated steers given HTM or STM (Genther and Hansen, 2015). As expected, total tract NDF digestibility of the medium-quality grass hay diet in the present study was less than NDF digestibilities observed in previous studies with lactating dairy cows (Faulkner and Weiss, 2017; Miller et al., 2020; Daniel et al., 2020), due to the lower quality forage utilized in the current experiment compared diets containing corn silage used by Faulkner and Weiss (2017), Miller et al. (2020), and Daniel et al. (2020). In contrast to our finding, ADF digestibility was not affected by TM source in dairy cows (Daniel et al., 2020). Dry matter ($P < 0.07$) and CP ($P < 0.06$) digestibility tended to be greater for HTM compared with STM-supplemented steers. In lactating dairy cows, DM and CP digestibility did not differ among cows supplemented with HTM or STM (Faulkner and Weiss, 2017; Daniel et al., 2020). Miller et al. (2020) reported that percent DM digestibility was not affected by TM source. However, because of greater DM intake in cows fed HTM, cows receiving HTM digested more DM, OM, and NDF than those supplemented with STM. Lambs supplemented with organic forms of Zn (Mallaki et al., 2015; Alimohamady et al., 2019) or Cu (Dezfoulian et al., 2012) had greater CP digestibility than those receiving sulfate forms of Zn or Cu.

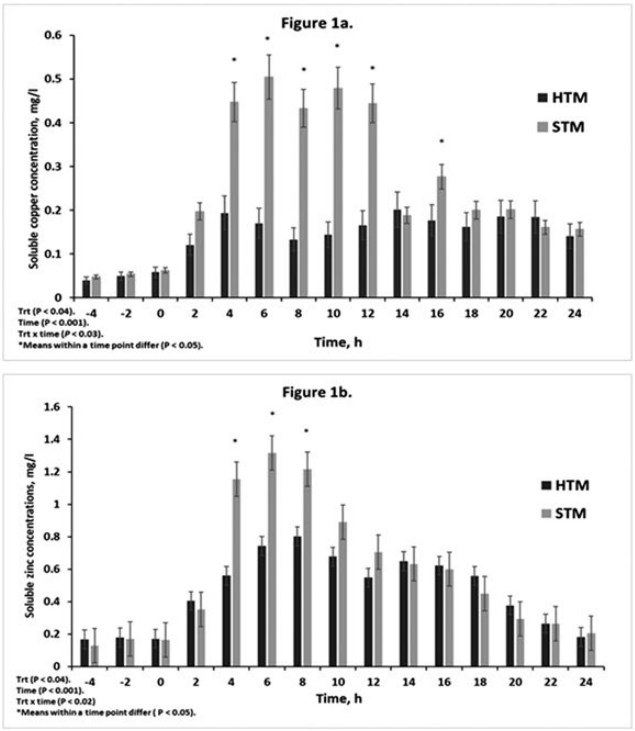
Ruminal pH was affected by time ($P < 0.01$) after feeding, but not by treatment or treatment x time (Table 3). There were no treatment x time interactions for total VFA concentrations or molar proportions of VFA (Table 3). Therefore, only main effect treatment means are presented. Steers receiving HTM had greater ($P < 0.05$) total VFA concentrations than those fed STM. The greater VFA concentrations in steers fed HTM is consistent with the greater fiber digestibility observed in this treatment. In growing steers fed a corn silage-based diet, total VFA concentrations were also greater in steers fed Zn hydroxychloride compared with those receiving $ZnSO_4$ (Shaeffer, 2006). Molar proportion of butyric acid was less ($P < 0.02$) in steers fed HTM than in steers fed STM. Lactating dairy cows fed STM also had a greater proportion of butyric acid than cows supplemented with HTM (Daniel et al., 2020). Other VFA molar proportions were not affected by treatment.

Experiment 2

Soluble trace mineral concentrations following a pulse dose of Cu, Mn, and Zn

The influence of trace mineral source on ruminal soluble Cu, Mn, and Zn concentrations in steers receiving a pulse dose of TM is presented in Figures 1a–c. There was a treatment x time interaction for Cu ($P < 0.03$) and Zn ($P < 0.02$). Ruminal soluble concentrations were greater ($P < 0.05$) for Cu at 4, 6, 8, 10, 12, and 16 h post-dosing in STM compared to HTM-supplemented steers (Figure 1a). Ruminal soluble Zn concentrations were greater in STM-supplemented steers at 4, 6, and 8 h post-dosing compared to HTM steers (Figure 1b). In agreement with the current experiment, Caldera et al. (2019) reported that ruminal soluble Cu and Zn concentrations were greater (at multiple time points over a 24-h period) in steers consuming a 50% corn silage, 50% steam flaked corn-based diet (DM basis) following a single bolus dose of Cu, Mn, and Zn [2× NASEM (2016) recommended concentration] from STM compared to HTM. Ruminal pH averaged 6.63 in the current experiment and 6.23 in the Caldera et al. (2019) experiment indicating that HTM forms of Cu and Zn are less soluble under slightly acidic pH conditions than STM forms of Cu and Zn.

The greater ruminal soluble concentrations of Zn and Cu in steers fed STM may explain the less fiber digestibility in STM- vs. HTM-supplemented steers. High-media Zn concentrations have been shown to result in Zn accumulation in the endoplasm of ciliated protozoa (Bonhomme et al., 1980), and to inhibit their growth in vitro (Bonhomme et al., 1979). Cellulolytic bacterial activity (Bonhomme et al., 1979) and concentrations of cellulolytic bacteria (Eryavug and Dehority, 2009) were also reduced by high Zn concentrations in vitro. The addition of 40 mg Zn/kg DM from $ZnSO_4$ to a diet that analyzed 22 mg Zn/kg did not affect NDF digestibility in one experiment with lambs (VanValin et al., 2018), but reduced NDF digestibility in a follow-up study (VanValin et al., 2020). High Cu concentrations have been reported to reduce bacterial populations through toxicity due to bioaccumulation of Cu within bacterial cells, reduction of Cu^{+2} to Cu^{+1} which is more toxic to bacteria, and/or causing the cell to activate Cu export pumps to alleviate Cu accumulation in bacteria (Osman and Cavet, 2008; Hernandez-Sanchez, 2018). The addition of low concentrations of Cu from $CuSO_4$ reduced in vitro cellulose digestion by ruminal microorganisms (Ward and Spears, 1993).



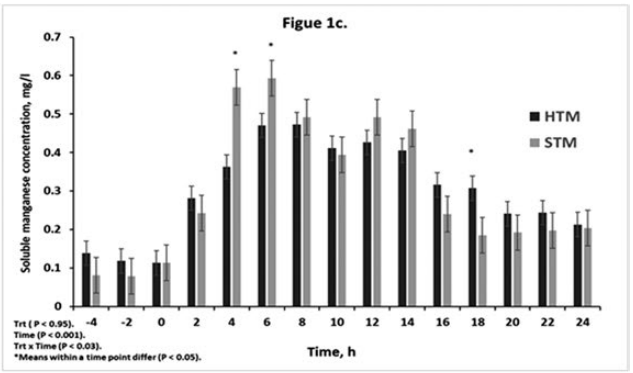


Figure 1. The influence of trace mineral source on copper (a), zinc (b), manganese (c), within the ruminal contents of steers receiving a pulse dose of either sulfate trace minerals (STM; 20 mg/kg DM from $CuSO_4$, 60 mg/kg DM from $ZnSO_4$, and 40 mg/kg DM from $MnSO_4$.) or hydroxy trace minerals (HTM; 20 mg/kg DM from CuOHCl, 60 mg/kg DM from ZnOHCl, and 40 mg/kg DM from MnOHCl. The x-axis denotes sampling time in hours and the y-axis denotes rumen soluble mineral concentration. Error bars represent standard errors.

There was a treatment x time interaction ($P < 0.03$) for ruminal soluble Mn (Figure 1c). Ruminal soluble Mn concentrations were greater for STM-supplemented steers at 4 and 6 h post-dosing and less at 18 h post-dosing when compared with HTM-supplemented steers. Ruminal soluble Mn from both sources were greater at 2 to16 h post-dosing compared to pre-dosing soluble Mn concentrations. Caldera et al. (2019) reported greater ruminal soluble Mn at 2 and 24 h post-dosing and less ruminal soluble Mn concentrations at 4 and 8 h post-dosing in HTM vs. STM-supplemented steers.

Binding strength of trace minerals in ruminal insoluble digesta

There was a treatment by time interaction for the concentration of Cu ($P < 0.01$), Mn ($P < 0.01$), and Zn ($P < 0.011$) in rumen solid digesta after a pulse dose of 20 mg Cu, 40 mg Mn, and 60 mg Zn/kg DM from either HTM or STM sources. Therefore, data are presented by time in Table 4. Initial (0 h) ruminal solid digesta Cu, Mn, and Zn concentrations were similar across treatments. At 12 h post-bolus dose administration, Cu ($P < 0.01$), Mn ($P < 0.03$), and Zn ($P < 0.01$) concentrations of ruminal solid digesta were greater in HTM compared to STM-supplemented steers. At 24 h post-dosing, Mn concentrations of ruminal solid digesta were less ($P < 0.03$) and Zn concentrations greater ($P < 0.01$) in HTM compared to STM-supplemented steers.

Research has shown that the ability of chelating agents to remove Zn from protein sources during dialysis may be useful in estimating in vivo bioavailability (Jones et al., 1985). Release of Cu ($P < 0.01$), Zn ($P < 0.01$), and Mn ($P < 0.01$) from the rumen solid digesta following dialysis against Tris-EDTA was affected by a treatment x time interaction. Following dialysis against Tris-EDTA, the percentages of Cu ($P < 0.69$), Mn ($P < 0.65$), and Zn ($P < 0.12$) released from ruminal solid digesta at 0 h were not different across treatments. At 12-h post-bolus dosing, the percentages of Cu ($P < 0.01$) and Zn ($P < 0.01$) released from solid digesta were greater and the percentage of Mn released was less ($P < 0.01$) in HTM vs. STM. At 24-h post-bolus dosing, the percentages of Cu ($P < 0.01$) and Zn ($P < 0.01$) released from ruminal solid digesta were greater in HTM compared to STM-supplemented steers. The percentage of Mn released at 24-h post-bolus dosing was similar ($P < 0.41$) across treatments. These data agree with dialysis data presented by Caldera et al. (2019) and indicate that Cu and Zn from HTM were less tightly associated with the solid digesta fraction in the rumen than STM. The tighter binding of Cu and Zn from STM to the ruminal solid fraction may reduce their absorption in the small intestine. Previous studies have reported greater bioavailability of Cu (Spears et al., 2004) and Zn (Shaeffer et al., 2017) from HTM vs. STM.

Trace minerals that become soluble in the rumen may bind to various feed components, microbial matter, or ruminal metabolites to form insoluble complexes. Previous studies in sheep (Bremner, 1970; Waghorn et al., 1990) have indicated that the majority of Cu, Zn, and Mn is associated with the solid digesta in the rumen, when trace minerals are derived from forages. In sheep fed dried grass, with no supplemental trace minerals, strength of binding to ruminal solid digesta was in the order of Cu > Zn > Mn, based on the extent of dissolution of the metals on treatment with acid or EDTA (Bremner, 1970). The order of binding strength to the solid digesta in steers receiving STM was also Cu > Zn > Mn when dialyzed against EDTA at 12 and 24 h post-dosing in the present study. However, in steers

Table 4. Influence of dialysis on copper, manganese, and zinc release from rumen solid digesta 0, 12, and 24 h after receiving a pulse dose of 20 mg copper, 40 mg manganese, and 60 mg zinc/kg DM from either hydroxy or sulfate trace mineral sources

	Treatment			
Item	HTM[1]	STM[2]	SEM	P <
Digesta, initial mineral concentration, mg/kg DM				
0 h				
Copper	0.92	0.99	0.10	0.92
Manganese	16.0	16.1	0.61	0.89
Zinc	14.5	13.5	0.47	0.61
12 h				
Copper	31.6	8.1	0.84	0.01
Manganese	38.2	35.3	1.27	0.03
Zinc	129.6	37.3	2.08	0.01
24 h				
Copper	1.13	1.37	0.10	0.73
Manganese	14.7	17.7	0.54	0.03
Zinc	23.1	16.3	0.52	0.01
Mineral released, %[3]				
0 h				
Copper	25.7	26.5	1.36	0.69
Manganese	30.2	31.4	1.70	0.65
Zinc	53.9	48.3	2.34	0.12
12 h				
Copper	59.2	26.5	1.71	0.01
Manganese	63.7	77.2	1.24	0.01
Zinc	87.8	34.3	2.45	0.01
24 h				
Copper	77.1	30.4	2.75	0.01
Manganese	90.9	93.1	1.94	0.41
Zinc	91.0	32.1	2.07	0.01

[1]Hydroxy trace minerals: 20 mg Cu/kg DM from CuOHCl; 40 mg Mn/kg DM from MnOHCl; 60 mg Zn/kg DM from ZnOHCl.
[2]Sulfate trace minerals: 20 mg Cu/kg DM from $CuSO_4$; 40 mg Mn/kg DM from $MnSO_4$; 60 mg Zn/kg DM from $ZnSO_4$.
[3]Dialyzed against Tris-EDTA (0.01M ethylenediaminetetraacetate in 0.05M tris-hydroxymethyl-aminomethane) at 4 °C for 22 h.

dosed with HTM, binding strength of Zn to the solid phase was similar to Mn.

In conclusion, steers supplemented with STM had less fiber digestibility and total ruminal VFA concentrations than steers receiving HTM. Steers receiving a pulse dose of STM had greater ruminal soluble Cu and Zn concentrations than those given HTM at multiple times after dosing. Based on their release following dialysis against Tris-EDTA, Cu and Zn from STM were more tightly bound to ruminal solid digesta than HTM. The stronger binding of STM to the ruminal solid digesta may indicate reduction on the bioavailability in the small intestine.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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