Comparison of trace mineral repletion strategies in feedlot steers to overcome diets containing high concentrations of sulfur and molybdenum

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ABSTRACT: To compare trace mineral (**TM**) repletion in feedlot steers after depletion by S and Mo, 72 Red Angus steers blocked by BW (253 ± 14 kg) were assigned (6 steers per pen, fed via GrowSafe bunks) to corn silage depletion diets (depletion, **DEP**) supplemented with NRC (1996) recommended concentrations of Cu, Mn, Se, and Zn (**CON**) or supplemented with 0.3% S ($CaSO_4$), 2 mg of Mo/kg dry matter (DM), and no added Cu, Mn, Zn, or Se (antagonist, **ANT**). Three 62 d TM repletion strategies (repletion, **REP**) were applied within DEP diets on day 89: 1) Multimin90 injection (contains Cu, Mn, Se, Zn) and 100% of recommended Cu, Mn, Zn, and Se from inorganic sources (**ITM**), 2) saline injection and 150% of recommended TM from inorganic sources (**ING**), or 3) saline injection and 150% of recommended TM provided as 25% organic and 75% inorganic sources (**BLEND**). Subcutaneous injections were given at 1 mL/68 kg BW. Inorganic sources were Cu, Mn, and Zn SO_4, and sodium selenite, and organic sources were Availa Cu, Mn and Zn, and SelPlex Se. Repletion period liver and blood were collected on day −10, 14, 28, and 42 and data were analyzed as a 2 × 3 factorial (n = 12 steers per treatment) using Proc Glimmix of SAS with plasma and liver analytes analyzed as repeated measures. Liver Cu, Se, and Mn were decreased ($P < 0.01$) by ANT during DEP. There were no DEP × REP × day interactions in liver TM ($P \geq 0.18$). A DEP × day effect was noted for liver Cu ($P < 0.01$) and Mn ($P = 0.07$), where ANT Cu increased linearly from day 0 to day 42, CON Cu was slightly increased on day 14 and day 28, and ANT Mn was lesser than CON Mn on all days except day 42. There were REP × day effects on liver Cu ($P < 0.01$) and Se ($P < 0.01$) where status was improved by ITM by day 14, increased in BLEND by day 28, and not different by day 42. Liver Se concentrations were lesser ($P < 0.01$) in ANT vs. CON throughout repletion. Liver Zn was greater ($P < 0.01$) on day 0 than day 14, 28, and 42, and concentrations were greater on day 42 than day 28. Glutathione peroxidase activity tended to be lesser ($P = 0.07$) on day 14 relative to other days. Manganese superoxide dismutase activity was lesser ($P < 0.01$) on day 14 and 28 compared to day 0 and 42, and tended to be lesser ($P = 0.06$) in ANT than CON during repletion. Final body weight (BW) and average daily gain (ADG) were not affected by treatment ($P \geq 0.60$), and ANT decreased dry matter intake (DMI) ($P = 0.04$) and improved G:F ($P < 0.01$) during repletion. All repletion strategies were effective at increasing TM status of steers, and ITM had the most rapid recovery of Cu and Se status, followed by BLEND, and ING.

Key words: antagonism, cattle, injectable trace mineral, sulfur, thiomolybdate, trace mineral

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INTRODUCTION

Trace minerals (**TM**) are required for adequate growth, health, and reproduction (Underwood and Suttle, 1999). Recently, greater inclusions of ethanol coproducts in cattle diets have increased dietary

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S concentrations (Drewnoski et al., 2014). Excessive S has been shown to decrease the availability of Cu and Se through antagonistic reactions in the gastrointestinal tract (Ivancic and Weiss, 2001; Spears et al., 2011), while the effects of increased S on Mn and Zn absorption are less well studied (Pogge et al., 2014). The effect of S on Cu is especially potent when Mo is included in the diet, as thiomolybdates form and cause an irreversible inhibition on Cu function (Suttle, 1974). These interactions create a unique challenge of supplementing TM to cattle in a method that is effective at bypassing rumen antagonisms but still available for absorption in the small intestine. Many organic TM are less ruminally soluble than inorganic TM, potentially supporting greater bioavailability in the small intestine (Spears, 2003). Samuelson et al. (2016) found a majority of consulting feedlot nutritionists supplement a combination of organic and inorganic TM at concentrations greater than those recommended by the NRC (1996). Though greater inclusions of dietary TM can increase status over time, alternative methods may cause more rapid improvements. Injectable TM provide TM directly to the tissues through a subcutaneous injection to quickly increase TM status (Pogge et al., 2012). Little research has examined how a high antagonist diet influences the ability of TM sources to increase TM status of cattle. The objective of this study was to compare the efficacy of various TM repletion strategies on TM status and performance of steers fed diets containing the antagonists S and Mo. The hypothesis was that TM status of steers would be improved most rapidly in those receiving injectable TM, followed by those receiving an organic/inorganic blend, and then those receiving inorganic TM alone.

MATERIALS AND METHODS

All procedures involving the use of animals were approved by the Iowa State University Institutional Animal Care and Use Committee (log # 10-15-8110-B).

Experimental Design and Sampling Procedures

This experiment was conducted at the Iowa State University Beef Nutrition Research Center (Ames, IA). Seventy-two Red Angus yearling steers were blocked by BW (254 ± 14 kg) and assigned to 1 of 2 corn-silage based depletion diets (**DEP**, n = 36 steers/treatment), either supplemented with Cu, Mn, Se, and Zn at NRC (1996) recommendations (control, **CON**), or not supplemented with these TM and supplemented with 0.3% S ($CaSO_4$) and 2 mg of Mo/kg DM (sodium molybdate, ACROS Organics, Janssen Pharmaceuticalaan, Belgium) to deplete TM status (antagonist, **ANT**). Both treatments received Co and I supplemented at NRC (1996) recommendations. Analyzed TM concentrations for depletion period diets are shown in Table 1. Steers were fed these diets for the entirety of the study. Steers were housed in pens (6 steers per pen) equipped with GrowSafe bunks to determine individual feed intake, and had ad libitum access to water. At the initiation of the study, steers received an electronic identification tag (Allflex US Inc., Dallas–Fort Worth Airport, TX) to assist in recording of individual intakes in the GrowSafe bunk system (GrowSafe Systems Ltd., Airdrie, AB, Canada). Steers were vaccinated against viral (Bovi Shield Gold 5; Zoetis Inc., Florham Park, NJ) and clostridial (Vision 7; Merck Animal Health, Summit, NJ) infections and dewormed with eprinomectin (Eprinex, Merial Ltd., Iselin, NJ). Steers were implanted on day 0 with a combination implant containing 200 mg progesterone USP, 20 mg estradiol benzoate, and 29 mg tylosin tartrate (Component E-S; Elanco Animal Health, Greenfield, IN), and reimplated with a combination implant containing 80 mg trenbolone acetate, 16 mg estradiol USP, and 29 mg tylosin tartrate (Component TE-IS; Elanco Animal Health, Greenfield, IN) at the start of repletion.

On day 88 and 89, respectively, the heavy and light blocks were assigned randomly within block to 1 of 3 TM repletion (**REP**; Table 2) strategies: 1) an injectable TM (Multimin90; Multimin USA, Fort Collins, CO) containing Cu, Mn, Zn, and Se, and dietary TM (Cu, Mn, Zn, and Se) supplemented at 100% of nationally recommended concentrations (NASEM, 2016) from strictly inorganic sources (**ITM**), 2) a sterilized saline injection and TM supplemented at 150% of NASEM (2016) recommendations from strictly inorganic sources (**ING**), 3) or a sterilized saline injection and TM supplemented at 150% of NASEM (2016) recommendations from a blend of 75% inorganic and 25% organic sources (**BLEND**). This 2 × 3 factorial resulted in 6 treatments for the 62 d repletion period (n = 12 steers per treatment). Multimin90 contains 15 mg Cu, 10 mg Mn, 5 mg Se, and 60 mg Zn per mL, and was dosed at a rate of 1 mL per 68 kg BW. Steers for ING and BLEND treatments were dosed with a sterilized saline solution at the same rate as ITM steers, and all injections were subcutaneous. Inorganic sources were Cu, Mn, and Zn sulfate, and sodium selenite, and organic sources were Availa Cu, Mn and Zn

Table 1. Ingredient composition of depletion diets (CON[1] or ANT[2]) fed throughout the trial (DM basis, %)

	Depletion Diet	
	CON	ANT
Ingredient		
Corn silage	40	40
Dry rolled corn	25	25
DDGS[3]	20	15
MDGS[4]	10	10
S and Mo premix[5]	0	5
Micro ingredients[6]	5	5
Calculated composition[7]		
CP, %	15.06	14.90
NDF, %	26.03	25.86
EE, %	5.16	5.11
Analyzed composition[8]		
S, % of DM	0.22	0.48
Cu, mg/kg DM	6.98	2.61
Mn, mg/kg DM	17.53	7.52
Mo, mg/kg DM	0.39	2.03
Zn, mg/kg DM	29.55	14.72

[1]Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu ($CuSO_4$), 30 mg of Zn ($ZnSO_4$), 20 mg of Mn ($MnSO_4$), 0.5 mg of I (ethylenediamine dihydroiodide), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

[2]Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (ethylenediamine dihydoiodide) and 0.1 mg of Co (cobalt carbonate).

[3]Dried distillers grains with solubles.

[4]Modified distillers grains with solubles.

[5]S and Mo premix use DDGS as a carrier and provide 0.3% S (as $CaSO_4$) and 2 mg of Mo/kg DM.

[6]Microingredients include trace minerals, Vitamin A, limestone, salt, and Rumensin, and use DDGS as a carrier.

[7]Calculated based on individual ingredient analysis from Dairyland Laboratories (Arcadia, WI). CP, crude protein; NDF, neutral detergent fiber; EE, ether extract.

[8]Analysis of TMR from depletion period.

(Zinpro Corp, Eden Prairie, MN), and SelPlex Se (Alltech, Nicholasville, KY). All treatments received Co and I supplemented at NASEM (2016) recommendations.

Steers were weighed on consecutive days on a single calibrated animal scale at the start (day −4, day −3) and end of the depletion period for the heavy (day 88, day 89) and light (day 89, day 90) groups, respectively. Liver biopsies were conducted on all steers and were collected over 2 d, thus, steers were stagger started for the repletion period, and the last day of depletion was determined to be day 0 for the repletion period. Steers were weighed on consecutive dates at the start of repletion (day −1, day 0) and end of repletion (final BW; day 61, day 62), and received treatment injections and started repletion diets on day 0. Liver biopsy samples were collected for TM analysis at the start (day −2 or day −1) and end (day 79 or day 80) of the depletion period, and during the repletion period on day 14, 28, and 42, using the methods of Engle and Spears (2000). Jugular blood samples were collected on these days 2 h postfeeding (day −10, 14, 28, 42) into 7 mL trace mineral potassium-EDTA vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) for analysis of plasma TM concentration, and red blood cell lysate (**RBCL**) glutathione peroxidase (**GSH-px**) and Mn superoxide dismutase (**Mn-SOD**) activity. One unit of GSH-px activity is defined as the amount of enzyme that is necessary for the oxidation of 1.0 nmol of reduced nicotinamide adenine dinucleotide phosphate to oxidized nicotinamide adenine dinucleotide phosphate per minute at 25°C. One unit of Mn-SOD is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide radical. All samples were transported to the laboratory on ice.

Tissue and Feed Analysis

Liver samples were dried in a forced air oven at 70°C for approximately 1 wk until they were dried completely. Liver tissues were then acid digested using trace metal grade nitric acid in preparation for TM determination. Blood samples were centrifuged at 1,000 × *g* for 10 min at 4°C and then plasma was removed and stored at −20°C until preparation for TM analysis. Plasma samples were diluted 1:20 with 1% nitric acid and vortexed before analysis. Liver and plasma samples were analyzed for Cu, Mn, Fe, Se, Mo, and Zn at the Iowa State University Veterinary Medicine Diagnostic Laboratory using an ICP-MS (Analytik Jena Inc. Woburn, MA). Several elements served as internal standards (Bi, Sc, In, Li, Y, and Tb), and all runs included serum (UTAK Laboratories, Inc., Valencia, CA) or bovine liver (National Institute of Standards and Technology, Gaithersburg, MD) standards as appropriate for verification of instrument accuracy. Packed red blood cells were lysed with 4 times their volume with ice-cold molecular grade water, vortexed, and centrifuged at 10,000 × g for 15 min at 4°C. The supernatant was removed carefully to avoid disturbing the pellet and stored at −80°C until further analysis. Red blood cell lysate GSH-px and RBCL Mn-SOD were analyzed using commercial assay kits (Cayman Chemical, Ann Arbor, Mo; catalog 706002 and 703102, respectively). Hemoglobin (**Hb**) analysis of RBCL was conducted using the methods of Hansen et al. (2010) using Drabkin's Reagent (Sigma Chemical Co., 1990) and

Table 2. Composition of repletion period dietary supplemental trace minerals (ITM[1] vs. BLEND[2] vs. ING[3]) applied within 2 depletion period diets (CON[4] vs. ANT[5]) fed to feedlot steers

Mineral, mg/kg DM	ITM	BLEND	ING
Co[6]	0.15	0.15	0.15
I[7]	0.5	0.5	0.5
Cu ($CuSO_4$)	10	11.25	15
Mn ($MnSO_4$)	20	22.5	30
Se (Na_2SeO_3)	0.1	0.1125	0.15
Zn ($ZnSO_4$)	30	33.75	45
Cu (Availa – Cu)	-	3.75	-
Mn (Availa – Mn)	-	7.5	-
Se (Selplex – Se)	-	0 .0375	-
Zn (Availa – Zn)	-	11.25	-
Analyzed composition[8]			
Cu, mg/kg DM	14.6	20.4	19.2
Mn, mg/kg DM	37.5	48.1	47.0
Zn, mg/kg DM	62.8	72.6	69.1

[1]ITM: Injectable trace mineral treatment supplemented inorganic forms of trace minerals at 100% of concentrations recommended by NASEM (2016).

[2]BLEND: Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from 25% organic and 75% inorganic sources.

[3]ING: Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from entirely inorganic sources.

[4]Control diet contained no supplemental Mo or S.

[5]Antagonist diet provided supplemental dietary minerals: 2 mg Mo/kg DM, and 0.3% S as $CaSO_4$.

[6]Cobalt supplemented as Co carbonate.

[7]Iodine supplemented as ethylenediamine dihydroiodide.

[8]Analysis of TMR from repletion period.

a hemoglobin standard (Pointe Scientific, Canton, MI), and Mn-SOD, and GSH-px activities are expressed per unit Hb. The CV for intra-assay and interassay were 3.05 and 4.08 for GSH-px and <10.0 and 6.73 for Mn-SOD, respectively.

Samples of the total mixed ration (**TMR**) were collected on a weekly basis, dried in an air forced oven at 70°C for 48 h, and TMR DM were calculated. Feed samples were composited within treatment by month, acid digested using trace metal grade nitric acid, and analyzed using ICP OES (PerkinElmer, Waltham, MA) for the analysis of S, Cu, Mn, and Zn concentrations as described by Pogge et al. (2014). In the depletion period, the S concentrations were 0.22% for CON and 0.48% for ANT. Dry matter intakes were calculated using GrowSafe intake data corrected for DM using the weekly TMR sample analyses. Average daily gain was calculated using the total weight gained in each period divided by the length of each period. Feed efficiency was calculated using the total gain and the total DMI for each period of interest.

Statistical Analysis

Data for the depletion period were analyzed using the GLIMMIX procedures of SAS 9.4 (SAS Institute, Cary, NC) with steer as experimental unit (n = 36 per treatment) and the fixed effect of the depletion period diet (DEP). After repletion period treatments (REP) were applied, data were analyzed as a 2 × 3 factorial using the GLIMMIX procedures of SAS 9.4 (SAS Institute) with steer as the experimental unit (n = 12 per treatment). The repletion period model included the fixed effects of depletion diet and repletion strategy and the interaction. Means were separated using the slice diff command in SAS. Plasma and liver TM concentrations assessed throughout the repletion period, as well as RBCL enzyme activity data, were analyzed as repeated measures to account for initial TM status, with day of sampling as the repeated effect. Covariance structures were selected based on the least Akaike information criterion; autoregressive 1 was used for liver data and variance component was used for blood data. Due to the pronounced effect of the antagonist diet during the depletion period on liver Cu and Zn concentrations, transformations were necessary for liver Cu and Zn data for both the depletion period and repletion periods, and were calculated using the exponential function and logarithmic function, respectively. Start of repletion BW was used as a covariate for DMI, G:F, and final repletion period BW. Data presented are LSMEANS and SEM, and back-transformed data are presented for liver Cu and Zn concentrations. Outlier analysis was conducted on all data and samples were removed if the Cook's D value was greater than 0.5 or if the value was determined to be physiological irrelevant. Nineteen data points were determined to be physiologically irrelevant across the 4 measurement days (1 Cu, 2 Mn, 5 Se, and 11 Zn) and were removed from analysis. Red blood cell lysate samples were not analyzed from d 42 for 13 steers in the ANT group (5 ANT-ING, 4 ANT-ITM, 4 ANT-BLEND) due to errors in initial sample processing, and no liver sample was collected for 1 steer (ANT-ING) on day 42 due to technical errors while sampling.

RESULTS

Depletion Period Liver and Plasma TM

Based on biopsies collected prior to start of the depletion period, steer liver mineral concentrations

(± SEM) were: 252 ± 15.0 Cu, 7.4 ± 0.20 Mn, 2.10 ± 0.069 Se, and 101 ± 2.9 mg Zn/kg DM.

Liver TM concentrations at the end of the depletion period are displayed in Table 3. At the end of the depletion period, liver concentrations of Cu, Mn, and Se were decreased ($P < 0.01$) in ANT compared to CON. Liver Cu decreased approximately 90% (± 4.9%) due to ANT, resulting in 23 ± 5.3 mg Cu/kg for ANT while CON maintained their status at 251 mg Cu/kg DM. Similarly, liver Se concentrations (mg/kg DM) were decreased by 40% such that ANT had 1.22 while CON maintained 2.00. Liver Mn concentrations were lesser ($P = 0.01$) in ANT than CON at the end of depletion, and liver Zn concentrations were unaffected ($P = 0.42$) by treatment. Liver TM concentrations at the end of depletion served as day 0 values for repeated measures analysis of liver TM concentrations during the repletion period.

At the end of the depletion period, plasma concentrations were decreased ($P \leq 0.03$) in ANT compared to CON for Cu (0.71 ± 0.092 mg/L vs. 0.81 ± 0.092 mg/L), Se (129 ± 1.7 µg/L vs. 135 ± 1.7 µg/L), and Zn (1.17 ± 0.019 mg/L vs. 1.26 ± 0.019 mg/L). Plasma concentrations of Mo were greater ($P < 0.0001$) in ANT than CON at the end of the depletion period such that ANT had 21.1 ± 0.47 µg/L while CON had 12.8 ± 0.45 µg/L.

Repletion Period Liver TM

Across the repletion period, there were no DEP × REP × day ($P \geq 0.19$) or DEP × REP effects ($P \geq 0.15$) on liver concentrations of Cu, Mn, Se, or Zn. Interactions of DEP × day and REP × day in liver TM concentrations through the repletion period are displayed in Fig. 1 and Fig. 2, respectively. Liver Cu concentrations across the repletion period were differentially affected by the DEP diet (DEP × day, $P < 0.0001$; Fig. 1A), where concentrations in ANT were greater at each subsequent sampling day, while within CON, concentrations were greater on day 14 and 28 relative to day 0 and 42. Liver Cu concentrations during the repletion period were also affected by REP × day ($P < 0.0001$; Fig. 2A), where Cu concentrations among REP strategies did not differ on day 0, were greater in ITM than BLEND and ING on day 14, were not different between ITM and BLEND and were greater in ITM and BLEND than ING on day 28, and were not different between treatments on day 42.

There was no DEP × day interaction ($P = 0.52$) for repletion period liver Se concentrations. There was a main effect of DEP on liver Se concentrations (mg/kg DM ± SEM) where ANT (1.47 ± 0.054) was lesser than CON (2.12 ± 0.053; $P < 0.0001$) in the repletion period. Similar to liver Cu concentrations, liver Se concentrations during the repletion period were affected by REP × day ($P < 0.0001$; Fig. 2B) where concentrations within REP strategy were not different on day 0 but were greater in ITM than BLEND and ING on day 14, liver Cu concentrations on day 28 were not different between ITM and BLEND and were greater than ING, and day 42 values were not different among REP treatments.

In the repletion period, there was a tendency for liver Mn concentrations to be affected by the DEP diet (DEP × day, $P = 0.07$; Fig. 1B) where ANT was less than CON on day 0 and day 14, tended to be lesser than CON on day 28, and was not different from CON on day 42. Liver Mn concentrations in the repletion period were also affected by day (REP × day, $P = 0.0001$; Fig. 2C) such that steers randomly assigned to receive ITM had lesser liver Mn concentrations than BLEND and ING on day 0, and ITM and ING were greater than BLEND on day 14; however, there were no differences in liver Mn concentrations due to treatment on day 28 and day 42.

There were no effects of DEP × day or REP × day ($P \geq 0.48$) on repletion period liver Zn concentrations. Additionally, there were no effects of DEP diet ($P = 0.14$) or REP strategy ($P = 0.64$) on liver Zn concentrations in the repletion period. There was a day effect during the repletion period ($P < 0.0001$) where liver Zn concentrations were greater on day 0 than day 14, 28, and 42, and concentrations were greater on day 42 than day 28. Average liver Zn concentrations (mg/kg DM ± SEM) during the repletion period were as follows: day 0 (121 ± 2.4), day 14 (104 ± 1.8), day 28 (102 ± 1.6), and day 42 (107 ± 2.0).

Repletion Period Plasma TM

There was a DEP × REP × day interaction ($P = 0.009$, Table 4) for plasma Mo concentrations in the repletion period. Due to random variation, on day 0 ANT had greater plasma Mo concentrations than all CON treatments such that ANT-BLEND was the greatest, followed by ANT-ITM, and lastly by ANT-ING ($P \leq 0.0001$). Plasma Mo was not different for any treatment on day 14 ($P \geq 0.23$), on day 28 ANT-ING was greater than all other treatments ($P \leq 0.05$), and on day 42 there was a cross-over where ANT treatments were lesser than CON ($P \leq 0.01$).

Table 3. Effect of depletion diet (CON[1] vs. ANT[2]) at the end of the depletion period on liver mineral concentrations, red blood cell lysate glutathione peroxidase activity, and red blood cell lysate manganese superoxide dismutase activity of feedlot steers

	Depletion diet			
	CON	ANT	SEM	*P* value
Liver mineral[3], mg/kg DM				
Cu[4]	251	23	-	0.0001
Mn	9.8	8.5	0.24	0.01
Se	2.00	1.22	0.052	0.0001
Zn	123	118	2.6	0.42
Red blood cell lysate[3]				
GSH-px[5], U × 10^3/g Hb	145.3	143.5	2.52	0.62
Mn-SOD[6], U × 10^3/g Hb	2.91	2.57	0.142	0.09

[1]Control diet provided supplemental trace minerals per kg diet DM: 10 mg of Cu (copper sulfate), 30 mg of Zn (zinc sulfate), 20 mg of Mn (manganese sulfate), 0.5 mg of I (ethylenediamine dihydroiodide), 0.1 mg of Se (sodium selenite), and 0.1 mg of Co (cobalt carbonate).

[2]Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (ethylenediamine dihydroiodide), 0.1 mg of Co (cobalt carbonate), and 2 mg Mo/kg DM, as well as 0.3% S (as $CaSO_4$).

[3]Liver and blood samples were collected after 79 or 80 d on depletion diets.

[4]CON liver Cu SEM is 8.1 mg/kg DM and ANT liver Cu SEM is 2.5 mg/kg DM.

[5]Glutathione peroxidase activity unit is defined as the amount of enzyme necessary for the oxidation of 1.0 nmol of reduced NADPH to $NADP^+$ per minute at 25°C.

[6]Manganese superoxide dismutase activity is defined as the amount of enzyme required to exhibit 50% dismutation of the superoxide radical.

There were no effects of DEP × REP × day, DEP × REP, DEP × day, or REP × day ($P \geq 0.21$) on plasma Se concentrations. In the repletion period, the DEP diet decreased ($P < 0.0001$) plasma Se concentrations in ANT (137 ± 1.4 µg Se/L) relative to CON (145 ± 1.4 µg Se/L), and there was an effect of day ($P < 0.0001$) where plasma Se concentrations (µg/L; ± 1.5) were less on day 0 (132) and day 14 (133) than on day 28 (151) and day 42 (152). There was also a tendency ($P = 0.07$) for REP strategy to affect Se plasma concentrations (µg/L; ± 1.7) such that ITM (144) were greater than BLEND (139) while ING was intermediate (142).

Depletion and Repletion Period Antioxidant Activity

At the end of the depletion period, there were no effects of DEP diet on RBCL GSH-px activity ($P = 0.62$), however there was a tendency ($P = 0.09$, Table 3) for RBCL Mn-SOD to have lesser activity for ANT relative to CON.

In the repletion period, there were no effects of DEP × REP × day, DEP × day, or REP × day on RBCL GSH-px activity ($P \geq 0.78$); however, there was a DEP × REP interaction ($P = 0.04$, Fig. 3) where ANT-ITM, ANT-BLEND, CON-ITM, and CON-ING were lesser than CON-BLEND ($P \leq 0.05$), and ANT-ITM was lesser than ANT-ING ($P = 0.04$). Additionally, day 14 GSH-px activity tended to be lesser ($P = 0.07$, Fig. 4A) than day 0, 28, and 42. There were no effects of DEP × REP × day, DEP × day, REP × day, or DEP × REP on RBCL Mn-SOD activity ($P \geq 0.99$). Repletion period Mn-SOD activity was lesser on day 14 and 28 than day 0 and 42, which did not differ ($P < 0.0001$, Fig. 4B). Activity of Mn-SOD also tended to be lesser for ANT (2.23 ± 0.070 U × 10^3/g Hb) relative to CON (2.42 ± 0.070 U × 10^3/g Hb) during the repletion period ($P = 0.06$).

Steer Growth Performance

At the end of the depletion period, there were no differences in BW due to DEP diet (472 ± 27.1 kg, $P = 0.73$). Body weights collected at the end of depletion were used as covariates for the analysis of repletion period DMI, G:F, and final BW data.

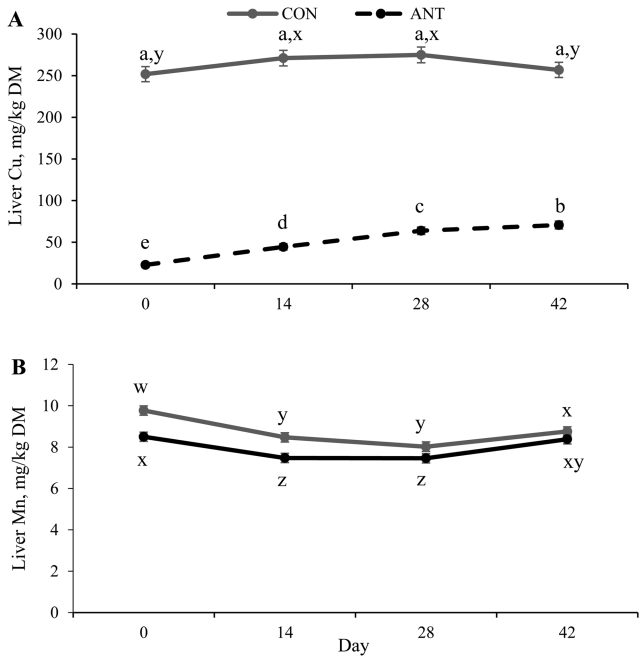

Figure 1. Effect of depletion diets × day of repletion period on liver Cu (Panel A, CON SEM ± 9.2 and ANT SEM ± 3.9; $P < 0.0001$) and liver Mn (Panel B, CON SEM ± 0.22 and ANT SEM ± 0.22; $P = 0.07$). CON diet contained no supplemental S and Mo while ANT supplemented 0.3% S as $CaSO_4$ and 2 mg Mo/kg diet DM (sodium molybdate). Within a panel, superscripts that differ are different (a,b, $P \leq 0.0001$) or tend to be different (x, y, $P \leq 0.09$).

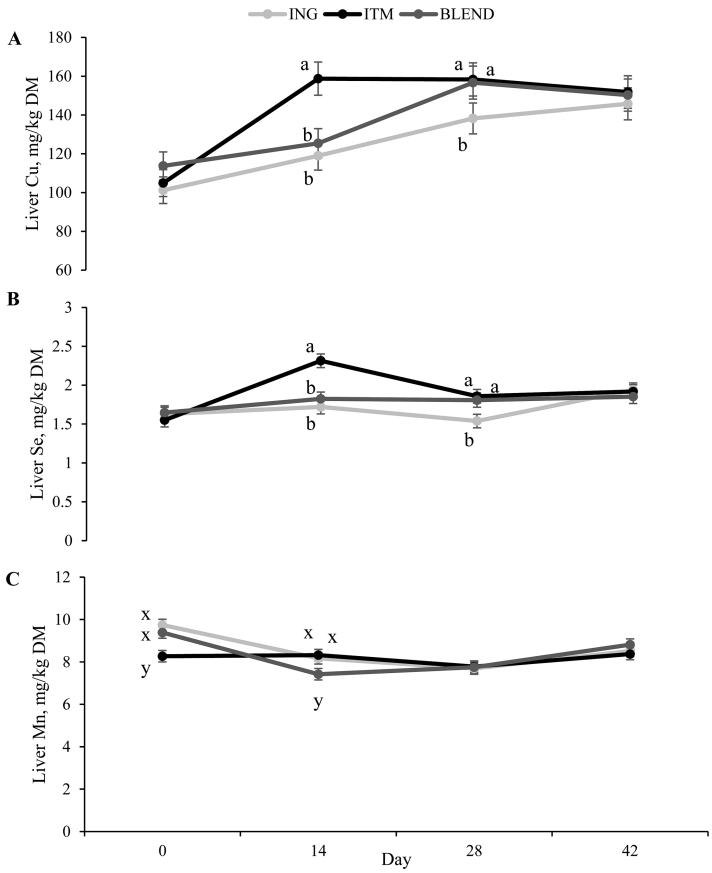

Figure 2. Effect of trace mineral repletion strategy × day of repletion period on liver Cu (Panel A, SEM ± 7.55, $P < 0.0001$), liver Se (Panel B, SEM ± 0.12, $P < 0.0001$), and liver Mn (Panel C, SEM ± 0.27, $P = 0.07$) concentrations. ITM is Multimin90 injection containing Cu, Mn, Se, and Zn and 100% NASEM (2016) supplemental dietary Cu, Mn, Se, and Zn from inorganic sources; BLEND is 150% of NASEM (2016) dietary Cu, Mn, Se, and Zn supplementation from 25% organic and 75% inorganic sources; ING is 150% of NASEM (2016) dietary Cu, Mn, Se, and Zn supplementation from entirely inorganic sources. Within a panel, within a day, superscripts that differ are different (a, b; $P \leq 0.0001$) or tend (x, y; $P \leq 0.06$) to differ.

There was no DEP × REP interaction for repletion period DMI, ADG, G:F, or final BW data ($P \geq 0.60$) and the main effects of DEP diet and REP strategy are shown in Table 5. There was no effect of DEP diet on ADG or final BW ($P = 0.11$); however, steers fed ANT consumed less DM ($P = 0.04$) than CON and had improved G:F in the repletion period ($P = 0.006$). There was no effect of REP strategy on final BW, ADG, or G:F; however, there was a tendency ($P = 0.09$) for repletion period DMI to be affected, where ITM consumed more than ING, while BLEND was intermediate.

DISCUSSION

High dietary S may result from the inclusion of a variety of feedstuffs including ethanol coproducts and byproducts such as molasses, and also through high sulfate water. This, in combination with the presence of forages containing high concentrations of Mo, especially in the western United States, creates likelihoods for unavoidable TM antagonisms in cattle. In the present study, consuming a diet containing high amounts of S and moderate amounts of Mo for 90 d decreased steer liver Cu concentrations by ~90%. Decreased liver Cu is consistent with previous literature when antagonists are fed (Underwood, 1962; Suttle, 1974; Ward, 1978), and it has been reported that dietary Mo can decrease plasma and liver Cu concentrations (Dias et al., 2013). Regardless of initial liver Cu concentrations of ANT steers (ranging 95 to 540 mg Cu/kg DM), the percentage decrease was extremely consistent (ranging 84% to 96%) after the addition of S and Mo to the diet. The average liver Cu concentration of the ANT group following depletion (23 ± 5.3 mg Cu/kg) classified these steers as deficient according to ranges suggested by Mills (1987) and Kincaid (1999). Often producers do not know the TM status of their cattle and may not realize cattle have been exposed to high S or Mo until symptoms of TM deficiency appear. Considering the essential role TM play in growth and immune function (Underwood and Suttle, 1999), it would be advantageous to determine a highly available TM source to rapidly improve TM status. As a result,

Table 4. Effect of repletion strategy (ITM[1] vs. BLEND[2] vs. ING[3]) within depletion diet (CON[4] vs. ANT[5]) by day (DEP × REP × day) on plasma Mo concentrations of feedlot steers during the repletion period

	CON			ANT				
Plasma Mineral	ITM	BLEND	ING	ITM	BLEND	ING	*P*-value	SEM
Mo, µg/L								
Day 0	13.3[d]	12.2[d]	12.7[d]	21.2[b]	23.6[a]	18.6[c]	0.0001	0.99
Day 14	6.6	5.5	6.6	6.7	6.6	6.0	0.23	0.97
Day 28	7.3[b]	6.7[b]	6.9[b]	6.4[b]	7.4[ab]	9.3[a]	0.05	0.97
Day 42	12.2[a]	10.9[a]	11.8[a]	8.4[b]	7.4[b]	7.6[b]	0.01	0.98

[1]ITM: Injectable trace mineral treatment supplemented inorganic forms of trace minerals at 100% of concentrations recommended by NASEM (2016).

[2]BLEND: Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from 25% organic and 75% inorganic sources.

[3]ING: Trace minerals supplemented at 150% of concentrations recommended by NASEM (2016) from entirely inorganic sources.

[4]Control diet contained no supplemental Mo or S.

[5]Antagonist diet provided supplemental dietary minerals: 2 mg Mo/kg DM, and 0.3% S as $CaSO_4$.

Within a day, superscripts that differ are different (a, b; $P \leq 0.01$).

this study was designed to examine the effectiveness of 3 common TM supplementation strategies on TM status and performance of beef steers with depleted TM status.

Injectable TM can improve TM status by avoiding ruminal interactions and competition for absorption in the intestine, providing TM directly to tissues (Pogge et al., 2012). Recent work by others has shown a dose of Multimin90 to increase liver Cu concentrations by approximately 50 mg Cu/kg DM, with advantages in liver Cu over saline-injected cattle maintained for at least 30 d postinjection (Genther and Hansen, 2014). In the present study, there were no interactions between DEP diet and REP strategy, suggesting that the pattern of TM repletion within a REP strategy was similar across diets with or without antagonists. This is clearly noted in the effect of ITM on steers fed ANT and CON diets in the repletion period, where although ANT had lesser Cu status (mg/kg DM) at the start of repletion (23 ± 5.3) than CON (251 ± 8.7), both treatments increased liver Cu by approximately 44 mg Cu/kg DM on day 14 (post-ITM, Fig. 2A). The rapid increase in liver Cu concentrations for steers receiving ITM relative to steers receiving only dietary TM suggests the metabolism of Cu in an injectable TM is not affected by the presence of dietary antagonists. Liver Cu was increased in ITM by day 14, while it took 28 and 42 d for Cu status to improve in steers supplemented with 150% of nationally recommended concentrations from BLEND and ING, respectively. These

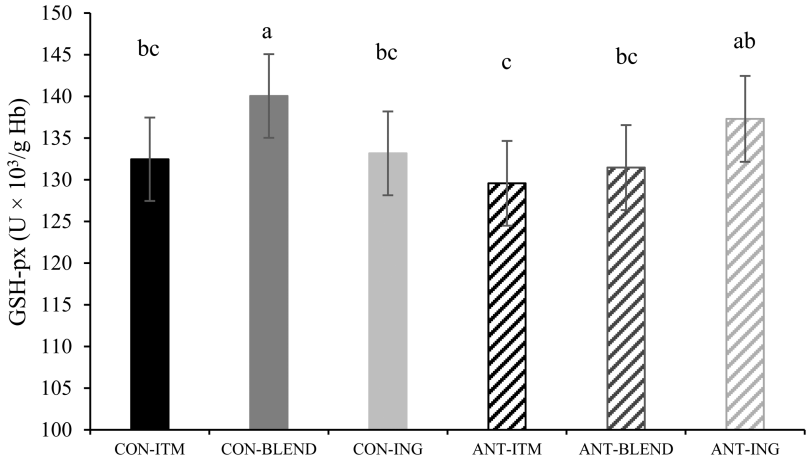

Figure 3. Effect of depletion diets × repletion strategy ($P = 0.04$, ± 3.6) on overall repletion period red blood cell lysate glutathione peroxidase activity. CON diet contained no supplemental S and Mo while ANT supplemented 0.3% S as $CaSO_4$ and 2 mg Mo/kg diet DM (sodium molybdate). ITM is Multimin90 injection containing Cu, Mn, Se, and Zn and 100% NASEM (2016) supplemental dietary Cu, Mn, Se, and Zn from inorganic sources; BLEND is 150% of NASEM (2016) dietary Cu, Mn, Se, and Zn supplementation from 25% organic and 75% inorganic sources; ING is 150% of NASEM (2016) dietary Cu, Mn, Se, and Zn supplementation from entirely inorganic sources. Superscripts that differ are different ($P \leq 0.05$).

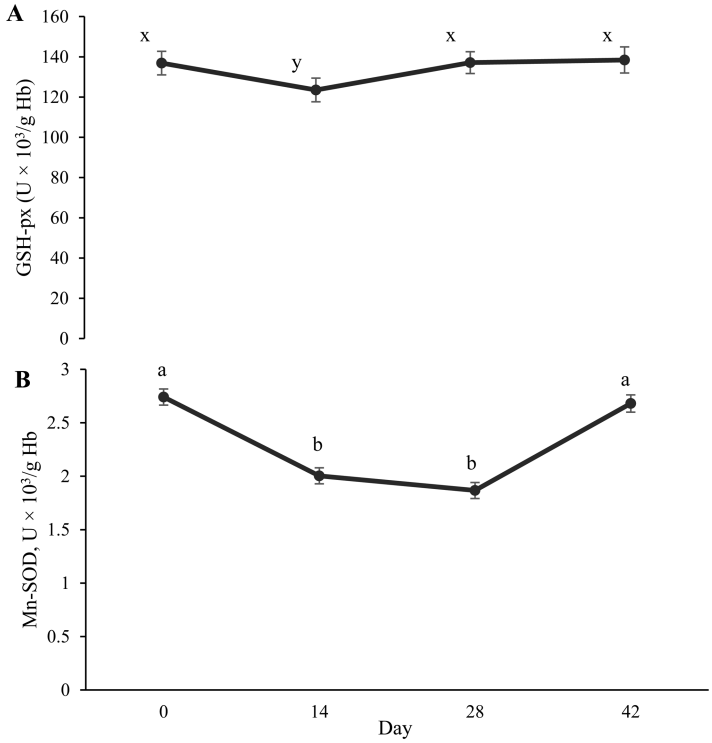

Figure 4. Effect of day of repletion period on red blood cell lysate glutathione peroxidase activity (Panel A, SEM ± 5.9, $P = 0.07$) and Mn superoxide dismutase activity (Panel B, SEM ± 0.77, $P < 0.0001$). Within a panel, superscripts that differ are different (a, b; $P \leq 0.0001$) or tend (x, y; $P \leq 0.1$) to differ.

data suggest any of these strategies would be sufficient depending on the urgency of TM repletion. Additionally, while the first biopsy sample collected during repletion in this study was on day 14, Pogge et al. (2012) collected liver biopsies 1 d post-Multimin90 injection from steers consuming at least 0.28% S and reported steers to have greater liver Cu concentrations on this day relative to saline-injected controls. These data suggest the increase in TM status is likely as rapid as 1 d post-ITM injection, and more work is needed to construct a metabolism curve for ITM early after injection when antagonists are present in the diet.

Research has shown plasma Cu concentrations do not decline until liver Cu stores are depleted below approximately 40 mg Cu/kg DM (Claypool et al., 1975), and plasma Cu has been shown to be an unreliable indicator of Cu status. Although ANT decreased plasma Cu and Se concentrations following depletion, the magnitude of difference in liver Cu between ANT and CON at the end of depletion was not translated to plasma Cu concentrations, thus further solidifying that plasma is a poor Cu status biomarker. In contrast, plasma Se has previously been shown to be a reliable indicator of Se status (Deagan et al., 1987; Kincaid, 1999). Research has shown injectable TM to increase liver Se compared to steers receiving a saline injection, and for the effect to last at least 30 d when all steers were supplemented with TM at 100% of NRC (1996) recommended concentrations (Genther and Hansen, 2014). In the present study, plasma concentrations of Se were lesser in ANT than CON during repletion, and increased over time such that day 28 and day 42 concentrations were greater than day 0 and day 14, regardless of DEP or REP treatment; this is consistent with previous literature (Spears et al., 1986). Additionally, plasma Se concentrations followed the trends of liver Se and tended to be greater in ITM than BLEND over the repletion period.

As suggested by Kincaid (1999), ANT steers were marginally Se deficient following depletion. Increased concentrations of dietary S have been shown to decrease Se status of ruminants; the addition of 0.2 or 0.4% dietary S with between 0.1 and 1.34 mg Se/kg (as sodium selenate) has been reported to decrease Se status in dairy cows (Ivancic and Weiss, 2001) and wethers (Van Ryssen et al., 1998) in a linear fashion. White et al. (1989) investigated the impact of dietary Mo alone on Se absorption by supplementing 10 mg Mo/kg DM to sheep, and reported no changes in blood or liver concentrations of Se. It appears dietary S may decrease Se absorption, as the combination of increased dietary S and no supplemental Se decreased Se status markedly in the 90 d depletion period, and the effect of dietary antagonists persisted across the repletion period, regardless of method of Se repletion.

Table 5. Effect of depletion diets (CON[1] vs. ANT[2]) and trace mineral repletion strategies (ITM[3] vs. BLEND[4] vs. ING[5]) on performance parameters of feedlot steers during the repletion period

	Depletion diet			Repletion strategy				
Performance	CON	ANT	*P*-value	ITM	BLEND	ING	*P*-value	SEM
Final BW, kg	531.3	536.1	0.11	537.8	531.8	531.6	0.16	2.58
ADG, kg	1.26	1.34	0.11	1.37	1.27	1.27	0.16	0.042
DMI, kg	10.4	10.0	0.04	10.5^{x}	10.2^{xy}	10.0^{y}	0.09	0.17
G:F, kg/kg	0.121	0.134	0.006	0.131	0.125	0.128	0.64	0.0040

[1]Control diet contained no supplemental Mo or S, plus 0.5 mg I/kg diet DM (ethylenediamine dihydroiodide), and 0.1 mg Co/kg diet DM (cobalt carbonate).

[2]Antagonist diet provided supplemental trace minerals per kg diet DM: 0.5 mg of I (ethylenediamine dihydroiodide), 0.1 mg of Co (cobalt carbonate), and 2 mg Mo/kg DM, as well as 0.3% S (as $CaSO_4$).

[3]Injectable trace mineral treatment received Multimin90 (1 mL/68 kg BW) on d 0 and inorganic forms of dietary Cu, Mn, Se, and Zn at 100% of concentrations recommended by NASEM (2016).

[4]Saline injection on day 0 and dietary Cu, Mn, Se, and Zn supplemented at 150% of concentrations recommended by NASEM (2016) from a blend of 25% organic and 75% inorganic sources.

[5]Saline injection on day 0 and dietary Cu, Mn, Se, and Zn supplemented at 150% of concentrations recommended by NASEM (2016) from entirely inorganic sources.

Within performance parameter, superscripts that differ tend (x, y; $P \leq 0.06$) to be different.

In the present study, bioavailability is defined as the amount of a substance that can be both absorbed and utilized in the body for physiological purposes (O'Dell, 1983). Previous research has shown some dietary organic TM to have superior absorption compared to inorganic TM when dietary S and Mo are included in cattle diets (Spears, 1989; Suttle, 1991). The increase in liver Cu and Se concentrations from BLEND by day 28 and ING by day 42 in the present trial support these data, and suggest the more rapid increase in liver Cu and Se concentrations in BLEND to be due to the improved bioavailability of the organic TM compared to ING. Hansen et al. (2008) supplemented steers either 5 or 10 mg Cu/kg DM from $CuSO_4$ or CuGly in diets with 2 mg supplemental Mo/kg DM and 0.15% supplemental S for 120 d, and reported the relative bioavailability of the organic source to be 131% compared to the inorganic source. Gao et al. (2014) reported Cu amino acid complex (Availa Cu) had greater absorption across Caco-2 cells when compared to $CaSO_4$, supporting the concept that organic sources often have greater bioavailability in the small intestine. Similarly, while inorganic Se can be easily reduced and made unavailable for absorption by rumen microbes (Wright and Bell, 1966), organic Se has greater bioavailability as it is incorporated into the body through active amino acid uptake (NRC, 2005). It appears that the supplementation of Cu and Se at 150% of nationally recommended concentrations (NASEM, 2016) from entirely inorganic TM is sufficient for TM repletion, however the response is slower when compared to ITM or BLEND strategies.

The mitochondrial antioxidant Mn-SOD is necessary for the conversion of reactive oxygen species to H_2O_2 (Weisiger and Fridovich, 1973). Furthermore, the Se-dependent enzyme GSH-px reduces H_2O_2 to H_2O (Meister, 1984), and has been used as a valuable estimator of Se status (Kincaid, 1995), although Kincaid (1999) has suggested GSH-px may be unreliable to compare between experiments. In the repletion period, ANT steers receiving ITM had lesser GSH-px activity than other treatments. This is in contrast to Pogge et al. (2012), who reported steers receiving an injectable TM had greater RBCL GSH-px activity than those steers receiving a saline injection. While both studies analyzed data as repeated measures, Pogge et al. (2012) reported the greatest GSH-px activity to occur on day 15, while in the present study GSH-px activity was least on day 14 compared to day 0, 28, or 42. A similar trend was noted for RBCL Mn-SOD activity, where lesser activity was recorded on day 14 and 28 compared to day 0 and 42. The decrease in Mn-SOD and GSH-px activity in the repletion period could potentially indicate a greater antioxidant utilization to counteract the increased ambient temperatures occurring during this time. This is consistent with previous literature by Alhidary et al. (2015) in which hot environmental temperatures decreased antioxidant status and promoted oxidative stress in sheep, regardless of the amount of Se supplement provided. Increased ambient temperatures can increase core body temperature, which may lead to greater production of free radicals and reactive oxygen species that in turn cause oxidative stress in the body (Ganaie et al., 2013). Additionally, the decrease in Mn-SOD in ANT

compared to CON could indicate a greater usage of the antioxidant Mn-SOD to counteract the effects oxidative stress caused by additional S and Mo in the ANT diet. However, the response of Mn-SOD during the repletion period could also be partially attributed to a carry over effect from the depletion period, where Mn-SOD activity tended to be lesser in ANT relative to CON. The lifespan of bovine erythrocytes is estimated to be approximately 115 d (Mizuno, 1959), thus it is probable the response noted during the repletion period is partially a response to the 90 d depletion period.

When concentrations of S and Mo are in excess in the rumen without sufficient ruminally soluble Cu to bind, thiomolybdates can be absorbed primarily in the di and tri forms and have inhibitory effects on circulating Cu (Kelleher et al., 1983). In the present study, plasma samples collected on day −10 showed ANT to have much greater plasma Mo concentrations compared to CON; however, after REP strategies began, the concentrations of plasma Mo decreased in ANT such that all steers had equal plasma Mo concentrations on day 14. Though this trial did not explicitly measure circulating thiomolybdates, it is possible the increased concentrations of dietary Cu were able to bind thiomolybdates in the rumen, and therefore decrease those absorbed into circulation. It is unclear why plasma Mo concentrations of CON on day 42 surpassed those of ANT, as dietary Mo supplementation to ANT continued throughout repletion.

In the present study, ANT steers had lesser DMI during repletion, which agrees with previous literature showing high S diets decrease DMI in feedlot cattle (Drewnoski et al., 2014). Interestingly, ANT steers also had more favorable G:F during the repletion period, which may be due to the improvement in TM status of these cattle during this period. Bottje et al. (2002) has reported highly feed efficient birds to have lesser mitochondrial reactive oxygen species and H_2O_2 production, and the TM assessed in the present study, Cu, Mn, Se, and Zn, are critically important in antioxidant activity in the body (Spears and Weiss, 2008; NASEM, 2016).

Less research has focused on how dietary antagonists such as S or Mo may affect Mn and Zn status of cattle. In the present study, dietary antagonists decreased liver Mn and plasma Zn assessed at the end of depletion; however, there were no effects of ANT on liver Zn concentrations at the end of depletion, which is in agreement with previous research (Daughtery et al., 2002). As defined by Kincaid (1999), steers at the end of depletion had adequate Zn status, as liver Zn concentrations were easily within the range of 25 to 200 mg Zn/kg DM. In repletion, due to random variation, steers assigned to receive ITM had lesser Mn concentrations on day 0. There was a decrease in liver Mn concentrations of BLEND and ING on day 14, which could have been related to the excessively warm summer temperatures shortly prior to that biopsy collection day. Interestingly, ITM Mn concentrations maintained their status during this time, suggesting ITM had additional available Mn to utilize in body functions.

In conclusion, greater concentrations of S and Mo greatly decreased Cu status in beef cattle, and had negative effects on Se, Mn, and Zn status. Through the repletion period, regardless of dietary antagonists, ITM had the most rapidly improved Cu and Se status, steers receiving BLEND reached similar status by day 28 and ING by day 42. Additionally, the improved G:F noted during the repletion period in ANT steers suggests some kind of compensational gain as TM status of the steers improved. There were very few DEP × REP interactions during the trial, indicating TM repletion is similar within REP strategy, regardless of presence of the antagonists S and Mo. The use of an injectable TM most rapidly improved TM status of steers from a deficient to mildly deficient, or adequate state. Excessive supplementation of TM may result in greater concentrations of excretion of TM in feces, which may introduce greater concentrations of TM into the environment and ultimately result in an economic loss for producers. The optimization of supplemental TM strategies to overcome dietary antagonists may assist in creating the greatest efficiency of production with the least environmental impact.

Conflict of interest statement. None declared.

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