Effect of a multielement trace mineral injection before transit stress on inflammatory response, growth performance, and carcass characteristics of beef steers[1]

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ABSTRACT: Weaned calves (n = 98; 256 ± 11.5 kg) were used to evaluate the impact of improving trace mineral (TM) status using a multielement TM injection 28 d before transit on markers of inflammatory and stress responses in response to transit and post-shipping growth performance. On d 0 of a 28-d preconditioning program, calves received subcutaneous TM injection (MM; n = 48) containing 15, 10, 5, and 60 mg/mL of Cu, Mn, Se, and Zn, respectively, or physiological saline injection (SAL; n = 48). On d 28, steers were weighed, half of the steers from each treatment were transported for a 20-h transit stress period (SHIP; n = 24 per injection treatment), and half of the steers were returned to their pens for 20 h of feed and water restriction without transit (NOSHIP; n = 24 per injection treatment). The SHIP steers were unloaded on d 29 and all steers (SHIP and NOSHIP) were immediately weighed and sorted into new pens (n = 4 steers per pen) for the growing period. At the start of finishing (d 113), steers received a second MM or SAL, resulting in a 2 × 2 × 2 factorial (n = 12 steers per treatment combination). Samples of blood were collected on d 28, 29, and 34 and liver on d 22 and 40. The initial MM increased liver Cu, Se, and Zn concentrations of cattle ($P \leq 0.02$) but did not affect ADG during preconditioning ($P = 0.89$) or BW shrink as a result of transit ($P \geq 0.52$). Plasma Fe concentrations were decreased after the transit stress period in SHIP calves ($P \leq 0.05$) relative to NOSHIP calves but recovered 5 d after transit, and serum IL-8 concentrations were greater in SAL–SHIP steers than MM–SHIP steers ($P = 0.04$). Altering TM status through MM caused steers to have lesser ADG ($P = 0.03$) during the 14-d period after transit (d 29 through 43) but did not affect growth during the growing period (d 5 through 112; $P \geq 0.40$). Minimal effects on finishing performance and carcass characteristics were noted, but there was a 3-way interaction ($P \leq 0.02$) in which SAL–NOSHIP–MM steers had the greatest yield grade (YG) and smallest ribeye area (REA) and SAL–SHIP–MM steers had the least YG and largest REA. Overall, a MM 28 d before transit or before feed and water restriction did not affect the inflammatory response or plasma TM concentrations but decreased ADG in the 14-d period after transit. Trace mineral injection had limited effects on overall growth performance and carcass characteristics, likely because steer initial TM status was well within the adequate range.

Key words: beef cattle, trace mineral, transportation

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INTRODUCTION

Feed and water deprivation and physiological stress associated with transit of cattle can cause BW loss (Hutcheson and Cole, 1986) and decrease growth after transit (Kegley et al., 1997; Marques et al., 2012). Chirase et al. (2004) demonstrated that transit increases oxidative stress and inflammation in cattle, potentially leaving them vulnerable to a disease challenge. Additionally, mildly trace mineral (**TM**)–deficient steers tended to lose more BW during transit than steers with adequate TM status, and shipping loss was negatively correlated with liver Cu and Se concentrations (Genther and Hansen, 2014b). Trace

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mineral injection rapidly increases liver Cu and Se concentrations (Genther and Hansen, 2014a) and has been shown to increase the activity of at least 2 critical TM-containing antioxidant enzymes, Mn-superoxide dismutase (Genther and Hansen, 2014a), and Se-containing glutathione peroxidase (Pogge et al., 2012).

Unfortunately, newly received cattle often eat less, and limited TM intake may decrease overall TM status, and the NRC (2000) recommends increasing receiving diet TM concentrations to approximately 150% of nonstressed cattle requirements to maintain adequate TM consumption. Preconditioning cattle may minimize the effects of transit (Duff and Galyean, 2007), preventing concurrent stresses of weaning and transit, and may be an optimal time to improve TM status through TM-fortified rations or TM injection. The objective of this study was to determine the influence of rapidly improving TM status of steers using a TM injection 28 d before transit on BW loss and markers of stress and inflammation in response to transit as well as post-transit growth. A secondary objective was to determine how a TM injection given at the beginning of the preconditioning period and a second injection at reimplant time (80 d before harvest) would affect carcass characteristics of growing and finishing steers.

Table 1. Experimental schedule

Day	Activity[1]	Experimental period
–1	BW	
0	BW and initial injection (MM[2] or SAL)	Preconditioning period (d 0 to 27)
22	Liver biopsies	
28	BW, blood, RT, and start of SHIP or NOSHIP	Transit stress period (d 28 to 29)
29	End of SHIP or NOSHIP, BW, and blood RT	14-d post-transit period (d 29 to 43)
34	BW and blood	
40	Liver biopsies	
43	Fasted BW (after 20-h fast)	
57	BW	Growing period (d 34 to 112)
85	BW	
112	BW	
113	BW and second injection (MM or SAL)	
141	BW	Finishing period (d 113 to 193)
169	BW	
192	BW	
193	BW and shipped to abattoir	

[1]MM = trace mineral injection; SAL = saline injection; RT = rectal temperature; SHIP = transported for a 20-h transit stress period; NOSHIP = 20 h of feed and water restriction without transit.

[2]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

MATERIALS AND METHODS

All procedures and protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (log number 7-13-7602-B).

Preconditioning Period

This experiment was conducted at the Iowa State University Beef Nutrition Research center in Ames, IA. The overall experimental schedule can be found in Table 1. Ninety-six Angus-crossbred steers were purchased from a single source, weaned, and immediately loaded onto a tractor-trailer and transported approximately 790 km (approximately 8 h on truck) to Ames, IA. Steers were vaccinated against clostridial bacterin-toxoid and *Haemophilus somnus* (Vision 7 Somnus; Merck Animal Health, Summit, NJ); infectious bovine rhinotracheitis virus, type I and II bovine virus diarrhea virus, parainfluenza-3 virus, and bovine respiratory syncytial virus (Bovishield Gold 5; Zoetis, Kalamazoo, MI); and *Mannheimia haemolytica* (Nuplura PH; Novartis Animal Health US, Inc., Greensboro, NC) 14 d before arrival. Forty-eight hours after arrival at Iowa State University, BW were measured on d –1 and 0, and on the second day (d 0 of the study) steers were stratified by BW (256 ± 11.5 kg initial BW) into 1 of 4 pens. Steers received 1 of 2 initial injection (**Inj1**) treatments (n = 2 pens and 48 steers per treatment), subcutaneous TM injection (**MM**; Multimin90; Multimin USA, Fort Collins, CO) or subcutaneous sterilized physiological saline injection (**SAL**) at a rate of 1 mL/45.4 kg of BW. The MM provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA). At the initiation of the study, steers were revaccinated with a multivalent respiratory vaccine (Bovishield Gold 5; Zoetis) and were dewormed with eprinomectin (Eprinex; Merial Ltd., Iselin, NJ) and identified with a unique ear tag. Steers were fed a common hay–based diet (Table 2) for 28 d (denoted as the preconditioning period). Steers were fed for ad libitum intake throughout the entire experiment. Bunk scores were recorded daily, and bunks were managed using a modified slick bunk system as detailed by Drewnoski et al. (2014). On d 22, liver biopsies for TM determination were collected from 48 randomly selected steers (24 per treatment; 12 per pen). Liver biopsies were also taken on d 1 from 14 steers purchased from the same source that were not enrolled in the experiment to establish an approximation of TM status of the steers before start of the study. Liver biopsy samples were collected for TM analysis using the method of Engle and Spears (2000).

Table 2. Diet composition for the preconditioning, growing, and finishing periods

	Experimental period[1]		
	Preconditioning	Growing	Finishing
Ingredient	% of diet DM		
Grass hay	54	–	–
Dry-rolled corn	14.5	15	55
Dried distillers grains	22.9	33.1	28.1
Molasses	5	–	–
Soybean meal	2	–	–
Corn silage	–	50	15
Limestone	1.2	1.47	1.47
Salt	0.31	0.31	0.31
Vitamin A premix[2]	0.11	0.11	0.11
Trace mineral premix[3]	0.024	0.024	0.024
Rumensin-90[4]	–	0.014	0.014
Calculated composition[5]			
CP, %	14.3	14.2	13.3
NDF, %	44.9	28.9	18.5
Ether extract, %	4.2	5.0	5.0
NEg, Mcal/kg	0.84	1.21	1.36
Se, mg/kg	0.30	0.33	0.31
Analyzed composition	mg/kg diet DM		
Cu	8.3	14.9	15.4
Fe	170.2	134.4	75.6
Mn	44.0	41.1	34.1
Zn	30.0	47.8	58.1

[1]The preconditioning period diet was fed d 0 through 27, the growing period diet was fed d 29 through 112, and the finishing diet was fed d 113 through 193.

[2]Vitamin A premix contained 4,400,000 IU vitamin A/kg.

[3]Provided per kilogram of diet: 30 mg Zn, 20 mg Mn, 0.5 mg I, 0.1 mg Se, 10 mg Cu, and 0.1 mg Co (all inorganic sources).

[4]Provided monensin at 30 mg/kg of diet DM (Elanco Animal Health, Greenfield, IN).

[5]Composition was calculated using values from the NRC (2000) and Corah and Dargatz (1996).

Transit Period

On d 28, steers were separated into additional treatments to create a 2 × 2 factorial design of Inj1 and shipping (transit) treatments (**Trans**). One pen from each treatment (n = 24 steers per treatment) was randomly selected for shipment and was transported for a 20-h transit stress period (**SHIP**; 1,675 km) while the remaining 48 steers (n = 24 per treatment) stayed at the Iowa State University Beef Nutrition Farm but were withheld from feed and water to serve as nonshipped controls (20 h of feed and water restriction without transit; **NOSHIP**). All pens received the same amount of feed at 0700 h on d 28 (approximately 25% of DMI). At approximately 1000 h, immediately before shipping, all steers were weighed and had rectal temperatures collected, and blood samples were collected from the same 48 steers that were previously biopsied for liver TM analysis (n = 24 per treatment; 12 per pen). After weights and samples were collected, SHIP steers were loaded onto a tractor-trailer (1100 h) and NOSHIP were steers returned to their pens, without access to feed and water. Steers were received back to the Iowa State University Beef Nutrition Research Center at 0700 h on d 29, BW and rectal temperatures were again collected from all steers, and blood was again collected from the same 48 sampling steers. Steers were then resorted into new pens in a separate barn, with 4 steers per pen (n = 6 pens per Inj1 × Trans treatment combination) and began receiving a growing diet (Table 2).

Fourteen-Day Postshipping Period and Growing Period

On d 34 (5 d postshipping), steers were weighed, and blood samples from the 48 sampler steers were collected. On d 40, liver biopsy samples for TM analysis were also collected from the same 48 sampler steers. To determine the effect of transit stress on short-term steer growth performance, all steers were withheld from feed and water for 20 h and weighed on the morning of d 43 (14 d postshipping). The growing period was considered the period from d 34 through 112. Steers were implanted with Component E-S with Tylan (Elanco Animal Health, Greenfield, IN) on d 57. Body weights during the growing period were collected on d 34, 57, 85, 112, and 113 of the experiment. On d 85, steers began the first of four 7-d step-up diets, in which corn gradually replaced corn silage, to transition steers to the final finishing diet, which began on d 113 (Table 2).

Finishing Period

On d 113, steers were randomly assigned within previous treatments to a second injection (**Inj2**) of another TM injection (MM; Multimin90) or sterilized saline (SAL). This created a 2 × 2 × 2 factorial design of Inj1, Trans, and Inj2 with 12 steers per treatment combination (3 pens per treatment combination; 4 steers per pen). Weights were measured on d 141, 169, 192, and 193, at the end of the experiment. Steers were implanted with Component TE-IS with Tylan (Elanco Animal Health) on d 113. On d 193, steers were shipped to a commercial abattoir in Denison, IA (Tyson Fresh Meats), where steers were harvested and HCW data were collected. After a 24-h chill, carcasses were ribbed between the 12th and 13th ribs and graded according to USDA standards. Carcass data were collected at the plant by representatives of the Tri-County Carcass Futurity (Iowa State University Beef Extension, Lewis, IA), who were masked to treatment. Data collected included ribeye area (**REA**), 12th rib

backfat (**BF**), KPH, marbling score, USDA quality grade (**QG**), and USDA yield grade (**YG**).

Feed and Tissue Sampling

Samples of the diet total mixed ration were collected weekly and dried in a forced air oven at 70°C for 48 h for DM determination. Steer DMI was calculated using as-fed intakes corrected for DM of weekly diet samples. Feed efficiency (G:F) was calculated from the total gain and total DMI monthly as determined by weighing intervals. Diet samples were ground through a 2-mm screen in a Wiley Mill (Thomas Scientific, Swedesboro, NJ), and samples were composited by treatment by month for TM analysis.

Jugular blood samples were collected into K_2EDTA vacuum tubes for plasma TM analysis (7 mL), K_2EDTA vacuum tubes for whole blood collection (4 mL), sodium heparin vacuum tubes for plasma collection (10 mL), and vacuum tubes without additive for serum collection (10 mL; Becton, Dickinson, and Co, Franklin Lakes, NJ).

Tissue Analysis

Blood samples were centrifuged at 1,200 × *g* for 10 min at 4°C and serum or plasma was harvested and stored at –80°C until analysis. Liver samples were dried in a forced-air oven at 70°C and digested using TM-grade nitric acid before mineral analysis (MARSXpress; CEMS, Matthews, NC) as described by Richter et al. (2012). Liver and plasma mineral concentrations were determined as described by Pogge et al. (2012) using inductively coupled plasma mass spectroscopy (PerkinElmer, Waltham, MA). Feed mineral concentrations were determined using inductively coupled plasma optic emission spectroscopy (PerkinElmer) as previously described (Richter et al., 2012). Whole blood samples were analyzed at the Clinical Pathology Laboratory at Iowa State University (Ames, IA) for a complete blood count and automated differentiation of white blood cells after collection. A serum aliquot was sent to the Kansas State University Diagnostic Laboratory (Manhattan, KS) for serum haptoglobin analysis using the method described by Smith et al. (1998). Serum IL-8 concentrations were analyzed using a commercial ELISA kit shown to cross-react with bovine samples (Quanitkine ELISA, Human CXCL8/IL-8; R&D Systems, Minneapolis, MN; catalog number D8000C). Plasma ceruloplasmin concentrations were analyzed using the method of Houchin (1958). Plasma cortisol concentrations were analyzed using a commercially available bovine cortisol ELISA kit (Bovine Cortisol ELISA test kit; Endocrine Technologies, Newark, CA; catalog number ERK A1004).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS 9.2 (SAS Inst., Inc, Cary, NC). Data for the preconditioning period were analyzed as a completely randomized design. The statistical model included the fixed effect of individual steer Inj1 (SAL or MM).

Data for the transit stress period, the 14-d post-transit period, and the growing period were analyzed as a 2 × 2 factorial, in which the statistical model included the fixed effects of Inj1 (SAL or MM) and Trans (NOSHIP or SHIP) and interactions. One steer died just before the start of the growing period (SAL–SHIP), of causes unrelated to treatment, and its data were excluded from the study. The models for 14-d postshipping period DMI and growing period DMI, BW, ADG, and G:F included day as the repeated effect, and the subject for the repeated statement was steer nested within Inj1 × Trans. Pretransit rectal temperature was used as a covariate in the analysis of post-transit rectal temperature.

Data for the finishing period were analyzed as a 2 × 2 × 2 factorial, in which the statistical model included the fixed effects of Inj1 (SAL or MM), Trans (NOSHIP or SHIP), and Inj2 (SAL or MM) and interactions. One steer died during the finishing period (MM–SHIP–MM), of causes unrelated to treatment, and its data were excluded from analysis. Day was the repeated effect for the finishing period DMI, BW, ADG, and G:F data analysis, and the subject was steer nested within Inj1 × Trans × Inj2.

The experimental unit for all phases was steer, except for DMI and G:F, where the experimental unit was pen. Antedependence 1 was selected as the covariance structure for analysis of all blood analyte data, and autoregressive 1 covariance structure was used for all performance variables based on the lowest Akaike information criterion. Data were checked for normalcy and homogeneity of variance and a natural log transformation was necessary for serum haptoglobin concentrations, but data presented are back-transformed and SEM presented were determined using the Delta method of approximation (Casella and Berger, 1990). Outliers were determined using Cook's D statistic and removed if Cook's D > 0.5. The data reported are least squares means ± SEM, and differences among means were determined using the PDIFF statement in SAS. Significance was declared at $P \leq 0.05$ and tendencies were declared from $P = 0.06$ to 0.10.

RESULTS

Preconditioning Period

Preconditioning period performance was unaffected by MM ($P \geq 0.28$; data not shown; average DMI, ADG, G:F, and ending BW were 3.56 ± 0.08 kg, 1.55 ± 0.054 kg/d, 0.240 ± 0.005 kg, and 299 ± 1.5 kg, respectively, across all treatments). Average liver TM concentrations of contemporary steers not included in the experiment were (per kg liver DM) 238 ± 67 mg Cu, 179 ± 37 mg Fe, 9.1 ± 1.2 mg Mn, 1.7 ± 0.32 mg Se, and 143 ± 35 mg Zn. Liver concentrations of Cu ($P = 0.005$; Table 3), Se ($P < 0.0001$), and Zn ($P = 0.02$) were increased by MM compared with SAL as measured 22 d postinjection. Liver Mn ($P = 0.22$) and Fe ($P = 0.98$) concentrations were not affected by injection (Table 3).

Table 3. Effect of a trace mineral injection (MM[1]) or saline injection (SAL) injection on liver trace mineral concentrations of steers assessed 22 d postinjection[2]

Mineral, mg/kg DM	Initial injection		SEM	*P*-value
	SAL	MM		
Cu	272	328	13.6	0.005
Fe	206	206	10.4	0.98
Mn	9.62	9.24	0.218	0.22
Se	2.14	3.91	0.079	<0.0001
Zn	129	151	6.5	0.02

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2]Liver biopsy samples were collected on d 22 of the experiment, 22 d after the initial injection, from 48 steers total ($n = 24$ per treatment).

Transit Stress Period

Neither Inj1 ($P = 0.89$) nor Trans ($P = 0.19$) nor the interaction ($P = 0.88$) affected BW following the transit stress period (Table 4). However, there was an interaction between Inj1 and Trans ($P = 0.05$) in percent shrink, where SAL–NOSHIP steers had less shrink than SAL–SHIP ($P < 0.0001$) and MM–SHIP steers ($P = 0.003$) but were not different from MM–NOSHIP steers ($P = 0.11$; Table 4). Interestingly, there was no difference in shrink between SHIP and NOSHIP steers that received MM ($P = 0.15$). There was no interaction between Inj1 and Trans on rectal temperature at the end of the transit stress period ($P = 0.95$; Table 4), but steers that received MM had greater rectal temperatures regardless of shipping treatment ($P = 0.03$) and SHIP steers had greater rectal temperatures than NOSHIP steers ($P = 0.01$). There was no effect of Inj1 on change in rectal temperature during the transit period, but SHIP cattle had less change than NOSHIP cattle ($P = 0.03$).

There was an interaction between Trans and Inj1 on liver Cu concentrations ($P = 0.009$; Table 5) measured 40 d postinjection (12 d post-transit). The Trans did not affect liver Cu concentrations of steers that initially received SAL ($P = 0.13$), but MM–SHIP steers had lesser liver Cu concentrations than MM–NOSHIP steers ($P = 0.02$). However, liver Cu concentrations of MM–SHIP steers were not different from either SAL–SHIP or SAL–NOSHIP steers ($P \geq 0.17$). A similar trend was observed within liver Se concentrations, where there was an interaction between Inj1 and Trans ($P = 0.02$; Table 5). The MM–SHIP steers tended to have lesser liver Se concentrations than MM–NOSHIP steers ($P = 0.097$), and there was a tendency for SAL–SHIP steers to have greater liver Se concentrations than SAL–NOSHIP steers ($P = 0.07$). The MM steers had greater liver Cu ($P = 0.01$) and Se ($P < 0.0001$) and tended to have greater liver Zn concentrations ($P = 0.07$) than SAL steers (Table 5).

Blood Trace Mineral Profile

Plasma TM concentration data are displayed in Fig. 1. There was a tendency for an interaction between Inj1, Trans, and day of sampling ($P \leq 0.08$) for plasma Cu, Fe, Se, and Zn concentrations (Fig. 1). There was a 2-way interaction in plasma Fe between Trans and day of sampling ($P < 0.0001$), where SHIP steers had decreased plasma Fe concentrations immediately after shipping relative to NOSHIP steers. Within plasma Se, initially there were differences between treatments, where MM–SHIP and MM–NOSHIP were not different ($P = 0.74$) and SAL–SHIP had lesser plasma Se concentrations than SAL–NOSHIP steers ($P = 0.001$), but there were no differences immediately post-transit and 5 d post-transit ($P \geq 0.18$). There was a similar trend within plasma Cu and Zn for a Trans × day effect ($P \leq 0.07$), where the SAL–SHIP steers tended to have lesser plasma Cu and Zn concentrations than other steers before shipping ($P \leq 0.10$), but there were no differences immediately post-transit or 5 d post-transit ($P \geq 0.17$). Plasma Mn concentrations (data not shown in Fig. 1) were unaffected by Inj1 (4.3 and 3.6 ± 0.40 μg/L for MM and SAL, respectively; $P = 0.22$) or transit (4.3 and 3.6 ± 0.40 μg/L for SHIP and NOSHIP, respectively; $P = 0.25$).

Complete Blood Count

There was a Trans × day interaction ($P = 0.001$; Table 6) for neutrophil numbers, where there was no difference between SHIP and NOSHIP steers preshipping ($P = 0.77$) and 5 d postshipping ($P = 0.12$) but greater blood neutrophils in SHIP vs. NOSHIP steers

Table 4. Effect of a trace mineral injection (MM[1]) or saline injection (SAL) on growth performance and rectal temperatures of steers as impacted by being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP)

	Injection 1	SAL		MM			*P*-value[2]		
Variable	Transit	NOSHIP	SHIP	NOSHIP	SHIP	SEM	Inj1	Trans	Inj1 × Trans
Initial BW,[3] kg		298	298	299	297	3.5	0.95	0.75	0.81
Ending BW,[4] kg		278	273	277	273	2.9	0.89	0.19	0.88
BW shrink,[5] %		7.2[c]	8.8[a]	7.8[bc]	8.3[ab]	0.27	0.79	0.0001	0.05
Rectal temperature change,[6] °C		–0.83	–0.51	–0.64	–0.59	0.085	0.52	0.03	0.12

[a,b]Within rows, means without a common superscript differ ($P \leq 0.05$).

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2] Inj1 = initial injection; Trans = transit treatment.

[3]Initial BW is the BW collected at the beginning of the transit stress period, where steers were either weighed and directly loaded onto a tractor-trailer for transit or were weighed and returned to their pens without access to feed and water.

[4]Ending BW is the BW collected at the end of the transit stress period, and steers were weighed either on return to the facility or on the end of the feed and water restriction.

[5] Calculated as (initial BW – ending BW)/initial BW × 100%.

[6]Rectal temperature change was calculated from initial and ending transit period rectal temperatures. A negative rectal temperature change indicates that temperature decreased over the transit stress period.

($P = 0.02$) immediately postshipping. This is reflected in the Trans × day interaction ($P = 0.02$; Table 6) within blood leukocyte count data, although individual means comparisons did not show any differences between treatments ($P = 0.15$). There was no effect of Inj1 or Trans or any associated interactions on blood lymphocyte or monocyte counts ($P \geq 0.26$; data not shown), but there was an effect of day, where, regardless of Inj1 or Trans, all steers had lesser blood lymphocytes immediately postshipping (data not shown in tabular form, 6.84 ± 0.236 cells × 10^3/μL) relative to preshipping (7.82 ± 0.239 cells × 10^3/μL) and 5 d postshipping numbers (7.58 ± 0.218 cells × 10^3/μL; $P < 0.0001$). There was an effect of day on blood monocytes ($P < 0.0001$), where all steers had lesser blood monocytes immediately postshipping (0.50 ± 0.018 cells × 10^3/μL; $P < 0.001$) relative to pretransit (0.54 ± 0.017 cells × 10^3/μL) and 5 d post-transit (0.63 ± 0.029

Table 5. Effect of a trace mineral injection (MM[1]) or saline injection (SAL) on liver trace mineral concentrations of steers 40 d postinjection and 11 d after being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP)[2]

	Injection 1					*P*-value[3]		
	SAL		MM					
	Transit							
Mineral, mg/kg DM	NOSHIP	SHIP	NOSHIP	SHIP	SEM	Inj1	Trans	Inj1 × Trans
Day 22, postinjection[4]								
Cu	256	287	345	312	19.1	0.005	0.99	0.11
Fe	198	215	193	222	14.7	0.95	0.13	0.69
Mn	9.4	9.7	9.5	9.0	0.31	0.21	0.80	0.23
Se	2.13	2.15	4.00	3.82	0.112	<0.0001	0.52	0.41
Zn	135	122	157	145	9.2	0.02	0.19	0.99
Day 40, postinjection								
Cu	279[b]	319[b]	378[a]	316[b]	18.6	0.01	0.55	0.009
Fe	160	178	184	182	12.2	0.25	0.51	0.41
Mn	7.6	7.3	7.9	7.3	0.26	0.58	0.17	0.61
Se	1.97[d]	2.25[c]	3.01[a]	2.75[b]	0.107	<0.0001	0.93	0.02
Zn	123	110	128	131	6.8	0.07	0.48	0.23

[a,b]Within rows, means without a common superscript differ ($P \leq 0.05$).

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2]12 steers per treatment.

[3]The main effects in statistical analysis were initial injection (Inj1), transit treatment (Trans), and their interaction (Inj1 × Trans).

[4]Day 22 postinjection liver biopsies were collected before transit; however, displayed values have been analyzed to include transit treatments for ease of comparison with post-transit values.

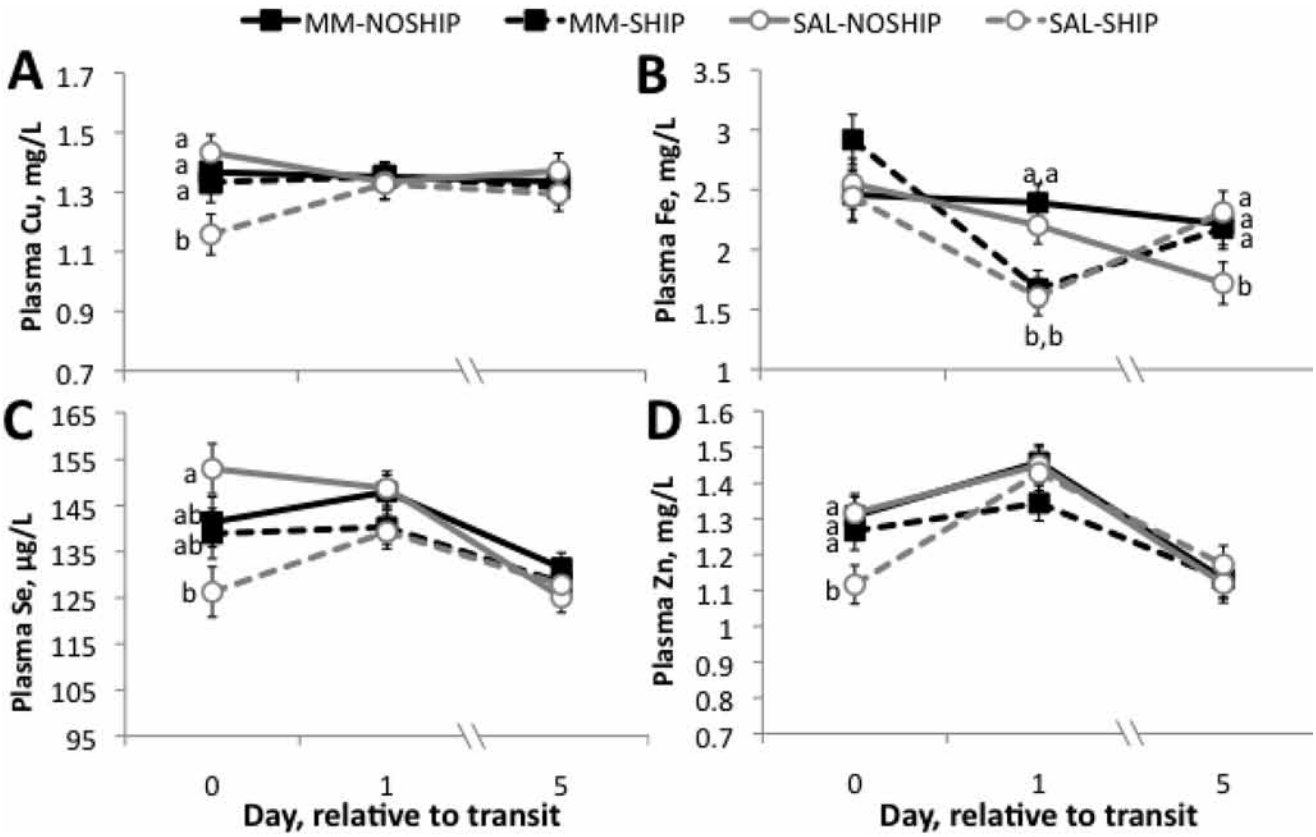

Figure 1. Effect of a trace mineral injection (MM) or saline injection (SAL) after being transported for a 20-h transit stress period (SHIP) or 20 h feed and water restriction without transit (NOSHIP), on plasma Cu concentrations (A), plasma Fe concentrations (B), plasma Se concentrations (C), and plasma Zn concentrations (D); values are means ± SEM; $n = 12$ per initial injection (Inj1) × transit treatment (Trans) combination. The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA). Within plasma Cu, there was an Inj1 × Trans × day interaction ($P = 0.06$). Within plasma Fe, there was an Inj1 × Trans × day interaction ($P = 0.08$). Within plasma Se, there was an Inj1 × Trans × day interaction ($P = 0.05$), and within plasma Zn there was an Inj1 × Trans × day interaction ($P = 0.07$). [a,b]Lowercase letters that differ denote differences ($P \leq 0.10$) between Inj1 and Trans within day.

cells × 10^3/µL). The SHIP steers had more blood eosinophils than NOSHIP steers ($P = 0.06$; Table 6), and there was a tendency for an Inj1 × day interaction ($P = 0.10$; data not shown) where MM steers had greater blood eosinophil numbers than SAL steers immediately after shipping ($P = 0.03$) but were not different preshipping ($P = 0.41$) or 5 d postshipping ($P = 0.60$). There was also an interaction between Trans and day in blood basophil counts ($P = 0.04$; Table 6), although individual mean comparisons did not reveal any differences between treatments ($P \geq 0.18$). The Inj1 × Trans × day interaction was similar within erythrocyte count, hemoglobin concentration, and hematocrit ($P \leq 0.01$), where all steers, regardless of treatment, had increased blood erythrocytes, hemoglobin, and hematocrit immediately post-transit ($P < 0.0001$; data not shown).

Inflammatory and Stress Response Markers

There was no effect of Inj1, Trans, or day on plasma ceruloplasmin concentrations ($P \geq 0.13$; data not shown). There was no effect of Inj1 or Trans on serum haptoglobin concentrations ($P \leq 0.51$; data not shown), but there was an effect of day ($P < 0.0001$), where all steers, regardless of treatment, had increased serum haptoglobin concentrations immediately postshipping (11.3 ± 0.66 mg/dL; $P = 0.0001$) relative to preshipping concentrations (3.8 ± 0.41 mg/dL) and seru haptoglobin concentrations were also decreased 5 d postshipping relative to immediately postshipping (7.6 ± 0.50 mg/dL; $P = 0.0001$). There was a tendency for an Inj1 × Trans interaction ($P = 0.06$) within serum IL-8 concentrations (Fig. 2), where throughout the transit and 5 d post-transit period, MM–SHIP steers had lesser serum IL8 concentrations than SAL–SHIP steers ($P = 0.04$) and tended to have lesser serum IL-8 concentrations than SAL–NOSHIP steers ($P = 0.07$). Finally, there was a tendency for a Trans × day interaction within plasma fibrinogen concentrations ($P = 0.10$), where SHIP steers had lesser plasma fibrinogen concentrations (375 ± 27.1 mg/dL) than NOSHIP steers (460 ± 28.5 mg/dL; $P = 0.04$) before shipping but were not different from NOSHIP steers immediately postshipping (729 ± 57.6 and 615 ± 57.6 mg/dL, respectively; $P = 0.17$) or 5 d postshipping (532 ± 38.3 and 579 ± 37.4 mg/dL, respectively; $P = 0.39$). All

Table 6. The effect of being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP) on blood leukocyte profile of steers

	Transit treatment			*P*-value[1]		
Cell number, $\times 10^3/\mu L$	NOSHIP	SHIP	SEM	Trans	Day	Trans × day
Leukocytes[2,3]	11.05	11.42	0.398	0.52	<0.0001	0.02
Day 0[4]	11.67	11.80	0.471	0.84	–	–
Day 1[5]	9.83	10.80	0.472	0.15	–	–
Day 5[6]	11.63	11.59	0.391	0.94	–	–
Neutrophils[2,3]	2.86	3.01	0.203	0.61	0.03	0.0001
Day 0[3]	3.12	3.01	0.270	0.77	–	–
Day 1[4]	2.26[a]	3.19[b]	0.272	0.02	–	–
Day 5[5]	3.21	2.84	0.170	0.12	–	–
Basophils[2,3]	0.15	0.15	0.0058	0.84	0.002	0.04
Day 0[3]	0.16	0.16	0.0070	0.37	–	–
Day 1[4]	0.15	0.13	0.0075	0.18	–	–
Day 5[5]	0.15	0.15	0.0071	0.76	–	–
Eosinophils[2]	0.08	0.11	0.0083	0.06	0.0005	0.51
Day 0[3]	0.06	0.09	0.013	0.15	–	–
Day 1[4]	0.07	0.10	0.011	0.05	–	–
Day 5[7]	0.12	0.13	0.011	0.53	–	–

[1]Trans = transit treatment.

[2]Data are based on repeated measures analysis.

[3]Inj1 = initial injection. Inj1: $P \geq 0.57$; Inj1 × Trans: $P \geq 0.29$; Inj1 × Day: $P \geq 0.78$; and Inj1 × Trans × Day: $P \geq 0.18$.

[4]Day-0 (experimental Day 28) samples were collected immediately before the beginning of the transit stress period, just before departure for SHIP steers, and just before beginning feed and water deprivation for NOSHIP steers.

[5]Day-1 (experimental Day 29) samples were collected at the end of the transit stress period, on arrival of the SHIP steers, and on the end of the feed and water restriction period for NOSHIP steers.

[6]Day-5 (experimental Day 34) samples were collected 5 d after the end of the transit stress period.

[7] Inj1: $P = 0.24$; Inj1 × Trans: $P = 0.55$; Inj1 × Day: $P = 0.10$ and Inj1 × Trans × Day: $P = 0.70$.

steers had increased plasma fibrinogen immediately postshipping (672 ± 40.8 mg/dL) relative to preshipping values (418 ± 19.7 mg/dL; $P = 0.0001$), and concentrations decreased from immediately postshipping to 5 d postshipping (556 ± 26.8 mg/dL; $P = 0.02$) but were still greater than d 0 values ($P = 0.0001$). There was also an Inj1 × Trans × day interaction ($P = 0.05$) for plasma cortisol (Fig. 2), where MM steers, regardless of Trans, as well as SAL–SHIP steers had relatively static plasma cortisol concentrations in response to transit or feed and water restriction ($P \geq 0.29$). In contrast, SAL–NOSHIP steers had increased plasma cortisol concentrations after the transit stress period relative to pretransit stress period values ($P = 0.003$).

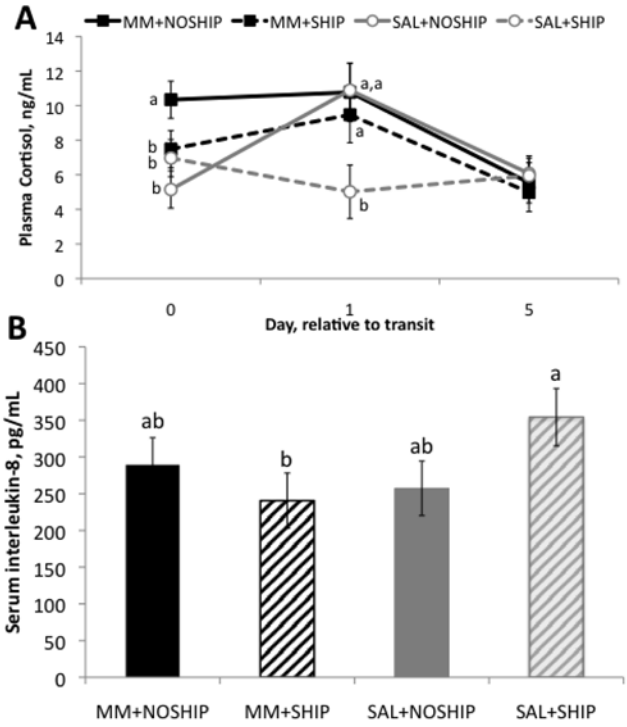

Figure 2. Effect of a trace mineral injection (MM) or saline injection (SAL) after being transported for a 20-h transit stress period (SHIP) or 20 h feed and water restriction without transit (NOSHIP), on plasma cortisol concentrations (A), and serum IL-8 concentrations (B); values are means ± SEM, $n = 12$ per initial injection (Inj1) × transit treatment (Trans) combination. The MM (Multimin90, Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA). Within plasma cortisol there was an Inj1 × Trans × day interaction ($P = 0.05$), and within serum IL-8 there was an Inj1 × Trans interaction ($P = 0.06$); lowercase letters that differ denote differences ($P \leq 0.10$) between Inj1 and Trans within day for plasma cortisol, lowercase letters that differ denote differences ($P \leq 0.05$) between Inj1 and Trans.

Fourteen-Day Post-Transit Performance

There was no difference in BW immediately after the transit period for any treatments ($P \geq 0.19$; Table 7). Although MM steers had smaller ADG ($P = 0.02$) than SAL steers, there was no difference in ending BW ($P = 0.19$; Table 7) as measured 14 d after the end of the transit period. The Trans treatment also did not affect 14-d post-transit ADG, DMI, or ending BW ($P \geq 0.42$). There was no difference in G:F between Inj1 or Trans treatments ($P \geq 0.17$).

Growing Period

There was no effect of either Inj1 or Trans on any growth performance variables ($P \geq 0.18$; Table 7). Ending BW, measured on d 112 and 113, was not different ($P = 0.62$) due to treatments.

Table 7. Effect of a trace mineral injection (MM[1]) or saline injection (SAL) on growth performance of steers during the 14-d period immediately following being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP), and the growing period (d 34 to 112)

	Injection 1							
	SAL		MM			*P*-value[2]		
	Transit							
Variable	NOSHIP	SHIP	NOSHIP	SHIP	SEM	Inj1	Trans	Inj1 × Trans
14-d post-transit performance								
Initial BW,[3] kg	278	273	277	273	2.9	0.89	0.19	0.88
Ending BW,[4] kg	300	297	296	293	3.4	0.19	0.42	0.88
ADG, kg	1.59	1.72	1.37	1.38	0.126	0.03	0.58	0.64
DMI, kg/d	6.85	6.89	6.82	6.41	0.159	0.12	0.25	0.18
G:F	0.234	0.249	0.199	0.220	0.0218	0.17	0.42	0.89
Growing period performance								
Initial BW,[5] kg	307	301	305	302	3.4	0.86	0.18	0.71
Ending BW,[6] kg	437	433	433	433	5.0	0.64	0.62	0.67
BW,[7] kg	369	364	366	364	3.8	0.74	0.40	0.64
ADG,[7] kg	1.80	1.75	1.78	1.76	0.052	0.93	0.47	0.80
DMI,[7] kg/d	8.4	8.4	8.4	8.2	0.097	0.43	0.41	0.42
G:F[7]	0.189	0.192	0.183	0.187	0.0050	0.27	0.44	0.87

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2]Inj1 = initial injection; Trans = transit treatment.

[3]Initial BW is the BW collected at the end of the transit stress period (experimental d 29), when steers were weighed either on return to the facility or on the end of the feed and water restriction.

[4]Ending BW is the BW measured 14 d (experimental Day 43) after 20 h of feed and water restriction of all steers.

[5]Initial BW for the growing period was measured on d 34 at 5 d post-transit.

[6]Ending BW for the growing period is based on the average of the consecutive day BW collected on d 112 and 113.

[7]Based on repeated measures. Day: $P < 0.001$ for all repeated measures, except ADG: $P = 0.64$; Inj1 × day: $P \geq 0.13$, Trans × day: $P \geq 0.12$, and Inj1 × Trans × day: $P \geq 0.11$ for all repeated measures for all variables.

Finishing Period

Similar to the growing period, there was no influence of Inj1 ($P \geq 0.41$), Trans ($P \geq 0.21$), or Inj2 as administered on d 113 ($P \geq 0.60$) on growth performance (Table 8). However, there was a tendency for an interaction ($P = 0.06$; Table 8) between Inj1 and Inj2 on finishing period ADG. The steers that received both MM or SAL for both Inj1 and Inj2 (MM–MM and SAL–SAL) tended to have greater ADG than steers that received SAL initially and MM as Inj2 (SAL–MM; $P \leq 0.08$), but no other differences between treatments were noted ($P \geq 0.32$).

Carcass Characteristics

Hot carcass weight was not affected by treatment ($P = 0.12$; Table 9). There was a tendency for a 3-way interaction ($P = 0.08$) in BF between Inj1, Trans, and Inj2, as the result of a tendency for less BF in SAL–MM steers that were shipped relative to SAL–MM steers that were not shipped ($P = 0.07$), whereas other treatment comparisons did not differ ($P > 0.10$). There was also a 3-way interaction in YG ($P = 0.007$) between Inj1, Trans, and Inj2. There was no difference in YG among MM–SHIP–MM, MM–SHIP–SAL, MM–NOSHIP–SAL, SAL–SHIP–SAL, and SAL–NOSHIP–MM, but all differed from SAL–SHIP–MM ($P \leq 0.05$) whereas other individual comparisons did not differ ($P \geq 0.10$). Unsurprisingly, this trend was reversed within REA, with a 3-way interaction ($P = 0.01$) between Inj1, Trans, and Inj1 where REA in MM–SHIP–MM, MM–NOSHIP–SAL, and SAL–NOSHIP–MM steers did not differ ($P > 0.10$) but all had smaller REA than SAL–SHIP–MM steers ($P \leq 0.05$), whereas other individual comparisons did not differ ($P > 0.10$). Interestingly, steers that received MM as the Inj2 had greater KPH than steers that received SAL as the Inj2 ($P = 0.05$). Injection and Trans did not impact marbling score or QG ($P \geq 0.17$).

DISCUSSION

Disparities among locations of cow–calf production and feedlots make shipping a necessity in the beef cattle industry. However, stress associated with transit, including feed and water deprivation, can cause shrink (Hutcheson and Cole, 1986; González et al., 2012), increase circulating acute phase proteins (Marques et al.,

Table 8. Effect of an initial (d 0) and second (d 113) trace mineral injection (MM[1]) or saline injection (SAL) after being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP) on performance of steers during the finishing period (d 113 through 193)

	Injection 1												
	SAL				MM								
	Transit												
	NOSHIP		SHIP		NOSHIP		SHIP			*P*-value[2]			
	Injection 2												
Variable	SAL	MM	SAL	MM	SAL	MM	SAL	MM	SEM	Inj1	Inj2	Trans	Inj1 × Inj2
Initial BW,[3] kg	438	437	434	433	434	432	431	433	7.2	0.60	0.92	0.58	0.93
Ending BW,[4] kg	589	580	589	579	585	589	580	586	9.2	0.94	0.71	0.73	0.27
BW,[5] kg	519	513	515	510	516	516	509	514	4.1	0.89	0.65	0.21	0.14
ADG,[5] kg	1.88^{xy}	1.78^{y}	1.93^{x}	1.83^{xy}	1.87^{xy}	1.94^{x}	1.85^{xy}	1.90^{xy}	0.060	0.41	0.60	0.80	0.06
DMI,[5] kg	11.3	11.4	11.5	11.4	11.4	11.4	11.3	11.5	0.17	0.87	0.77	0.93	0.91
G:F[5]	0.167	0.155	0.170	0.161	0.164	0.170	0.164	0.166	0.0068	0.60	0.60	0.78	0.15

$^{x–z}$Within rows, means without a common superscript tend to differ ($P \leq 0.10$).

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2]The main effects in statistical analysis included initial injection (Inj1), transit treatment (Trans), and second injection (Inj2) and the corresponding interactions.

[3]Initial BW represents weights collected on d 112 and 113 at the beginning of the finishing period. Inj1 × Trans: $P = 0.74$; Inj2 × Trans: $P = 0.80$; Inj1 × Inj2 × Trans: $P = 0.85$.

[4]Ending BW represents weights collected on d 192 and 193 at the end of the finishing period. Inj1 × Trans: $P = 0.79$; Inj2 × Trans: $P = 0.92$; Inj1 × Inj2 × Trans: $P = 0.95$.

[5]Based on repeated measures, Day: $P < 0.0001$ for all variables; all interactions not shown in table: $P \geq 0.15$ for all variables.

Table 9. Effect of an initial (d 0) and second (d 113) trace mineral injection (MM[1]) or saline injection (SAL) after being transported for a 20-h transit stress period (SHIP) or 20 h of feed and water restriction without transit (NOSHIP) on carcass characteristics of steers

	Injection 1													
	SAL				MM									
	Transit													
	NOSHIP		SHIP		NOSHIP		SHIP			*P*-value[2]				
	Injection 2													
Variable	SAL	MM	SAL	MM	SAL	MM	SAL	MM	SEM	Inj1	Inj2	Trans	Inj1 × Inj2	Inj1 × Inj2 × Trans
HCW, kg	365	353	360	351	359	361	357	359	5.5	0.68	0.29	0.43	0.12	0.85
Backfat, cm	1.35^{xy}	1.55^{y}	1.49^{xy}	1.31^{x}	1.44^{xy}	1.36^{xy}	1.47^{xy}	1.47^{xy}	0.089	0.86	0.83	0.86	0.66	0.08
REA,[3] cm^2	83.6^{ab}	79.6^{b}	81.7^{ab}	86.2^{a}	80.6^{b}	84.0^{ab}	82.1^{ab}	81.2^{b}	1.74	0.52	0.54	0.52	0.69	0.01
Marbling score[4]	452	435	448	458	436	474	430	413	22.0	0.52	0.83	0.46	0.66	0.19
KPH, %	2.29	2.64	2.36	2.42	2.29	2.36	2.38	2.41	0.088	0.28	0.05	0.94	0.25	0.31
YG[5]	3.21^{ab}	3.57^{a}	3.41^{a}	2.96^{b}	3.40^{a}	3.19^{ab}	3.35^{a}	3.42^{a}	0.141	0.61	0.55	0.59	0.89	0.007
QG[6]	3.08	2.82	3.09	3.17	2.92	3.27	2.92	3.00	0.212	0.93	0.68	0.89	0.30	0.31

^{a,b}Within rows, means without a common superscript differ ($P \leq 0.05$).

$^{x–z}$Within rows, means without a common superscript tend to differ ($P \leq 0.10$).

[1]The MM (Multimin90; Multimin USA, Fort Collins, CO) provided 15 mg Cu/mL (as copper disodium EDTA), 10 mg Mn/mL (as manganese disodium EDTA), 5 mg Se/mL (as sodium selenite), and 60 mg Zn/mL (as zinc disodium EDTA).

[2]The main effects in statistical analysis included initial injection (Inj1), transit treatment (Trans), and second injection (Inj2) and the corresponding interactions. Inj1 × Trans: $P \geq 0.14$; Inj2 × Trans: $P \geq 0.19$ for all carcass measurements.

[3]REA = ribeye area.

[4]Marbling scores: slight: 300, small: 400, modest: 500.

[5]YG = yield grade.

[6]QG = quality grade: Select^{+}: 2, Choice^{-}: 3, and Choice: 4.

2012; Cooke et al., 2013), and increase circulating markers of oxidative stress (Chirase et al., 2004). Stressed cattle have less DMI on arrival at the feedlot (Ceciliani et al., 2012) and are more susceptible to bovine respiratory disease, also known as "shipping fever" (Camp et al., 1981), which is the greatest health problem in the U.S. beef cattle industry (Duff and Galyean, 2007). Because of the expected decreased DMI of stressed calves, the NRC (2000) recommends increasing TM concentrations in receiving diets to approximately 150% of nonstressed cattle requirements to maintain adequate TM consumption. Preconditioning of cattle, including weaning, vaccination, and becoming accustomed to bunk-style feeding and water troughs, can be an effective way to decrease morbidity in the feedlot and increase value of calves (Duff and Galyean, 2007). The use of injectable TM concurrent with vaccination can increase neutralizing antibody titers in response to vaccination (Arthington and Havenga, 2012), suggesting that this may also be an ideal time to improve the TM status of cattle before shipment to the feedlot, potentially improving feedlot performance.

Previous research revealed that mild TM deficiency induced during a 90-d period where steers were not supplemented with Cu, Mn, Se, and Zn caused steers to lose more weight after a transit period of 20 h compared with steers that had adequate dietary TM supplementation (Genther and Hansen, 2014b). After transit, those mildly deficient steers that received a MM had improved growth performance and carcass characteristics relative to mildly deficient steers that did not receive MM. It was hypothesized that providing TM through injection before transit may improve TM status, thereby potentially increasing performance and decreasing BW loss in response to transit. Steers in the current experiment experienced BW shrink similar to calves experiencing long haul transport according to González et al. (2012). However, there was no impact of Inj1 on BW shrink for SHIP steers. However, contrary to the previously mentioned study, steers in this experiment had adequate Cu status and above-adequate Se status based on liver concentrations, which may explain the difference in responses between Genther and Hansen (2014b) and this study. It appears that deficiencies of Cu and Se in cattle influence the stress response to transit; however, the relationship between Se status and stress has not been investigated and the impact of Cu and Se cannot be separated due to experimental design. As the value of MM is to rapidly improve TM status, the greatest benefit would likely be observed when used in cattle with mild to moderate deficiencies.

Liver TM concentrations from steers in the present study indicate that these cattle were well within the adequate or high adequate range of status for Cu, Mn, Se, and Zn, as reported by both Kincaid (2000) and Herdt and Hoff (2011), before the start of the trial. Previous research has shown that MM rapidly increases liver concentrations of Cu and Se in cattle through at least 30 d postinjection relative to steers treated with saline (Pogge et al., 2012; Genther and Hansen, 2014a), which is consistent with this experiment. Surprisingly, MM also increased liver Zn concentrations. Liver Zn is generally a poor indicator of Zn status (Herdt and Hoff, 2011), and previous research with the same MM has produced inconsistent results, with one study finding a slight increase in liver Zn concentration (based on repeated measures from 1, 8, and 15 d postinjection; Pogge et al., 2012) and another finding no difference due to injection (Genther and Hansen, 2014a).

The MM–SHIP steers had lesser liver Cu and Se concentrations than MM–NOSHIP steers post-transit. However, this is likely because cattle randomly assigned to Trans within the MM group had slightly different liver Cu and Se concentrations before transit. The liver concentrations of Fe, Mn, Se, and Zn were numerically lesser after transit stress relative to pretransit concentrations. Although MM steers appear to have lost more TM during the transit period, this is likely an artifact of the length of time from injection. The post-transit biopsy samples were collected 40 d postinjection, and previous work has indicated that cattle given a MM had greater liver Cu and Se concentrations than saline treated calves at 30 d postinjection but were not different from saline-treated calves 60 d postinjection (Genther and Hansen, 2014a). Liver TM concentrations likely decrease over time as stores are used for biological functions or are excreted (Pogge et al., 2012; Genther and Hansen, 2014a).

Steers overall had relatively high rectal temperatures before shipping, perhaps related to the time of truck loading and ambient temperature (1100 h; 25°C), which led to a rectal temperature decrease over the transit period. Overall steer rectal temperature decreased 0.65 ± 0.42°C during the transit stress period and was greater in NOSHIP steers, but rectal temperature change was not affected by increased TM status.

Plasma cortisol was unchanged in response to transit or feed and water restriction in all treatments except for SAL–NOSHIP steers, which had increased plasma cortisol after the transit period. In general, plasma cortisol will increase in response to a stressor such as transit or feed restriction (Marques et al., 2012), although other studies have also found a lack of cortisol response to transit (Galyean et al., 1981). The minimal changes in plasma cortisol concentrations appear to be unrelated to steer TM status, as affected by MM. Despite the lack of plasma cortisol changes,

calves had a near 3-fold increase in plasma haptoglobin and a 75% increase in plasma fibrinogen, indicating that they experienced a physiological response to transit or feed and water restriction. Calves in the current study had previously experienced both weaning and transit stress (approximately 8 h) 30 d before the transit stress experienced in this experiment. Research has demonstrated that cattle experiencing repeated handling have decreased plasma cortisol concentrations relative to nonhandled control cattle (Cooke et al., 2009). The previous exposure to stress may have prepared calves for the subsequent transit stress period in this experiment, resulting in the variable and somewhat limited response observed in this study.

The majority of the acute phase proteins measured in this study were increased in response to both transit and feed and water restriction, which is consistent with other studies (Marques et al., 2012; Cooke et al., 2013). There was no evidence to suggest that improving TM status with a MM 28 d before transit had an impact on the inflammatory or stress response in cattle subjected to either transit stress or feed and water restriction. Previous research has indicated that Cu-deficient heifers had an altered acute phase response when challenged intranasally with live bovine herpesvirus-1, including decreased plasma ceruloplasmin and an increase of plasma fibrinogen that was 66% greater than Cu-adequate control heifers (Arthington et al., 1996). The lack of differences in the current study could be explained by the difference in challenge type (transit vs. viral challenge) or the lesser difference in TM status relative to cattle used by Arthington et al. (1996), as both MM and SAL calves were well within the adequate ranges for all measured TM.

Increasing TM status of steers resulted in decreased ADG during the 14-d period postshipping. Although unexpected, a similar response was reported by Clark et al. (2006), who found that calves (266 kg initial BW) that received a MM on arrival to the experimental facility had lesser ADG during a 28-d receiving period than non-TM-injected cattle. Similarly, Arthington et al. (2014) reported that heifers that had previously received a MM at 100 and 200 d of age and were subjected to transit stress (1,600 km) and weaning stress at 250 d of age followed by a third MM on arrival back to the farm had negative ADG in the 14-d period postshipping. The authors hypothesized that the decrease in ADG postshipping was due to the redirection of nutrients in support of the greater acute phase protein response observed in those calves as previously discussed (Arthington et al., 2014), although they suggest that this was mainly due to a response to the latest MM and not due to a response to transit. Although the MM steers in our study did have lesser ADG relative to SAL in the 14-d period postshipping, the inflammatory markers measured do not support a differential inflammatory response to transit stress in calves with increased TM status from MM. However, it is possible, due to limited sampling, that the peak concentrations of inflammatory markers were missed. Additionally, the model in the current experiment was different from that used by Arthington et al. (2014), as those researchers were interested in the inflammatory response to MM and not the influence of TM status on response to transit. However, detecting differences in ruminant performance during a 14-d window is difficult with ruminant animals due to potential differences in gut fill, and data accuracy increases as test length increases (Archer and Bergh, 2000). Overall, the decreased ADG did not affect BW at the end of the 14-d post-transit period, and no subsequent effects of MM to improve TM status or Trans on cattle growth were noted in the growing period, suggesting this effect was short lived.

Previous research indicates that a MM approximately 90 d before harvest will not influence growth in calves with adequate TM status (Genther and Hansen, 2014b). However, in the present study, in steers that received the same injection at both initial and Inj2 (SAL–SAL or MM–MM), ADG tended to be greater than steers that received SAL–MM or MM–SAL. Trace mineral injection can increase the activity of glutathione peroxidase (Pogge et al., 2012) and Mn-superoxide dismutase (Genther and Hansen, 2014a), which may come at an energetic cost, diverting energy from growth to enzyme synthesis. It is possible that SAL–MM steers were increasing translation of TM-containing proteins, decreasing growth. Although the mineral status of the steers at the time of Inj2 was not determined, the diet analyzed to contain TM concentrations above the NRC (2000) recommendations, so steers were likely still within the adequate range.

Interestingly, within the carcass characteristic data, a differential response to MM (Inj2) within steers that received SAL as the Inj1 was noted, where shipped steers had the least overall BF and the largest overall REA whereas nonshipped steers had the greatest BF and least REA. These treatment groups are identical with the exception of the shipping treatment, suggesting that the presence of the transit stress may have caused a repartitioning effect in response to increased available TM, resulting in improved body composition relative to nonshipped steers. It is unclear what signal may have been initiated by transit stress, 85 d before Inj2 and reimplant time, but further research is warranted.

Overall, administering a MM to increase TM status at the beginning of a 28-d preconditioning period to cattle with adequate TM status had little impact on the physical and physiological response to the stress

of transit or feed and water restriction. Transit stressed steers experienced greater shrink and lesser rectal temperature change relative to feed- and water-restricted steers. As noted by others, steers that had received injectable TM had lesser ADG in the 14 d immediately following stress, but growth performance was not affected long term. Trace mineral injection 192 and 80 d before harvest had little impact on overall growth performance and carcass characteristics. Any potential impact that improving TM status using a MM in steers with mild to moderate TM deficiencies may have on transit stress response remains to be elucidated.

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