Effects of trace mineral injections on measures of performance and trace mineral status of pre- and postweaned beef calves[1]

J. D. Arthington,*[2] P. Moriel,*[3] P. G. M. A. Martins,* G. C. Lamb,† and L. J. Havenga‡

*Range Cattle Research and Education Center, Ona 33865; †North Florida Research and Education Center, Marianna 32446, University of Florida, Institute of Food and Agricultural Sciences; and ‡MultiMin USA, Inc., Fort Collins, CO

ABSTRACT: Three experiments were conducted to examine the effects of injectable trace minerals (ITM) on measures of trace mineral status and performance in pre- and postweaned Brangus-crossbred beef calves. In Exp. 1, calves were assigned to treatments in alternating birth order (n = 150; 75/treatment), consisting of a 1-mL subcutaneous injection of ITM (MultiMin 90; MultiMin USA, Inc., Fort Collins, CO) or sterile saline. The ITM formulation consisted of 60, 10, 15, and 5 mg/mL of Zn, Mn, Cu, and Se. Treatments were readministered at 100 and 200 d of age. Calf BW was recorded at birth and on d 100, 150, 200, and 250 (weaning). Trace mineral status was assessed in liver biopsy samples (n = 12/treatment) collected on d 150, 200, and 250. Administration of ITM had no impact on BW gain ($P \geq 0.55$) but did result in greater ($P \leq 0.02$) concentrations of liver Cu and Se and lesser ($P = 0.05$) liver Fe concentrations compared to saline-injected calves. In Exp. 2, 24 heifers were selected from the weaned calves of Exp. 1 (n = 12/treatment) and transported 1,600 km. Remaining on their original treatments, heifers were administered 5 mL of ITM or saline following transport (d 0). Blood samples, for acute phase protein (APP) analysis, were collected on d 0, 1, 3, 6, 9, and 13 and liver biopsy samples for assessment of trace mineral status on d 13. Plasma APP concentrations increased in all calves following weaning and transport but concentrations were greatest ($P < 0.05$) in ITM- vs. saline-injected heifers on d 6 and 9. Liver concentrations of Cu, Se, and Zn were greater ($P \leq 0.04$) but ADG lesser ($P = 0.05$) for heifers receiving ITM vs. saline. In Exp. 3, 34 heifers, without previous exposure to ITM, were enrolled in a 177-d development study (n = 17/treatment). Treatments consisted of 2.5-mL injections of ITM or sterile saline on d 0, 51, and 127. Humoral immune response to an injection of porcine red blood cells (PRBC) was evaluated on d 51. Trace mineral status was evaluated in liver biopsy samples collected on d 177. Overall heifer ADG, PRBC antibody titers, and liver Se concentrations were greatest ($P \leq 0.06$) for ITM vs. control heifers. Collectively, these studies demonstrate an increased trace mineral status, a greater humoral response to novel antigen, and a heightened APP response to weaning and transport stress in pre- and postweaned beef calves administered ITM.

Key words: acute phase protein, antibody, calves, heifer, injectable trace minerals

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INTRODUCTION

Trace mineral nutrition supports physiological functions related to growth, reproduction, and immunity in livestock (Underwood and Suttle, 1999). Forage is the primary source of trace minerals for grazing cattle with secondary sources being water and ingested soil. In many environments, these sources will not fully supply the trace mineral requirement of cattle resulting in a need for trace mineral supplementation. Supplementation of trace minerals may occur through a variety of means, including free-choice loose mineral mixes, trace-mineral-fortified salt blocks, and trace-mineral-fortified energy/protein supplements. Trace minerals administered through injection (injectable trace minerals [**ITM**]) is another method of supplementation. An advantage of ITM, compared with traditional oral supplementation methods, is the targeted delivery of a known amount of trace minerals to individual animals. This removes the

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[2]Corresponding author: jarth@ufl.edu
[3]Present address: North Carolina State University, Mountain Research Station, Waynesville 28785.
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variability associated with annual fluctuations in voluntary intake, which is common among cattle provided a free-choice mineral mix (Arthington and Swenson, 2004). In addition, ITM can be used within production environments that might experience difficulty managing the routine delivery of free-choice mineral mixes, such as extensive rangeland systems, seasonal grazing of mountain meadows, and seasonally flooded pastures. Furthermore, the contribution of wildlife to the overall consumption and disappearance of free-choice mineral mixes also can cause complications in these production environments and add further value to the use of ITM.

Sources of ITM containing EDTA-chelated sources Cu, Mn, and Zn (combined with sodium selenite) have been shown to increase mineral status in cattle (Pogge et al., 2012) without causing injection site reactions (Arthington and Havenga, 2012) that were common in earlier ITM preparations, particularly Cu-containing injectable supplements (Boila et al., 1984; Chirase et al., 1994). Recent research efforts in beef cattle have reported positive effects of ITM on feed efficiency (Clark et al., 2006), improved humoral responses to vaccination (Arthington and Havenga, 2012), reduced treatments for illness (Berry et al., 2000), and overall reduced morbidity treatment costs (Richeson and Kegley, 2011). The objective of the current study was to investigate the influence of ITM in beef calves throughout a traditional cow/calf production cycle, including pre- and postweaned calf performance.

Table 1. Nutrient composition of feedstuffs (Exp. 1, 2, and 3)[1]

Item	Pasture (Exp. 1)[2]	Hay (Exp. 2)[3]	Supplement (Exp. 2 and 3)[4]	Fall pasture (Exp. 3)[5]	Winter pasture (Exp. 3)[5]
	% (DM basis)				
CP	14.4	7.4	17.9	16.2	5.8
ADF	34.2	41.4	34.1	34.5	47.6
NDF	66.9	75.4	52.4	59.7	78.1
Ca	0.34	0.30	0.70	0.50	0.26
P	0.29	0.19	0.59	0.30	0.15
Mg	0.23	0.27	0.39	0.25	0.18
K	1.75	1.48	1.22	2.32	0.86
Na	0.015	0.017	0.098	0.119	0.051
	mg/kg (DM basis)				
Fe	73	74	385	120	127
Zn	33	45	60	28	35
Mn	50	52	60	63	47
Cu	5.0	10.0	10.3	10.7	7.9
Mo	1.4	1.0	1.2	0.41	0.43
Se	0.02	0.01	0.22	0.02	0.01

[1]Values represent an average of 3 analyses from a composited mixture of several hand-grab samples.

[2]Hand-grab bahiagrass (*Paspalum notatum*) pasture samples collected in mid June.

[3]Long-stem bahiagrass hay provided in a large-round bale.

[4]Grain concentrate composed of soybean hulls (49.0%), wheat middlings (30.3%), dried distillers grains (12.3%), molasses (4.5%), canola (3.2%), and limestone (0.7%) was offered to heifers at a rate of 4.50 and 2.25 kg daily (as-fed) throughout Exp. 2 and 3, respectively.

[5]Hand grab limpograss (*Hemarthria altissima*) pastures collected during the vegetative growing period (September) and after stockpiling (February).

MATERIALS AND METHODS

Calves were cared for in accordance with acceptable practices as outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 2010). In addition, the research protocol was reviewed and approved by the University of Florida Institute of Food and Agricultural Sciences (**UF-IFAS**) Animal Research Committee (number 08-10ONA). All cattle used in these experiments were derived from the resident Brangus-crossbred beef herds of the UF-IFAS Range Cattle Research and Education Center, Ona, FL.

Animals, Treatments, and Sample Collection

Experiment 1. Two treatments were administered to November-born calves within 24 h of birth in alternating birth order. Treatments (n = 75/treatment) consisted of 1 mL subcutaneous injection of trace minerals (MultiMin 90; MultiMin USA, Inc., Fort Collins, CO) or 1 mL subcutaneous injection of sterile physiological saline. The ITM contained 60, 10, and 15 mg/mL of Zn, Mn, and Cu, as disodium EDTA chelates, and 5 mg/mL of Se, as sodium selenite. All 150 treatment administrations were delivered within a 25-d calving period. Within 24 h of birth, calves were assigned their treatment and provided individual identification, and sex and BW were recorded. Treatments were readministered to all calves 2 additional times before weaning for a total of 3 treatment administrations. The second and third treatment administration corresponded to approximately 100 and 200 d of age. Throughout the entire study, cow and calf pairs grazed summer bahiagrass pastures (*Paspalum notatum*; Table 1) with free-choice access to a salt-based mineral supplement (Cattle Select Essentials Range; Lakeland Animal Nutrition, Lakeland, FL; 6.0, 0.10, 0.10, 0.30, 63, and 1.0% of Ca, K, Mg, S, NaCl, and P, respectively, and 50, 1,500, 800, 210, 500, 40, and 3,000 mg/kg of Co, Cu, Fe, I, Mn, Se, and Zn, respectively). Multiple hand-plucked samples of pasture forage were collected on a single day in mid June (approximately 30 d before weaning), composited into a single sample, dried at 60°C for 5 d, and ground and stored at room temperature for later analysis for nutrient content (Table 1). Individual calf BW was recorded at birth (d 0) and on d 100, 150, 200, and 250 (weaning). Trace mineral status of calves was assessed in liver biopsy samples collected from 12 random heifer calves per treatment on approximately d 150, 200, and weaning (d 250).

Experiment 2. The 24 heifer calves, selected for liver biopsy at weaning in Exp. 1 (n = 12/treatment), were held in a drylot pen overnight (d –1; weaning) and transported 1,600 km in a commercial livestock trailer over a 24-h period (d 0). Heifers remained on the trailer for the full 24-h period, although the truck was not moving the entire time. To avoid unnecessary heat stress on the heifers, the truck remained moving during daylight with short stops totaling <6 h during the night and early morning. Following transport and entry into the research feedlot (d 1), heifers were readministered treatments (according to preweaning assignments; 5 mL). In the interval between weaning and transport and for the 13-d post-transport evaluation period, heifers remained in a single group in a drylot pen with free-choice access to long-stem bahiagrass hay and water. In addition, heifers were provided 4.5 kg/d of a concentrate supplement (as-fed basis). Multiple hand-grab samples of hay and supplement were collected on a single day, composited into a single sample (hay and supplement separately), dried at 60°C for 5 d, and ground in a Wiley mill (Model 4, Thomas-Wiley Laboratory Mill; Thomas Scientific, Swedesboro, NJ) to pass a 1-mm stainless steel screen and stored at room temperature for later analysis for nutrient content (Table 1). Shrunk BW was collected on d 1 (immediately following transport) and 14 (16 h of feed and water withdrawal) for calculation of ADG. Jugular blood samples were collected on d 0 (before loading onto truck), 1, 3, 6, 9, and 13 into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing 158 containing freeze-dried Na heparin. Samples were placed on ice immediately following collection and centrifuged at 1,200 × g for 30 min at 4°C for plasma collection. Plasma was frozen at –20°C on the same day of collection until later analysis for concentrations of the acute phase proteins (**APP**): ceruloplasmin, haptoglobin, and acid-soluble protein. Trace mineral status was evaluated in liver biopsy samples collected on d 1 (immediately before treatment administration) and 13.

Experiment 3. Thirty-four weaned heifers, not previously enrolled in Exp. 1 or 2, were stratified by BW and randomly assigned to 1 of 2 treatments for (n = 17/treatment) for a 177-d development study. Treatments consisted of 2.5 mL subcutaneous injections of ITM (approximately one-half of the recommended dosage) or sterile saline on d 0, 51, and 127. Shrunk BW (16 h of feed and water withdrawal) was measured at the start (d 0) and end of the study (d 177) for calculation of ADG. The humoral immune response to a 10-mL intramuscular injection of a porcine red blood cell (**PRBC**; Lampire Biological Laboratories, Pipersville, PA) solution (25% PRBC and 75% sterile PBS; Amresco, Solon, OH) was evaluated on d 51 following the second treatment administration. Anti-PRBC antibody titers were determined in blood samples collected on d 0, 3, 7, 14, and 21, relative to PRBC injection, and attainment of puberty was determined once monthly by progesterone analysis of 2 blood samples collected 10 d apart. Blood samples were collected, processed, and stored using the same procedures as Exp. 1. Trace mineral status was evaluated in liver samples collected at the end of the study (d 177). Throughout the experiment, heifers grazed of single pasture consisting of limpograss (*Hemarthria altissima*) and were provided supplement (same as Exp. 2; Table 1) on Monday, Wednesday, and Friday in amounts equivalent to 2.25 kg/d (as-fed basis; Table 1). Heifers were provided free-choice access to common white stock salt without trace mineral fortification. Hand-grab samples of supplement and pasture were collected on a single day during the vegetative growing period (September) and after stockpiling (February). Samples were composited into a single sample (vegetative and stockpiled separately), dried at 60°C for 5 d, and ground and stored at room temperature for later analysis for nutrient content (Table 1). Heifers were exposed to 2 mature Angus bulls from d 83 to 177 (end of study) and subsequent pregnancy status was determined by transrectal ultrasonography (5-MHz linear array transducer, Aloka 500V; Corimetrics Medical Systems, Inc., Wallingford, CT) approximately 60 d later.

Sample Analysis

Feed, Forage, and Liver (Experiments 1, 2, and 3). Composited feed and forage samples were submitted in triplicate to a commercial laboratory (Dairy One Laboratory, Ithaca, NY) for analysis of all nutrients, except Se, which was analyzed at the Michigan State University Diagnostic Center for Population & Animal Health (Lansing, MI). Liver biopsy samples were collected using procedures previously described (Arthington and Corah, 1995) and assessed for trace mineral content at the Michigan State University Diagnostic Center for Population & Animal Health (Braselton et al., 1997).

Acute Phase Proteins (Experiment 2). Plasma ceruloplasmin oxidase activity was measured in duplicate samples using the colorimetric procedures described by Demetriou et al. (1974). Ceruloplasmin concentrations were expressed as milligrams per deciliter as described by King (1965). Inter- and intra-assay CV were 4.95 and 2.26%, respectively.

Plasma haptoglobin concentrations were determined in duplicate samples by a biochemical assay measuring haptoglobin-hemoglobin complexing by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982). Results were obtained as arbitrary units resulting from the absorption reading at 450 nm (inter- and intra-assay CV = 7.20 and 3.81%, respectively), which were converted to milligrams per milliliter using a

standard curve generated from haptoglobin values confirmed via an commercial bovine ELISA assay (bovine haptoglobin ELISA test kit; Life Diagnostics, Inc., West Chester, PA; Cooke and Arthington, 2013). The detectable concentration of was 0.03 mg/mL with a maximum of 0.95 mg/mL (intra-assay CV of standards = 1.26%).

Plasma acid soluble protein concentrations were analyzed according to Nakajima et al. (1982) with modification. Briefly, 0.1 mL of plasma sample was mixed with 1.0 mL of 0.6 *M* perchloric acid and incubated at room temperature for 20 min. Following centrifugation at 1,200 × *g* for 20 min at 4°C, 0.2 mL supernatant was mixed with 1.0 mL Coomassie Brilliant Blue G-250 solution and incubated for 20 min. Absorbance was then read at 590 nm. A standard curve was generated using a commercial protein standard (Sigma, bovine serum albumin [P0914; Sigma-Aldrich, Inc., St. Louis, MO]). Inter- and intra-assay CV were 12.70 and 2.28%, respectively.

Porcine Red Blood Cell Titers (Experiment 3). Hemagglutination to PRBC was determined by the procedure described in cattle by Engle et al. (1999) based on previously established methods (Ferket and Qureshi, 1992). Results were recorded as $\log_2$ PRBC titers, corresponding to the total anti-PRBC immunoglobulin titers. Inter- and intra-assay CV were 9.07 and 4.17%, respectively.

Progesterone (Experiment 3). Concentrations of plasma progesterone were analyzed by ELISA. The ELISA procedure was adopted from that previously described by Rasmussen et al. (1996). Quality controls were established using 100 µL plasma with a known concentration of progesterone of 2.5 ng/mL. Standards were determined with 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 ng/mL concentrations with a duplicate of each respective standard. All samples were analyzed in duplicate. The inter- and intra-assay CV were 5.42 and 6.12% for 5 plates, respectively. Puberty was defined as the first sampling day when concentrations of progesterone were ≥1.5 ng/mL (Cooke and Arthington, 2009) followed by a progesterone pattern consistent with normal estrous cycles (Perry et al., 1991).

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.2) with Satterthwaite approximation to determine the denominator df for the test of fixed effects. The model statement for the analysis of growth performance in Exp. 1 contained the fixed effects of treatment, sex, and the interaction and calf (sex × treatment) as the random variable. The model statement for the analysis of growth performance (Exp. 2 and 3), trace mineral status (Exp. 2 only), age at puberty, and attainment of pregnancy (Exp. 3 only) included the fixed effect of treatment and the random effect of heifer (treatment). Liver trace minerals (Exp. 1 and 2), plasma APP, and hemagglutination titers were analyzed as repeated measures with the model statement containing the effects of treatment, time, and the interaction and heifer(treatment) as the random variable. Compound symmetry was used as the covariance structure, which was selected based on the least Akaike information criteria. For all analyses, individual animal was the experimental unit, results are reported as least square means and were separated using PDIFF, significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $P \leq 0.10$.

Table 2. Growth performance of preweaned calves (Exp. 1)[1]

Item	ITM	Saline	SEM	*P* =
Birth BW, kg	33.7	32.5	0.73	0.23
BW, kg				
d 100	113	110	2.1	0.28
d 150	141	137	2.6	0.26
d 200	196	192	3.6	0.39
d 250	234	230	3.9	0.40
ADG, kg/d				
d 100	0.78	0.78	0.019	0.74
d 150	0.72	0.70	0.016	0.59
d 200	0.83	0.82	0.018	0.71
d 250	0.79	0.78	0.015	0.67
ADG (birth to d 250), kg/d	0.79	0.78	0.015	0.55
Age at weaning, d	253	252	0.9	0.50

[1]Calves administered 1.0 mL of injectable trace mineral (ITM) or sterile saline at birth and at 100 and 200 d of age. Treatments were assigned in alternating birth order resulting in a sex distribution of 32 and 43 male and 39 and 36 female calves provided ITM and saline, respectively. There was no treatment × sex effect for calf BW at any of the calf weigh dates ($P \geq 0.56$); however, male calves were heavier ($P < 0.001$) at birth and at weaning (d 250; $P = 0.058$).

RESULTS

Experiment 1

Administration of ITM had no impact ($P = 0.55$) on overall ADG of preweaned calves (Table 2). Although there were differences in birth BW between steers and heifers (35.4 and 30.8 kg, respectively; SEM = 0.73), there were no gender × treatment interactions ($P > 0.25$). There were no treatment × sampling day interactions ($P > 0.10$) for liver concentrations of Co, Cu, Fe, Mn, Mo, or Zn; however, pooled across both treatments, there were changes in liver tissue concentrations of Cu, Mn, Mo, and Se across the 3 sampling days (Fig. 1). Most notably among this finding was the >60% decrease in liver Se concentrations in calves from d 150 to 250. Averaged across all 3 sampling times, administration of ITM resulted in greater ($P = 0.001$) concentrations of liver Cu (34%) and a lesser ($P = 0.05$) liver concentration of Fe (–13%) compared to saline-injected calves (Table 3). There was a treatment × sampling day interaction ($P < 0.001$) for liver Se concentrations,

with calves provided ITM having over 5 times greater ($P < 0.001$) liver Se concentrations compared to saline-injected calves on d 150 (Table 3). The magnitude of this difference lessened on subsequent sampling dates.

Experiment 2

Initial (d 0) and final (d 14) shrunk heifer BW did not differ among treatments ($P \geq 0.92$); however, heifer calves administered ITM had a lesser ($P = 0.05$) ADG than saline-injected heifers over the 14-d evaluation period (Table 4).

Liver trace mineral concentrations at d 0 of Exp. 2 are the same values as those presented at weaning for heifers in Exp. 1. A treatment × time effect was detected ($P \leq 0.03$) for liver concentrations of Cu, Se, and Zn. Liver concentrations of Cu, Se, and Zn increased following ITM injection and were greater ($P < 0.05$) than saline-injected heifers on d 13 (Fig. 2). Concentrations of liver Fe (average of d 0 and 13) tended ($P = 0.10$) to be less for ITM- vs. saline-injected heifers (358 and 414 mg/kg; SEM = 23.3), and there were no treatment or treatment × time interactions for liver concentrations of Co, Mn, and Mo ($P \geq 0.45$).

Administration of ITM resulted in a greater APP response during the 14 d post-transport period (Fig. 3). Plasma ceruloplasmin, haptoglobin, and acid soluble protein concentrations increased ($P < 0.01$) in both treatments following transport and were greater ($P < 0.05$) in ITM- vs. saline-injected heifers on d 6 and 9 for all proteins and d 13 for plasma ceruloplasmin and acid soluble protein concentrations (Fig. 3).

Experiment 3

Initial (d 0) and final (d 177) shrunk heifer BW did not differ ($P \geq 0.64$) among treatments; however, heifers

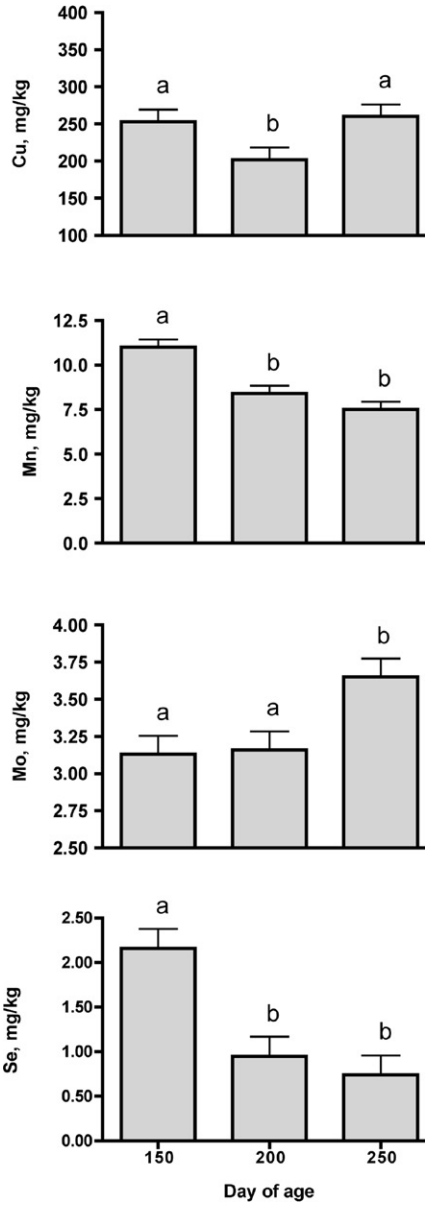

Figure 1. Effect of sampling date on liver trace mineral concentrations; Exp. 1. Calves administered 1.0 mL of injectable trace mineral or sterile saline within 24 h of birth and at 100 and 200 d of age. Values are presented on a DM basis and are pooled across treatments. [a,b]Means with unlike superscripts differ ($P < 0.05$).

Table 3. Liver trace mineral concentrations of preweaned calves (Exp. 1)[1]

Item	ITM	Saline	SEM	*P* =
	mg/kg (DM basis)			
Co	0.19	0.20	0.013	0.50
Cu	273	204	13.3	0.001
Fe	310	355	15.7	0.05
Mn	9.15	8.81	0.393	0.55
Mo	3.36	3.26	0.102	0.51
Zn	262	272	11.0	0.53
Se				
d 150	3.66	0.65	0.437	<0.001
d 200	1.29	0.61	0.428	0.12
d 250	0.99	0.49	0.428	0.25

[1]Calves administered 1.0 mL of injectable trace mineral (ITM) or sterile saline at birth and at 100 and 200 d of age. Values for Co, Cu, Fe, Mn, Mo, and Zn are an average of d 150, 200, and 250, whereas treatment comparisons for Se is presented within sampling day; treatment × sampling day, $P < 0.001$.

Table 4. Growth performance of postweaned, transport-stressed heifers (Exp. 2)[1]

Item	ITM	Saline	SEM	*P* =
	– kg –			
Weaning wt.	240	241	3.6	0.92
Post-transport BW, d 0	220	220	3.7	0.97
Shrunk BW,[2] d 14	216	221	3.7	0.34
ADG, d 14[3]	–0.27	0.14	0.143	0.05

[1]Heifers were selected from Exp. 1 and remained on their previously assigned treatment. At weaning (d –2), heifers remained in a drylot pen overnight before transport for 1,600 km (d –1), returning to the research feedyard the following day (d 0). After transport, heifers were administered 5 mL of injectable trace mineral (ITM) or sterile saline.

[2]Shrunk BW collected following 16 h of feed and water withdrawal.

[3]ADG calculated from the difference of shrunk BW at d 14 less post-transport BW (d 0).

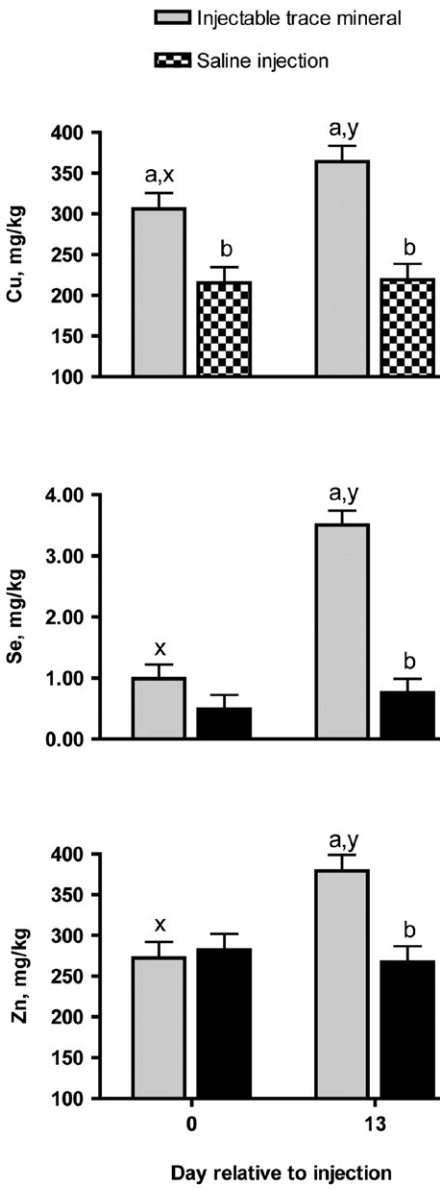

Figure 2. Effect of injectable trace minerals (ITM) on liver concentrations of Cu, Se, and Zn; Exp. 2. Treatments consisted of a 5.0 mL injection of ITM or sterile saline (n = 12 heifers/treatment) delivered immediately following a transport stress (d 0), which began 24 h following weaning. All heifers had previously received the same treatments (1.0 mL of ITM or sterile saline) at birth and at 100 and 200 d of age. Average age at weaning = 253 d. Values are presented on a DM basis. [a,b]Means across treatments within sampling day differ (P < 0.05). [x,y]Means within treatment and across sampling day differ (P < 0.05).

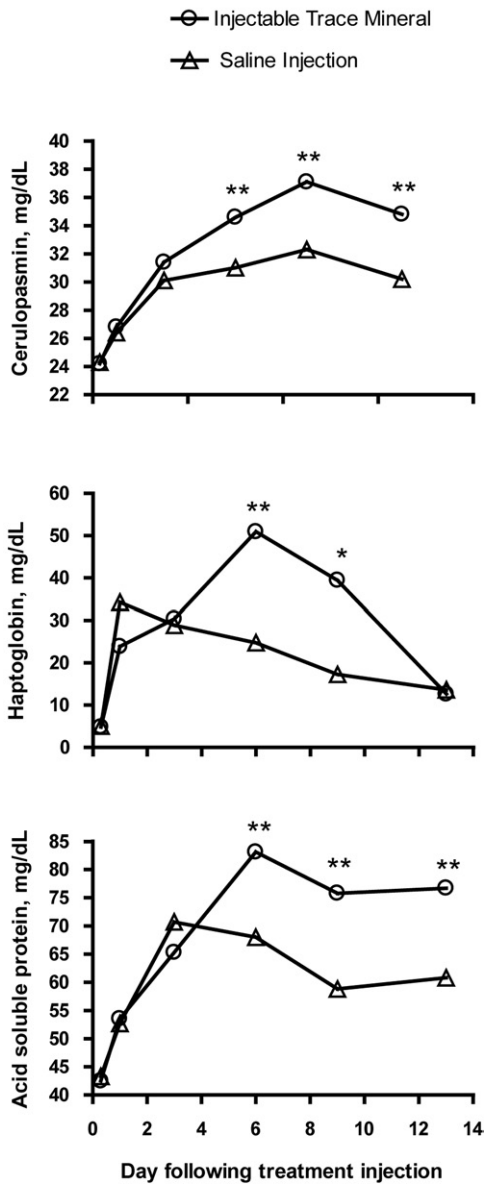

Figure 3. Effect of injectable trace minerals (ITM) on plasma concentrations of ceruloplasmin, haptoglobin, and acid soluble protein; Exp. 2. Treatments consisted of a 5.0 mL injection of ITM or sterile saline (n = 12 heifers/treatment) delivered immediately following a transport stress (d 0), which began 24 h following weaning. All heifers had previously received the same treatments (1.0 mL of ITM or sterile saline) at birth and at 100 and 200 d of age. Average age at weaning = 253 d. Treatment means within sampling day differ (**P < 0.05) or tend to differ (*P < 0.10). Pooled SEM = 0.74, 0.060, and 2.77 for ceruloplasmin, haptoglobin, and acid soluble protein, respectively.

administered ITM tended to have greater (P = 0.06) ADG than saline-injected heifers over the entire development period (Table 5).

Increased PRBC titers were observed in both treatments on d 3 (tendency; P = 0.10) and 7 and 14 (P < 0.03) relative to preinjection values (d 0). The treatment × time interaction was not significant (P = 0.24), reflecting a lack in change in treatment order over time. However, the difference in magnitude of overall change in PRBC titer over time was greater (P < 0.05) in ITM- compared to saline-injected control heifers (Fig. 4); therefore, mean separations were performed. Additionally, liver Se concentrations were 83% greater (P < 0.01) in ITM- vs. saline-injected heifers on conclusion of the study (d 177; Table 6). Although the number of heifers enrolled was minimal, there were no treatment differences in age at puberty (P = 0.26) or attainment of pregnancy (P = 0.89) in this study (Table 5).

Table 5. Body weight, age at puberty, and pregnancy attainment in growing heifers (Exp. 3)[1]

Item	ITM	Saline	SEM	*P* =
BW, d 0,[2] kg	207	211	8.0	0.70
BW, d 177,[2] kg	262	256	8.4	0.64
ADG, kg	0.31	0.26	0.020	0.06
Age at puberty,[3] d	437	417	12.4	0.26
Pregnant,[4] %	41.1	43.8	–	0.89

[1]Heifers administered 2.5 mL of injectable trace mineral (ITM; approximately one-half recommended dosage rate) or sterile saline on d 0, 51, and 127.

[2]Shrunk BW (16 h of feed and water withdrawal) recorded at the start (d 0) and end of the study (d 177).

[3]Age at puberty determined by assessment of progesterone concentrations collected once monthly on a 10-d interval.

[4]Heifers exposed to 2 mature Angus bulls from d 83 to 177 (end of study). Pregnancy was determined by transrectal ultrasonography at approximately 60 d after the conclusion of the breeding season.

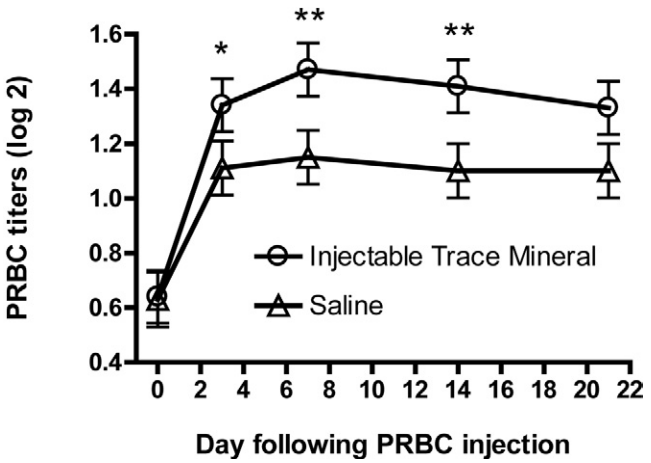

Figure 4. Effect of injectable trace minerals (ITM) on humoral immune response to porcine red blood cell (PRBC) injection; Exp. 3. Treatments consisted of ITM (2.5 mL; approximately one-half of the recommended dosage on d 0, 51, and 127) or 2.5 mL of saline on the same days (n = 17 heifers/treatment). Humoral immune response to PRBC injection was evaluated on d 51 following the second treatment administration. Blood samples, for the assessment of anti-PRBC antibody titers, were collected on d 0, 3, 7, 14, and 21 relative to PRBC injection. Treatment means differ (**$P < 0.03$) on d 7 and 14 and tend to differ (*$P < 0.10$) on d 3.

DISCUSSION

Experiment 1

The lack of an effect of ITM on BW gain of preweaned calves is likely the result of adequate trace mineral nutrition available to the calves. Although pasture concentrations of Se and Cu were less than the suggested requirement for beef cattle (0.10 and 10 mg/kg, respectively; NRC, 1996), calves also had access to a trace-mineral-fortified, salt-based supplement, although levels of intake were not estimated. In addition, calves were also nursing their dams, which would provide an additional source of trace minerals, although with exception of Zn, this source was likely of minimal influence on the total intake of Cu, Se, and Mn (Salih et al., 1987). Therefore, averaged across all sampling dates, calves in both treatments had adequate liver tissue concentrations of all trace minerals assessed (Puls, 1988), with the exception of Se. The liver Se concentration of saline-injected control calves was 0.65 and 0.61 mg/kg (DM basis) on d 150 and 200, respectively. This value is on the low end of adequacy and might be considered deficient depending on the reference used. Puls (1988) suggests deficiency of Se in ruminants to be ≤0.17 mg/kg (wet weight basis), converting to 0.61 mg/kg (DM basis), assuming an average liver tissue DM of 28% (Herdt and Hoff, 2011). Although not statistically different at the time of weaning (d 250), saline-injected control calves were Se deficient (0.49 mg/kg; DM basis), whereas calves receiving ITM had liver Se concentrations within the range of adequacy (0.99 mg/kg; DM basis; Puls, 1988). It is important to note that all calves in Exp. 1 had free-choice access to a trace mineral-fortified, salt-based supplement containing 40 mg/kg Se from sodium selenite. Although intake was not estimated, another study at the same location, using the same supplemental salt-based mineral and conducted during the same season of the year, reported an average intake of 20 ± 5 g/calf daily during a 100-d period before weaning (Moriel and Arthington, 2013).

The decrease in liver Se concentrations from d 150 to 250 for all calves is a reflection of the dam's ability to partition maternal Se to the fetus during gestation (Van Saun et al., 1989). This maternal supply in combination with ITM administration at birth and d 100 resulted in a significant accumulation of Se in the liver of ITM-treated calves on d 150 (the first sampling day). Using reference tables from Puls (1988) and a 28% correction for expected liver DM (Herdt and Hoff, 2011), the range for liver Se concentrations considered "high" but not "toxic" in cattle range between 2.78 and 4.46 mg/kg (DM basis). Thus, the ITM management protocol for calves in Exp. 1 resulted in excessive but not toxic liver Se concentrations on d 150 and adequate liver Se concentrations on d 200 and at weaning (d 250). These findings imply that

Table 6. Liver trace mineral concentrations of heifers (Exp. 3; d 177, end of study)[1]

Item	ITM	Saline	SEM	*P* =
	– mg/kg (DM basis) –			
Co	0.16	0.16	0.011	0.96
Cu	172	125	27.5	0.23
Fe	293	284	17.9	0.51
Mn	10.6	9.8	0.55	0.32
Mo	4.0	4.1	0.21	0.71
Se	0.88	0.48	0.045	<0.01
Zn	120	129	4.6	0.18

[1]Heifers administered 2.5 mL of injectable trace mineral (ITM; approximately one-half recommended dosage rate) or sterile saline on d 0, 51, and 127.

ITM treatment may be beneficial to avoid Se deficiency in beef calves at weaning. Treatment with ITM is likely most effective after 100 to 150 d of age when the maternal supply has waned and intake from forage, milk, and supplemental mineral may be inadequate to support sufficient tissue Se stores.

The increased liver Cu concentrations, concurrent to lesser liver Fe concentrations for ITM- vs. saline-treated calves, has biological relevance, even though they are both within normal adequacy ranges for cattle (Puls, 1988). Ceruloplasmin, the primary Cu transport protein in blood, is also an important mediator for the mobilization of iron stores within the body (Roeser et al., 1970). Therefore, although both ITM- and saline-treated calves had adequate liver Cu concentrations at each of the 3 sampling dates, the greater liver Cu values of the ITM-treated calves was likely linked to enhanced Cu-dependent biological processes in ITM- vs. saline-treated calves, as presented by less liver Fe concentrations in these calves.

A lack of treatment differences for liver Mn and Zn may be due to individual element metabolism, storage, and homeostasis. In early deficiency, small decreases in Zn concentrations of plasma and soft tissue can be observed; however, pronounced reductions are typically found in bone and hair (Underwood and Suttle, 1999). Sampling of these sites has particular limitations, such as collection in live animals (i.e., bone) and sampling variation (i.e., hair). Of the 4 trace minerals delivered in the ITM, Mn is the least concentrated in the liver. Although large increases in liver Mn concentrations can be achieved with supernutritional supplementation levels (liver Mn = 7.2 and 26.7 mg/kg DM for diets containing 40 and 1,000 mg/kg Mn, respectively; Jenkins and Hidiroglou, 1991), only minor increases in are observed when cattle consume diets within a moderate Mn supplementation range (liver Mn = 8.2 and 9.4 mg/kg DM for diets containing 16 and 66 mg/kg Mn, respectively; Hansen et al., 2006). In further support of the current findings, a previous study using the same ITM product reported increases in liver Cu and Se concentrations of 56 and 265% vs. only 13 and 10% for Zn and Mn at 15 d following injection (Pogge et al., 2012).

Experiment 2

The transportation process, in combination with abrupt weaning, resulted in increased concentrations of APP in heifers assigned to both treatments. This reaction is part of a normal physiological process involving the activation of the acute phase reaction, which is an immunological response to stress stimuli, such as weaning, transportation, and vaccination (Arthington et al., 2003, 2008, 2013). This reaction is characterized by increased concentrations of circulating proinflammatory cytokines (Klasing and Korver, 1997) and APP (Petersen et al., 2004), which affect nutrient metabolism and animal growth (Johnson, 1997; Carroll and Forsberg, 2007). In the current study, heifers receiving ITM experienced greater peak concentrations of plasma ceruloplasmin, haptoglobin, and acid soluble protein, which were sustained for 3 to 7 d. This enhanced proinflammatory reaction corresponded to a reduced ADG over the 13-d post-transport evaluation period, compared to saline-injected heifers. Although the negative correlation between plasma APP concentrations and BW gain in cattle is not surprising (Qiu et al., 2007; Gifford et al., 2012), the increased APP concentrations of heifers receiving ITM was unexpected. A partial explanation relates to the Cu-dependent APP ceruloplasmin. Ceruloplasmin concentrations are reflective of Cu status and cattle with greater Cu status have greater net changes (baseline to peak) in plasma ceruloplasmin concentrations following a stress stimulus (Arthington et al., 1996). Thus, the increased Cu status of ITM- vs. saline-injected heifers is the likely explanation for the increased post-transport ceruloplasmin concentrations in ITM- vs. saline-injected heifers. However, the influence of ITM administration on increased concentrations of haptoglobin and acid-soluble protein are more difficult to explain. To our knowledge, neither protein has a direct link to the trace minerals found in the ITM product used in this study (i.e., Cu, Mn, Se, and Zn). It is possible that administration of the ITM product resulted in local tissue inflammation, which will produce an APP response in cattle when elicited by a nonspecific immunogen (Conner et al., 1988) or commercial vaccine preparation (Arthington et al., 2013). Moderate to severe injection site reactions have been previously reported in other ITM preparations (Boila et al., 1984; Chirase et al., 1994). If this were a contributing factor in the current study, then the tissue inflammation was not readily visible, as no signs of injection-site reactions were observed in the current study or another study involving the same ITM product (Arthington and Havenga, 2012). Another consideration is the potential impact of EDTA, an ingredient of the ITM product, on electing an inflammatory reaction. Since EDTA was not evaluated as a standalone treatment in the current study, its potential contribution to these response variables cannot be excluded. However, a Institue for Health and Consumer Products (2004) report found no evidence of inflammatory distress among rodents receiving high doses of EDTA orally and generally concluded EDTA to be exceedingly safe for a variety of industrial and pharmaceutical applications. Furthermore, EDTA has also been shown to possess anti-inflammatory actions by inducing antioxidant enzyme activity and reducing lipid peroxidation in rodents challenged with a carcinogenic substance (González-Cuevas et al., 2011).

Liver concentrations of Cu, Se, and Zn increased in ITM- but not saline-injected heifers on d 13, relative to d 0

values, and each was greatest in ITM- vs. saline-injected heifers on d 13 (Fig. 2). These elements are recognized as essential nutrients for the support of optimal immune function, particularly in stressed calves. Many stressors accompany normal beef production management, such as weaning, commingling, transportation, castration, vaccination, and feedlot entry. Each of these stressors may exasperate trace mineral balances leading to special considerations relative to the trace mineral nutrition of these calves (NRC, 1996). Management systems focused on improving the trace mineral status of stressed feeder calves have the potential for decreasing morbidity and improving performance. Previous studies have reported potential advantages of injectable combinations of Zn, Cu, Mn, and Se. These benefits have been associated with improved feed efficiency (Clark et al., 2006), reduced treatments for illness (Berry et al., 2000), and overall reduced morbidity treatment costs.

Experiment 3

Unlike the calves in Exp. 1, the heifers in Exp. 3 did not have access to free-choice supplemental trace minerals. Instead, 2.5 mL of ITM or sterile saline was administered (approximately one-half the recommended dosage rate) on d 0, 51, and 127 of the 177-d experiment. In addition, common white stock salt, without mineral fortification, was provided to all heifers in amounts to ensure free-choice consumption. A single evaluation of trace mineral status at the end of the study revealed differences only for liver Se concentrations. Assuming a voluntary DMI of 2.5% BW, heifers would have been consuming approximately 2.0 and 3.9 kg of supplement and fall forage DM daily, thus achieving calculated dietary concentrations of 0.09, 10.6, 39, and 62 mg/kg for Se, Cu, Zn, and Mn, respectively. Within this estimate, the dietary Cu and Se requirements were only minimally achieved considering recommendations for growing beef cattle (NRC, 1996) of 0.10, 10, 30, and 20 mg/kg for Se, Cu, Zn, and Mn, respectively. Based on liver Se concentrations at the end of the 177-d evaluation, saline-injected but not ITM-injected heifers were classified as Se deficient (0.48 vs. 0.88 mg/kg, DM basis), based on minimum adequacy guidelines suggested by Puls (1988; <0.61 mg/kg, DM basis). Because these heifers were reared together in the same pasture, the observed difference in Se status is most likely attributed to ITM injection. In addition, the increased ADG of ITM- vs. saline-injected heifers over the 177-d evaluation period could also be attributed to increased Se status of the ITM-injected heifers or Se deficiency of the saline-injected heifers. In another study at the same location (Arthington, 2008), growing beef steers consumed a basal diet with a Se concentration similar to the current study (approximately 0.10 mg/kg Se; DM basis) and had a 90-d ADG that did not differ ($P = 0.96$) from steers consuming the same basal diet fortified with Se (0.46 mg/kg; DM basis). In that study, liver Se concentrations at the end of the evaluation period (d 90) were greater for Se-supplemented vs. Se-unsupplemented steers (2.14 vs. 0.97 mg/kg; DM basis). In comparison to the current study, Se-unsupplemented steers had nearly 2 times the liver Se concentration than saline-injected heifers in the current study (0.97 vs. 0.48 mg/kg, DM basis). These differences are likely the result of the length of the study's evaluation period (90 vs. 177 d for the comparison study and the current study, respectively). Thus, the longer period of inadequate Se intake depleted tissue reserves and likely impacted the growth performance of heifers in the current study.

The increased humoral response of ITM- vs. saline-injected heifers has been reported previously (Arthington and Havenga, 2012). In that study, a commercially available modified live virus vaccine was used. Steers, previously seronegative for bovine herpesvirus – 1, experienced an increase in serum neutralizing antibody titers when injected with ITM vs. saline at the same time as vaccination. In the current study, heifers were injected with a novel immunogen (PRBC), which would not be considered a normally occurring antigen in beef production systems. Nonetheless, the heightened humoral immune response, as noted by increased PRBC titers in ITM-injected heifers, was surprisingly similar to the aforementioned study. The reasons for these responses are not well understood. It is possible that Se status may be a contributing factor. In the earlier study, serum but not liver mineral status was evaluated. Although serum Se status was greater in ITM- vs. saline-injected calves, both were within the range of adequacy as defined by Puls (1988) and Herdt and Hoff (2011). In comparison, liver tissue was used to characterize Se status in the heifers of the current study, which is a preferred sampling depot for the estimation of Se status (Sunde, 1997). These results revealed inadequate (saline injected) and marginally adequate (ITM injected) Se status classifications, thus indicating that Se status may be an important factor impacting humoral responses to antigen challenge. In further support, injectable Se, with or without vitamin E, has been shown to enhance humoral responses to antigens such as *Escherichia coli* and *Mannheimia haemolytica* (Droke and Loerch, 1989; Panousis et al., 2001). Collectively, these findings indicate that ITM or, more specifically, injectable Se may be beneficial to the humoral immune response in cattle. Alternatively, another explanation may be related to the heightened pro-inflammatory response realized in Exp. 2. Similar to the function of an adjuvant in a vaccine, ITM administration may be promoting an immune response through a heightened inflammatory reaction (Botrel et al., 1994), independent of the presence of trace minerals.

In conclusion, these studies indicate that ITM administration at birth and at 100 and 200 d of age results in increased Cu and Se status without affecting calf BW gain. Readministered at the time of weaning, ITM injection results in an increase in Cu, Se, and Zn status, a heightened APP reaction, and reduced BW gain over a 13-d postweaning period. When administered 3 times at a partial-dose rate over a 177-d growing period, heifers experienced increased Se status and BW gain and a heightened humoral immune response to PRBC injection.

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